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Assessment of Heavy Metal Concentrations in Different Processed Fish Species: Implications for Human Health

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ABSTRACT

In this study, the concentrations of sixteen heavy metals (Al, Ba, Cd, Cr, Sb, As, Cu, Pb, Mn, Ni, Se, V, Zn, Sn, Fe, Co.) were estimated in raw, fried, and smoked different thirteen commercial fish species. The concentration of the detected metals in samples of raw fish were in the following order: Fe > Al > Zn > Cu > Cr > Pb > Ni> Mn > Ba > Co according to the highest value for each element. Other heavy metals were undetected (Sb, As, Cd, Se, Sn and V) in all fish species. The Fe, Cr, Ni and Pb levels in raw fish exceeded their permitted limits except Pb in the thumbprint emperor and grass carp. The impact of thermal processing methods on the concentration of trace elements in fish was determined showing various effects where the maximum values of Fe, Zn, Pb and Ba were recorded in fried fish sample while the maximum values of Al, Cr, Co, Ni and Mn were recorded in smoked fish sample. On the other hand, higher Cu content was in raw fish sample. Smoking and frying processing methods had varied effects on heavy metals accumulations. However, customers must be aware that excessive consumption of fried and smoked fish may raise health problems.

INTRODUCTION

Fish and their products are essential for maintaining a balanced diet, especially for those who avoid red meat, are pregnant, immune-compromised, breastfeeding, or malnourished **(Rørvik, 2000)**. Fish consumption can reduce the risk of heart disease, cancer, Alzheimer's, high blood pressure, and inflammation **(Talab *et al.*, 2016)**. Seafood contains important nutrients and metals for human health at low amounts but can be hazardous at higher levels. Additionally, heavy elements in fish have significant functions for fish and humans alike. However, several of these elements are poisonous at low doses and are categorized as a health risk **(Ju *et al.*, 2017)**. The rising pollution of aquatic habitats due to exposure to trace metals has raised concerns during the last decades **(Ulaganathan *et al.*, 2022)**. Possible risks are due to their bio-accumulative and non-biodegradable properties, as well as their extended biological half-lives **(Anna & Kamila, 2013)**.

Raw fish may be eaten, but it is typically treated by various heat methods such as grilling, smoking, and frying before consumption. Thermal processing improves flavor, kills pathogens, and extends shelf life **(Ersoy *et al.*, 2006)**. Frying is the oldest food processing method, being quick and more convenient for meals to be prepared, as well as preserving the sensory features including taste and distinct flavor. Moreover, it is a low-cost and rapid process of mass transfer and heat that alters nutritional properties and sensory because of the intricate interactions between oil and food **(Ersoy *et al.*, 2006)**. Frying increases the concentration of macro- and microelements, as well as heavy metals (Cu, Pb, Cd, Fe, Zn, and Hg) to a great extent **(Huong, 2014)**.

On the other hand, fish smoking is a traditional way for preserving fish, as it reduces lipid oxidation and bacterial growth, potentially increasing shelf life. Smoking is the exposure of fish to smoke caused by incomplete combustion of plant wood or sawdust. It involves salting, drying, followed by placing in a smoking oven **(EOS, 2005)**. Toxin generation in products can be inhibited by smoking **(Abbas *et al.*, 2021)**, and bacterial growth can be reduced by lowering water activity through salting and drying, creating a physical barrier **(Takarina *et al.*, 2025)**. Quality of smoked fish is impacted by raw materials, salting techniques, smoke composition, processing conditions, brining level, and smoking technique **(Abbas *et al.*, 2021)**. Furthermore, Cooking methods include boiling, grilling, microwaving, smoking, and frying, and the latter is internationally the most preferred **(JUsero *et al.*, 2005)**.

In addition, cooking preferences vary according to individual favor, and it is important to determine their effect on fish. Therefore, the purpose of the current study was to investigate the influence of different thermal processing (smoking and frying) on heavy metals in thirteen fish species namely the grass carp (*Ctenopharyngodon idella*), bluefish (*Pomatomus saltatrix*), sharpfin barracuda (*Sphyraena acutipinnis*), golden pompano (*Trachinotus ovatus*), horse mackerel (*Trachurus trachurus*), shrimp scad (*Alepes djedaba*), threadfin bream (*Nemipterus japonicus*), thumbprint emperor (*Lethrinus harak*), haffara seabream (*Rhabosargus haffara*), brushtooth lizardfish (*Saurida undosquamis*), flathead grey mullet (*Mugil cephalus*), sardinella (*Sardinella* sp.) and sand smelt (*Atherina hepsetus*). Specifically, the study focused on (1) Assessing the levels of (Sb, As, Al, Cd, Ba, Cr, Pb, Co, Mn, Ni, Sn, V, Se, Fe, Zn, Cu) heavy metals in fillets samples of these different fish species; (2) assessing the heavy metals concentrations in smoked and fried samples; and (3) determining the values of Metal Pollution Index (MPI) for these different samples of all fish species.

MATERIALS AND METHODS

Fish samples collection

The grass carp (*Ctenopharyngodon idella*) fish species were purchased from El-Serew Fish Research Station, NIOF, Egypt, while other seafood's fish constituted of 12 species [bluefish (*Pomatomus saltatrix*), sharpfin barracuda (*Sphyraena acutipinnis*), golden pompano (*Trachinotus ovatus*), horse mackerel (*Trachurus trachurus*), shrimp scad (*Alepes djedaba*), threadfin bream (*Nemipterus japonicus*), thumbprint emperor (*Lethrinus harak*), haffara seabream (*Rhabosargus haffara*), brushtooth lizardfish (*Saurida undosquamis*), flathead grey mullet (*Mugil cephalus*), sardinella (*Sardinella* sp.) and sand smelt (*Atherina hepsetus*). Those 12 species were collected from the Gulf of Suez during January 2022, Suez City, Egypt. Fresh fish samples were carefully washed with potable water, packed in ice boxes, and transported to Fish Processing and Technology Laboratory, National Institute of Oceanography and Fisheries, El-Kanater El-Khiria City, El-Qaluobia Governorate, Egypt. Fish samples were carefully washed with tap water, manually beheaded, gutted, filleted, carefully rewashed and drained. Fresh fish samples were thoroughly cleansed with water, stored in ice boxes, and transferred to laboratory of the Fish Processing and Technology of the National Institute of Oceanography and Fisheries in El-Kanater El-Khiria City, El-Qaluobia Governorate, Egypt. Samples of fish were properly washed using tap water, beheaded, gutted, and filleted, and then carefully rewashed and drained.

Morphometric measurements of the studied fish samples

The studied 13 different fish species (grass carp, bluefish, sharpfin, barracuda, golden pompano, horse mackerel, shrimp scad, threadfin bream, thumbprint emperor, haffara seabream, brushtooth lizardfish, flathead grey mullet, sardinella and sand smelt) are listed in Table (1) along with their weight, average length, and scientific name. The fish's body measurements varied widely, with statistically significant range. Fish body weight, length, and condition factor vary depending on species and size.

Table 1. Morphometric measurements of the studied fish species, the average of total weight and length (Mean ± SD) of collected fish samples in Egypt

No.	English common name	Scientific name	Weight (g)	Length (cm)
1	Grass carp	*(Ctenopharyngodon idella)*	6575±991.52	76.12±2.00
2	Sardinella	*(Sardinella* sp.*)*	100.96±14.11	21.52±1.23
3	Flathead Grey Mullet	*(Mugil cephalus)*	260.41±32.23	30.79±2.67
4	Horse mackerel	*(Trachurus trachurus)*	140.68±39.93	23.58±2.53
5	Thumbprint emperor	*(Lethrinus harak)*	135.70±25.57	20.44±1.36
6	Sand smelt	*(Atherina hepsetus)*	5.46±1.26	9.27±0.74
7	Threadfin bream	*(Nemipterus japonicus)*	106.17±11.73	19.64±1.17
8	Haffara seabream	*(Rhabosargus haffara)*	148.43±44.76	23.01±2.34
9	Brushtooth lizardfish	*(Saurida undosquamis)*	103.75±26.01	23.89±2.52
10	Shrimp scad	*(Alepes djedaba)*	38.39±14.62	13.31±2.12
11	Sharpfin barracuda	*(Sphyraena acutipinnis)*	202.54±35.53	35.03±2.69
12	Golden pompano	*(Trachinotus ovatus)*	247.63±44.28	23.64±1.77
13	Bluefish	*(Pomatomus saltatrix)*	1028.26±178.26	44.00±1.41

Thermal processing methods

Frying process

Fish fillets samples were deeply fried in sunflower oil preheated at 160°C for 5-6 minutes.

Smoking process

Frozen fish fillets were left at room temperature, cleaned with tap water, wet salted with a 10% brine solution for two hours, then thoroughly rinsed with tap water for one minute to eliminate any remaining salt. Salted samples were partially dried on net shelves at 20-23ºC for two hours before cold smoking. Salted samples were smoked in a laboratory smokehouse at NIOF, Egypt. The smoking process lasted for 10-11 hours at 35-45°C. After smoking, the smoked samples were cooled at room temperature, sealed in polyethylene bags, and stored until analysis.

Heavy metals analysis

Fish fillets were processed for analysis of heavy metals using the methodology outlined by **Meche *et al.* (2010)** as follows: the fish fillets were dried in an oven at 105°C. The samples were gathered from the oven, cool, and then ground in a clean mortar. Each sample (0.5g) was put in a Teflon microwave digestion bomb with 10mL of concentrated HNO_3. The samples were heated to 180°C for 5 minutes, digested for 9.5 minutes, and cooled for 5 minutes in a microwave (model Milestone, MLS1200 mega, Germany) digestion system. After that, the samples were put into volumetric flasks and diluted to 10ml of water. Until they were prepared for metal analysis, the samples were kept at 5°C. The samples were measured against a multi-element standard curve to determine the ppm of each element in the digested samples. The materials were examined three separate times using the Perkin Elmer Optima 3000 model of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The detection of metals were taken at different wavelengths as follows: As 193.696; Al 396.153; Ba 455.403; Co 228.616; Cr 267.716; Cd 214.440; Fe 259.993; Mn 257.610; Cu 324.752; Ni 231.604; Sb 217.582; Pb 220.353; Se 203.985; Zn 213.857; V 310.230 and Sn 189.927 nm.

Metal pollution index (MPI)

The total metal content of the fish samples of each fish species was compared using the Metal Pollution Index (MPI), adopted by multiplying the measured heavy metal concentrations as follows **(Abou-Arab *et al.*, 1996; USPHS, 2000)**. MPI = (CM1 × CM2 × CM3 × . . . × CMn) 1/n

Where CM1 is the content of the first concerning metal, CM2 is the content of the second metal, CM3 is level of the third metal, and CMn is the content of the nth metal (mg/kg) in a sample of a certain fish species, and n represents the number of metals tested.

RESULTS

Heavy metals concentrations in raw fish samples

Table (2) denotes the levels of heavy metals (Sb, Al, Cd, As, Cr, Co, Cu, Ba, Pb, Sn, Mn, Ni, Se, V, Fe, Zn) in 13 different species of raw fish samples (grass carp, bluefish, sharpfin, barracuda, golden pompano, horse mackerel, shrimp scad, threadfin bream, thumbprint emperor, haffara seabream, brushtooth lizardfish, flathead grey mullet, sardinella and sand smelt) obtained during January 2022 (in mg/kg).

Table 2. Heavy metals concentrations (mg/kg) in raw samples of some commercial fish species

Metal	Fish samples numbers												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Al	120.2	102.3	128.9	83.6	79	62	87.7	102.9	132.1	87.1	89.8	83	102.7
Sb	<DL*	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
As	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Ba	0.6	1	2.7	0.4	<DL	<DL	2.2	1.4	<DL	1.5	0.8	<DL	0.5
Cd	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cr	16.7	16.6	13.8	14	13.5	9.3	14.2	12.3	14.6	12	11.6	11.8	14.1
Co	0.6	0.4	0.4	0.5	0.3	0.4	0.5	0.4	0.88	0.4	0.5	0.36	0.9
Cu	4.2	8.6	39.7	20.8	16.6	35.7	11.7	50.8	9.1	29.5	32.2	14.2	30.9
Fe	147.7	129.4	154.5	138.8	114.9	129.1	140.3	130.2	103.8	118.4	115.5	90.6	143.3
Pb	2	2.2	3.2	2.6	0.7	2.6	2.4	5.4	2.2	7.9	2.9	3.6	11.7
Mn	5.6	3.1	4.9	3.1	1.9	3.1	9.2	6.9	6.4	7.1	3.7	4	5.8
Ni	9.9	9.8	8.9	8.3	8.1	5.7	8.3	8.1	8.6	7.6	7.2	7.2	8.4
Se	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Sn	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
V	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Zn	28.7	34.2	59.4	33.3	30.7	63.3	112.9	52.6	23	71.3	39.8	61.8	70.8

<DL*: below detection limits.

The higher level of Fe (154.50mg/ kg) was recorded in the flathead grey mullet while Zn and Mn were (112.90 and 9.20mg/ kg, respectively) recorded in the threadfin, on the other hand, Cu was registered with a high level (50.8mg/ kg) in the haffara seabream. Remarkably, the highest values of Cr and Ni were (16.70 and 9.90mg/ kg, respectively) recorded in the grass carp while the highest value of Al (132.10mg/ kg) was detected in the brushtooth lizardfish and Ba (2.70mg/ kg) in the flathead grey mullet. The higher value of Pb and Co content was (11.7 and 0.9 mg/kg, respectively) recorded in the bluefish.

Effect of different thermal processing treatments on heavy metals in various fish samples

The results of the effect of thermal processing such as frying and smoking on sixteen heavy metals in 13 different fish species during January 2022 (in mg/kg) are illustrated in Tables (3, 4). There were ten metals detected and the others remained undetected. In addition, the changes in the detected metals (Fe, Al, Cr, Cu, Pb, Ba, Zn, Ni, Co, and Mn) after frying and smoking of fish samples compared to raw fish samples is shown in Tables (3, 4). The lowest value of Al was (18.19mg/ kg) in the smoked sample of mackerel but the maximum level was (152.11 mg/kg) recorded in the smoked lizardfish.

Table 3. Effect of thermal processing treatments on heavy metals concentrations (mg/kg) of some commercial fish species

Fish species		Al	Sb	As	Ba	Cd	Cr	Co	Cu
Grass carp	R	120.20	<DL	<DL	0.60	<DL	16.70	0.60	4.20
	F	100.80	<DL	<DL	<DL	<DL	15.60	0.50	2.40
	S	91.02	<DL	<DL	<DL	<DL	11.21	0.52	3.11
Sardinella	R	102.30	<DL	<DL	1.00	<DL	16.60	0.40	8.60
	F	99.20	<DL	<DL	<DL	<DL	12.20	0.40	3.00
	S	78.01	<DL	<DL	<DL	<DL	13.35	0.21	5.71
Flathead grey mullet	R	128.90	<DL	<DL	2.70	<DL	13.80	0.40	39.70
	F	78.50	<DL	<DL	<DL	<DL	17.00	0.40	16.90
	S	92.51	<DL	<DL	<DL	<DL	15.11	0.61	28.21
Horse mackerel	R	83.60	<DL	<DL	0.40	<DL	14.00	0.50	20.80
	F	80.90	<DL	<DL	<DL	<DL	13.70	0.30	19.10
	S	18.19	<DL	<DL	<DL	<DL	14.55	0.37	22.11
Thumbprint emperor	R	79.00	<DL	<DL	<DL	<DL	13.50	0.30	16.60
	F	94.30	<DL	<DL	2.60	<DL	11.30	0.50	21.10
	S	95.25	<DL	<DL	<DL	<DL	12.27	0.49	25.01
Sand smelt	R	62.00	<DL	<DL	<DL	<DL	9.30	0.40	35.70
	F	84.60	<DL	<DL	<DL	<DL	13.00	0.40	25.50
	S	78.33	<DL	<DL	<DL	<DL	15.11	0.71	22.11
Threadfin bream	R	87.70	<DL	<DL	2.20	<DL	14.20	0.50	11.70
	F	96.50	<DL	<DL	0.90	<DL	12.90	0.40	43.40
	S	112.35	<DL	<DL	<DL	<DL	16.37	0.33	33.15
Haffara seabream	R	102.90	<DL	<DL	1.40	<DL	12.30	0.40	50.80
	F	91.30	<DL	<DL	<DL	<DL	13.20	0.50	44.70
	S	111.24	<DL	<DL	<DL	<DL	17.33	0.25	43.13
Brushtooth lizardfish	R	132.10	<DL	<DL	<DL	<DL	14.60	0.88	9.10
	F	136.40	<DL	<DL	<DL	<DL	12.70	0.62	3.60
	S	152.11	<DL	<DL	<DL	<DL	16.21	0.75	11.02
Shrimp scad	R	87.10	<DL	<DL	1.5	<DL	12.00	0.40	29.50
	F	117.60	<DL	<DL	<DL	<DL	13.80	0.40	12.10

	S	110.12	<DL	<DL	<DL	<DL	15.27	15.13	22.03
Sharpfin barracuda	R	89.80	<DL	<DL	0.80	<DL	11.60	0.50	32.20
	F	91.00	<DL	<DL	<DL	<DL	14.20	0.50	21.80
	S	112.23	<DL	<DL	<DL	<DL	17.12	17.11	17.25
Golden pompano	R	83.00	<DL	<DL	<DL	<DL	11.80	0.36	14.20
	F	90.80	<DL	<DL	<DL	<DL	12.20	0.75	21.30
	S	115.15	<DL	<DL	<DL	<DL	15.11	0.74	19.03
Bluefish	R	102.70	<DL	<DL	0.50	<DL	14.10	0.90	30.90
	F	118.60	<DL	<DL	7.70	<DL	13.40	0.50	39.30
	S	122.13	<DL	<DL	<DL	<DL	17.23	0.83	35.11

<DL*: below detection limits; R: raw samples; F: fried samples and S: smoked samples.

Table 4. Effect of thermal processing treatments on heavy metals concentrations (mg/kg) of some commercial fish species

Fish species		Fe	Pb	Mn	Ni	Se	Sn	V	Zn
Grass carp	R	147.70	2.00	5.60	9.90	<DL	<DL	<DL	28.70
	F	101.70	1.00	5.70	12.40	<DL	<DL	<DL	25.10
	S	91.11	1.31	8.51	17.31	<DL	<DL	<DL	22.11
Sardinella	R	129.40	2.20	3.10	9.80	<DL	<DL	<DL	34.20
	F	144.30	3.00	10.20	7.60	<DL	<DL	<DL	31.40
	S	120.01	2.70	9.71	10.11	<DL	<DL	<DL	33.51
Flathead grey mullet	R	154.50	3.20	4.90	8.90	<DL	<DL	<DL	59.40
	F	161.00	2.50	3.50	9.70	<DL	<DL	<DL	59.00
	S	162.11	4.20	3.77	8.31	<DL	<DL	<DL	60.35
Horse mackerel	R	138.80	2.60	3.10	8.30	<DL	<DL	<DL	33.30
	F	86.50	2.20	2.30	8.30	<DL	<DL	<DL	40.70
	S	102.05	1.10	2.23	9.11	<DL	<DL	<DL	37.78
Thumbprint emperor	R	114.90	0.70	1.90	8.10	<DL	<DL	<DL	30.70
	F	98.40	1.40	15.10	6.70	<DL	<DL	<DL	102.40
	S	105.01	1.31	19.33	7.71	<DL	<DL	<DL	87.35
Sand smelt	R	129.10	2.60	3.10	5.70	<DL	<DL	<DL	63.30
	F	122.40	1.90	2.30	8.10	<DL	<DL	<DL	31.80
	S	133.17	3.20	5.60	8.94	<DL	<DL	<DL	75.14
Threadfin bream	R	140.30	2.40	9.20	8.30	<DL	<DL	<DL	112.90
	F	119.60	15.50	5.60	8.00	<DL	<DL	<DL	135.80
	S	116.11	13.50	13.65	11.75	<DL	<DL	<DL	129.70
Haffara seabream	R	130.20	5.40	6.90	8.10	<DL	<DL	<DL	52.60
	F	89.80	1.70	2.60	8.20	<DL	<DL	<DL	26.60
	S	119.20	8.70	8.22	11.12	<DL	<DL	<DL	72.10
Brushtooth lizardfish	R	103.80	2.20	6.40	8.60	<DL	<DL	<DL	23.00
	F	105.80	2.80	3.60	8.10	<DL	<DL	<DL	32.70
	S	108.15	5.20	4.30	7.15	<DL	<DL	<DL	29.38
Shrimp scad	R	118.40	7.90	7.10	7.60	<DL	<DL	<DL	71.30
	F	140.60	3.40	4.20	8.50	<DL	<DL	<DL	25.00

	S	135.20	7.15	9.45	8.35	<DL	<DL	<DL	82.11
Sharpfin barracuda	R	115.50	2.90	3.70	7.20	<DL	<DL	<DL	39.80
	F	98.20	5.00	4.50	8.00	<DL	<DL	<DL	36.20
	S	120.15	4.33	4.41	9.21	<DL	<DL	<DL	44.41
Golden pompano	R	90.60	3.60	4.00	7.20	<DL	<DL	<DL	61.80
	F	138.30	2.10	2.90	7.30	<DL	<DL	<DL	43.00
	S	113.15	7.11	9.25	5.41	<DL	<DL	<DL	77.38
Bluefish	R	143.30	11.70	5.80	8.40	<DL	<DL	<DL	70.80
	F	166.60	10.20	16.30	8.80	<DL	<DL	<DL	63.90
	S	155.20	15.37	9.30	11.20	<DL	<DL	<DL	82.71

<DL*: below detection limits; R: raw samples; F: fried samples and S: smoked samples.

In raw fish species samples, the maximum Ba value was (2.70mg/ kg) in the flathead grey mullet, but the minimum level was (0.40 mg/kg) in the horse mackerel. Ba was detected in the fried bluefish (7.70mg/ kg), thumbprint emperor (2.60mg/ kg), and threadfin bream (0.90 mg/kg). The maximum value of Cr was (17.33 mg/kg) detected in the smoked haffara seabream. Notably, Co values in smoked samples of shrimp scad (15.13mg/kg) and sharpfin barracuda (17.11mg/ kg) were the highest. The maximum value of Cu was (50.8mg/ kg) that found in the raw sample of the haffara seabream. Additionally, the highest value of Fe was (166.60mg/ kg) recorded in the fried sample of the bluefish while the lowest value was (86.50 mg/kg) in the mackerel. For Pb, the highest level (15.5mg/ kg) was found in the fried sample of the threadfin bream, but the lowest value was (0.7 mg/kg) obtained in the raw sample of the thumbprint emperor. Additionally, the raw sample of the thumbprint emperor was recorded with the lowest Mn value (1.90mg/ kg) followed by the smoked and fried samples of the horse mackerel. The highest content of Ni was (17.31) recorded in the smoked sample of the blue fish and the lowest was (5.41) in the golden pompano. Furthermore, the maximum Zn value was (135.80) recorded in the fried sample, followed by the smoked sample of the threadfin bream.

Metal pollution index (MPI)

The MPI was used to compare the total concentrations of heavy metal accumulations in the examined fishes (Table 5 & Fig. 1).

Table 5. The metal pollution index (MPI) of different thermal processing treatments in the examined fish species

Fish species	Raw	Fried	Smoked
Grass carp	8.231	7.132	7.598
Sardinella	8.390	8.289	8.174
Flathead grey Mullet	12.560	9.687	11.197
Horse mackerel	8.275	7.893	6.715
Thumbprint emperor	6.769	11.535	10.988

Sand smelt	8.892	8.297	11.153
Threadfin bream	11.662	13.618	15.195
Haffara seabream	12.223	8.645	13.039
Brushtooth lizardfish	9.225	8.014	10.035
Shrimp scad	12.087	8.964	17.969
Sharpfin barracuda	9.285	9.897	14.880
Golden pompano	8.838	9.269	12.254
Bluefish	12.782	18.086	16.220

The MPI values varied from 6.715- 18.086. The overall concentration distribution of heavy metal accumulations (MPI) in our studied raw fish follows the order: bluefish> flathead grey mullet > haffara seabream > shrimp scad > threadfin bream > sharpfin barracuda > brushtooth lizardfish > sand smelt > golden pompano > sardinella > horse mackerel > grass carp > thumbprint emperor (Table 5).

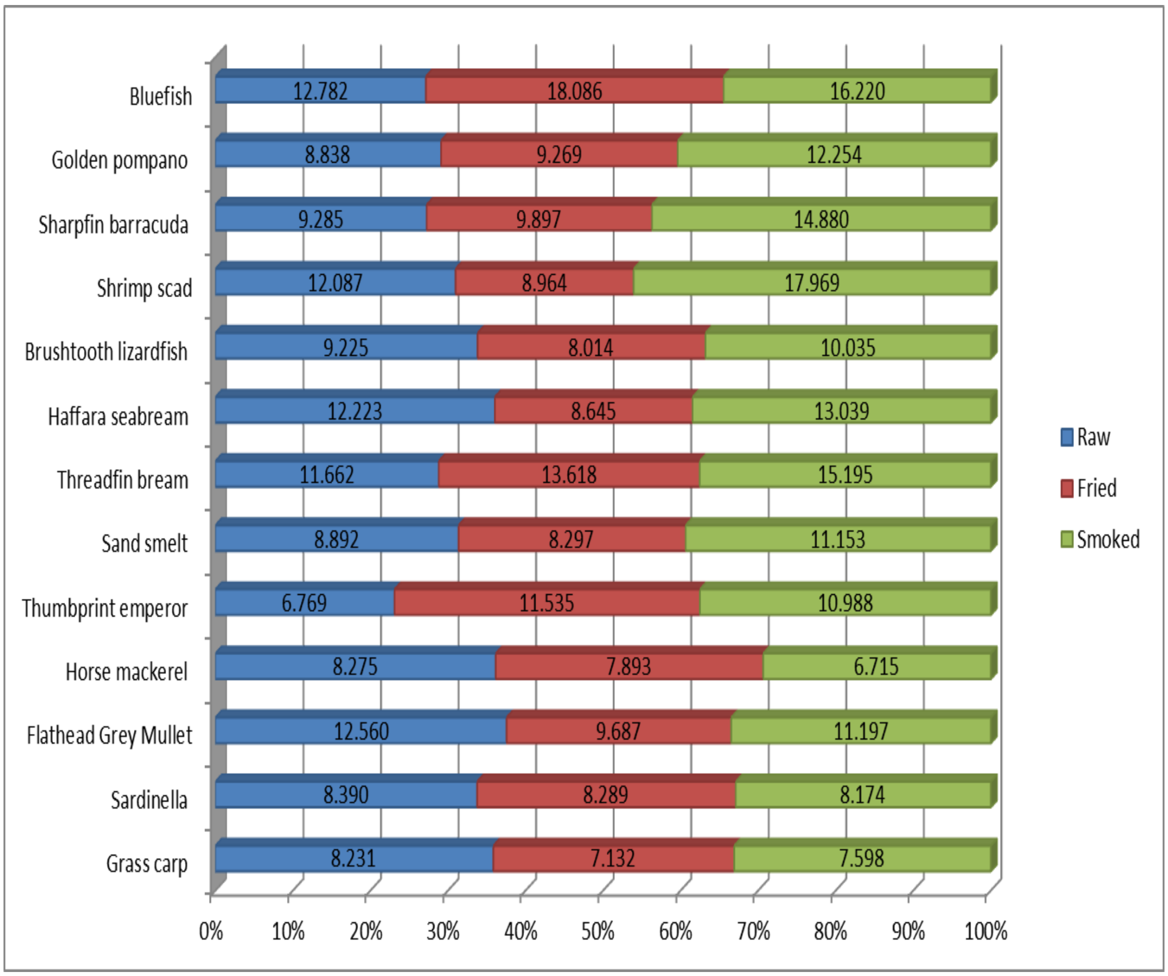

Fig. 1. The MPI concentration in the different fish species

However, the distribution pattern of MPI in the fried and smoked fish species did not follow any regular pattern where it follows the order (Fig 1): raw > fried > smoked in the horse mackerel and sardinella, while it was ordered as raw > smoked > fried in the flathead grey mullet and grass carp. On the other hand, it follows the order of: fried > smoked > raw samples in the bluefish and thumbprint emperor, while the order: smoked > fried > raw samples was obtained in the golden pompano, sharpfin barracuda and threadfin bream. In the shrimp scad, brushtooth lizardfish, haffara seabream and sand smelt, the following order was recorded: smoked > raw > fried.

DISCUSSION

Heavy metals concentrations in raw fish samples

The levels of 16 heavy metals were analyzed in 13 different species of raw fish samples obtained during January 2022 (in mg/kg). The concentration of the detected trace metals in raw fish fillets often followed the order Fe > Al > Zn > Cu > Cr > Pb > Ni > Mn > Ba > Co according to the highest value for each element in all fish species. Other heavy metals were less than the detection limit (Sb, As, Cd, Se, Sn and V) so they were not detected. This result agrees with that of **Iniaghe *et al.* (2024)**, who stated that the average level of trace elements in the samples of raw fish followed the order: Zn > Cu > Ni > Mn.

Essential elements such as Fe, Zn, Cu and Mn were more abundant in fish since they have impact roles in biological system than toxic and unnecessary elements such as lead and cadmium. The Fe concentrations were higher than the maximum permissible limit (30mg/ kg), set by the Egyptian Organization for Standardization and Quality **(Ersoy & Ozeren, 2009)** in all fish species under study. This high level of iron and zinc in fish fillets may be ascribed to the organism's automatic adsorption for these metals. In addition, high accumulation of zinc in fish may be attributed to its impact on the physiological function of fish **(Meche *et al*., 2010)**.

The highest values of Cr and Ni were recorded in the grass carp. The Cr and Ni levels in all studied samples were more than MPLs set by the **EOS (2005)**. The highest value of Al was recorded in the brushtooth lizardfish and that of Ba was detected in the flathead grey mullet. The current outputs coincide with those recorded in prior studies **(Ziaiifar *et al*., 2008; Talab *et al*., 2016; Abbas *et al*., 2021)**.

It is worthy to mention that, lead (Pb) has harmful effects on the kidney, cardiovascular and nervous system. It has no known physiological or biochemical purpose but its impact on children's cognitive and behavioral development, even at relatively low exposure levels, is especially concerning the **USPHS (2000)**. The higher value of Pb and Co content was (11.7 and 0.9, respectively) recorded in the bluefish. The Pb concentration was higher than the permissible limit in all raw species, except for the thumbprint emperor and grass carp which exhibited the lower levels. The high Pb levels match those of **Abou-Arab *et al.* (1996)**, who reported that Pb in sardine and mackerel in the Egyptian governorates was greater than the permissible limits. Furthermore, **Abd El-aziz and Zaky (2010)** postulated that Pb concentration in the imported filleted fish in Assiut City, Egypt, exceeded its permissible limits.

Effect of different thermal processing treatments on heavy metals in various fish samples

The effect of thermal processing such as frying and smoking on sixteen heavy metals in 13 different fish species during January 2022 (in mg/kg) were assessed. For fried fish samples, the detected heavy metals levels followed the order: Fe > Al > Zn > Cu > Cr > Mn > Pb > Ni > Ba > Co according to the highest value for each element in all fish species. For smoked fish samples, the trace metals levels followed the order: Fe > Al > Zn > Cu > Mn > Cr > Ni > Co > Pb.

In the current study, thermal processing decreased Al levels in the grass carp, sardinella, flathead grey mullet, horse mackerel while increased levels were detected in other fish species compared to raw fish samples. A decrease was detected in Ba values of the fried samples compared to raw samples for all fish species, except the fried samples of the thumbprint emperor and bluefish. Eminently, Ba wasn't detected in all smoked fish samples. Cr decreased in fried and smoked samples in the grass carp, sardinella and thumbprint while it increased in the sand smelt and other fish samples compared to the raw samples. Moreover, the maximum two values of Co were in the smoked samples of the shrimp scad and sharpfin barracuda. This may be due to the size of fish, as the smaller the fish, the more increment of metals levels measured in cooked samples as mentioned by **Kalogeropoulos *et al.* (2012)**.

On the other hand, Cu increased in fried and smoked samples compared to the raw in blufish, golden pampano and threadfin while it decreased in other species viz. the grass carp, sardinella, sand smelt, and grey mullet. Consequently, the thermal treatments affected the Cu levels in fish samples. Fe levels was less in almost fried and smoked samples than the raw sample, except for the blue fish, shrimp scad and flathead grey mullet. This result is in accordance with that of **Ersoy and Ozeren (2009)**, who noted that the content of iron in fried samples increased significantly while an insignificant increase was recorded in the grilled cooked and baked samples.

The Pb level increased in the smoked sample of the blue fish, golden pampano and haffara but decreased in the fried sample of the same fish species when compared to the raw sample. Besides, the raw sample of the thumbprint emperor had the lowest Mn value followed by smoked and fried samples of the horse mackerel. This is similar to the finding of **Iniaghe *et al.* (2024)**, who found that Mn was present in the Atlantic horse mackerel, with levels that varied from 0.60 to 1.05mg/ kg.

Ni content in smoked samples was higher than the fried sample in all fish species except the golden pompano, flathead grey and lizard fish. Zn concentration increased in the fried and smoked samples compared to the raw one in the threadfin and thumbprint.

Zn concentration increased in almost all smoked samples compared to the raw, except for the grass carp. The increasing Zn level concurs with the outcome of the study of **Ersoy and Ozeren (2009)**, who stated that following baking, frying, and microwave cooking, the zinc content of fish increased significantly ($P < 0.05$).

The impact of thermal processing on the levels of trace elements in fish had various effects where the maximum value of Fe, Zn, Pb and Ba was recorded in the fried sample and the maximum value of Al, Cr, Co, Ni and Mn was recorded in the smoked one, while higher Cu content was in the raw fish sample. These various results may be due to many factors such as temperature, presence of pollutants in the thermal processing medium, type of frying oil, cooking water, duration of cooking, initial content of metals in the fish, etc., as confirmed by **Iniaghe *et al.* (2024)**.

Metal pollution index (MPI)

The MPI is typically used to determine the level of heavy metal pollution in fish tissues. Higher estimated MPI values are thought to indicate a higher level of contamination in fish **(Kalogeropoulos *et al.*, 2012)**. The maximum MPI level was obtained in the fried sample of the bluefish while the lowest value was in the smoked sample of the horse mackerel. The elevation in some metals may be traced back to the decrease in the moisture content that happen through frying, as confirmed by **Ersoy and Ozeren (2009)**, who found that losses of water in fried fish are higher than those in baked and grilled fish. These results agree with those of **Ersoy *et al.* (2006)**, who postulated that the frying and microwaving methods have significantly increased concentrations of metals, thus these methods were found to be inappropriate. Heavy metal accumulation in fish varies by species, size, and environment, as well as factors like the fish's lifespan and physiological processes **(Huong, 2014)**.

CONCLUSION

The levels of heavy metals varied between the frying and smoking techniques. The observed heavy metal levels in fried samples were in the following order: Fe > Al > Zn > Cu > Cr > Mn > Pb > Ni > Ba > Co while, for the smoked sample, the order of Fe > Al > Zn > Cu > Mn > Cr > Ni > Co > Pb was arranged according to the highest value for each element in all fish species. The distribution pattern of MPI in our studied raw fish species follows the order: bluefish> flathead grey mullet > haffara seabream > shrimp scad > threadfin bream > sharpfin barracuda > brushtooth lizardfish > sand smelt > golden pompano > sardinella > horse mackerel > grass carp > thumbprint emperor. Also, the distribution pattern of MPI in the fried and smoked fish species did not follow any regular pattern where, fried > smoked > raw samples in the bluefish and thumbprint emperor but the order: smoked > fried > raw samples was obtained in the golden pompano, sharpfin barracuda and threadfin bream.

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