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DETERMINATION OF THE LEVEL OF ZINC, SELENIUM, ARSENIC AND MERCURY IN SOME MARINE SPECIES FISH

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I. ABSTRACT

The present study assessed the concentration of Zinc (Zn) , Selenium (Se),Arsenic(As), and Mercury(Hg) in skeletal muscles of six marine fish species. Thirty (30) marine fish collected, including three fresh species **(***Pampus argenteus*, *Epinephelus tauvina*, and *Acanthopagrus latus*) from Al- Faw region, Basrah, Iraq, and three imported frozen species(*Sparus aurata, Scomber scombrus, and Mullus surmuletus)*obtained from markets in Baghdad ,five samples each species .Analysis of elements was conducted by using Atomic Absorption Spectrophotometer, while mercury was determined by the cold vapor technique. The results showed noticeable differences in the concentrations of the examined heavy metals among the studied species Zinc concentrations were highest in *Pampus argenteus* (4.508 ppm) and lowest in *Epinephelus tauvina* (1.834 ppm), while Selenium levels were highest in *Scomber scombrus* (0.066 ppm) and lowest in *Epinephelus tauvina* (0.024 ppm). Arsenic concentrations reached the highest value in *Epinephelus tauvina* (1.792 ppm), whereas the lowest level was observed in *Scomber scombrus* (0.051 ppm). Mercury levels were below the detection limit (0.005 ppm) in all examined samples .Although arsenic showed significant variation among species, zinc and selenium did not differ significantly. All recorded values were within internationally accepted safety limits, indicating that the examined fish species are safe for human consumption with respect to the analyzed elements

Keywords *: Marine fish, Zinc, selenium , Arsenic ,Mercury*

II. Introduction

Fish are an important part of the human diet because they provide high-quality protein, beneficial omega-3 fatty acids, and essential micronutrients. In many regions, particularly in developing countries, fish represent an economical and readily available source of animal protein. However, in addition to their nutritional value, fish tissues may accumulate heavy metals as a result of exposure to the surrounding aquatic environment. Heavy metals, defined as elements with densities greater than 5 g/cm³ include, essential trace elements such as zinc (Zn), cobalt (Co) and selenium (Se), as well as toxic metals such as mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb). Although zinc and selenium are essential for physiological functions, they can be harmful when their concentrations exceed safe limits [1, 2]. These elements accumulate in water and sediments and subsequently enter fish through dietary intake the digestive system or direct absorption across the gills and other permeable tissue, resulting in bioaccumulation within fish tissues and transferred to human through the food chain [3]. Zinc is a naturally occurring essential trace element that plays a critical role in the biological functions of all living organisms [4]. It is required for the activity of more than 300 enzymes involved in key metabolic processes, including carbohydrate, lipid, and protein metabolism, as well as cell division and the synthesis of DNA and proteins, which are fundamental for growth and tissue repair [5]**.** Selenium is an essential trace element and plays a crucial role in various physiological processes, including antioxidant defense through selenoproteins such as glutathione peroxidase, DNA synthesis, immune system function, and reproduction. Although required only in trace amounts, selenium is vital for maintaining cellular health and protecting tissues from oxidative stress [6-8].

In addition to its antioxidant and metabolic functions, selenium has been associated with anti-cancer effects, cardiovascular protection, enhancement of reproductive capacity, and anti-inflammatory properties.. However, the range between beneficial and toxic intake levels is narrow[9]. From an environmental perspective, selenium is a metalloid that enters ecosystems primarily through anthropogenic activities such as mining, metal smelting, and fossil fuel combustion. Once released, selenium can accumulate in aquatic environments, posing ecological and public health risks. It has the ability to bioaccumulate in aquatic food chains and become toxic at elevated concentrations, especially in areas with ongoing industrial pollution(Uddin et al.,2024). Among aquatic organisms, fish are of particular concern because of their importance in human diets. The accumulation of selenium in fish muscle tissue is especially relevant for food safety, as muscle represents the primary edible portion consumed by humans [10]. Moreover, selenium interacts with mercury in fish tissues, where adequate selenium levels may mitigate mercury toxicity through the formation of stable Hg–Se complexes and by influencing the selenium-to-mercury (Se:Hg) molar ratio, which is widely used as an indicator of mercury-related health risks associated with seafood consumption [11].Therefore, monitoring the levels of these elements in fish is important from both a nutritional and health perspective.

Accordingly, this study aimed to determine and compare the concentrations of zinc, selenium, arsenic, and mercury in six selected marine fish species to assess their safety for human consumption and to investigate potential differences between species.

III. Materials and Methods

2.1 Collection of Samples

A total of thirty marine fish samples were collected, including fresh and imported frozen species. The fresh fish (*Acanthopagrus latus*, *Pampus argenteus*, and *Epinephelus tauvina*) were obtained from Al-Faw District, Basrah, Iraq. The imported frozen fish (*Sparus aurata*, *Scomber scombrus*, and *Mullus surmuletus*) were procured from local markets in Baghdad. The samples were divided into six groups according to species , with five samples per species. The fish were dissected, and muscle samples were collected from one side of the body, especially from the dorsal muscle region. For the determination of zinc (Zn), selenium (Se), and arsenic (As), samples were analyzed using graphite furnace atomic absorption spectrophotometry (GF-AAS). Mercury (Hg) concentration was measured separately using cold vapor atomic absorption spectrophotometry (CV-AAS), which provides high sensitivity for mercury detection at very low concentrations

2.2 Bioaccumulation study of heavy metal

To evaluate the bioaccumulation of four metals(zinc ,selenium arsenic and mercury) in the skeletal muscle of the studied fish species Approximately of one gram were weighed and placed in clean plastic containers of 50 ml and kept in a freezer until taken to the examination in the laboratory. Then the samples were subjected to the following procedure [12].

A: Sample Preparation and Tissues Digestion

One gram of tissue was weighed and placed in a 100 ml conical flask. Five milliliters of concentrated nitric acid HNO_3, 70% and(1 m)l of perchloric acid $HClO_4$ were added, and the mixture was left at room temperature for one hour. The samples were then heated on a hot plate at 100°C until violet vapors appeared. After that, the temperature was gradually increased to 150–200°C until white fumes were observed. The appearance of a pale yellow solution indicated the completion of the digestion process.

B: Determination of Zn ,Se and As

Four working standard solutions of Zinc (0.5, 1, 1.5 and 2 µg/ ml) were prepared by serial dilution of the stock solution. Similarly , four standard solutions of Selenium (2, 4, 6, and 8 µg/ml) and arsenic (1, 5, 10, and 15 µg/ml) were prepared. Calibration curves were constructed for each element, and the concentrations in the digested samples were determined using atomic absorption spectrophotometry .

C: Determination of Hg

Mercury concentration was determined using cold vapor atomic absorption spectrometry (CVG-AAS) .Five standard working solutions of mercury (2, 4, 6, 8, and 10 ng/mL) were prepared to plot the calibration curve.

A portion of the digested sample solution (10 mL) was transferred to a conical flask, and 2 mL of 10% stannous chloride ($SnCl_2$) solution was added as a reducing agent to convert mercury(II) ions (Hg^{2+}) to elemental mercury vapor (Hg^0). The resulting mercury vapor was transferred by a gas stream to a quartz cell connected to the atomic absorption spectrometer.Absorption was measured at a wavelength of 253.7 nm using a 0.5 nm slit and a 3.0 mA lamp current. Mercury concentrations were calculated based on the calibration curve [13].

2.3 Statistical analysis

The statistical analysis of the biochemical concentrations of heavy metals was conducted using Microsoft Excel and IBM SPSS Statistics version 28. Descriptive statistics were expressed as mean ± standard deviation (SD). To evaluate differences in heavy metal levels across sources, both the one-sample independent t-test and one-way analysis of variance (ANOVA) were applied. A significance threshold of $p < 0.05$ was adopted to determine the presence of statistically significant differences among the tested groups.

IV. Results and Discussion

3.1 Zinc in marine fish muscle tissue

The results of examined samples revealed variation in zinc (Zn) concentrations among both fresh and frozen fish species, as presented in(Table 1) and illustrated in the (Figure 1). Although differences were observed among the studied species, these variations were not statistically significant at 0.05 level.

The highest Zn concentration was found in *Pampus argenetus* (4.508 ppm), followed by *Sparus auarta* (3.556 ppm),while moderate Zn values were observed in *Scomber scombrus* (3.218 ppm), *Mullus surmuletus* (3.110 ppm), and *Acanthopagrus latus* (2.642 ppm). The lowest Zn concentration was measured in *Epinephelus tauvina* (1.834 ppm). Despite these interspecific differences , all recorded values remained below the maximum permissible limit for zinc in fish set by Agriculture Organization(FAO), and World Health Organization (WHO) (50 ppm or mg/kg) [14],indicating that the analyzed fish species are safe for human consumption with respect to zinc content. The observed differences among species may attributed variability influenced by habitat , feeding ecology and trophic level .These results are line with [15, 16],and Zhang et al. (2020),,who reported that Zn levels in marine fish vary depending on ecological conditions and feeding strategies. [17] similarly noted that *P. argenteus* tends to accumulate higher zinc levels ,supporting the current results. In addition, [18] reported that zinc concentrations in fish muscle are generally low and remain well below regulatory limits, reflecting differences in bioaccumulation capacity and supporting the present findings that fish muscle is less prone to excessive zinc accumulation.

The imported frozen fish species exhibited relatively uniform zinc concentrations that uniformity may be attributed to the effects of freezing and storage, which slow biochemical activity and stabilize tissue composition. These findings are consistent with those of [19, 20], who reported stable Zn levels in frozen fish during storage. Additionally, [21], who reported that freezing conditions cause only minor fluctuations in trace metal concentrations, suggesting that the bioavailability and structural binding of zinc in fish tissues remain largely unaffected by storage temperature.

Table (1): Effect of fish species on Zn content (Mean ± SD) with LSD test at 0.05 level

Type of fish	Mean	SD	SE	Range (Minimum - Maximum)	LSD$_{0.05}$
Mullus surmulteus	3.110 b	1.551	0.694	3.55 (1.27 - 4.82)	1.834*
Scomber scombrus	3.218 b	0.643	0.287	1.62 (2.33 - 3.95)	
Sparus auarta	3.556 ab	1.297	0.580	3.48 (2.19 - 5.67)	
Acanthopagrus latum	2.642 b	1.792	0.801	4.15 (0.95 - 5.1)	
Epinephelus tauvina	1.834 b	1.071	0.479	2.28 (0.91 - 3.19)	
Pampaus argentus	4.508 a	1.708	0.764	3.7 (2.72 - 6.42)	

***There is significant difference between groups (p value < 0.05)**
SD: standard deviation, SE: standard error
-Groups with different letters are statistically different.

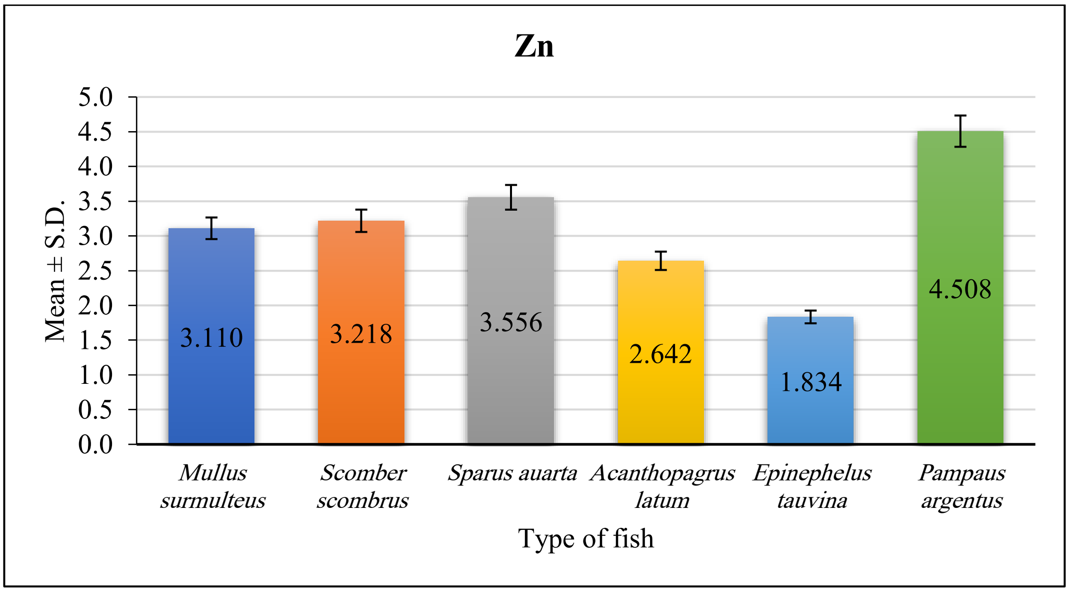

Figure (1): Zn content in different fish species

3.2 Selenium in meat fish

The results showed that selenium (Se) concentrations in both fresh and frozen fish species, as presented in (Table 2) and illustrated in the (Figure 2). Although the differences were not statistically significant, observable variations in Se concentrations were noted among the examined species .

The highest Se concentrations were recorded in *Scomber scombrus* (0.066 ppm) and *Acanthopagrus latus* (0.065 ppm),while comparable values were also found in *Sparus aurata* (0.056 pmm), *Pampus argenteus* (0.057 ppm), and *Mullus surmuletus* (0.047 ppm), all clustered. In contrast, the lowest Se concentration was observed in *Epinephelus tauvina* (0.024 ppm).Importantly, all recorded values fall within the natural selenium range typically found in fish muscle (0.18–0.68 ppm) and are considered safe for human consumption (FAO/WHO, 2024).

The absence of statistically significant differences among species, despite minor numerical variation, can be explained by the essential physiological role of selenium in fish. Selenium is incorporated into selenoproteins, such as selenocysteine, which are involved in antioxidant defense and thyroid hormone metabolism [22]. These proteins are conserved across fish species, resulting in relatively stable Se levels in muscle tissue, especially when fish inhabit similar environments or have comparable dietary exposure.

These findings are in agreement with previous studies reporting that selenium concentrations in fish muscle are generally maintained within narrow ranges due to physiological regulation, and tend not to vary significantly among species unless exposed to markedly different environmental conditions [23, 24]. Moreover, Se plays a protective role against mercury (Hg) toxicity, and the molar Se/Hg ratio is considered a critical bio indicator for Hg-related risks, further supporting the biological significance of stable Se concentrations in fish tissues(Peterson et al., 2009).

Table (2): Effect of fish species on Se content (Mean ± SD) with LSD test at 0.05 level

Type of fish	Mean	SD	SE	Range (Minimum - Maximum)	$LSD_{0.05}$
Mullus surmulteus	0.047 a	0.004	0.002	0.011 (0.042 - 0.053)	0.041 *
Scomber scombrus	0.066 a	0.031	0.014	0.076 (0.013 - 0.089)	
Sparus auarta	0.056 a	0.014	0.006	0.035 (0.043 - 0.078)	
Acanthopagrus latum	0.065 a	0.031	0.014	0.072 (0.03 - 0.102)	
Epinephelus tauvina	0.024 b	0.007	0.003	0.015 (0.016 - 0.031)	
Pampaus argentus	0.057 a	0.037	0.016	0.081 (0.015 - 0.096)	

***There is significant difference between groups (p value < 0.05)**
SD: standard deviation, SE: standard error
-Groups with different letters are statistically different.

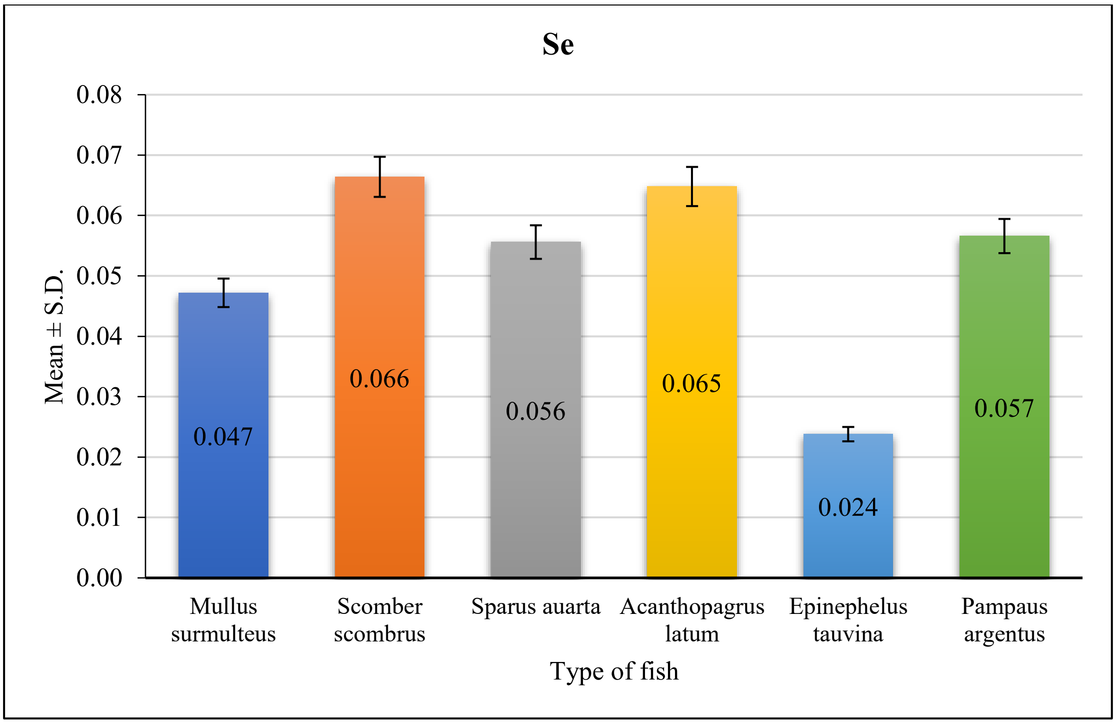

Figure (2): Se content in different fish species

3.3 Arsenic in meat fish

The analysis of arsenic levels demonstrated that variation in arsenic (As) concentrations among both fresh and frozen fish species, as presented in (Table 3) and illustrated in the (Figure3). The ANOVA results revealed a highly statistically significant difference between groups ($p < 0.05$). The highest arsenic (As) concentration was observed in *Epinephelus tauvina* (1.792 ppm), clearly distinguish it from all other species. *Acanthopagrus latus* (0.624 ppm) showed an intermediate level, while *Sparus aurata* (0.395 ppm) showed overlapping values between these two species , indicating moderate accumulation. In contrast, the lowest concentrations were measured in *Mullus surmuletus* (0.131 mg/kg), *Pampus argenteus* (0.110 ppm), and *Scomber scombrus* (0.051 ppm). Despite these interspecific, most recorded values remained below the maximum permissible limit for arsenic in fish established by FAO/ WHO (1.4 ppm or mg/kg; [25]. However *Epinephelus tauvina* ,as showed a higher concentration exceeding the recommended threshold . The results highlight a broad range of interspecies variability in arsenic accumulation, likely reflecting differences in feeding habits, habitat exposure, and detoxification capacity. These outcomes are consistent with prior studies reporting that certain predatory marine fish tend to accumulate higher As concentrations due to biomagnification in the food web [26].

The relatively high arsenic concentration of *E. tauvina* may be linked to its benthic feeding habits and its habitat near the seabed, where arsenic-rich sediments are more abundant. This explanation aligns with the findings of [27], who reported that bottom-dwelling fish such as *E. areolatus*, tend to accumulate higher arsenic levels compared to pelagic species in the Red Sea and Gulf of Aden. Moreover, [28] observed that arsenic levels in *E. tauvina* and other marine fish species from Egyptian markets remained below the European Commission safety thresholds set by the, confirming that natural environmental exposure rarely leads to toxic accumulation in edible fish tissue . In addition , [29] highlighted that most of the arsenic found in marine organisms exits in organic forms- primarily arsenobetaine, which are considered non-toxic and significantly less harmful than inorganic arsenic.

Table (3): Effect of fish species on As content (Mean ± SD) with LSD test at 0.05 level

Type of fish	Mean	SD	SE	Range (Minimum - Maximum)	$LSD_{0.05}$
Mullus surmulteus	0.131 c	0.034	0.015	0.084 (0.106 - 0.19)	0.409*
Scomber scombrus	0.051 c	0.024	0.011	0.055 (0.038 - 0.093)	
Sparus auarta	0.395 ab	0.160	0.071	0.348 (0.179 - 0.527)	
Acanthopagrus latum	0.624 b	0.179	0.080	0.39 (0.42 - 0.81)	
Epinephelus tauvina	1.792 a	0.722	0.323	1.71 (1.06 - 2.77)	
Pampaus argentus	0.110 c	0.078	0.035	0.166 (0.037 - 0.203)	

***There is significant difference between groups (p value < 0.05)**
SD: standard deviation, SE: standard error
-Groups with different letters are statistically different.

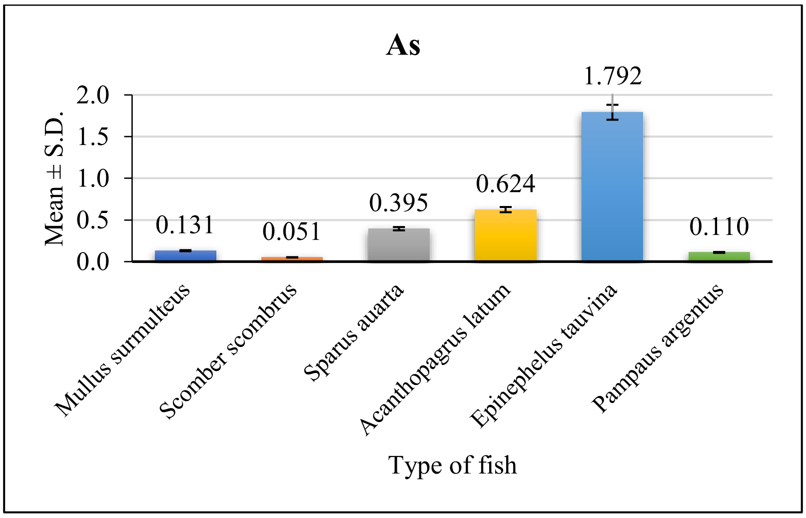

Figure (6): As content in different fish species

3.4 Mercury in meat fish

The results revealed that mercury (Hg) concentration in all examined fish including , the fresh samples species(*Acanthopagrus latus, Epinephelus tauvina* and *Pampus argenteus)* and the frozen samples(*Mullus surmuletus ,Sparus aurata* and *Scomber scombrus*) were below the detection limit (BDL = 0.005ppm) , as shown in(Table 7-3). This indicates that mercury levels in all samples was lower than the quantification capability of the analytical method used. As all measured values were below the detection limit, no statistical comparison was conducted. According to FAO/WHO, the maximum permissible limit for mercury in fish is 0.5ppm or mg/kg [25] . Since all analyzed samples were below this limit, the results confirm that the examined fish species are safe for human consumption and pose no health risk related to mercury exposure. The absence of detectable Hg across all species indicates that the suggests that the investigated fish are free from mercury contamination at levels of toxicological concern. This finding is particularly important given that mercury,especially in its methylmercury form is considered one of the most hazardous heavy metals due to its strong tendency to bioaccumulate and biomagnify within aquatic food webs. The lack of measurable Hg further indicates that the studied marine environment is relatively unpolluted with respect to mercury, and that these fish can be considered safe within the detection limits of the present study. These findings are in agreement with previous reports which showed that fish from less industrially impacted coastal waters often exhibit mercury concentrations below international safety thresholds [30].

Table (4): Accumulation of Mercury (Hg) in the six fish species

Type of fish	Hg
Mullus surmulteus	**B.D.L**
Scomber scombru	**B.D.L**
Sparus auarta	**B.D.L**
canthopagrus latmmu	**B.D.L**
,Epinephelus tauvina	**B.D.L**
Pampaus argentus	**B.D.L**

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