

Bioaccumulation and Health Risk Potential of Heavy Metals Including Cancer in Wild and Farmed Meagre *Argyrosomus regius* (Asso, 1801), Mediterranean Sea, Egypt

Ahmed Md. Salem*, Mohamed E. A. El-Metwally

National Institute of Oceanography & Fisheries, NIOF, Egypt

*Corresponding Author: drsalemniofaqua@gmail.com, am.salem@niof.sci.eg

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ABSTRACT

The sciaenid meagre (*Argyrosomus regius*, Asso 1801) is a significant organism in fisheries and a promising candidate for the Egyptian aquaculture. This study presented a comparative analysis of wild and aquacultured meagre. Two size groups of fish, small (S) and large (L), from the Egyptian Mediterranean Sea and a local farm in Maryut Valley were examined. Oxidative stress markers and antioxidant responses, including superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase (CAT), were measured in the liver. Additionally, variations in muscle composition of lipids, total proteins, moisture, and ash content were evaluated. The muscle heavy metals (lead (Pb), cadmium (Cd) mercury (Hg), manganese (Mn) and nickel (Ni)) concentrations were analyzed, and total accumulation was assessed using the metal pollution index (MPI). Furthermore, assessments of hazard associated with fish ingestion were conducted based on estimated dietary intakes (EDI and EWI), the target hazard quotient (THQ) and the carcinogenic risk (CR). The muscle contents of moisture, ash, total lipids, and total protein revealed insignificant results. The liver of small farmed fish (SFM) exhibited higher levels of SOD, CAT, and TAC compared to other groups. Analysis of heavy metals showed a non-significant trend of higher accumulation of Ni in farmed and small-sized fish, while Pb, Cd and Hg levels were higher in wild fish. However all recorded heavy metals did not exceed the international guideline of maximum permissible limit (MPL) for human consumption. Metal estimated dietary intake was calculated, and non-carcinogenic and carcinogenic health risk was assessed for different studied groups. The calculated THQ for Pb, Cd, Mn and Ni were less than 1, indicating lack of health risks from consumption of single metal up to seven times per week. The CR level indicated safe levels of Pb, Cd, Ni and Hg in the flesh of meagre in the Egyptian water.

INTRODUCTION

Fisheries and aquaculture sustain food, income, and livelihood for millions of population around the world (FAO, 2018). The average annual fish intake has increased over the past decades, which has accounted for 20kg per year, as an important contributor in the world food security (FAO, 2018). Hence, ensuring the quality and safety of fish is of paramount importance for human health (Noman *et al.*, 2022). Heavy metals

contamination from industrial, agricultural discharges and mining activities poses significant ecological and health risk in the aquatic environment. With high persistence and non-biodegradable nature, heavy metals accumulate in sediments, aquatic environment and enter the food chain (**El-Metwally et al., 2022**). Uptake of toxic heavy metals as Cd, Pb and Hg in edible muscles of the fish consumed can have severe implications for human health (**El-Moselhy et al., 2014**). Meagre (*Argyrosomus regius*, Asso, 1801) is a piscivorous fish which inhabits the Atlantic coast of Europe (**Grigorakis et al., 2011**), east coast of Africa, the Mediterranean water and the Black Sea (**Abou Shabana et al., 2012**). This species is becoming more prominent in the expansion of aquaculture in Europe due to its appealing biological characteristics. The features of this species include its resistance to infections (**Foata et al., 2009**), and its low feed conversion ratio, as well as its zootechnical performance (**Kružić et al., 2016**), with a rapid growth rate in moderate temperatures. It also has good flesh value (**Costa et al., 2013**) characterized by little fat content and good taste. That is why this species is economically important (**Monfort, 2010**) in the Mediterranean countries. Moreover, it is among the most abundant fish (**Faliex et al., 2008; Antonelli et al., 2016**).

Wild and farmed fish have varied biochemical, sensory, and physical traits due to their differing diets. For instance, the composition of fatty acids (**Grigorakis, 2007; Chaguri et al., 2017**) and the macro- and microminerals levels (**Minganti et al., 2010; Custodio et al., 2011**) differ greatly. The indicated changes can be utilized to provide traceability (**Rasmussen et al., 2000; Busetto et al., 2008**). The authenticity of the fish can be evaluated using various parameters, such as fatty acid profiles (**Gonzalez, 2006; Thomas et al., 2008**), forms of pigments (**Moretti et al., 2006**) and various contaminants occurrence (**Fallah et al., 2011**). Heavy metals, among other stressors, can disrupt essential physiological processes in fish, produce reactive oxygen species (ROS), and prompt oxidative stress. Monitoring antioxidant defense mechanisms in fish can serve as a biomarker for fish health and ecosystem stress (**Mahmood et al., 2021; El-Metwally et al., 2023**).

In the human diet, the fish are considered an indispensable part because of its high protein, omega-3 fatty acid, and vitamin content, which reduce inflammation and cardiovascular disease risk. Given these benefits, the American Heart Association endorses including fish in the diet two-times per week to meet omega-3 fatty acid needs (**El-Moselhy et al., 2014**). However, in polluted environment, fish can accumulate heavy metals in edible tissues and becoming a serious source of human exposure to toxic chemicals (**Miri et al., 2017**). In severe exposure, they could impose adverse consequences such as impaired kidney function, lung and liver malfunctioning, in addition to long-term injury in the nervous system (**Yu et al., 2016**). Thus, monitoring heavy metals in food is crucial, particularly in fish which is essential in human diet. Meager recently become a favorable candidate for farming in the Mediterranean region.

Nevertheless, few researches have examined its nutritional and ecotoxicological values. Meagre muscles heavy metal accumulation has been assessed in Portugal (Carvalho *et al.*, 2005), and in the Gironde estuary (Durrieu *et al.*, 2005). Moreover, the nutritional composition of edible part was studied in samples from Greece (Grigorakis *et al.*, 2011), Portugal (Costa *et al.*, 2013), Italy (Martelli *et al.*, 2013), and Spain (García Mesa *et al.*, 2014). Thus, this study aimed to reveal the accumulation of heavy metals in two different size categories of fish from Egypt's Mediterranean water and from locally farmed fish, in addition to estimating the potential harm to human health through food intake.

MATERIALS AND METHODS

Wild meagre were captured by local fishermen from the Egyptian Mediterranean coast, specifically, the region facing Alexandria, afterward it was landed in El-Meadyia fishing port, Behira, Egypt. Aquacultured fish were collected from a local farm located in Maryut valley, Alexandria, Egypt (Fig. 1), where they fed trash fish such as small sardines and the tilapia in net cages. All samples were carefully cleaned and quickly transported in ice to the laboratory to execute further analysis.



Fig. 1. Sampling sites: a) fisheries location and b) fish farms in Maryut valley

Fish sampling and tissue preparation

The morphological characteristics of farmed and wild meagre were recorded before dividing fish into large and small category, constituting four experimental groups, namely large wild (LWM), small wild (SWM), large farmed (LFM) and small farmed (SFM), as shown in Table (1). Prewashed fish were dismembered on ice, and samples from the hepatic tissue and dorsal muscles were obtained. Muscle was distributed into two aliquot for heavy metal determination and biochemical analysis, while liver was used for the antioxidant determination. For biochemical analysis, obtained tissue was weighed after rinsing in isotonic saline. Then, 1g of the sample was used to prepare a homogenate in 9 volumes of ice-cold (0.1M) phosphate buffer (pH 7.4). The mixture was centrifuged at 15,000g for 15min using a cooling centrifuge (4°C). The supernatant was collected and

utilized for the analysis of muscle's biochemical composition and liver's antioxidant response employing a UV/Vis spectrophotometer (JENWAY 6505, UK).

Table 1. The recorded weight of studied fish groups (average \pm standard deviation)

Weight (gm)	LWM n= 5	SWM n= 5	LFM n= 5	SFM n= 5
Mean \pm Sd	1997.00 \pm 154.76	985.80 \pm 77.22	1978.50 \pm 138.20	996.50 \pm 72.32
Minimum	1735	875	1785	875
Maximum	2210	1095	2300	1115

LWM: Large wild meagre, SWM: Small wild meagre, LFM: Large farmed meagre; SFM: Small farmed meagre.

Biochemical composition of the muscle

The muscle supernatant was analyzed for total protein and lipid content following the methods outlined by **Gornall *et al.* (1949)** and **Zollner *et al.* (1962)**, respectively. The **AOAC (1995)** method was used to analyze moisture and ash contents. The moisture content was assessed by subjecting the samples to a drying process in a 135°C oven for duration of two hours. Subsequently, the samples were cooled in a desiccator and reweighed. The ash content was determined by weighing pre-dried tissue before and after 6 hours of heating at 600- 650°C in a muffle furnace. The percentage of weight ratio is the ash content.

Quantification of antioxidants biomarkers in liver

The chemicals for this analysis were purchased from Biodiagnostic Company kits, Cairo, Egypt. Fish liver's SOD and CAT activities as well as TAC were measured as stated by **Nishikimi *et al.* (1972)**, **Aebi (1984)** and **Koracevic *et al.* (2001)** protocols, respectively. Specific enzymatic activities were estimated from dividing the obtained activity by the total protein content (**Salem & Ibrahim, 2022**).

Determination of metal concentration

To study the levels of heavy metals in the white dorsal muscles, one gram of the sample was treated with a ratio of 2 concentrated nitric acid (HNO₃) and 1 perchloric acid (HClO₄), and left overnight before being heated up to 100°C until a complete digestion was achieved. After cooling to room temperature, the sample was diluted with deionized water, filtered, and adjusted to required volume with deionized water. This solution was subjected for the analysis of Cd, Pb, Hg, Mn, and Ni utilizing a PerkinElmer Atomic Absorption Spectrometer (AAS Perkin Elmer, Pinnacle 900T, USA). The results were expressed in mg/kg wet weight. All measurements were carried out in triplicate, blank and standard solutions were created for the calibration curves, following the concentrations indicated by **AOAC (2015)**. In addition, heavy metals were extracted from seawater sample according to **Brewer *et al.* (1969)**, analyzed with the AAS, and the concentrations were expressed in µg/l.

Metal pollution index (MPI)

The MPI was calculated to evaluate the overall extent of metal buildup in fish muscle utilizing the equation of **Usero *et al.* (1997)**:

$$\text{MPI} = (M_1 \times M_2 \times M_3 \times \dots \times M_n)^{1/n} \quad (1)$$

M_n is the metal concentration (mg/Kg wet weight), and n is the metals number.

Evaluation of the potential health hazards

Numerous metrics were adopted to evaluate the potential impact on human health from consuming contaminated meagre. Initially, the overall consumed metals were measured through the estimated daily (EDI) and weekly intake (EWI). Then, we evaluated the probability of non-carcinogenic (Target Hazard Quotient (THQ)) and carcinogenic risk (CR) resulted from the metals. To set benchmarks for safe levels of weekly metal intake, we calculated the provisional tolerable weekly intakes (PTWIs) and tolerable weekly intakes (TWIs) set by health authorities. For the calculation of EDI ($\mu\text{g}/\text{kg}$ bw/day) of fish for the Egyptian individuals, the following equation was employed:

$$(\text{EDI}) = (\text{MC} \times \text{DI}) \times \text{BW}^{-1} \quad (2)$$

Where MC is the metal concentration in fish muscle (mg/Kg wet weight), DI is the daily average intake of fish (62.25 g/person/day) for the Egyptian population (**GAFRD, 2021**), and BW is the average body weight (70 kg for an adult, approximately 45 kg for the young (youth), and children approximately 15 kg).

The EWI ($\mu\text{g}/\text{kg}$ bw/week) was assessed following the formula:-

$$(\text{EWI}) = \text{EDI} \times 7 \text{ days} \quad (3)$$

The result of EWI was evaluated by comparing with PTWI described in FAO/WHO food safety standards. Meagre consumption has no significant risk when the calculated EWI is smaller than the international PTWI (**FAO/WHO, 2004**).

$$\text{PTWI \%} = (\text{EWI}/\text{PTWI}) \times 100 \quad (4)$$

Additionally, the weekly safety intake of meagre that should be consumed by children, adolescents, and adults used to calculate the maximum daily intake (MDI) to achieve the PTWI (**FAO/WHO, 2004**).

$$\text{MDI} = (\text{PTWI} \times \text{BW})/(\text{C} \times 7) \quad (5)$$

$$\text{MWI} = \text{MDI} \times 7 \quad (6)$$

Non-carcinogenic risk

THQ and hazard index (HI) are two common indices which describe the prospective of health risk that do not include the carcinogenic potential, and is estimated from the following equation (USEPA, 1989):

$$THQ = (EF \times ED \times FIR \times MC) / (RFD \times BW \times AT) \times 10^{-3} \quad (7)$$

To evaluate the potential health risks from heavy metals in fish, several parameters are considered. The concentration of heavy metals (MC) in the fish is compared against reference oral dosages (RFDs), which are the safe daily exposure levels: mercury (Hg) at $1.6 \times 10^{-4} \mu\text{g/g/day}$, lead (Pb) at $3.5 \times 10^{-4} \mu\text{g/g/day}$, cadmium (Cd) at $1 \times 10^{-3} \mu\text{g/g/day}$, manganese (Mn) at $1.4 \times 10^{-2} \mu\text{g/g/day}$, and nickel (Ni) at $2 \times 10^{-2} \mu\text{g/g/day}$ (USEPA, 2010, 2014, 2017; Jooste *et al.*, 2014; Silva *et al.*, 2020). The fish ingestion rate (FIR), assumed to be 62.25 grams per day (GAFRD, 2021), and the body weight (BW) of an average adult, typically 70 kg, are also taken into account. The average time of non-carcinogenic exposure (AT) is calculated as 365 days/year \times ED 365 days/year \times ED, with an exposure duration (ED) considered to be 30 years. These parameters help estimate the potential exposure levels and assess whether they exceed the established safety thresholds. Fish consumption is considered safe if the THQ is less than 1 (Sallam *et al.*, 2019).

$$HI = HQ(\text{Cd}) + HQ(\text{Pb}) + HQ(\text{Hg}) + HQ(\text{Mn}) + HQ(\text{Ni}) \quad (8)$$

Carcinogenic risk

The CR is a developing cancer probability measure due to probable carcinogens exposure over individual's lifetime. It could be assessed with the cancer slope factors (CSF) (USEPA, 2012), considering the CSF of Cd, Pb and Ni values are 15, 0.0085 and 1.7 mg/kg/day, correspondingly according to this formula:

$$CR = CSF \times EDI \quad (9)$$

Target carcinogenic risk (TCR) (USEPA, 2012) has been estimated according to this equation:

$$TCR = (EF \times ED \times IR \times C \times CSF / WAB \times ATc) \times 10^{-3} \quad (10)$$

USEPA (2012) defined CR values as negligible if they are lower than 1×10^{-6} (risk of 1 in 1,000,000 over 70 years), unacceptable when they are above 1×10^{-4} (risk of 1 in 10,000), acceptable between 10^{-6} and 10^{-4} , if $CR \leq 10^{-3}$ to 10^{-1} it's high risk, and at $CR \geq 10^{-1}$, it's very high risk (US EPA, 1989; NYSDOH 2007).

Statistical analysis

SPSS software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA) were employed to analyze all data. Statistical significance was determined depending on one-way and two-way analysis of variance (ANOVA) with a confidence limit of 95%, and

followed by Duncan's multiple-range as post hoc test ($P < 0.05$). The heavy metals accumulation association with fish groups as well as other measured parameters were estimated using the principal component analysis (PCA).

RESULTS

Muscle biochemical composition

Table (2) displays the biochemical composition of the meagre white muscles. We found no significant differences ($P > 0.05$) in the moisture, ash, total lipid, and total protein levels among the different sizes and sources of the examined meagre.

Table 2. Wild and farmed meagre proximate analysis % on dry matter basis (average \pm standard deviation)

%	LWM	SWM	LFM	SFM
Moisture	77.75 \pm 0.58	78.02 \pm 0.17	78.17 \pm 0.42	77.99 \pm 0.21
Fat	7.23 \pm 0.50	7.15 \pm 0.18	7.80 \pm 0.19	7.26 \pm 0.22
Ash	1.28 \pm 0.08	1.28 \pm 0.06	1.33 \pm 0.04	1.31 \pm 0.05
Crude protein	18.49 \pm 0.73	18.25 \pm 0.75	19.07 \pm 0.09	18.79 \pm 0.25

Antioxidant biomarkers

The current investigation demonstrated a considerable increase ($P < 0.05$) in liver SOD and CAT total and specific activities in aquacultured fish as compared to wild fish. Among the groups, the small farmed meagre (SFM) recorded the highest activities of SOD and CAT. However, the variations in total antioxidant capacity (TAC) were not significant among the studied groups (Fig. 2).

Heavy metal concentrations and metal pollution index

The water characteristics of the open sea compared to the farm are presented in Table (3). Except for Mn, the Pb, Cd, Hg, and Ni levels between the two environments were insignificant ($P > 0.05$). The Mn levels in the farm water were substantially greater ($P < 0.05$) compared to the saltwater. On the other hand, all physical characteristics were significantly different ($P < 0.05$), with higher dissolved oxygen (DO), pH, and turbidity, and lower salinity in the aquaculture environment compared to seawater.

Table 3. Water quality and trace elements content of seawater and aquaculture water in mg/L (average \pm standard deviation)

Parameter	Seawater (WM)	Aquaculture farm (FM)
DO%	101.91 \pm 7.73	117.19 \pm 2.42
pH	7.94 \pm 0.09	8.14 \pm 0.09
TDS g/l	60.80 \pm 3.35	36.41 \pm 2.47
Salinity	35.31 \pm 0.57	14.86 \pm 0.60
EC ms/cm	86.22 \pm 8.09	44.74 \pm 9.62
Turbidity	56.50 \pm 3.56	76.49 \pm 10.72
Minerals		
Pb	0.043 \pm 0.004	0.036 \pm 0.01
Cd	0.060 \pm 0.02	0.061 \pm 0.02
Hg	0.075 \pm 0.02	0.076 \pm 0.02
Mn	0.104 ^b \pm 0.06	0.380 ^a \pm 0.06
Ni	0.302 \pm 0.19	0.432 \pm 0.18

Muscles heavy metals bioaccumulation are presented in Table (4). Pb recorded the lowest levels with concentrations from 0.020 to 0.029 μ g/ g, followed by Cd with levels from 0.036 to 0.048 μ g/ g, then Hg recorded 0.045 to 0.061 μ g/ g, and Mn recorded 0.06 to 0.345 μ g/ g, and finally Ni recorded 0.146 to 0.4112 μ g/ g. The two-way ANOVA indicated that Ni varied significantly among the size-group and the fish-source ($P < 0.05$), where aquacultured and smaller size individuals exhibited a higher tendency to accumulate Ni. Contrariwise, Pb, Cd and Hg displayed an inconsequential trend ($P > 0.05$) of higher levels in wild compared to farmed fish muscles. In total, the MPI indicated higher degree of contamination in the smaller fish, where the values were in the following order SFM > SWM > LFM > LWM (Table 4).

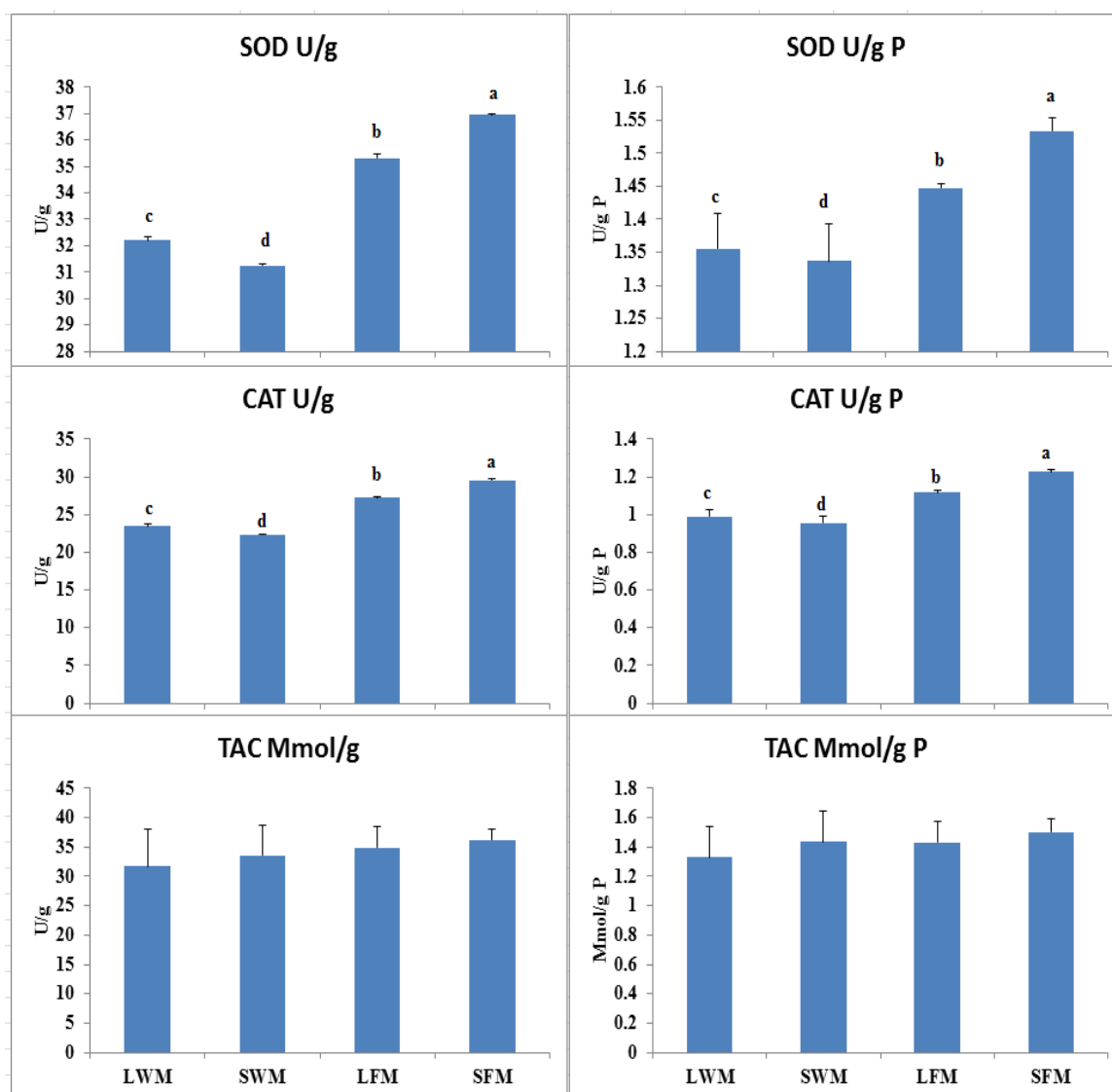


Fig. 2. Fish liver total and specific catalase (CAT), superoxide dismutase (SOD) activities and total antioxidant capacity (TAC) in different fish groups. Letters indicate significance at ($P < 0.05$).

The association among heavy metals accumulation and antioxidant response is illustrated by the PCA (bi-plot, Fig. 3). The major three components accounted for 83.5% of the variances. PC1 was principally related to Ni, Mn, SOD and CAT, and mainly associated with samples from aquaculture. The 2nd PC reveals high connection between Cd and Hg, while, the 3rd PC describe only Pb. PC2 and PC3 highly associated with samples from the wild.

Evaluation of health risk connected with consuming meagre

Table (5) shows EDI ($\mu\text{g}/\text{kg bw}/\text{w}$), EWI ($\mu\text{g}/\text{kg bw}/\text{w}$), also Table (6) displays PTWI%, MDI and MWI that should be consumed by children, teenagers, and adults.

Generally, the wild fish exhibited higher values with Pb, Cd and Hg in the three age-groups. While, Ni recorded higher values in the aquacultured fish.

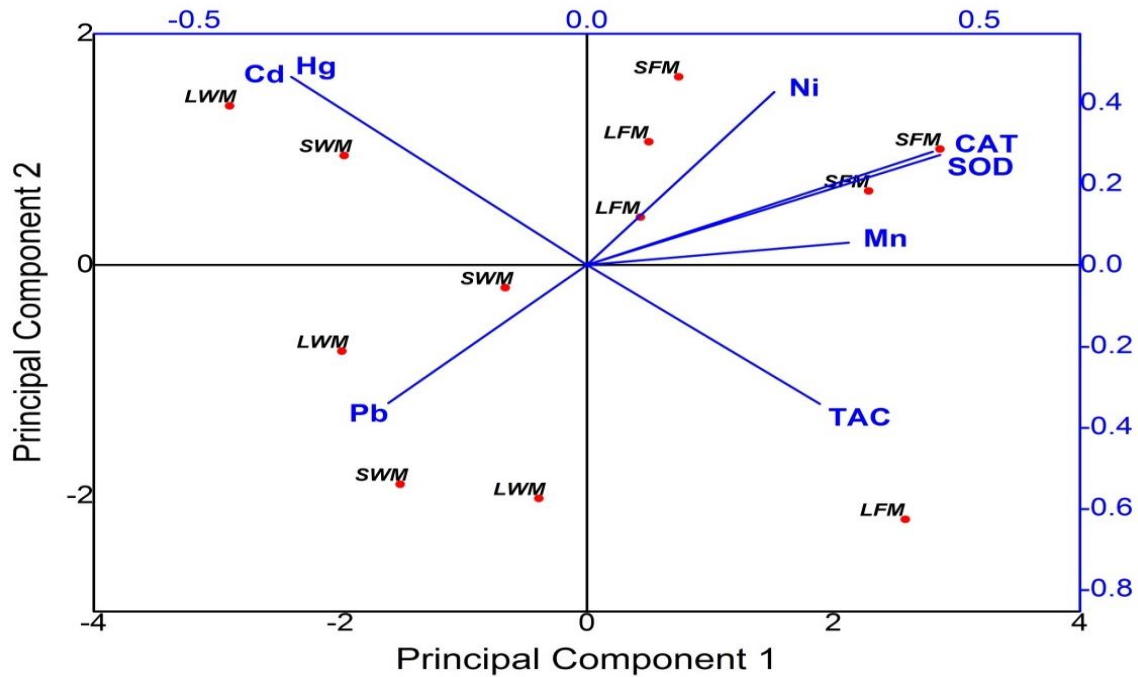


Fig. 3. Principal component analysis (PCA) of heavy metals accumulation and antioxidant response in different fish groups

Non carcinogenic risk

In Table (7), the THQ for the five metals in the wild and farmed meagre of different sizes (small and large) consumed 1, 3, and 7 times per week are presented. The THQ for Pb, Cd, Mn, and Ni were all less than 1, demonstrating that there are no health risks correlated with consuming a single heavy metal through the meagre consumption from both sources and sizes, regardless of the frequency of consumption. However, the THQ for Hg are less than 1 if people consumed the meagre one time per week from all sources and sizes or three times per week for the meagre from LWM, LFM and SFM but not from SWM which accounted more than 1 (1.01295 ± 0.16353). This indicates that consuming these species may pose health hazards related to Hg if consuming SWM 3 times per week and also if meagre from all sources were consumed 7 times per week. HI for the five metals were less than 1 if people consumed the meagre one time per week from all sources and sizes, and if they consumed the meagre three times per week from LFM, there were no risks in both cases. SFM were considerably ($P < 0.05$) higher in Mn and Ni contents if the meagre consumed one, three or seven times per week compared with other sources and sizes of the meagre (Fig. 4).

Table 4. Heavy metals accumulation in the muscles and metal pollution index (MPI) in different fish groups

	Pb	Cd	Hg	Mn	Ni	MPI
LWM	0.0296 ± 0.006	0.0479 ± 0.018	0.0599 ± 0.021	0.0551 ^c ± 0.0052	0.1460 ^b ± 0.0029	0.0576 ± 0.0076
SWM	0.0287 ± 0.008	0.0486 ± 0.008	0.0608 ± 0.010	0.2897 ^{bc} ± 0.2476	0.2309 ^b ± 0.1319	0.0828 ± 0.0247
LFM	0.0200 ± 0.007	0.0358 ± 0.017	0.0447 ± 0.021	0.3445 ^b ± 0.1679	0.1670 ^b ± 0.0171	0.0671 ± 0.0073
SFM	0.0228 ± 0.008	0.0407 ± 0.015	0.0509 ± 0.018	0.3258 ^a ± 0.0513	0.4112 ^a ± 0.0286	0.0897 ± 0.0141
FAO/WHO 1989	0.5	0.1	0.5	1.0		
(USFDA 1993)					70-80	
EC 1881/2006	0.3	0.05	0.5			

Different letters within the same column indicates significance at $P < 0.05$.

Table 5. The estimated daily and weekly dietary intakes (EDI and EWI) in different fish groups

Parameter	LWM		SWM		LFM		SFM		
	EDI	EWI	EDI	EWI	EDI	EWI	EDI	EWI	
Pb	Child	0.123 ± 0.025	0.860 ± 0.174	0.119 ± 0.033	0.834 ± 0.232	0.083 ± 0.028	0.581 ± 0.197	0.094 ± 0.032	0.663 ± 0.225
		Young	0.041 ± 0.008	0.287 ± 0.058	0.040 ± 0.011	0.278 ± 0.077	0.028 ± 0.009	0.194 ± 0.066	0.032 ± 0.011
	Adult	0.026 ± 0.005	0.184 ± 0.037	0.026 ± 0.007	0.179 ± 0.050	0.018 ± 0.006	0.124 ± 0.042	0.020 ± 0.007	0.142 ± 0.048
Cd	Child	0.199 ± 0.073	1.393 ± 0.513	0.202 ± 0.033	1.412 ± 0.228	0.148 ± 0.070	1.039 ± 0.492	0.169 ± 0.061	1.184 ± 0.424
		Young	0.066 ± 0.024	0.464 ± 0.171	0.067 ± 0.011	0.471 ± 0.076	0.050 ± 0.023	0.346 ± 0.164	0.056 ± 0.020
	Adult	0.043 ± 0.016	0.298 ± 0.110	0.043 ± 0.007	0.303 ± 0.049	0.032 ± 0.015	0.223 ± 0.106	0.036 ± 0.013	0.254 ± 0.091
Hg	Child	0.249 ± 0.092	1.741 ± 0.642	0.252 ± 0.041	1.765 ± 0.285	0.186 ± 0.088	1.299 ± 0.615	0.211 ± 0.076	1.479 ± 0.531
		Young	0.083 ± 0.031	0.580 ± 0.214	0.084 ± 0.014	0.588 ± 0.095	0.062 ± 0.029	0.433 ± 0.205	0.070 ± 0.025
	Adult	0.053 ± 0.020	0.373 ± 0.138	0.054 ± 0.009	0.378 ± 0.061	0.040 ± 0.019	0.278 ± 0.132	0.045 ± 0.016	0.317 ± 0.114
Mn	Child	0.229 ^b ± 0.022	1.601 ^a ± 0.150	1.202 ^{ab} ± 1.028	8.414 ^{ab} ± 7.193	1.430 ^{ab} ± 0.697	10.008 ^{ab} ± 4.878	1.352 ^a ± 0.213	9.465 ^a ± 1.491
		Young	0.076 ^b ± 0.007	0.534 ^b ± 0.050	0.401 ^{ab} ± 0.343	2.805 ^{ab} ± 2.398	0.477 ^{ab} ± 0.232	3.336 ^{ab} ± 1.626	0.451 ^a ± 0.071
	Adult	0.049 ^b ± 0.005	0.343 ^b ± 0.032	0.258 ^{ab} ± 0.220	1.803 ^{ab} ± 1.542	0.306 ^{ab} ± 0.149	2.145 ^{ab} ± 1.045	0.290 ^a ± 0.046	2.028 ^a ± 0.319
Ni	Child	0.606 ^b ± 0.012	4.241 ^b ± 0.086	0.958 ^{ab} ± 0.548	6.707 ^{ab} ± 3.835	0.693 ^b ± 0.071	4.852 ^b ± 0.497	1.707 ^a ± 0.119	11.946 ^a ± ± 0.831
		Young	0.202 ^b ± 0.004	1.414 ^b ± 0.029	0.319 ^{ab} ± 0.183	2.236 ^{ab} ± 1.278	0.231 ^b ± 0.024	1.617 ^b ± 0.1656	0.569 ^a ± 0.040
	Adult	0.130 ^b ± 0.003	0.909 ^b ± 0.018	0.205 ^{ab} ± 0.117	1.437 ^{ab} ± 0.821	0.149 ^b ± 0.015	1.040 ^b ± 0.107	0.366 ^a ± 0.026	2.560 ^a ± 0.178

Different letters within the same row for each metal, parameter, and each age are for significance of treatments' effects ($P < 0.05$).

Carcinogenic risk (CR)

Table (8) shows CR and TCR values of Pb, Cd, and Ni. The current study found that the CR level for Pb in all the investigated meagre for children, youth, and adults ranges between 10^{-6} and 10^{-7} . If people consumed the meagre one, three or seven times per week from various sources and sizes, there would be no risk of cancer for these consumers. TCR level of Pb were 10^{-8} , indicating no carcinogenic risk from the Pb-contaminated meagre. Regarding Cd, the CR levels ranged between 10^{-3} and 10^{-4} , indicating moderate risk if people consumed the Cd-contaminated meagre one, three or seven times per week. Similarly, TCR level were between 10^{-3} and 10^{-4} , suggesting moderate carcinogenic risk from the Cd-contaminated meagre. The CR levels for Ni were between 10^{-3} and 10^{-4} . These values can pose moderate danger from Ni-contaminated fish if the meagre is consumed one, three or seven times per week. However, TCR of Ni suggested no carcinogenicity with levels ranging between 10^{-9} and 10^{-10} .

DISCUSSION

The biochemical composition examination of the white muscles in the meagre conducted in this work showed inconsequential variations ($P > 0.05$) in moisture, ash, total lipid, and total protein values among the various sizes and sources of the meagre. These findings are different from the study of **Martelli *et al.* (2013)**, who found that the chemical composition of the meagre raw fillets changed with time. More precisely, they noted that the lipid content correlated positively with the size of the fish. Furthermore, **Shearer (1994)** suggested that the composition of the flesh underwent alterations in accordance with its growth. The restricted deposition of lipids in muscle tissue in the meagre is corroborated with earlier studies conducted on both adult individuals (**Poli *et al.*, 2003; Piccolo *et al.*, 2008; Hernandez *et al.*, 2009**) and juvenile (**Chatzifotis *et al.*, 2010**). This feature sets it apart from other regularly eaten European aquacultured species, like the sea bass and sea bream, which often have more lipid content (**Grigorakis *et al.*, 2002; Yildiz *et al.*, 2008**).

Contrariwise, the farmed, in particular SFM, exhibited higher liver activities of SOD and CAT compared to the wild fish. This outcome disagrees with the results of **Ferreira *et al.* (2008)**, who observed higher levels of antioxidant enzymes in the liver of the wild white-seabream contrasted with the cultured fish. They attributed their observation to the wild fish higher heavy metals accumulation. However, our finding suggests that other water quality parameters (as salinity, pH, and DO) may influence the levels of antioxidant enzymes in fish liver. Other factors are also suggested to influence the antioxidant capacity and lipid peroxidation such as the marine synbiotic (**Salem & Ibrahim, 2022**) and diet (**Saavedra *et al.*, 2023**).

Table 6. The standard provisional tolerable weekly intakes (PTWIs), maximum daily intake MDI (in grams) and maximum weekly intake WDI (in grams) based on the weekly safety intake of different fish groups in children, teenagers, and adults

Parameter		LWM	SWM	LFM	SFM	
Pb	PTWI%	3.441 ± 0.697	3.338 ± 0.926	2.323 ± 0.787	2.653 ± 0.899	
	MDI	Child	0.224 ± 0.089	0.216 ± 0.109	0.107 ± 0.068	0.140 ± 0.088
		Young	0.523 ± 0.207	0.504 ± 0.255	0.250 ± 0.158	0.326 ± 0.206
		Adult	1.047 ± 0.414	1.008 ± 0.509	0.499 ± 0.316	0.652 ± 0.412
	MWI	Child	1.570 ± 0.620	1.512 ± 0.764	0.750 ± 0.474	0.978 ± 0.619
		Young	3.663 ± 1.447	3.528 ± 1.782	1.749 ± 1.107	2.282 ± 1.444
		Adult	7.327 ± 2.894	7.055 ± 3.563	3.500 ± 2.214	4.565 ± 2.888
	Cd	PTWI%	4.096 ± 0.829	3.973 ± 1.103	2.766 ± 0.937	3.158 ± 1.070
		MDI	Child	0.406 ± 0.101	0.415 ± 0.133	0.192 ± 0.069
Young			0.948 ± 0.235	0.968 ± 0.309	0.448 ± 0.163	0.688 ± 0.466
Adult			12.350 ± 9.595	19.360 ± 6.184	8.956 ± 3.256	13.753 ± 9.324
MWI		Child	2.845 ± 0.704	2.904 ± 0.928	1.343 ± 0.488	2.063 ± 1.399
		Young	6.638 ± 1.642	6.776 ± 2.164	3.135 ± 1.139	4.814 ± 3.263
		Adult	86.451 ± 67.168	135.523 ± 43.287	62.691 ± 22.791	96.271 ± 65.265
Hg		PTWI%	11.520 ± 2.334	11.175 ± 3.101	7.778 ± 2.636	8.883 ± 3.011
		MDI	Child	1.429 ± 0.353	1.459 ± 0.466	0.675 ± 0.245
	Young		3.334 ± 0.825	3.403 ± 1.087	1.574 ± 0.572	2.418 ± 1.639
	Adult		6.667 ± 1.649	6.806 ± 2.174	3.149 ± 1.145	4.835 ± 3.278
	MWI	Child	10.001 ± 2.474	10.210 ± 3.261	4.723 ± 1.717	7.253 ± 4.917
		Young	23.335 ± 5.772	23.822 ± 7.609	11.020 ± 4.006	16.923 ± 11.472
		Adult	46.670 ± 11.544	47.645 ± 15.218	22.039 ± 8.012	33.845 ± 22.945
	Mn	PTWI%	0.038 ± 0.014	0.039 ± 0.006	0.028 ± 0.0135	0.0323 ± 0.0116
		MDI	Child	0.005 ^b ± 0.002	0.024 ^{ab} ± 0.019	0.0177 ^a ± 0.003
Young			0.011 ^b ± 0.005	0.056 ^a ± 0.045	0.041 ^{ab} ± 0.006	0.051 ^{ab} ± 0.011
Adult			0.021 ^b ± 0.010	0.112 ^{ab} ± 0.089	0.083 ^a ± 0.012	0.102 ^a ± 0.022
MWI		Child	0.032 ^b ± 0.014	0.169 ^{ab} ± 0.134	0.124 ^a ± 0.018	0.153 ^a ± 0.033
		Young	0.075 ^b ± 0.034	0.393 ^{ab} ± 0.313	0.289 ^a ± 0.042	0.357 ^a ± 0.076
		Adult	0.149 ^b ± 0.067	0.787 ^{ab} ± 0.626	0.579 ^a ± 0.083	0.714 ^a ± 0.153
Ni		PTWI%	100.086 ^b ± 9.396	525.896 ^{ab} ± 449.590	625.483 ^a ± 304.857	591.531 ^{ab} ± 93.142
		MDI	Child	31.359 ^b ± 3.572	263.405 ^{ab} ± 205.869	217.468 ^{ab} ± 84.200
	Young		73.157 ^b ± 8.335	614.612 ^{ab} ± 480.362	507.426 ^{ab} ± 196.468	1225.102 ^a ± 276.259
	Adult		146.313 ^b ± 16.669	1229.224 ^{ab} ± 960.724	1014.852 ^{ab} ± 392.935	2450.205 ^a ± 552.518
	MWI	Child	219.470 ^b ± 25.004	1843.836 ^{ab} ± 1441.086	1522.279 ^{ab} ± 589.403	3675.307 ^a ± 828.777
		Young	512.096 ^b ± 58.342	4302.284 ^{ab} ± 3362.535	3551.983 ^{ab} ± 1375.274	8575.716 ^a ± 1933.813
		Adult	1024.192 ^b ± 116.683	8604.568 ^{ab} ± 6725.070	7103.966 ^{ab} ± 2750.548	17151.432 ^a ± 3867.625

Different letters within the same row for each metal, parameter, and each age are for significance of treatments' effects ($P < 0.05$).

Heavy metals concentrations in the present study showed different accumulation potential between the wild and farmed fish. The muscle content of Ni changed significantly among the size-group and the fish-source. Higher levels of Ni were observed in the smaller-size as well as in the farmed fish, which coincide with the higher levels of Ni in the aquaculture water. Alternatively, the accumulation of Pb, Cd and Hg showed a non-significant trend of higher levels in the muscle of wild. Similar trend of higher Cu, Cd, As and Pb in the wild seabream compared to the cultivated counterparts was

observed by **Ferreira et al. (2008)**. In fact, some studies recorded 4 up to 50 times higher levels of Cd, As, Pb, Zn, and Fe in the wild fish flesh (**Chaguri et al., 2017**). The reason may be related to multiple external influences including nutrition type and geographic distribution, which could vary greatly in relation to the local environmental conditions (**Orban et al., 2003**). In total, the MPI based on the studied metals (i.e., Cd, Pb, Hg, Ni and Mn) ranged from 0.058 to 0.090 and showed general higher value in the small-sized meagre. As an average indication of pollution, the MPI describes the lower contamination condition of fish in the present study with regard to previous work. The MPI estimated for the captured meagre (*A. regius*) from the westAtlantic ocean was 0.1 (**Elaarabi et al., 2022**). The MPI values for sardines and anchovies were reported to range from 0.46 to 0.76 and 0.65 to 0.89, respectively (**Sofoulaki et al., 2019**). According to **Ahmed et al. (2019)**, the demersal and pelagic fish species values were 3.65 to 4.70, respectively. In addition, the average MPI was 2.5 in the muscles of the tilapia (*Oreochromis niloticus*) from the Egyptian fishponds (**Abbas et al., 2023**), while it ranged between 2.63 and 4.63 in eight fish species from the Red Sea (**El-Shorbagy et al., 2024**).

Additionally, all recorded heavy metals concentration in the flesh of the meagre in the current study were below the MPL in line with the international standards of the **FAO/WHO (1989)**, **USFDA (1993)** and the **European Commission (EC) (1881/2006)**. **Elaarabi et al. (2022)** found that Pb concentrations in the muscles of the captured meagre (*A. regius*) from the Moroccan-side of the Atlantic waters were 0.004mg.kg^{-1} compared to $0.02 - 0.03\text{mg.kg}^{-1}$ in the present study. **Baki et al. (2018)** reported that Pb values in fish muscles varied between <0.06 to 8.92mg.kg^{-1} . Whereas, the international safety guidelines of Pb in fish are limited to 0.3mg/ kg (**European Union, 2006**). Publications recorded Cd accumulation in the fish muscles with 0.002 and 0.480mg/ kg (**Giri & Singh, 2015**). **Chahid et al. (2014)**, recorded Cd in various fish types from the Atlantic between 0.005 and 0.036mg/ kg . The safety criteria recommended for Cd in fish by international organization is 0.05mg.kg^{-1} . However, the **European Union (2014)** established differing safety limits for *Scombridae* (0.1mg.kg^{-1}) as well as the swordfish, anchovies and the European pilchard (0.25mg.kg^{-1}). The Cd concentrations in the present study (0.036 to 0.049mg.kg^{-1}) were lower than the previous fish consumption safety limits. However it was higher than the Cd content recorded by **Elaarabi et al. (2022)** in the captured meagre (*A. regius*) from the Moroccan Atlantic waters, with a value of 0.0002mg.kg^{-1} .

Hg is environmentally extremely toxic, non-essential and persistent heavy metal (**Korbas et al., 2011**). Its accumulation can be harmful and fatal because it's similar in important peptides and proteins to thiol groups (**Mieiro et al., 2010**). The meagre accumulation of Hg could be related to its food, trophic level, and regional dispersal. Several studies such as **Afonso et al. (2015)** and **Di Lena et al. (2017)** detected an accumulation of Hg in the meager muscles higher than the recommended regulatory

standards, with levels ranging between 0.255 and 0.251mg kg⁻¹ since it is relatively on the top of the marine trophic chain (Amoussou *et al.*, 2019). However, the Hg accumulation recorded values of 0.02 to 0.03mg.kg⁻¹ in the current study. The meagre is a highly adaptive species to the environmental fluctuations (Monfort, 2010). It can reduce its accumulation potential under stress such as heat and can minimize the accumulation of Hg (Sampaio *et al.*, 2018).

Table 7. The target hazard quotient (THQ) to the health of people consuming fish in different fish groups

THQ	Meagre	LWM	SWM	LFM	SFM
Pb	Once a week	0.0075 ± 0.0015	0.0073 ± 0.0020	0.0051 ± 0.0017	0.0058 ± 0.0020
	3 times a week	0.0226 ± 0.0046	0.0219 ± 0.0061	0.0152 ± 0.0052	0.0174 ± 0.0059
	7 times a week	0.0527 ± 0.0107	0.0511 ± 0.0142	0.0356 ± 0.0121	0.0406 ± 0.0138
Cd	Once a week	0.0426 ± 0.0157	0.0432 ± 0.0070	0.0318 ± 0.0151	0.0362 ± 0.0130
	3 times a week	0.1279 ± 0.0472	0.1297 ± 0.0209	0.0954 ± 0.0452	0.1087 ± 0.0390
	7 times a week	0.2984 ± 0.1100	0.3025 ± 0.0488	0.2226 ± 0.1055	0.2536 ± 0.0909
Hg	Once a week	0.3331 ± 0.1228	0.3377 ± 0.0545	0.2484 ± 0.1178	0.2830 ± 0.1015
	3 times a week	0.9992 ± 0.3684	1.0130 ± 0.1635	0.7453 ± 0.3532	0.8491 ± 0.3045
	7 times a week	2.3315 ± 0.8595	2.3636 ± 0.3816	1.7391 ± 0.8242	1.9813 ± 0.7104
Mn	Once a week	0.0004 ^b ± 0.0001	0.0018 ^{ab} ± 0.0016	0.0022 ^{ab} ± 0.0011	0.0021 ^a ± 0.0003
	3 times a week	0.0011 ^b ± 0.0001	0.0055 ^{ab} ± 0.0047	0.0066 ^{ab} ± 0.0032	0.0062 ^a ± 0.0010
	7 times a week	0.0025 ^b ± 0.0002	0.0129 ^{ab} ± 0.0111	0.0153 ^{ab} ± 0.0075	0.0145 ^a ± 0.0023
Ni	Once a week	0.0065 ^b ± 0.0001	0.0103 ^{ab} ± 0.0059	0.0074 ^b ± 0.0008	0.0183 ^a ± 0.0013
	3 times a week	0.0195 ^b ± 0.0004	0.0308 ^{ab} ± 0.0176	0.0223 ^b ± 0.0023	0.0549 ^a ± 0.0038
	7 times a week	0.0454 ^b ± 0.0009	0.0719 ^{ab} ± 0.0411	0.0520 ^b ± 0.0053	0.1280 ^a ± 0.0089
HI	Once a week	0.3901 ± 0.1376	0.4003 ± 0.0676	0.2949 ± 0.1309	0.3454 ± 0.1148
	3 times a week	1.1702 ± 0.4127	1.2008 ± 0.2029	0.8848 ± 0.3927	1.0363 ± 0.3444
	7 times a week	2.7304 ± 0.9630	2.8019 ± 0.4734	2.0646 ± 0.9164	2.4180 ± 0.8036

Different letters within the same row for each metal and each time are for significance of treatments' effects ($P < 0.05$).

Ni is also non-essential that can be a very toxic heavy metal for human and aquatic organisms. Ni concentration in the present study (0.15 to 0.41mg.kg⁻¹) was equal to the recorded concentrations in the meagre from the north-western Atlantic ocean, as measured at 0.35mg.kg⁻¹ (Elaarabi *et al.*, 2022). However, it was greater than the levels of Ni reported by Makedonski *et al.* (2017) of 0.008 - 0.07mg.kg⁻¹. In general, Ni levels in the fish ranges from 0.14 to 0.61mg.kg⁻¹, while crustaceans had an Ni content ranging from 0.24 to 0.46mg.kg⁻¹ (Gu *et al.*, 2016). The FDA established the greatest concentration of Ni at 70- 80mg.kg⁻¹ (USFDA, 1993).

Mn is an essential element and poses less toxicity for human and aquatic species. The Turkish Food Codex (TFC, 2002) established a safety limit of 20mg.kg⁻¹ for Mn. The flesh of the meagre in the current study recorded Mn concentrations between 0.02 to 0.03mg.kg⁻¹, as well as 2.52mg.kg⁻¹ from the Moroccan Atlantic meagre (Elaarabi *et al.*, 2022). The literature reported a wide range of Mn in fish tissues with levels from 0.025 to 9.1mg.kg⁻¹ (Tuzen, 2009; Copat *et al.*, 2018).

To prevent health risks related to excessive seafood consumption, the average levels of heavy metals in the flesh are utilized to determine safe dietary intake indices

such as EDI, EWI, PTWI%, MDI, and MWI. Based on estimated intake by children, young and adult human, our results revealed that Pb, Cd and Hg from wild fish can pose more hazard in all human age groups, while Ni poses more hazard in aquacultured fish. Additionally, the hazard of dietary intake was predominantly in the following order children > young > adult.

Table 8. The carcinogenic risk (CR) to the health of people consuming fish in different fish groups

Parameter		LWM	SWM	LFM	SFM	
Pb	CR Once a week	Child	0.000001045	0.000001013	0.000000705	0.000000805
		Young	0.000000348	0.000000338	0.000000235	0.000000269
		Adult	0.00000022	0.000000217	0.000000151	0.000000173
	CR 3 times a week	Child	0.000003134	0.000003040	0.000002116	0.000002416
		Young	0.000001045	0.000001013	0.000000705	0.000000805
		Adult	0.00000067	0.00000065	0.000000453	0.000000518
	CR 7 times a week	Child	0.000007312	0.000007092	0.000004936	0.000005638
		Young	0.00000244	0.000002364	0.000001645	0.000001879
		Adult	0.000001567	0.000001520	0.000001058	0.000001208
TCR		1.56674E-08	1.51979E-08	1.05779E-08	1.20803E-08	
Cd	CR Once a week	Child	0.002984	0.003025	0.00222	0.002536
		Young	0.000995	0.001009	0.00074	0.000845
		Adult	0.0006395	0.000648	0.000477	0.0005434
	CR 3 times a week	Child	0.0089528	0.009076	0.006679	0.007608
		Young	0.0029843	0.003025	0.002227	0.002536
		Adult	0.00191846	0.001945	0.001431	0.0016303
	CR 7 times a week	Child	0.0020889	0.021178	0.01558	0.017753
		Young	0.0069633	0.007059	0.00519	0.005918
		Adult	0.0044764	0.004538	0.00334	0.0038041
TCR		0.00023385	0.000045	0.000033	0.00003804	
Ni	CR Once a week	Child	0.001030048 ^b	0.001628911 ^{ab}	0.001178309 ^b	0.00290109 ^a
		Young	0.000343349 ^b	0.000542970 ^{ab}	0.000392770 ^b	0.00096703 ^a
		Adult	0.000220725 ^b	0.000349052 ^{ab}	0.000252495 ^b	0.000621661 ^a
	CR 3 times a week	Child	0.003090143 ^b	0.004886734 ^{ab}	0.003534925 ^b	0.00870326 ^a
		Young	0.001030048 ^b	0.001628911 ^{ab}	0.001178309 ^b	0.00290109 ^a
		Adult	0.000662174 ^b	0.001047157 ^{ab}	0.000757484 ^b	0.001864984 ^a
	CR 7 times a week	Child	0.007210334 ^b	0.01140238 ^{ab}	0.008248159 ^b	0.02030761 ^a
		Young	0.002403445 ^b	0.003800793 ^{ab}	0.002749386 ^b	0.00676921 ^a
		Adult	0.001545072 ^b	0.002443367 ^{ab}	0.001767463 ^b	0.004351630 ^a
TCR		3.08838E-10	1.62276E-09	1.93006E-09	1.82529E-09	

Different letters within the same row for each metal, time and each age are for significance of treatments' effects ($P < 0.05$).

The present values of THQ for heavy metals from small and large sizes wild and farmed meagre consumed 1, 3, 7 times per week for Pb, Cd, Mn and Ni were a smaller than 1, demonstrating no health risks for intake of one heavy metal through eating the meagre once, thrice or seven times per week. In dissimilarity, the Hg THQ are less than 1 if people consumed any kind of the meagre one time per week or three times per week for the meagre from LWM, LFM and SFM. Nevertheless, eating the meagre from SWM three times per week or from any source seven times per week may have health risks associated with Hg uptake. HI for the five elements were less than 1 if people consumed meagre one time per week from all sources and sizes also if they consumed meagre three times per week from LFM, there were no risk. SFM were expressively ($P < 0.05$) greater

in Mn and Ni content of meagre consumed one, three or seven times per week compared with other sources and sizes of meagre. The THQ values below the threshold of unity show that consuming seafood does not have any non-carcinogenic health consequences. However, values that surpass the established limit are likely to have negative health consequences (USEPA, 1989; Saha & Zaman, 2013). Estimating THQ in different fish species showed values below one assuring no potential impact on the health of the customer (Alipour *et al.*, 2014; Gu *et al.*, 2016; Elaarabi *et al.*, 2022). Conversely, THQ more than one for Cd indicates that consuming the examined species at high rates poses a health risk to the consumer (Baki *et al.*, 2018; Elaarabi *et al.*, 2022). Our results are consistent with other investigations indicating acceptable THQ values in the popular fish species from the Egyptian market, such as *Sparus aurata* (Abbas, 2024), *Clarias gariepinus*, and *Oreochromis niloticus* (Afifi *et al.*, 2024), suggesting no potential harm for different human age groups.

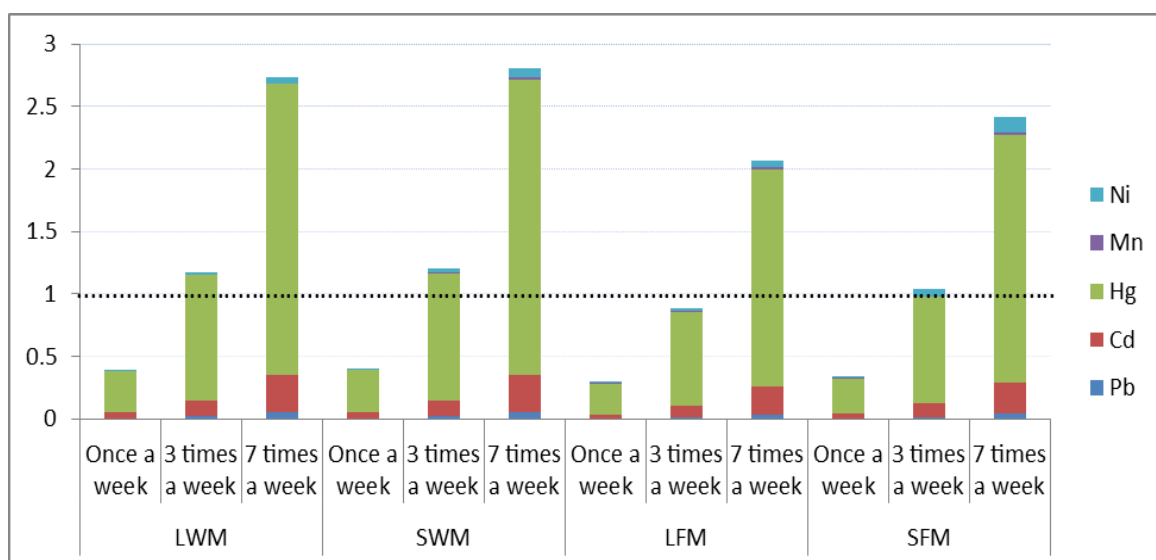


Fig. 4. The target hazard quotient (THQ) to the health of people consuming fish in different fish groups

In the current study, the values of CR and TCR for Pb in children, youth and adults were 10^{-6} - 10^{-7} and 10^{-8} , respectively, in all the meagre groups, indicating no potential danger of cancer for consumers. Consumption of the Cd-contaminated meagre showed moderate danger of cancer for consumers with CR values between 10^{-3} and 10^{-4} and TCR level between 10^{-3} and 10^{-4} . Concerning Ni uptake, the TCR levels of Ni were between 10^{-9} and 10^{-10} , suggesting no risk of cancer for people who consume the Ni-contaminated meagre.

CONCLUSION

The study assessed the flesh quality and heavy metal accumulation in the small and large meagre collected from both wild and aquaculture sources in Egypt. The findings showed that muscle composition—comprising lipid, total protein, moisture, and ash content—remained largely consistent across size groups and between wild and farmed fish. However, there was a notable difference in liver antioxidant responses: small farmed fish exhibited significantly higher catalase (CAT) and superoxide dismutase (SOD) activities, indicating a greater stress exposure. Wild fish from the Mediterranean displayed elevated levels of cadmium (Cd), lead (Pb), and mercury (Hg) in their muscle tissue, while farmed fish had higher nickel (Ni) concentrations. Despite these differences, the heavy metal levels in the meagre were below the maximum permissible limits (MPL) set by the international guidelines. The health risk assessment revealed no significant non-carcinogenic or carcinogenic risks associated with consuming the meagre, as long as consumption stays within the recommended daily and weekly limits. However, it is advisable to exercise caution, particularly regarding the consumption of wild fish by children due to the potential risks of Cd, Pb, and Hg, especially if there is an industrial contamination in the area.

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