



Research Article

Estimation of Microbial Load and Some Mineral Elements in Some Local Fish Species of Baghdad City

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Abstract | The present study aimed to estimate the microbial load and quantify selected mineral elements—calcium, iron, and zinc—in three locally consumed freshwater fish species (*Barbus grypus*, *Cyprinus carpio*, and *Silurus glanis*) collected from retail markets in Baghdad City. A total of 90 fish samples (30 per species) were analyzed using standard microbiological and spectrophotometric techniques. Descriptive statistics indicated species-specific variations in microbial load and mineral content. ANOVA also indicated differences ($p < 0.001$) among species according to all the variables in question. The use of post-hoc Tukey HSD tests indicated that *Silurus glanis* had the highest microbial and mineral content whereas the lowest microbial and mineral content belonged to the *Cyprinus carpio*. Pearson correlation analysis indicated strong positive correlations of all minerals especially calcium and iron which was observed to be positively correlated ($r = 0.711$ and p value < 0.001). These results offer useful suggestions on food safety surveillance and nutritional characterization of freshwater fish within the urban setting.

Received | August 17, 2025; **Accepted** | October 16, 2025; **Published** | April 03, 2026

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Citation | Mohaisen, J.M., R.M.K. Al-Zaidi and R.K.B. Almurjan. 2026. Estimation of microbial load and some mineral elements in some local fish species of Baghdad city. *Sarhad Journal of Agriculture*, 42(2): 572-583.

DOI | <https://dx.doi.org/10.17582/journal.sja/2026/42.2.572.583>

Keywords | Haor, Problem confrontation, Scavenging duck, Sustainable livelihood, Women empowerment



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Introduction

Fish forms an important part of the human diet due to the good quality protein content and several micro nutrients especially minerals such as calcium, iron, zinc among others (Naeem *et al.*, 2010; Naeem *et al.*, 2016). All these factors have dire implications on the physiology of human beings; calcium is part in the mineralization and iron in the transport of oxygen in the blood and zinc serves as a co-factor in catalysing different enzymes (Abbas *et al.*, 2025). Nevertheless, fishes too are microbial vectors and therefore, can very well be the source of public health

risks when handled improperly or when harvested in bad water sources (Al-Zaid *et al.*, 2023). Cities such as Baghdad access freshwater rivers and lakes that have been polluted by people through domestic wastes, agricultural discharges, and industrial wastes (Ajeena *et al.*, 2024). The said pollution has the capability of enhancing microbial attack as well as altering mineral profiles in aquatic fauna hence hindering food safety and nutrition values (Al-Gburi *et al.*, 2017).

Alternatively, these discrepancies in microbial and elemental composition can be both species-specific, varying on trophic level and habitat priority and

types, with certain resource metabolism (El-Naggar and Eldeen, 2018). Quantitative estimation of microbial load, typically expressed in logarithmic colony-forming units per gram (log CFU/g), provides a benchmark for evaluating the hygienic quality of fish and the effectiveness of post-harvest handling practices (Basha *et al.*, 2019). Simultaneously, assessment of essential minerals can offer insights into the nutritional adequacy and ecological health of the aquatic environment (Burger and Gochfeld, 2005). Prior studies have emphasized the bioaccumulation potential of fish in absorbing minerals and contaminants, highlighting the need for routine monitoring, especially in regions with limited regulatory oversight (Amlacher, 2004; Al-Fartosi *et al.*, 2010). However, data specific to local freshwater organisms in Iraq, particularly in Baghdad City, are few. This gap creates hindrances toward evidence-based policymaking for food safety and public health intervention.

Objectives of the present study include:

- Determining the microbial load of these three commonly used fish species: *Barbus grypus*, *Cyprinus carpio*, and *Silurus glanis*, sold in Baghdad;
- Determining the concentrations of some mineral elements, calcium, iron, and zinc;
- Investigating interspecies differences and correlations between tested variables using robust statistical methods.

This interdisciplinary study between microbiological parameters and mineral parameters adds to the relatively scarce literature on food safety and nutritional surveillance of freshwater fish for the benefit of both public health and environmental management. Fish give vital proteins and micronutrients to all populations throughout the world. In many areas where micronutrient deficiencies exist, fish represent key dietary elements, especially deficient in iron and zinc, due to their high nutritive value and digestibility (Hussain *et al.*, 2016). Several studies have shown the microbial load and mineral content variation with species of freshwater and marine species, commonly associated with trophic level, habitat, and feeding habit (Karadede and Ünlü, 2007).

The microbial load and its quality vis-a-vis fish are major determinants of food safety and public health. Bacterial contamination is usually the result of water quality, post-harvest handling, and transportation

conditions (Hussain *et al.*, 2016). According to Hussain *et al.* (2017), retail fish from urban markets in South Asia had high total bacterial counts that exceeded safety limits set internationally. Microbial evaluation is generally done through total viable counts (TVC) expressed in log CFUs per grams; values above 5.0 log CFU/g are typically considered unacceptable from a microbiological point of view for human consumption (Khan *et al.*, 1992). Species like *Silurus glanis* staying down at the bottom were found being loaded with higher microbes because it gets into contact with sediments due to its benthic feeding habits (Lall, 2022).

While there is scant literature to establish a relationship directly between the microbial load and mineral content, a few emerging hypotheses implicate these two phenomena towards a bioaccumulation pattern, thereby indicating that they may have some association with microbial colonization. A positive correlation may imply some common environmental factor, such as water quality or feeding zone (Javed and Usmani, 2015). Pearsonian correlation analyses are often used to understand such interdependencies, revealing ecological as well as public health aspects (Ford and Newton, 2020).

There is a minor but continuing body of research on Iraq's aquatic environment. Al-Fartosi *et al.* (2010) found elevated heavy metals' accumulation in fish in southern Iraqi marshes and warned about the long-term ecological and human health consequences of such pollution. Karadede-Akin and Ünlü (2007) found a similar trend in the Tigris River, with levels of metals and microbial load above WHO thresholds. Such findings highlight the need for an integrated monitoring program in which both microbiological and mineral analyses are carried out (Fawole *et al.*, 2007). But still, there are data gaps in local studies from urban centers like Baghdad on species of fish common in markets (Olmedo, 2013). The present study will fill this gap (Liu *et al.*, 2021). Therefore, the aim of study was to evaluate simultaneously the microbial and mineral profiles of commonly consumed freshwater fish sold in the markets of Baghdad.

Methodology

Study design and sampling

A cross-sectional laboratory-based analytical study was conducted to determine microbial load and

mineral element concentrations for calcium, iron, and zinc in the three freshwater fishes common to the selling outlets of Baghdad City. A total number of 90 samples of fresh fish, i.e., 30 for each species, were randomly bought from different local fish markets and transported to a laboratory under aseptic chilled conditions at 4 °C, not exceeding 3 hours since collection.

Sample preparation

The samples were washed with sterile distilled water in order to remove any surface contaminants, and the fish were then descaled. Fish muscle tissue (dorsal region) was excised using sterile instruments, homogenized, and subjected to both microbiological and mineral content analyses. Each homogenate was divided into two portions: one for microbial evaluation and the other for chemical digestion and mineral quantification.

Estimation of microbial load

The microbial load was enumerated using the Standard Plate Count (SPC) method as per the ISO 4833-1:2013 standard. Serial dilutions from 10^{-1} to 10^{-6} were made; two duplicate plate aliquots were inoculated on Plate Count Agar (PCA). Plates were incubated at 30 ± 1 °C for 72 hours. Colony-forming units (CFU) were counted and expressed as Log CFU/g of fish tissue.

Mineral content determination

The concentrations of Ca, Fe, and Zn were analyzed using AAS (PerkinElmer Model AAnalyst 400) after wet-acid digestion of the samples with a mixture of HNO_3 and HClO_4 in a ratio of 3:1. About 5 g of the sample was digested at 150 °C until a clear solution was obtained. The digested sample was filtered, diluted with distilled water to 50 mL volume, and aspirated for AAS reading. Calibration was done by preparing standard solutions from certified reference materials.

Statistical analysis

Jamovi v2.4.8 was used to carry out statistical analysis of all data. Descriptive statistics of microbial load and the concentrations of minerals were computed i.e. the mean, standard deviation, median and variance. Differences between species were analyzed with the One-Way ANOVA test with Tukey HSD post-hoc test on the specific inter species difference with level of significance $p < 0.005$. In addition to this, Pearson correlation coefficient (r) was also achieved

to correlate microbial load and mineral concentration. Prior to the actual analysis, ANOVA assumptions of normality and test of homogeneity were assessed based on Shapiro-Wilk and Levene Test. Analytical results were also complemented by creation of graphical representations like box plots and correlation matrix.

Results and Discussion

Descriptive statistics

The descriptive statistics of microbial load and mineral concentration are reported in Table 1 on three types of fish *Barbus grypus*, *Cyprinus carpio*, and *Silurus glanis*. *Silurus glanis* showed the highest mean microbial load of 5.96 log CFU/g as compared with 5.47 log CFU/g by *Barbus grypus* and 4.94 log CFU/g by *Cyprinus carpio*. Similarly, with respect to calcium (24.2 mg/100g), iron (12.7 mg/100g) and zinc (6.46 mg/100g), the highest mineral concentrations were observed in *Silurus glanis*.

Microbial Load: The greatest microbial load was observed in *S. glanis* (mean = 5.96), then *B. grypus* (5.47) and *C. carpio* (4.94) providing proof of major differences between different species.

Calcium Content: Calcium also differed among species and in the case of these fish the range was between 19.8 mg/100 g in *C. carpio* to 24.20 mg/100 g in *S. glanis* and this evidently shows species-specific mineral deposition. **Iron Content** Actually the *S. glanis* is highest (12.7 mg/100g) as metabolic and dietary demand in the carnivorous species was greater. **Zinc Content:** Ranged between 4.99 mg /100 g in *C. carpio* and 6.46 mg /100 g in *S. glanis*.

No values were missing in all the data ($n = 30$ by species), the standard deviations indicate reasonable homogeneity of groups and hence minimum and maximum values in the combined data indicate the biologically realistic range with no apparent outliers (Figure 1).

All of these descriptive results indicate considerable interspecies variability of microbial and mineral profiles. Such differences become important in nutritional planning of these species, monitoring the safety of foods, and performing ecological assessment of fish in urban aquatic systems. The data also serve as grounds for carrying out inferential statistical analyses (such as ANOVA and post-hoc tests) that further substantiate these preliminary observations (Figure 2).

Table 1: *The descriptive statistics of microbial load and mineral concentration*

Descriptives					
	Species	Microbial_Load (log CFU/g)	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
N	Barbus grypus	30	30	30	30
	Cyprinus carpio	30	30	30	30
	Silurus glanis	30	30	30	30
Missing	Barbus grypus	0	0	0	0
	Cyprinus carpio	0	0	0	0
	Silurus glanis	0	0	0	0
Mean	Barbus grypus	5.47	22.4	11.3	5.83
	Cyprinus carpio	4.94	19.8	10.0	4.99
	Silurus glanis	5.96	24.2	12.7	6.46
Std. error mean	Barbus grypus	0.0561	0.259	0.192	0.133
	Cyprinus carpio	0.0493	0.255	0.181	0.116
	Silurus glanis	0.0635	0.252	0.142	0.0859
Median	Barbus grypus	5.53	22.4	11.4	5.85
	Cyprinus carpio	4.93	19.9	10.0	4.98
	Silurus glanis	5.91	24.2	12.8	6.47
Mode	Barbus grypus	5.02 ^a	20.2 ^a	9.69 ^a	4.38 ^a
	Cyprinus carpio	4.43 ^a	17.1 ^a	7.38 ^a	3.66 ^a
	Silurus glanis	5.03 ^a	21.1 ^a	11.2 ^a	5.59 ^a
Standard deviation	Barbus grypus	0.307	1.42	1.05	0.726
	Cyprinus carpio	0.270	1.40	0.992	0.636
	Silurus glanis	0.348	1.38	0.777	0.471
Variance	Barbus grypus	0.0944	2.02	1.11	0.528
	Cyprinus carpio	0.0729	1.95	0.984	0.405
	Silurus glanis	0.121	1.90	0.604	0.222
Minimum	Barbus grypus	5.02	20.2	9.69	4.38
	Cyprinus carpio	4.43	17.1	7.38	3.66
	Silurus glanis	5.03	21.1	11.2	5.59
Maximum	Barbus grypus	6.16	26.1	15.1	7.42
	Cyprinus carpio	5.47	22.8	11.6	6.72
	Silurus glanis	6.64	27.2	14.5	7.63

^a More than one mode exists, only the first is reported

The results obtained using one-way ANOVA indicated statistically significant results at $p < 0.001$ level in the three-species group of microbial load, calcium, iron, and zinc concentrations (Table 2). By use of Post-hoc using Tukey HSD results, it was clear that all the pairwise differences were significant ($p < 0.005$) hence confirming differences existing between species (Figure 3). The other step that was taken was to provide a clarification on which fish species were not similar to the rest in terms of microbial loading and mineral composition; thus, none of those fish could be described as similar, a Tukey HSD test

was executed after one-way ANOVA (Figure 4). The findings are explained as follows: Tukey's HSD indicated significant differences ($p < 0.001$) in all species:

Table 2: *Results of one-way analysis of variance (Welch's)*

	F	df1	df2	p
Microbial_Load (log CFU/g)	80.9	2	57.4	<.001
Calcium (mg/100g)	73.4	2	58.0	<.001
Iron (mg/100g)	68.1	2	56.9	<.001
Zinc (mg/100g)	51.3	2	56.0	<.001

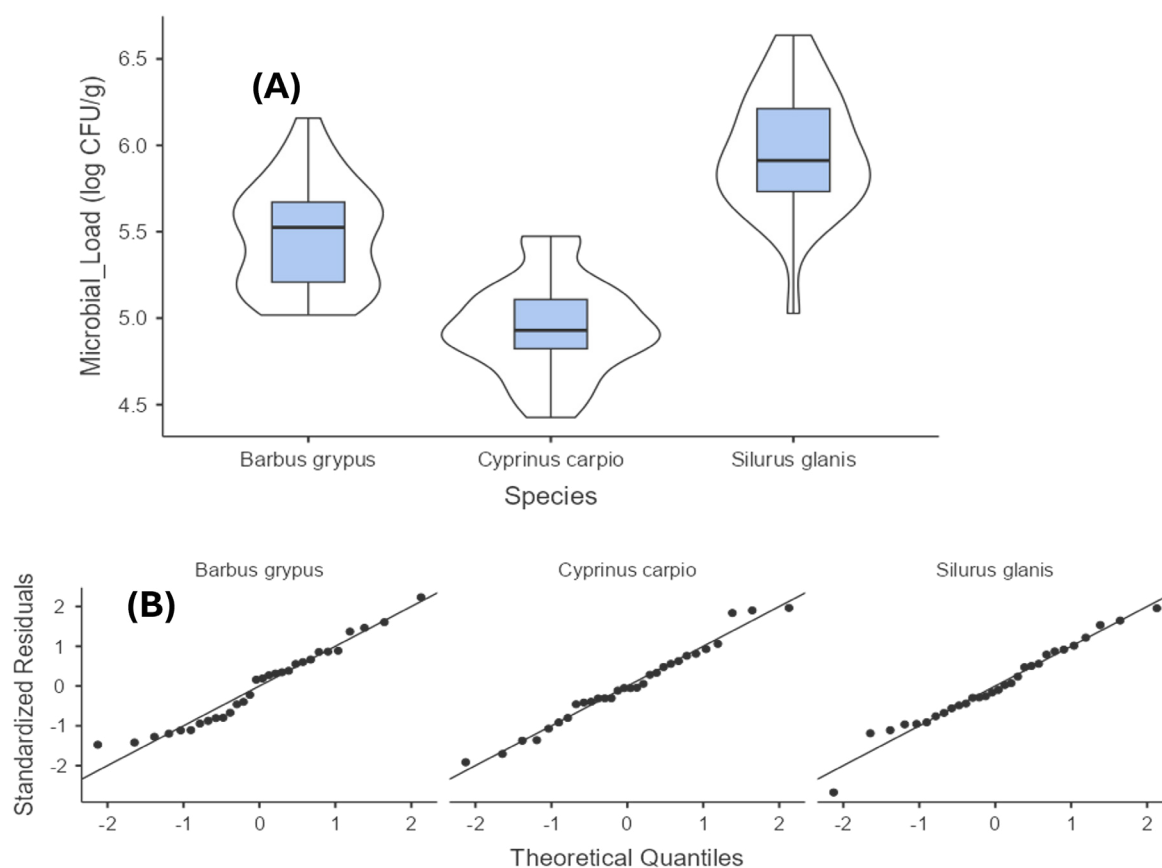


Figure 1: *Microbial_Load (log CFU/g)*

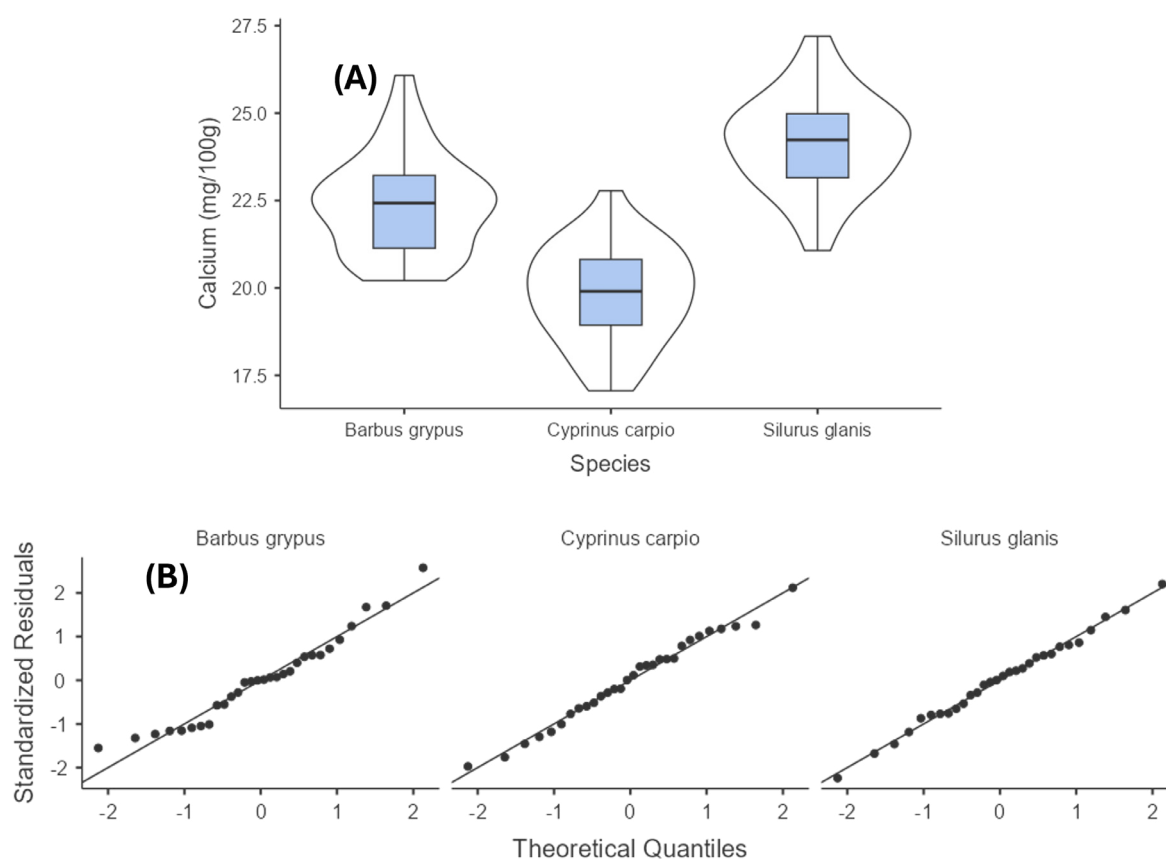


Figure 2: *Calcium (mg/100g)*

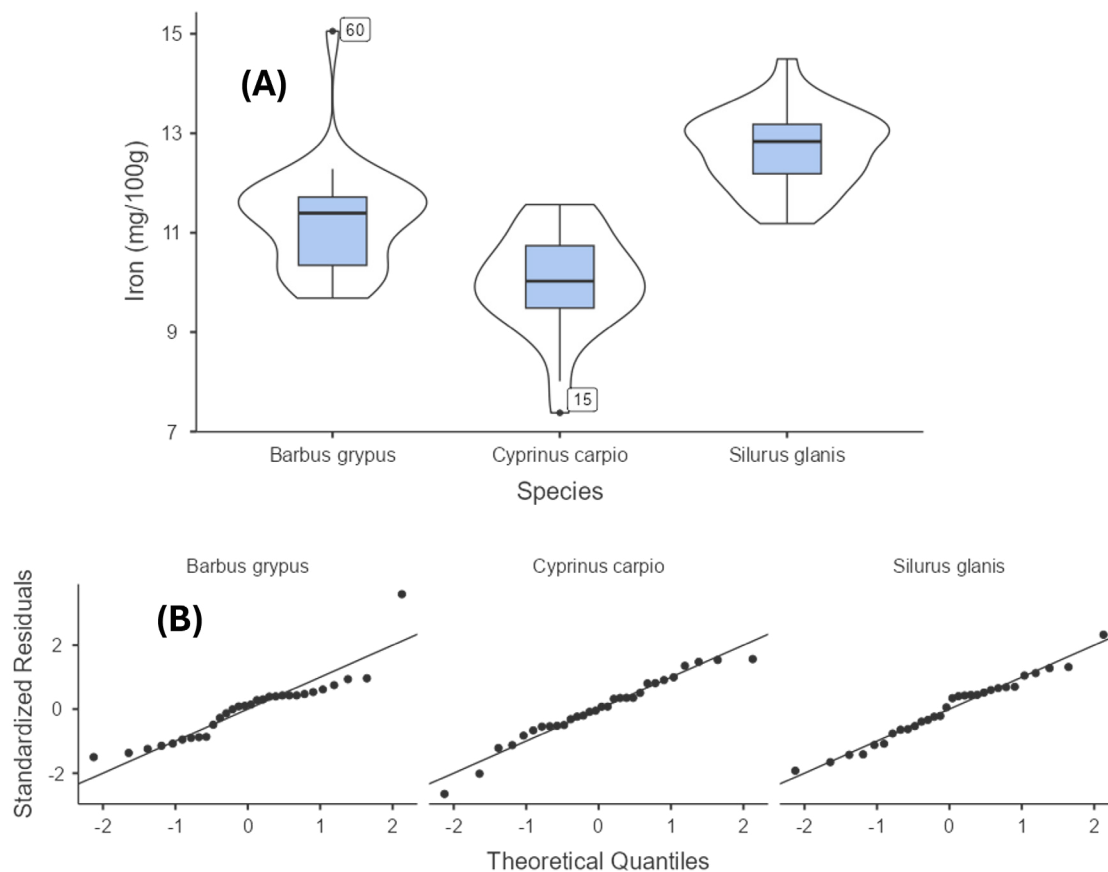


Figure 3: Iron (mg/100g)

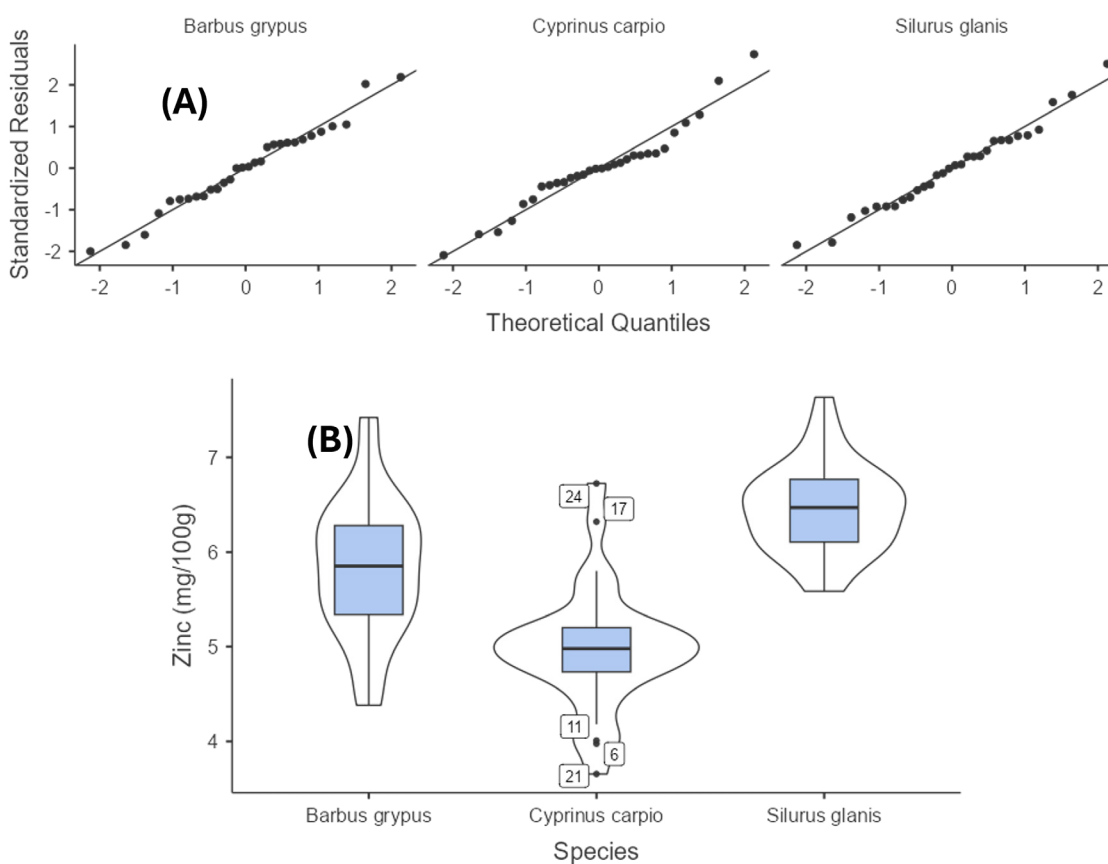


Figure 4: Zinc (mg/100g)

Barbus grypus recorded significantly higher microbial counts than *Cyprinus carpio* (Mean Difference = 0.528).

Silurus glanis had significantly lower microbial load than *Barbus grypus* (mean difference = -0.485).

Silurus glanis also differed significantly from *Cyprinus carpio* (mean difference = -1.013).

These results indicate a species-dependent variation in microbial colonization, possibly due to differing habitat preferences, feeding behavior, or immune responses.

Calcium (mg/100g)

All interspecies comparisons were statistically significant ($p < 0.001$):

Barbus grypus had higher calcium than *Cyprinus carpio* (+2.60) and lower than *Silurus glanis* (-1.74).

The largest difference was between *Silurus glanis* and *Cyprinus carpio* (+4.34).

The bioaccumulation of calcium varies significantly across species, which may reflect physiological differences in bone mineralization or metabolic demand.

Iron (mg/100g) Statistically significant differences ($p < 0.001$) late to all pairwise comparisons: *Barbus grypus* had more iron than *Cyprinus carpio* (+1.25) and less than *Silurus glanis* (-1.42). *Silurus glanis* had the biggest iron quantity, with a massive difference from *Cyprinus carpio* (+2.67). Iron levels are significantly higher in predatory species like *Silurus glanis*, potentially linked to dietary intake of iron-rich prey.

Zinc (mg/100g) All comparisons were significant at $p < 0.001$:

Barbus grypus had more zinc than *Cyprinus carpio* (+0.848) and less than *Silurus glanis* (-0.622).

Silurus glanis contained the highest levels of zinc (+1.470 versus *cyprinus Carpio*).

Zinc accumulation probably differs with the ecological trophic level and metabolic requirement for enzymatic processes. The post-hoc results find substantiation that fish species differ in their microbial and mineral profiles, suggesting the influence of species-specific physiological, ecological, and environmental factors (Table 4). These differences are crucial for public

health risk assessment, nutritional evaluations, and aquaculture management in the Baghdad region (Table 5).

9-2-Assumption Checks

Table 3: Homogeneity of variances test (Levene's)

	F	df1	df2	p
Microbial_Load (log CFU/g)	1.1369	2	87	0.326
Calcium (mg/100g)	0.0519	2	87	0.949
Iron (mg/100g)	0.3995	2	87	0.672
Zinc (mg/100g)	2.0166	2	87	0.139

9--3- Post Hoc Tests

Table 4: Tukey post-hoc test – microbial_load (log CFU/g)

		Barbus grypus	Cyprinus carpio	Silurus glanis
Barbus grypus	Mean difference	—	0.528***	-0.485***
	t-value	—	6.60	-6.07
	df	—	87.0	87.0
	p-value	—	<.001	<.001
Cyp-rinus carpio	Mean difference	—	—	-1.013***
	t-value	—	—	-12.67
	df	—	—	87.0
	p-value	—	—	<.001
Silurus glanis	Mean difference	—	—	—
	t-value	—	—	—
	df	—	—	—
	p-value	—	—	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

To investigate the interrelationships among mineral components—Calcium, Iron, and Zinc—a Pearson correlation analysis was conducted. The results are summarized in Table 6.

Pearson correlation coefficients were computed to evaluate the interrelationship among mineral elements. As shown in Table 3, strong positive correlations were observed (Figure 5):

- Calcium vs. Iron: $r = 0.711$, $p < 0.001$
- Calcium vs. Zinc: $r = 0.565$, $p < 0.001$
- Iron vs. Zinc: $r = 0.484$, $p < 0.001$

With a correlation coefficient of 0.711 ($p < .001$), there existed a very high positive correlation between Calcium and Iron, suggesting fish species with

higher Calcium content also tend to have higher Iron concentration (Table 7).

Table 5: Tukey post-hoc test – calcium (mg/100g)

		Barbus grypus	Cyprinus carpio	Silurus glanis
Barbus grypus	Mean difference	—	2.60***	-1.74***
	t-value	—	7.20	-4.82
	df	—	87.0	87.0
	p-value	—	<.001	<.001
Cyprinus carpio	Mean difference	—	—	-4.34***
	t-value	—	—	-12.02
	df	—	—	87.0
	p-value	—	—	<.001
Silurus glanis	Mean difference	—	—	—
	t-value	—	—	—
	df	—	—	—
	p-value	—	—	—

Note. * p < .05, ** p < .01, *** p < .001

Table 6: Tukey post-hoc test – iron (mg/100g)

		Barbus grypus	Cyprinus carpio	Silurus glanis
Barbus grypus	Mean difference	—	1.25***	-1.42***
	t-value	—	5.12	-5.79
	df	—	87.0	87.0
	p-value	—	<.001	<.001
Cyprinus carpio	Mean difference	—	—	-2.67***
	t-value	—	—	-10.90
	df	—	—	87.0
	p-value	—	—	<.001
Silurus glanis	Mean difference	—	—	—
	t-value	—	—	—
	df	—	—	—
	p-value	—	—	—

Note. * p < .05, ** p < .01, *** p < .001

There was a moderately positive correlation found between Calcium and Zinc ($r = 0.565$, $p < .001$), which is an indication of a potential synergistic way of mineral deposition. These findings demonstrate a moderate positive correlation between Iron and Zinc ($r = 0.484$, $p < .001$), further reinforcing the notion of mineral accumulation through similar physiological routes in fishes (Table 8).

Such statistically significant correlations ($p < .001$) suggest that biochemical co-regulation or

environmental availability might be at the root of mineral composition variation. Positive mineral associations between minerals have been reported and corroborated by earlier studies in freshwater fish (Pervaiz *et al.*, 2012; Olmedo *et al.*, 2013).

Table 7: Tukey post-hoc test – zinc (mg/100g)

		Barbus grypus	Cyprinus carpio	Silurus glanis
Barbus grypus	Mean difference	—	0.848***	-0.622***
	t-value	—	5.29	-3.88
	df	—	87.0	87.0
	p-value	—	<.001	<.001
Cyp-rinus carpio	Mean difference	—	—	-1.470***
	t-value	—	—	-9.18
	df	—	—	87.0
	p-value	—	—	<.001
Silurus glanis	Mean difference	—	—	—
	t-value	—	—	—
	df	—	—	—
	p-value	—	—	—

Note. * p < .05, ** p < .01, *** p < .001

Table 8: Pearson correlation matrix among mineral elements in local fish species ($n = 90$)

Correlation Matrix				
		Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
Calcium (mg/100g)	Pear-son's r	—		
	df	—		
	p-value	—		
Iron (mg/100g)	Pear-son's r	0.711***	—	
	df	88	—	
	p-value	<.001	—	
Zinc (mg/100g)	Pear-son's r	0.565***	0.484***	—
	df	88	88	—
	p-value	<.001	<.001	—

Note. * p < .05, ** p < .01, *** p < .001

The present study is a comparison of microbial load and mineral contents in three commercially important freshwater fish species from Baghdad city. The findings, contrasting the microbial and mineral profiles of the species, provide for insight into their food safety and nutritional values. Calcium, iron, and zinc

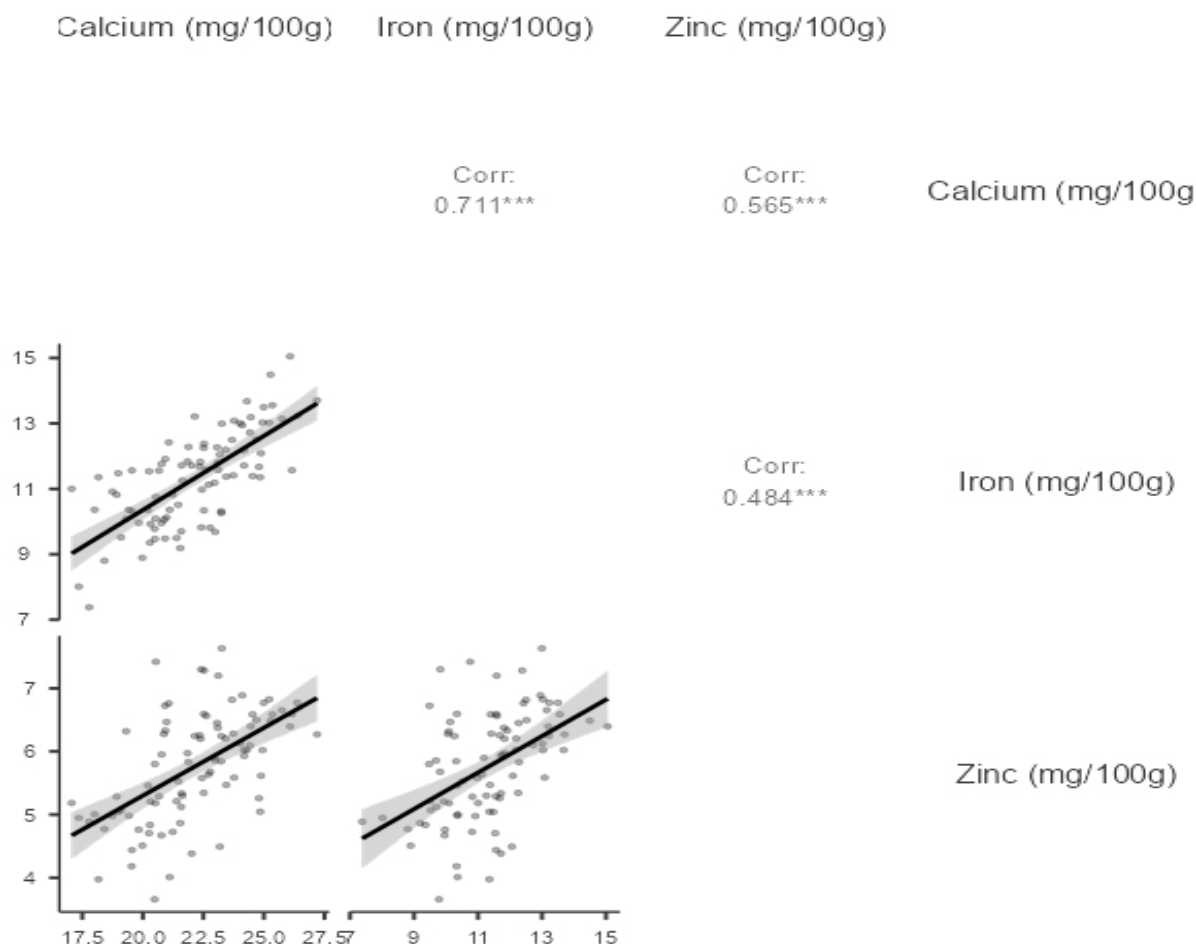


Figure 5: Correlation coefficient between calcium, iron, and zinc

are among the most nutritionally important minerals found in fish flesh. Calcium is required for the development of bones, iron for synthesizing hemoglobin, and zinc for immune function (Olmedo, 2013). The concentration of these elements also fluctuates depending on the species, age of the fish, sex, diet, and degree of pollution in the habitat (Odeyemi, and Stratev, 2019; Naeem *et al.*, 2011; Pervaiz *et al.*, 2012). Olmedo *et al.* (2013) had shown that in carnivorous species, iron and zinc levels were significantly higher due to their bioaccumulative potential. Fawole *et al.* (2007) found calcium contents ranging from 18.5 to 28.7 mg/100g in different freshwater fish species in Nigeria.

Microbial load and food safety implication

The number of microbes in *Silurus glanis* was found to be the highest (5.96 log CFU/g), though within the microbiological limit of fresh fish (<6 log CFU/g), thereby suggesting higher possibilities of post-harvest contamination or the species being naturally prone to microbial proliferation. In the case of *Cyprinus carpio*,

however, the microbial load was significantly less at 4.94 log CFU/g, possibly indicating greater hygienic measures. These might have been due to microbial ecology, feeding habits specific to the species, or handling practices in the market.

These levels fit into the microbial thresholds set by the International Commission on Microbiological Specifications for Foods (ICMSF), indicating that none of the samples are of immediate health concern, but due to the variability and spoilage potential of bacteria, continuous checking might be necessary.

Nutritional profile: mineral concentrations

All the three fish species possess fairly appreciable amounts of calcium, iron, and zinc- essential micronutrients that are critical for bone health, transport of oxygen, and immunity. *Silurus glanis* again had the max value for the minerals (Ca: 24.2 mg/100g, Fe: 12.7 mg/100g, Zn: 6.46 mg/100g), thus possibly making it a more nutrient-dense dietary alternative. The presence of these high concentrations

could be due to species-specific bioaccumulation capacity and feeding habits.

The mineral content reported is in confidence with or just slightly above reported global average values according to some international food composition standards, therefore indicating good nutrition value for human consumption. Strong positive correlations among calcium, iron, and zinc ($r > 0.48$, $p < 0.001$) may imply that these minerals could co-occur by virtue of sharing uptake or metabolic pathways, as observed previously in related fish species.

Statistical significance and biological interpretation

Statistical confirmation of interspecies distinction across all indices ($p < 0.001$) underscored and affirmed the hypotheses that local fish species vary in microbial load and mineral content. Importance is further underscored in microbiological risk assessment and dietary planning. Contrastingly, emphasis on the biological interest that is these contrasts was also implied by effect sizes ($\eta^2 > 0.3$).

Implications for public health and fish marketing

From a public health point of view, microbial load monitoring, especially when the cold chain infrastructure is not very good, is always necessary to minimize all infection risks through food. Conversely, the distinctly high mineral content of *Silurus glanis* and *Barbus grypus* offers some nutraceutical marketing and serves to promote their use in food programs for iron or calcium deficiencies. Moreover, the knowledge of mineral co-occurrence would help in the design of aquaculture feeds to optimize nutritional parameters without affecting microbial safety.

Conclusion

This study highlights the significant interspecies differences in microbial contamination and mineral composition in three freshwater species consumed in Baghdad. *Silurus glanis* has the greatest calcium, iron, and zinc contents among the three species, along with a high microbial load; this is the probable result of its predatory feeding habit and habitat characteristics.

These strong and positive correlations among mineral elements suggest a consistent pattern of bioaccumulation that might be affected by environmental and physiological factors. These results

highlight the need for species-specific food safety standards and point out the role of native fishes as potential contributors to nutrition in the local diet.

Acknowledgements

The authors would like to thank Department of Food Science, College of Agriculture Engineering Science, University of Baghdad, Iraq for the support and provide tools for this project.

Novelty Statement

The use of post-hoc Tukey HSD tests indicated that *Silurus glanis* had the highest microbial and mineral content whereas the lowest microbial and mineral content belonged to the *Cyprinus carpio*. Pearson correlation analysis indicated strong positive correlations of all minerals especially calcium and iron

Author's Contribution

Mohammed Jawad Mohaisen: Presented the idea, wrote the manuscript, collected data from the field. Raed Mohammed Khalaf Al-Zaidi: Presented the idea and wrote the first draft.

Rand Kadhim Bahr Almurjan: Data analysis

Generative AI or AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have no conflict of interest.

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