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RESEARCH ARTICLE **ENVIRONMENTAL SCIENCE**

Evaluation of the Concentration of Some Heavy Metals in Some Fish Species

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ABSTRACT

This study aimed to evaluate the concentration of iron (Fe), copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd), zinc (Zn), and chromium (Cr) in the tissue muscle of seven fish species samples collected from a local market of Sebha, including Sardina pilchardus, Scomber scombrus, Pagrus pagrus, Carangoides praeustus, Diplodus vulgaris, Boops Boops and Mullus barbatus, using atomic absorption spectroscopy (AAS). The results show that the concentrations of Fe, Cu, Ni, Pb, Cd, Zn, and Cr ranged from 1.17 to 7.87, 2.03 to 6.50, 1.43 to 4.00, 1.17 to 2.47, 1.43 to 9.33, 2.17 to 3.60, and 2.23 to 5.17 µg/g, respectively. dry weight, in descending order. The results show that the concentration of Fe, Cu, and Zn were within the permitted limits; however, the concentration of Ni, Pb, Cd, and Cr exceeded the allowed limits. The evaluation of metal sources in fish tissue was conducted using correlation analysis, the principal component analysis (PCA), and cluster analysis. The findings demonstrate that the presence of these contaminants is linked to human activities and the discharge of lubricants in fishing areas.

تقييم تركيز بعض المعادن الثقيلة في بعض أنواع الأسماك

سعيدة معتوق علي محمد[1،*]، منصور عويدات سالم[2]

الكلمات المفتاحية
انواع الاسماك
المعادن الثقيلة
التركيز
تحليل المكون الاساسي

الملخص

هدفت هذه الدراسة إلى تقييم تركيزات الحديد (Fe)، والنحاس (Cu)، والنيكل (Ni)، والرصاص (Pb)، والكادميوم (Cd)، والزنك (Zn)، والكروم (Cr) في عينات الأنسجة العضلية لسبعة أنواع من الأسماك تم جمعها من السوق المحلي في مدينة سبها، وشملت هذه الدراسة، Sardina pilchardus Scomber scombrus, Pagrus pagrus, Carangoides praeustus, Diplodus vulgaris, Boops Boops and Mullus barbatus ، وذلك باستخدام مطيافية الامتصاص الذري (AAS). أظهرت النتائج أن تركيزات Fe وCu وNi وPb وCd وZn وCr تراوحت من 1.17 -7.87، 2.03 - 6.50، 1.43- 4.00، 1.17 - 2.47، 1.43 - 9.33، 2.17 - 3.60، 2.23 - 5.17 ميكروغرام/غرام، على التوالي. الوزن الجاف، بترتيب تنازلي. أظهرت النتائج أن مستويات Fe وCu وZn كانت ضمن الحدود المسموح بها؛ إلا أن مستويات Ni وPb وCd وCr تجاوزت الحدود المسموح بها. أُجري تقييم مصادر المعادن في أنسجة الأسماك باستخدام تحليل الارتباط، وتحليل المكونات الرئيسية (PCA)، وتحليل المجموعات. أظهرت النتائج أن وجود هذه الملوثات مرتبط بالأنشطة البشرية وتصريف مواد التشحيم في مناطق الصيد.

Introduction

Aquatic ecosystems play a vital role in environmental research as they host over 90% of the planet's living organisms. Fish are especially vulnerable to pollution. They serve as a crucial source of protein that should be incorporated into our diets. Fish provide essential fatty acids that may help lower the risk of heart disease and stroke. Eating fish can aid in reducing blood cholesterol levels and supplying important vitamins and minerals [1 - 3]. In various regions worldwide, fish constitute a key food source that addresses food shortages in about 90% of cases. The significant protein content, fat-soluble vitamins, polyunsaturated fatty acids, and vital minerals found in fish make it an essential part of a balanced diet. As awareness of its nutritional advantages increases, so has fish consumption [4,5]. To acquire essential vitamins, minerals, and omega-3 fatty acids, consuming fish at least twice a week is advisable. Fish naturally contain metals such as Fe, Zn, and Cu. However, they can also harbor harmful metals like Hg, Cd, and Pb, which typically exist in small quantities [6]. The contamination of aquatic ecosystems from diverse sources, such as industrial effluents, agricultural runoff, landfill leakage, and natural geological activities, presents a considerable health threat due to toxic metals [7]. Heavy metals can accumulate in the fish tissues, leading to public health issues for those who consume seafood. Fish can absorb heavy metals from sediments, which serve as reservoirs, consequently impacting the whole food web. Gaining insight into these matters is essential for safeguarding public health and aquatic ecosystems [8,9]. Several environmental factors, including the release of significant amounts of sewage into the ocean, can lead to fish contamination. Heavy metals like Pb, Cd, Cr, Ni, and Zn are commonly found in high levels in

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sewage [10,11]. The decomposition of industrial and domestic waste results in the buildup of heavy metals in aquatic environments as sediments. Heavy metals break down slowly and have a long half-life, which makes them a persistent danger to water quality. Due to their non-biodegradable nature, they can act as potential bio-accumulators and biomagnifiers in the food chain. The buildup of heavy metals in biological systems, especially in fish, can arise from both acute and chronic exposure, resulting in their accumulation in critical organs [12-15]. Several factors like the concentration of metals in the water, exposure duration, and ecological aspects such as fish species and feeding behavior, affect the bioaccumulation of heavy metals in fish tissues. Research has shown that fish muscles typically have lower concentrations of heavy metals in comparison to other organs, such as the liver [16–20]. The degree of heavy metal accumulation in fish species and various tissues is affected by several factors, including the biological environment, fish mobility, feeding patterns, and trophic positions [21]. To assess potential health dangers and pollution levels in contaminated areas, it is necessary to evaluate the bioavailability of heavy metals in aquatic species, as they indicate environmental contamination. Moreover, the accumulation of heavy metals in fish tissues can negatively impact their health [22]. Libya's Mediterranean coastline measures 1,950 km, and the lagoons are vital for maintaining marine biodiversity and productivity [23]. Numerous aquatic species benefit from these areas as they provide food and nursery grounds due to the rich marine vegetation present. Additionally, this location is noted as one of the most lucrative commercial fishing zones in North Africa. Near Tripoli port, B. boops, H. distant, S. aurita, S. undosquamis, and S. japonicus collected exhibited low levels of Zn, Cu, Fe, and Cd. However, mercury concentrations exceeded permissible limits for P. radiata, S. scriba, and E. marginatus. Pearl oysters, cuttlefish sediments, and marine plants found along the Farwa seashore have attributed the presence of these metals in the lagoon to chemical waste disposed of near the island's coast [24 - 26]. In 2017, four fish species from Misrata, Libya, displayed cadmium, lead, zinc, and copper levels above the allowable values [27]. People in southern Libya often buy fish caught in the Mediterranean Sea, which are then sold at local marketplaces; however, these fish have not been analyzed for their heavy metal content to our knowledge. Therefore, this study aimed to evaluate the concentrations of Zn, Cu, Fe, Cr, Co, Cd, Pb, and Ni in the muscle tissues of different fish species frequently sold in the Sebha market.

Materials and Methods

Sample collection

Seven fish species, including P. pagrus, D. vulgaris, S. pilchardus, S. scombrus, B. boops, C. paeustus, and M. barbatus, were obtained from a local market in Sebha City in the summer of 2024. These samples were sourced from the Mediterranean shores of Libya and placed in clean polyethylene bags prior to being transported to the laboratory, where they were kept frozen until further analysis.

Extraction and evaluation of heavy metals in fish tissue

The extraction of heavy metals from the fish muscles was performed following the method described in [28]. After thawing, the fish samples were thoroughly rinsed with distilled water to eliminate any contaminant particles. The muscles were then removed and cut into small pieces using a ceramic knife that had been cleaned and sterilized according to established procedures. The fish muscles were dried overnight in a drying oven set at 120–125 °C. A precise 2.0 g of the dried fish muscle was weighed into a porcelain crucible and heated in a muffle furnace at 200 °C for 90 minutes. The temperature was gradually increased until it reached 350 °C, where it remained constant for 4 hours. The dried samples were treated with a 10 ml mixture of HCl and HNO_3 (1:1) and heated on a hot plate using an electric device. Once the solution had evaporated to near dryness, the filtrate was filtered through Whatman No. 40 filter paper and then placed into a 25 mL volumetric flask, which was filled with 3% HNO_3. The heavy metal content was quantified by analyzing the extracted samples with an atomic absorption spectrophotometer (Analytik Jena, NOVA-A400, Germany). All standard solutions were prepared using analytical-grade chemicals and ultrapure water to evaluate the heavy metals present in the samples. The standard solutions were supplied by BDH UK with a purity level of 99.9%.

Multivariate Statistical Analysis

The data were analyzed using SPSS version 26.01 to determine statistical significance. The mean and standard deviation were calculated by assessing the concentrations of heavy metals across various fish species. A one-way ANOVA was utilized to identify significant differences in metal concentrations. In addition, multiple variables were applied to compute post-hoc Duncan tests and Pearson correlation coefficients. Statistical significance was determined with a $p< 0.05$.

Results and discussion

Heavy metals concentration in fish muscle tissue

Fish muscles were chosen for heavy metal analysis because they are the only edible tissue that can accumulate harmful substances that could harm consumers. Table 1 demonstrate the concentration of heavy metals in the muscles tissue of the examined fish species. The results show that the concentrations of Fe, Cu, Ni, Pb, Cd, Zn, and Cr ranged from 1.17 to 7.87, 2.03 to 6.50, 1.43 to 4.00, 1.17 to 2.47, 1.43 to 9.33, 2.17 to 3.60, and 2.23 to 5.17 µg/g. dwt, respectively, with a mean ±SD of 4.98±3.12, 4.13±1.86, 2.45±0.85, 1.60±0.54, 4.50±3.01, 3.04±0.56 and 3.28±1.31 g/g. dwt, respectively. The results shown that the concentrations of Fe, Cu, and Zn were within the permissible limits, while the concentration of Ni, Pb, Cd, and Cr were above the recommended permissible limits [29-32]. It is shown that D. vulgaris, S. pilchardus, S. scombrus, and C. paeustus accumulated relatively high concentrations of these heavy metals. Concentrations of heavy metals can differ significantly between fish species because of variations in size, growth cycles, and feeding habits [33, 34]. High concentrations of heavy metals were previously reported in fish tissue from the northeastern Mediterranean Sea [35-37]. Specifically, high concentrations of Pb, Cd, and Cr that exceeded the limits set by EC regulations were detected in fish samples collected from the Siracusa area in Sicily, Italy, located 20 km south of the industrial complex in Augusta-Priolo-Melilli [38 – 41]. Similarly, high levels were found in three fish species collected from the Barekese reservoir in Kumasi, Ghana [42-44]. Variations in concentrations of copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), and lead (Pb) were observed among four fish species in Kuala Terengganu, Malaysia, during both non-monsoon and monsoon seasons. These differences were attributed to changes in temperature, tides, and strong winds associated with the monsoon season [45-46]. The high concentrations of

Table 1: The concentration of heavy metal mean ± SD (µg/g. dwt) in the muscles of fish species

Heavy metals	Fish species (n=3)							Range	Mean ± SD	Permissible limit
	P. pagrus	*D. vulgari*	*S. pilchardus*	*S.Scombrus*	*B. boops*	*C. praeustus*	*M. barbatus*			
Fe	1.73	7.07	7.07	7.87	1.17	7.83	2.13	1.17- 7.87	4.98±3.12	30[b]
Cu	3.17	6.10	3.73	2.07	5.33	2.03	6.50	2.03 - 6.50	4.13±1.86	30[a]
Ni	2.03	2.20	4.00	3.13	1.43	2.23	2.10	1.43 - 4.00	2.45±0.85	0.5-0.6[c]
Pb	2.47	1.43	1.23	1.30	1.17	2.27	1.30	1.17 - 2.47	1.60±0.54	0.5[a]
Cd	7.43	9.33	1.43	3.20	3.23	5.37	1.53	1.43 - 9.33	4.50±3.01	0.5[a]
Zn	3.23	3.33	3.60	2.37	3.10	2.17	3.50	2.17 - 3.60	3.04±0.56	40[a]
Cr	5.17	5.03	3.43	2.60	2.23	2.23	2.30	2.23 - 5.17	3.28±1.31	0. 5[b]

(a)= [39]; (b)= [38]; (c)= [37].

Ni in the fishing area may be linked to the combustion of diesel and lubricant oils, as well as brake abrasion [47 - 49]. Our findings are consistent with those previously reported [44, 50 -55].

Statistical analysis

Correlation coefficients

The correlation coefficients of heavy metals found in fish samples are summarised in Table 2. The evaluation of the relationships between different metals was done by calculating these coefficients. The results indicate significant positive correlations between Cr and Ni (r = 0.640), Cu and Zn (r = 0.678), and Cd and Cr (r = 0.747) at $p < 0.01$. A positive correlation was also observed between Pb and Cd (r = 0.534) at $p < 0.05$. The relationship between Fe and Cu was negatively correlated (r = -0.475), and Cu and Pb (r = -0.480). Fe and Cr did not show any correlation. The positive correlations between the metals suggest that they are interconnected and may have originated from the same sources in the study area [56-60]. The strong correlations between heavy metals suggest that they are interdependent, likely because they share similar sources and behaviours when exposed to environmental influences [61-64]. This pattern is likely a result of discharges from non-point sources that contribute heavy metals to the fishing area [12]. Our findings are consistent with those previously reported [65 – 67].

Table 2: The Pearson correlation coefficient matrix for heavy metal concentrations.

Heavy metals	Correlation coefficient						
	Fe	Cu	Ni	Pb	Cd	Zn	Cr
Fe	1						
Cu	-.475*	1					
Ni	.640**	-.376	1				
Pb	-.021	-.480*	-.220	1			
Cd	.109	.006	-.377	.534*	1		
Zn	-.354	.678**	.111	-.282	-.090	1	
Cr	.000	.104	.032	.366	.747**	.413	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Principal component analysis (PCA) and Factor analysis (FA)

The Kaiser Criterion was employed to perform Principal Component Analysis (PCA) and identify components with eigenvalues exceeding 1.0. The analysis revealed that 86.36% of the examined fish species had potential sources of heavy metals that were elucidated. The observed connections between the heavy metals indicate that they may have shared origins and pathways, providing a basis for analyzing the similarity of the pollution sources investigated [68- 71]. The variances were 33.50%, 31.71%, and 21.15% for the first three principal components, as explained in Table 3. The first factor, which included Cd, Cr, and Pb, accounted for 33.50% of the variance and was primarily correlated with human activities, suggesting a shared source of pollution [53]. The second factor, which accounted for 31.71% of the variance, consisted of zinc (Zn) and copper (Cu). The third factor, comprising 22.1% of the data, was composed of Ni and Fe, which exhibited a strong positive correlation (Table 2). These two heavy metals likely originate from similar pollution sources. Cr, Cd, and Ni levels could be increased by inadequate waste disposal [43]. Our findings align with those from previous studies [49].

Cluster analysis

Cluster analysis is a method that can effectively understand and detect patterns in data by highlighting existing similarities. The heavy metals found in the muscle tissues of the fish species studied were classified into two primary clusters according to this study, as depicted in a dendrogram (Fig. 1). Cluster 1 consists of cadmium (Cd) and iron (Fe). Similarly, Cluster 2 is split into two sub-clusters. Sub-cluster 2A consists of Ni, Zn, 19 Cr, and Pb, whilst Sub-cluster 2B only has Cu. The arrangement suggests that the heavy metals may come from a common source of pollution, likely connected to human activities such as vessel discharges and oil spills [12].

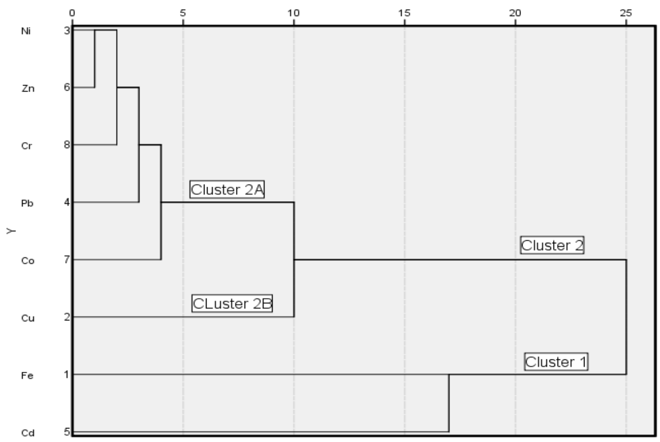

Fig. 1: Dendrogram depicting the grouping of heavy metals among fish species

Conclusion

This research has presented data on the levels of heavy metals in the muscle tissues of various fish species sold in the

Table 3: Principal Component Analysis (PCA) of Heavy Metals in Fish Species

Comp.	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Cd	2.345	33.496	33.496	2.345	33.496	33.496	2.152	30.747	30.747
Cr	2.220	31.712	65.208	2.220	31.712	65.208	2.060	29.435	60.182
Pb	1.480	21.149	86.357	1.480	21.149	86.357	1.832	26.176	86.357
Zn	.665	9.493	95.850						
Cu	.216	3.092	98.942						
Ni	.062	.890	99.832						
Fe	.012	.168	100.000						

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 5 iterations.

Heavy metals	Components		
	1	2	3
Cd	.919	-.089	-.140
Cr	.894	.362	.145
Pb	.675	-.523	-.148
Zn	.098	.937	.007
Cu	-.085	.814	-.435
Ni	-.178	.090	.946
Fe	.069	-.316	.828

Extraction Method: Principal Component Analysis.

Sebha City market, Libya. The results obtained indicate that the concentrations of Fe, Cu, and Zn were within the permissible limits. In contrast, the concentrations of Ni, Pb, Cd, and Cr exceeded the FAO/WHO permissible limit in some fish species, which can pose a danger to consumers of these fish. Long-term exposure can lead to deleterious effects. The sum of mean concentrations of all seven HMs in the fish species was found in the descending order of D. vulgaris > P. pagrus > S. pilchardus > C. praeustus > S. scombrus > M. barbatus > B. boops. The extent of heavy metal accumulation was found in the descending order of Fe > Cd > Cu > Cr > Zn > Ni > Pb in all fish species. The positive and significant correlations ($p < 0.05$) among the metals in the studied fish species indicate the possibility of contamination from a common source. Although the results reveal higher heavy metal content in fish, it is essential to monitor the metal concentration in fish continuously.

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