Research Article

Bioaccumulation of Heavy Metals in Bones, and Brains of Some Aquatic Species at the Northwestern Part of Suez Gulf

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ABSTRACT

Fish species have attracted considerable interest in studies assessing biological responses to environmental contaminants. In this study, the attention has been focused on fishbone and brain as a tool to detect bioaccumulation of metals, so ten fish species were collected from Suez Gulf to evaluate if toxicant elements has an effect on bone and brain which suffered negative effects due to trace element levels, detected by flame atomic absorption spectrometry. The concentrations of Hg, Al, Zn, Pb, Cu, Fe, Mn, Ni, V, Cr, Co, and Ca were determined in the bone and brain of *Sauridaundo squamis; Euthynnus affinis; Rhabdosargus haffara; Argyrops spinifer; Nemipterus japonicus; Oreochromis niloticus; Trachurus indicus; Peneus japonicas; Scomber japonicus and Pomadasys stridens.* The results show total concentrations for metals in order of decreasing content was as follows Ca>Fe>Co>Cr>Zn>Cu>V>Mn>Al>Pb>Ni. However, mercury ranged from 0.0069 to 0.2095 µg/g wet wt. (mean value 0.1040 µg/g wet wt.), which is lower than the prescribed limits (0.4 µg/g wet wt.). Species-specific and spatially heterogeneous patterns of tissues metals loads were apparent within the pelagic and demersal fish species for the area of study. Metal selectivity index (MSI) obtained for all the metals except Hg and Ca showed that both pelagic and demersal fish species have almost same kind of affinity towards the metals, irrespective of their feeding habit. The MSI values also indicate that the fishes have the potential to accumulate metals. High tissue selectivity index (TSI) values were reported for bone and brain for all metals suggests that the metal concentration in these tissues can serve as an indication of metal polluted environment. Fishes like *Nemipterus japonicus* being a favorite's food of people in this region; the high consumption of it can lead to chronic disorders as this fish has high concentration of metals.

Keywords: Suez Gulf, metals, Fish, bone and brain, Feeding habits, TSI.

INTRODUCTION

A harmful class of aquatic pollutants is represented by trace elements owing to their toxicity, carcinogenicity, persistence, together with their bioaccumulative and non-biodegradable properties in food chains. Trace element pollution in marine ecosystems has increased, principally due to numerous anthropogenic inputs, direct consequence of mining, industrial, urban and agricultural activities[1]. Fish represent a sensitive group to trace element pollution especially they belong to high trophic levels of food webs. Indeed, they tend to bioaccumulate trace elements especially in tissues with high fat content, which becoming good bioindicators of trace element availability and potential stress to marine ecosystems[2]. At the same time, as fish represent an important food source, human consumption of fish tissues exceeding trace element acceptable levels poses a severe risk to human health[3]. Moreover, fish exposed to high contamination levels can suffer from major alterations of biochemical pathways[4]. Although the damages of pollutant exposure have been analyzed in several fish tissues, such as muscle, gill, brain and liver[5] among others, to our knowledge, the effect of trace element pollution on fish bone and brain has received relatively little attention[6]. Nevertheless, bones may be used as proxies for evaluating contamination levels in fish. Previous insights obtained in mammals suggest a clear relationship between bone mineralogical features and environmental pollution[7]. Bone is one of the most sophisticated biological structures developed by nature[8]. The major constituent of bone is a calcium phosphate mineral that is similar in composition and structure to minerals within the apatite group, which are naturally formed in the Earth's crust[9]. In particular, the inorganic components of both bone and enamel have been likened to the mineral hydroxylapatite (HAP)[10], which is an OH-containing mineral with a very specific structure and composition. There is an increasing interest in medical and toxicological research related to the factors altering bone mineralization[11]. Some others[12] have reviewed the effects of Hg accumulation in the fish brain, describing "reduced neurosecretory material, hypothalamic neuron degeneration, and alterations in parameters of monoaminergic neurotransmission". The potential neurotoxic effects of Hg can be assessed by comparing brain Hg concentrations of fish to levels that can affect the central nervous system (CNS). Despite that, we do not have studies of fish-brain Hg concentrations. There is a scarcity of knowledge about fish biomarkers of MeHg neurotoxicity for use in aquatic environment studies. We need to learn fish neurotoxin thresholds. With the largest diversity of fresh-water fish in the planet, there is a lack of data regarding Hg concentrations in brain of fish from Amazonian tropical rivers. Various toxicological studies have proved how many toxicants, like trace elements and organochlorines, may cause skeletal damages and malformations, inducing alterations in bone formation and composition[13]. During the last century, this system has been particularly deteriorated by direct discharge of industrial contaminants as well as by navigation and dredging of polluted sediments, which presumably spread xenobiotics, especially Hg, along the entire coastal area and in the surrounding offshore system. Here, several skeletal malformations of fishbone, such as scoliosis, abnormal thickening, y-shaped column, and fin deformities have been documented in two fish species (*Sarda sard*a and *Pagellus erythrinus*), and are presumably correlated with the elevated Cd and Zn concentrations measured in their tissues[14]. Suez Gulf is recognized as a chronically polluted marine area, especially due to a heavy industrialization (chemical and petrochemical plants together with oil refineries), with resulting risks to ecosystem and human health[15]. The aims of the present study are (i) analysis the chemical content in fish bone and brain of ten fish species collected from Suez Gulf, (ii) assessment the trace element concentrations (Hg, Al, Zn, Pb, Cu, Fe, Mn, Ni, V, Cr, Co, and Ca) in fishbone and brain and (iii) Evaluation any possible a response to pollution in fishbone and brain.

MATERIALS AND METHODS

Study area

The Gulf of Suez is a large semi-closed area. Its length reaches 346 km, with an average width of 54.2 km, and average depth of 40 m[16]. It has a total surface area of about 10,510 km^2. For this study, ten stations were selected at the North western part of Suez Gulf **Fig.(1),** they are exposed to the different sources of pollution including, domestic drainage, oil refineries and ship's effluents, and coastal oil pollution in the vicinity of the SUMED pipeline company terminals. Localized pollution problems are mainly due to increasing coastal development activities including large urbanization, tourist centers and industrial development.

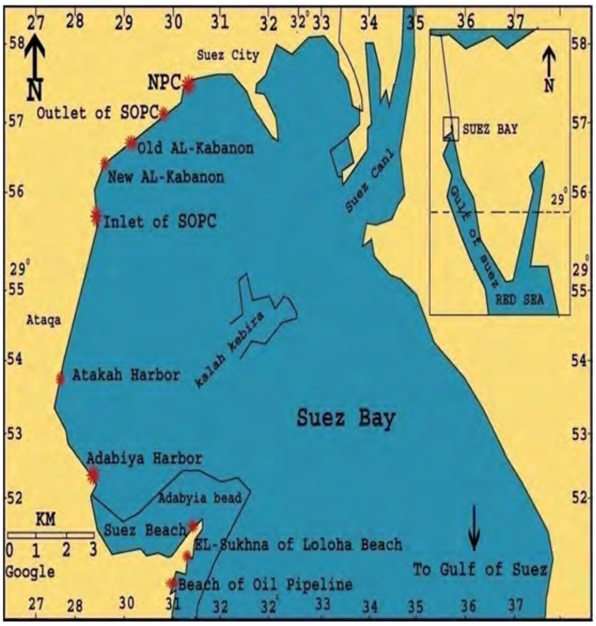

Figure 1: Study area showing sampling locations.

Sample collection and preservation

Ten fish species **Fig. (2**) Were collected from the coastal waters of the Suez Gulf by using cast or throw fishing net from different water bodies and geographical regions.

The general characteristics and morphometric data of the fish species collected are shown in **Table (1)**. They were first identified, and the number of individual fish species, total lengths (cm) and the body-wet weights (g) were determined, labeled, stored in ice at - 20°C in polyethylene bags into isolated container of polystyrene box.

In laboratory, bone and brain organs were separated, weighed, and then deep frozen. Subsequently, dissected tissues of the fish specimens were dried in an oven at 105°C and stored in vacuum desiccators.

Digestion and analytical procedures

Dried tissues were ground, sieved and weighed accurately to the fourth decimal in a digestion vessel of 15 ml capacity kjldahl digestion tubes.

4 c.c. of concentrated HNO_3 was added to the samples. The vessel was placed in a digestion block on a hot plate at 120°C for two hours. The block was then left over night for cooling[17]. It was insured that the digestion conditions were followed exactly; this is being achieved when the solution was being clear after cooling.

The solution was filtered using Wattman No. 42 filters paper and kept in a polyethylene bottle previously cleaned with nitric acid and rinsed with deionized water. The contents of each tube was then transferred to a measuring flask and diluted with deionized water.

Concentrations of heavy metals were analyzed in the studied samples by atomic absorption spectrometry according to ASTMD4961 using atomic absorption spectrometer ZEEnit 700P, Germany. Fe, Cu, Pb, Cr, Co, Ni, Ca, Mn, and Zn were determined by flame absorption using acetylene/air flame, at 248.8 nm, 324.8nm, 217 nm, 357.9 nm, 240.7 nm, 232 nm, 422.7 nm, 279.5 nm, and 213.9 nm, respectively.

Al and V were determined by flame absorption using acetylene/nitrous flame, at 309.3 nm, and 318.5 nm, respectively. Hg was analyzed using cold vapor atomic absorption spectrometer (CV-AAS) attached to hydride system. This system is usually used for quantitative determination of mercury in liquid samples due to its high sensitivity and selectivity as well as its extremely low detection limits (1 µg/L). Briefly, Hg was reduced using Na B H_4 in presence of argon as a carrier gas, moved through a gas liquid separator. The gaseous mercury is enriched on a gold collector, and then heated to release elemental mercury which was re-carried out to a quartz cell inside the atomic measurement unit where the concentration of elemental mercury is measured. All experiments were measured in triplicate and average values were calculated.

Table 1: General characteristics and morphometric data of some Aquatic Species collected along Suez Gulf.

Code	Sites	Scientific name	Common name	Feeding habits(Main food)	Habitat type (Environment)	No.	Fish length (cm)	Fish wet. wt (g)
1	AL- Nasr Oil Company (NPC)	***Sauridaundo squamis***	Brushtooth lizard fish	**Carnivore** (small fish)	Demersal, (benthic).	**7**	14	400
2	Outlet of Suez Oil Petroleum Company (SOPC)	***Euthynnus affinis***	Kawakawa	Feeds on small fish, squids, and sometimes zooplankton (**Omnivore).**	Found in open waters but always close to the shoreline.	1	40	350
3	Old Al-Kabanon	***Rhabdosargus haffara***	Haffara sea bream	Feeds on benthic invertebrates. Consumed fresh **(Predator)**.	Inhabits shallow waters, mainly around coral reefs, and over sandy or mud-sandy bottoms.	4	13.5	420
4	New Al-Kabanon	***Argyrops spinifer***	Porgies	Feeds on benthic invertebrates, mainly mollusks, important food fish (**Predator**).	Inhabits a wide range of bottoms. Young fish occur in very shallow waters of sheltered bays; larger individuals in deeper water.	4	15	480
5	Inlet of Suez Oil Petroleum Company (SOPC)	***Nemipterus japonicus***	Japanese threadfin bream	Carnivore on small fish, invertebrate's polychates (Predator).	Demersal.	5	18	425
6	Atakah Harbor	***Oreochromis niloticus***	Nile Tilapia	**Herbivorous,** feed on phytoplankton (**Omnivore**).	Benthic and pelagic due to air bladder.	3	14.5	389
7	Adabiya Harbor	***Trachurus indicus***	Horse Mackerel	Carnivore (invertebrates and fish) **(Predator).**	Pelagic.	4	24	431
8	Suez Beach	***Peneus japonicas***	Red mullets	Prey of small fish and crustaceans **(Predator)**.	Inhabit the inshore area and coral reefs, can be found on a range of sea beds including sand, mud and coarse gravel.	8	11.5	450
9	El- Sukhna of Loloha Beach	***Scomber japonicus***	Chub mackerel, Pacific mackerel or blue mackerel	Feed on copepods and other crustacean, fishes and squids **(Predator).**	A coastal pelagic species, to a lesser extent epipelagic to mesopelagic over the continental slope.	4	21	470
10	Beach of oil pipeline	***Pomadasys stridens***	Striped piggy	Feeding on a variety of crustaceans, mollusks and small juvenile fishes, called a predator **(Predator**).	Living in the reef environment and sandy.	3	13.5	495

No: number of sample

Sauridaundo squamis/
Brushtooth lizard fish

Euthynnus affinis/
Kawakawa

Rhabdosargus haffara/
Haffara Sea bream

Argyrops spinifer /
Porgies

Nemipterus japonicus/
Japanese Threadfin bream

/Oreochromis niloticus
Nile tilapia

Trachurus indicus/
Horse mackerel

/Peneus japonicas
Red mullets

Scomber japonicus/
Chub Mackerel, Pacific mackerel or Blue mackerel

Pomadasys stridens/
Striped piggy

Figure 2: Photographs of ten Aquatic Species collected along the Suez Gulf.

Table 2: Accumulation of T Hg (µg /g wet wt.) in bone and brain of fishes along Suez Gulf

Fish species↓	Element→	Hg	T Hg
Sauridaundo squamis	**Bone**	0.0337	0.1554
	Brain	0.1217	
Euthynnus affinis	**Bone**	0.0540	0.1000
	Brain	0.0460	
Rhabdosargus haffara	**Bone**	0.0409	0.1490
	Brain	0.1081	
Argyrops spinifer	**Bone**	0.0776	0.2095
	Brain	0.1283	
Nemipterus japonicus	**Bone**	0.0843	0.1680
	Brain	0.0837	
Oreochromis niloticus	**Bone**	0.0043	0.0069
	Brain	0.0026	
Trachurus indicus	**Bone**	0.0458	0.1028
	Brain	0.0570	
Peneus japonicas	**Bone**	0.0229	0.0532
	Brain	0.0303	
Scomber japonicas	**Bone**	0.0126	0.0498
	Brain	0.0372	
Pomadasys stridens	**Bone**	0.0299	0.0491
	Brain	0.0192	
Range of Bone		**0.0043-0.0843**	**T.R =0.0069-0.2095**
Mean		**0.0406**	
Range of Brain		**0.0026-0.1283**	**T.M = 0.1040**
Mean		**0.0634**	

Hg: Mercury; **T Hg***;* Total mercury; **T.R =** Total range; **T.M =** Total mean.

RESULTS AND DISCUSSION

Accumulation of metals in bone and brain of aquatic species comparable with reported literature values

The concentration of accumulated heavy metals in bones, and brain of selected fishes are given in **Tables (2) & (3).** All results are expressed as µg/g wet wt. using converting factor 0.3; since the moisture is usually about 70%[18].

Mercury is one of the most toxic heavy metals in the environment[19]. Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage bone, brain and developing fetus[20]. The results **Table (2)** indicated that the concentration of Hg in bone ranged from 0.0043 to 0.0843 µg/g wet wt, and in brain from 0.0026 to 0.1283 µg/g wet wt, respectively. *Argyrols spinifer* and Predatory *Nemipterus japonicus* recorded 0.2095 µg/g wet wt and 0.1680 µg/g wet wt, the highest levels of total mercury than the other species. Total Hg ranged from 0.0069 to 0.2095 µg/g wet wt. (mean value 0.1040 µg/g wet wt. i.e. 0.350 µg/g dry wt.), which are lower than those reported from other marine areas, varying from N.D-0.925 and N.D-1.236µg/g dry wt.[21] in Southwest India for bone and brain respectively; 1.400-7.176µg/g wet wt. in illegal fish farm in Al-Minufiya Province, Egypt[22]; 0.23-1.2 µg/g dry wt. in North Sea coast[23]; 0.46-3.4 µg/g wet wt.[24] in Mediterranean, Scotland, United Kingdom.

Data obtained from this study are consistent with those reported from the Madeira River; Brazilian Amazon 0.03-0.37µg/g wet wt.[12] and with previous findings in the USA, 0.09 µg/g wet wt.[25]; 0.2537µg/g wet wt.[26]; in Ivory Coast and Comparable Hg levels found in Brazil 0.02 µg/g wet wt.[27]; and in Italy 0.16 µg/g wet wt.[28]; Lake Mead, USA 0.08 µg/g wet wt.[29]. Mercury also occurs naturally in seawater, and coastal waters receive mercury runoff from land, input from rivers, and airborne deposition. Regarding the existing law for Hg, the European Community, Canada, and the FDA establish a total Hg level of 1 µg/g wet wt.[30-32]; Japan sets forth limit values of 0.4 µg/g wet wt.[33] whereas, in Brazil limit values are 0.5 µg/g wet wt. for non predator fishes and 1.0 µg/g for predator ones[34].

Aluminum despite some reports for a role of Al in physiological processes, there is no clear evidence that it plays an essential function in organisms[35]. Moreover it is well known that Al can be potentially neurotoxic and there have been concerns in recent years that dietary and environmental exposure to this element could cause developmental toxicity in organisms. Indeed, there is unequivocal evidence that Al induces neurofibrillary degeneration in animal brains following intracerebral Ainjections and systemic Al exposure[36]. Normally, Suez fish maintain low Al concentrations in their bones ranged from N.D to 2.199 µg/g wet wt. compared to the brain from N.D to 14.63 µg/g wet wt. as indicated in **Table (3)**. However, it is now recognized that toxicity can occur, either if accumulation is markedly increased or renal clearance is impaired. We found a high variability in Al concentrations; in particular, *Nemipterus japonicus* showed low Al level in the bone 1.513 µg/g wet wt. compared to the brain level 14.639 µg/g wet wt. beside, *Oreochromis niloticus* and *Scomber japonicus* recorded high levels in bone 2.199, 0.909 µg/g wet wt. but low in the brain 1.331 & 0.4006 µg/g wet wt. respectively. As shown in **Table (3)**, the mean values of Aluminum are 1.117 and 1.720 µg/g wet wt. for bone and brain respectively. The concentration of Al can be ordered in bone as follows: *Oreochromis niloticus> Peneus japonicas> Argyrops spinifer> Nemipterus japonicus> Trachurus indicus> Rhabdosargus haffara> Scomber japonicus> Euthynnus affinis> Sauridaundo squamis* µg/g wet wt. and *Pomadasys stridens* was under the limit of detection *and* In brain as follows: *Nemipterus japonicus>Oreochromis niloticus> Pomadasys stridens> Scomber japonicus***>** *Sauridaundo squamis=Euthynnus affinis = Rhabdosargus haffara* **=** *Argyrops spinifer = Trachurus indicus=Peneus japonicas*. Al levels have been reported in the range of 4.098-8.339µg/g wet wt. in Al-Minufiyah Province, Egypt [21]; N.D-12.61 µg/g wet wt. in bone at Mediterranean, Scotland, United Kingdom[23]; and 1.38- 2.73 µg/g wet wt. in brain fish at Adriatic coast of Southern Italy[37], levels in analyzed samples were found to be higher than those reported in the literatures. High levels of Al are toxic to fish, so that bio-concentration generally is not significant[38]. In another study revealed that Al exposure disturbs osmoregulation in fish[39]. The freshwater criterion to protect aquatic life is 87µg/L chronic and 750 µg/L acute[40].

Zinc being a heavy metal has a tendency to get bioaccumulation in the fatty tissues of aquatic organisms, including fish and is known as a reproductive physiology effect in fishes[41]. Some authors reported that chronic exposure to Zn and Cu is associated with Parkinson's disease[42] and these elements might act alone or together over time to induce the disease[43]. Fishes are known to have a high threshold level of Zn. From **Table (3)**, there was a great variation in Zn concentrations among the studied tissues, ranged from 5.963 to 17.246, and from 10.197 to 286.872 in bone and brain respectively. The brain of all the fishes showed considerable amount of Zn accumulation, Nemipterus *japonicus* contain the highest amount of zinc in its brain 286.872 µg/g wet wt. and the lowest Zn in *Pomadasys stridens* 10.197 µg/g wet wt. among the ten species of fish. The high levels of Zn are likely attributed to the high Zn levels in the local habitat, as industrial and anthropogenic input of metals from Suez City and the maritime activities through the Suez Canal[17]. The concentration of Zn in bone can be ordered as follows: *Oreochromis niloticus> Rhabdosargus haffara> Trachurus indicus***>** *Peneus japonicas> Euthynnus affinis> Scomber japonicus***>** *Sauridaundo squamis***>***Pomadasys stridens> Argyrops spinifer> Nemipterus japonicus*. Whereas, in brain as follows: *Nemipterus japonicus> Oreochromis niloticus> Sauridaundo squamis>Scomber japonicus> Rhabdosargus haffara>Trachurus indicus> Peneus japonicas> Euthynnus affinis> Argyrops spinifer> Pomadasys stridens.* Zn levels have been reported in the range of 97.6 - 98.4 and 53.7 µg/g dry wt. at North Sea coast[22] for bone and brain respectively; 13.88-24.12 µg/g wet wt. in bone[23]; 14.23 - 16.52 µg/g wet wt.[37] in brain fish at Adriatic coast of Southern Italy; in addition to that 0.800±0.037µg/g wet wt. at Tajan River, Iran[44]; 8.29±0.38 µg/g wet wt. in Caspian Sea[45]; 56.01-109.9&185.55-415.52 µg/g dry wt.[20] in bone and brain respectively; 44.43 µg/g dry wt.[46] for bone in Augusta Bay Italy, Central Mediterranean; and 6.06&6.63 µg/g wet wt.[47] for bone and brain respectively in Eastern Mediterranean, Italy. In our study the amount of Zn determined in the fish samples were far below the standard of 1000 µg/g set by ANHMRC[48] and WHO[49].

Lead is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity, and many others adverse health effects[50]. The details of lead concentration detected for individual fish of both bone and brain were given in **Table (3)**. Among individual fish species, *Sauridaundo squamis* contains the highest lead concentration whereas; *Pomadasys stridens* was under the limit of detection in brain, and recorded 1.204µg/g wet wt. for bone. Concentration of Pb in bone in decreasing order was as follows: *Scomber japonicus***>***Trachurus indicus> Pomadasys stridens> Rhabdosargus haffara>Euthynnus affinis> Oreochromis niloticus> Peneus japonicas>Sauridaundo squamis = Argyrops spinifer = Nemipterus japonicus*. However, in brain as follows: *Sauridaundo squamis>Argyrops spinifer> Rhabdosargus haffara>Euthynnus affinis>Nemipterus japonicus> Trachurus indicus>Peneus japonicas> Oreochromis niloticus> Scomber japonicus* and *Pomadasys stridens* below the detection limit.

Table 3: Accumulation of metals (µg /g wet wt.) in bone and brain of different fishes along Suez Gulf

Elements **Fish species**		**Al**	**Zn**	**Pb**	**Cu**	**Fe**	**Mn**	**Ni**	**V**	**Cr**	**Co**	**Ca**
Sauridaundo squamis	**Bone**	0.0206	11.885	N.D.	0.5861	11.652	1.398	N.D.	N.D.	0.9321	3.612	9197.157
	Brain	N.D.	30.501	5.430	17.011	33.216	N.D.	0.9582	N.D.	8.144	N.D.	14640.689
Euthynnus affinis	**Bone**	0.8470	12.960	0.5784	7.696	40.266	0.5784	0.5785	N.D.	1.736	3.939	10565.026
	Brain	N.D.	12.00i	2.021	0.0112	0.6135	N.D.	0.2245	N.D.	3.929	N.D.	4533.787
Rhabdosargus haffara	**Bone**	1.015	16.537	1.0123	0.3127	10.462	2.925	0.5625	N.D.	1.913	5.625	10461.976
	Brain	N.D.	19.085	2.508	0.0414	15.595	0.2181	0.2181	1.016	5.346	N.D.	9337.647
Argyrops spinifer	**Bone**	1.514	6.393	N.D.	0.1639	18.975	0.8136	0.9299	N.D.	2.557	6.509	13378.535
	Brain	N.D.	11.841	2.723	0. 6749	24.866	N.D.	0.4736	3.529	5.565	N.D.	7675.245
Nemipterus japonicas	**Bone**	1.513	5.963	N.D.	1.137	10.468	7.023	1.060	N.D.	6.095	8.746	17557.42
	Brain	14.639	286.872	0.9006	1.659	18.111	4.603	1.4008	11.407	9.005	N.D.	24094.456
Oreochromis niloticus	**Bone**	2.199	17.246	0.5187	1.273	14.004	3.761	N.D.	6.613	2.982	9.336	12149.896
	Brain	1.331	32.712	0.1128	0.7197	30.681	0.1128	0.4512	N.D.	1.466	N.D.	6986.021
Trachurus indicus	**Bone**	1.283	14.537	1.179	2.120	27.501	1.310	0.7858	N.D.	3.536	9.167	12334.992
	Brain	N.D.	18.390	0.7755	1.601	16.023	N.D.	0.4431	N.D.	5.317	N.D.	10578.982
Peneus japonicas	**Bone**	1.865	13.948	0.3984	0.002	12.752	2.391	1.395	N.D.	4.384	11.955	15308.846
	Brain	N.D.	15.845	0.3685	11.980	22.601	N.D.	0.9826	8.684	6.633	N.D.	11042.417
Scomber japonicas	**Bone**	0.9099	12.311	1.204	0.047	15.254	2.141	0.8029	5.888	3.479	10.169	11185.102
	Brain	0.4006	20.134	0.1060	1.090	13.458	0.2120	0.5298	N.D.	2.861	1.696	9158.954
Pomadasys stridens	**Bone**	N.D.	9.504	1.071	0.3213	10.709	2.142	0.6693	13.613	5.622	11.110	11909.244
	Brain	0.8280	10.197	N.D.	7.020	19.884	0.5100	0.1020	N.D.	1.224	2.855	5537.050
Total conc. (bone + brain)		**28.37**	**57.89**	**20.92**	**55.46**	**367.09**	**30.14**	**12.57**	**50.75**	**82.73**	**84.72**	**227636.43**
Bone Range		**N.D-2.199**	**5.963-17.246**	**N.D-1.204**	**0.002-7.696**	**10.462-40.266**	**0.5784-7.023**	**N.D.-1.395**	**N.D.-13.613**	**0.9321-6.095**	**3.612-11.110**	**9197.157-17557.42**
Mean		**1.117**	**12.1284**	**0.5962**	**1.3659**	**17.2043**	**2.4483**	**0.6784**	**2.6114**	**3.3236**	**8.0168**	**12404.819**
Brain Range		**N.D-14.639**	**10.197-286.872**	**N.D-5.430**	**0.0112-17.011**	**0.6135-33.216**	**N.D- 4.603**	**0.1020-1.401**	**N.D.-11.407**	**1.224-9.005**	**N.D-2.855**	**4533.787-24094.46**
Mean		**1.720**	**45.7582**	**1.4945**	**4.1803**	**19.5049**	**0.5656**	**0.5784**	**2.4636**	**4.949**	**0.4551**	**10358.824**

N.D: Under the limit of detection; **Total conc.: Total concentrations; Al**: Aluminum; **Zn**: Zinc**, Pb**: Lead; **Cu**: Copper; **Fe**: Iron; **Mn**: Manganese; **Ni:** Nickel; **V:** Vanadium; **Cr**: Chromium; **Co**: Cobalt**; Ca:** Calcium.

Pb contents in the literature have been reported in the range of 1.842-10.359 µg/g dry wt. from illegal fish farm in Al-Minufiya Province, Egypt[21]; 0.112-0.116&0.015 µg/g dry wt. for bone and brain respectively at North Sea coast[22]; N.D -0.66 µg/g wet wt. in Mediterranean, Scotland, United Kingdom[23]; Another similar study also shown results, 3.450±0.132 µg /g wet wt.[44]; 3.96 ±2.16µg /g dry wt.[51]; 3.73±0.18 µg/g wet wt.[45] in brain; in addition to 1.29 ± 0.28 µg/g dry wt.[46]; and 1.775-22.52 µg/g dry wt.[20]at Augusta Bay, Italy, Central Mediterranean and Southwest India. In general, Pb bioaccumulation levels in bone and brain in this study agreed well with the findings in aquatic and terrestrial vertebrates in hard tissues such as bone and teeth[52]. The Brazilian legislation[53] recommends a maximum Pb level of 2.0 µg/g and the European Community (EC) regulates 0.2 µg/g as maximum limit in this study the determined values were found to be higher than the maximum limit. The present observation showed that level of Pb in *Sauridaundo squamis* among the ten species was beyond the proposed not acceptable limit for human consumption. From the literature survey, it was apparent that *Sauridaundo squamis* was Demersal, (benthic) bottom living and therefore, sediments could be the major sources of Pb contamination in that fish. Moreover, lead is a ubiquitous pollutant which could finds its way into the Gulf through discharge of industrial effluents from various industries such as printing, dyeing, oil refineries, textile, and other sources.

Copper is an essential part of several enzymes. It is essential for hemoglobin synthesis[54]. However, accumulation of Cu has been recognized to cause adverse health problems which leads to death of neurons and resulting neurological symptoms in brain[41]. As indicated in **Table (3**), Cu concentration in bone of *Euthynnus affinis* was significantly higher than all other species followed by *Trachurus indicus* while, in brain, *Sauridaundo squamis* has the highest Cu concentration followed by *Peneus japonicas*. In brain, the pattern of the Cu concentration in decreasing order was as follows: *Sauridaundo squamis***>** *Peneus japonicas***>** *Pomadasys stridens>Nemipterus japonicus> Trachurus indicus>Scomber japonicus> Oreochromis niloticus>Argyrops spinifer>Rhabdosargus haffara> Euthynnus affinis*. Whereas, in bone was as follows: *Euthynnus affinis>Trachurus indicus>Oreochromis niloticus> Nemipterus japonicus>Sauridaundo squamis> Pomadasys stridens>Rhabdosargus haffara>Argyrops spinifer> Scomber japonicus>Peneus japonicas*. In the literature, Cu levels in fish samples have been reported in the range of 0.941-1.08 &18.1µg/g dry wt.[22] in bone and brain respectively; N.D - 0.35 µg/g wet wt. in bone fish at Mediterranean, Scotland, United Kingdom[23]; 4.470-127.2&15.77-88.31µg/g dry wt. in bone and brain respectively for fish species from Southwest India[20]. In another studies also results ranged 2.43-2.64 µg/g wet wt.[37]; 2.940 ± 0.138 µg /g wet wt.[44]; 5.15 ± 0.25 µg /g wet wt.[45]; and 1.64 & 2.91 µg/g wet wt.[47] for bone and brain respectively. FAO/WHO put a limit of Cu (30 µg/g wet wt.), and the UK and Saudi Arabia have a limit of 20 µg/g wet wt.

Iron is the most abundant element examined in the tissues of the various fishes. Fish is a major source for iron in adults and children, iron deficiency causes anemia. **Table (3**), shows large interspecific variation in metal iron for all species and ranged from 10.462 to 40.266 and from 0.6135 to 33.216 µg/g wet wt. for bone and brain, respectively. The maximum Fe content was observed in *Euthynnus affinis* and minimum in *Rhabdosargus haffara* of fishbone, while *Saurida squamous* exhibited a maximum Fe content in brain and minimum value of *Euthynnus affinis*. Fe concentration had the highest values in all the species studied. In fish brain, the decreasing order was as follows: *Sauridaundo squamis>Oreochromis niloticus>Argyrops spinifer>Peneus japonicas> Pomadasys stridens>Nemipterus japonicus> Trachurus indicus> Rhabdosargus haffara> Scomber japonicus> Euthynnus affinis,* while in bone was as follows: *Euthynnus affinis> Trachurus indicus>Argyrops spinifer> Scomber japonicus> Oreochromis niloticus> Peneus japonicas> Sauridaundo squamis>*Rhabdosargus *haffara*. Fe contents in the literature have been reported in the range of 6.71-21.78 µg/g wet wt.[23]; 44.68-56.57µg/g wet wt.[37] for bone and brain respectively, in another similar study concentration of 15.20 ± 0.59[45] and 9.200 ± 0.404 µg/L wet wt.[44] for bone and brain respectively. Generally Fe levels in the studied samples were found to be in agreement with levels reported.

Manganese is an element of low toxicity, it has considerable biological significance. The concentration of Mn in the analyzed samples ranged from 0.57843 to 7.023 and from N.D to 4.603 µg/g wet wt. in bone and brain respectively, **Table (3**). *Nemipterus japonicus* exhibited the maximum Mn concentration for bone and brain, and *Euthynnus affinis* showed the minimum for bone. Mn contents in the literature have been reported in the range of 10.48 to 27.32 µg/g dry wt.[46]; 0.653-0.716µg/g dry wt.[22] for bone. Also some authors reported concentrations for brain 0.260±0.009 µg/g wet wt.[44]; 0.44 ± 0.02 µg/g wet wt.[45]; 0.38-0.56 µg/g wet wt.[37] at Adriatic coast of Southern Italy; and 0.12 µg/g wet wt.[47] at Eastern Mediterranean, Italy. Another studies reported showed that fish's hard tissues had consistently higher accumulations of Mn than soft tissues[55]. Normally, water contains low level 0.05 µg/g of Mn[56], but the studied fishes contained higher concentration of Mn might be due to the tendency of various species of fish to concentrate certain elements in their tissues more than the surrounding medium. Manganese is used in dry battery cells, iron alloys, glass ceramics, and electric coils that could be considered as the major sources of Mn pollution.

Nickel normally occurs at very low levels in the environment and it can cause variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema and tumors[57]. The present study, investigates the highest amount of nickel in bone fish of *Peneus japonicas* but *Oreochromis niloticus* was under the limit of detection. However, in brain fish, the highest value at *Nemipterus japonicus* and the lowest value at *Pomadasys stridens* **Table (3)**.The results showed that there was a considerable variation in the concentration of the Ni from one sample to another, ranged from N.D to 1.395 and from 0.1020 to 1.4008 for bone and brain, respectively. The elevated nickel content might be attributed to the extensive upwelling occurring in the area, which brings nickel rich subsurface waters to the surface[58]. Ni contents in the literature have been reported in the range of 2.105-8.646 µg/g wet wt.[21]; 0.6-0.97 µg/g wet wt.[37] from illegal bone fish farm in Al-Minufiya Province, Egypt and Mediterranean, Scotland, United Kingdom respectively. However, according with some literature in brain as; 0.02-0.04 µg/g wet wt.[23]; 3.300 ± 0.153µg/g wet wt.[44]; 7.99 ± 1.94 µg/g dry wt.[51]; and 3.66 ± 0.18 µg/g wet wt.[45]. Ni concentrations in Suez water fishes were below the established safe level of 5.5 µg/g by Western Australian Food and Drug Regulations[48]. Nickel and its salts are used in several industrial applications such as in electroplating, storage batteries, auto-mobiles, aircraft parts, spark, electrodes, cooking utensils, pigments, lacquer cosmetics, water and printing fabrics. The industrial effluents were the main sources of nickel contamination of the aquatic environment in the Gulf. While, the present study depicted that the examined fish species were not contaminated by Ni, a long term discharge of untreated industrial wastes could pollute the marine organisms. Since, nickel is a cumulative body poison so its concentration should remain as low as possible.

Vanadium was detectable in Suez fish, and ranged from N.D to 13.613 and from N.D to 11.407, for bone and brain respectively. The concentration of V in bone can be ordered as follows: *Peneus japonicas>Scomber japonicus> Trachurus indicus> Pomadasys stridens> Sauridaundo squamis = Euthynnus affinis = Rhabdosargus haffara = Argyrops spinifer = Nemipterus japonicus* and in brain: *Nemipterus japonicus> Peneus japonicas> Argyrops spinifer> Rhabdosargus haffara> Sauridaundo squamis = Euthynnus affinis = Oreochromis niloticus = Trachurus indicus = Scomber japonicus = Pomadasys stridens* and the results were shown in **Table (3)**. V levels have been reported in the range of 0.043-0.1& 0.04-2.75 µg/g wet wt.[22,23] for bone fish and 0.012&0.01-0.02µg/g dry wt.[22,37] for brain. Similar results for vanadium as were found in seafood from Kuwait[59]. Vanadium is an uncommon constituent of the Earth's crust (about 100 µg /g) both natural and anthropogenic sources contribute to the levels of vanadium in water, and it occurs at trace levels in many foods, particularly seafood[60]. V is found in a wide variety of vertebrate bone and brain in variable concentrations, related to different dietary and background levels. Acute ingestion of vanadium compounds has caused nausea, diarrhea, and stomach cramps at doses of about 13 µg/g vanadium/day. High dose oral studies in rodents have caused anemia, hypertension, and neurotoxicity[60].

Chromium accumulated in fish species **Table (3)** and the highest level of Cr were detected in bone and brain of *Nemipterus japonicus* and the lowest in *Sauridaundo squamis* and *Pomadasys stridens*. Cr levels determined in bone were as follows: *Nemipterus japonicus>Pomadasys stridens>Peneus japonicas>Trachurus indicus>Scomber japonicus>Oreochromis niloticus>Argyrops spinifer> Rhabdosargus haffara>Euthynnus affinis>Sauridaundo squamis*, and in brain as follows*: Nemipterus japonicus> Sauridaundo squamis> Peneus japonicas> Argyrops spinifer> Rhabdosargus haffara>Trachurus indicus> Euthynnus affinis> Scomber japonicus> Oreochromis niloticus> Pomadasys stridens*. Cr contents in the literature have been reported in the range of 0.088-0.10 µg/g dry wt.[22]; 1.81± 0.35 µg/g dry wt.[46]; and 0.05-0.11µg/g wet wt.[23] in bonefish. Another similar study also showed concentrations of 0.01-0.02 µg/g wet wt.[37]; 0.37µg/g wet wt.[22]; and 0.260± 0.009 µg/g dry wt.[44] for brain. In Brazil, the maximum Cr level recommended by the legislation is 0.1 µg /g while in the United States there is no tolerable level for Cr according to the Washington Medicine Institute in New York[1], which was lower than our values. The higher level of Cr in *Nemipterus japonicus* than others due to its living close to the floor[61] in contact with sediment, so sediments may be the major sources of Cr contamination. In this study, high Cr levels may be attributed to the chromites deposits[62] beside, the wastewater coming from various industries such as tanning industries, photography, textile, manufacturing green varnish, paints and river run-off from upstream agricultural fields. Exposure to chromium occurs by intake of contaminated food and water and breathing contaminated air. It leads to various disorders, including cancer, allergic disease, liver damage and lung irritation.

Cobalt is beneficial for humans because it is a part of vitamin B12, however, exposure to high levels of cobalt can result in lung and heart defects and dermatitis[63] **Table (3)**, shows that Cobalt concentration in brain is under the limit of detection in all fish species except of *Pomadasys stridens* and *Scomber japonicus*, whereas in bone ranged from 3.612 to 11.110, can be ordered as follows: *Peneus japonicas> Pomadasys stridens>Scomber japonicus> Oreochromis niloticus>Trachurus indicus> Nemipterus japonicus>Argyrops spinifer>Rhabdosargus haffara>Euthynnus affinis> Sauridaundo squamis.* Co levels have been reported in bone 0.42 ± 0.07 µg/g dry wt.[46] 0.13-0.16 µg/g wet wt.[23]. Moreover, fish brain has displayed 0.027&0.088 - 0.10 µg/g dry wt.[22]. Turkish Standards do not cover information about maximum Co levels in fish samples.

Calcium belongs in to alkaline earth metals[64], elevated concentrations of Ca **Table (3)** which accumulated in bone ranged from 9197.157 to 17557.42 and from 4533.787 to 24094.456 in brain respectively, *Nemipterus japonicus* exhibit the maximum values 17557.42 and

24094.456 µg/g wet wt. in bone and brain, respectively, while, *Sauridaundo squamis* has the minimum values 9197.157 in bone and *Euthynnus affinis* has the minimum value of 4533.787µg/g wet wt. in brain. The decreasing order was as follows: *Nemipterus japonicus>Peneus japonicas> Argyrops spinifer> Trachurus indicus> Oreochromis niloticus> Scomber japonicus> Euthynnus affinis> Rhabdosargus haffara> Sauridaundo squamis* in bone fish. The pattern in brain was as follows: *Nemipterus japonicus> Sauridaundo squamis> Peneus japonicas> Trachurus indicus> Rhabdosargus haffara> Scomber japonicus> Argyrops spinifer> Oreochromis niloticus> Pomadasys stridens> Euthynnus affinis*.

What are the effects of metal bioaccumulation, the authors added that metals are bio-available and bio-accumulative substances in concentrations that cause physiological effects in vertebrates[65]. The metals Hg and Pb possess chemical properties that mimic trace metals such as Ca and Zn. Pb have a similar ionic radius to Ca. Therefore, we can expect that these metals are stored and affect the kidneys and bone tissues and active transport through calcium channels of the nervous system[66]. The mechanism of lead toxicity is by replacing cations Ca and Zn within cells. This replacement allows interactions with the compounds that coordinate binding of multivalent cations affecting survivor ship, growth, learning, reproduction, development, and metabolism[67]. Another study[68] confirmed that Pb can be biomagnified when the top predators are invertebrates, other authors[69] reported that Ca concentrations in the *rotifers* when exposed to 1 mg/L of Pb for 24h and compared to Ca concentrations in the control animals, show Ca reduction this is probably due to decalcification of the *rotifer cuticle* by Pb substitution for Ca. Decalcification by Pb has been reported in other invertebrates. For instance, Pb bioaccumulation on skeleton morphogenesis in the common asteroid *Asterias rubens* affected morphogenesis.

Selectivity indices of metals in fish bone and brain

Fishes are able to regulate metal concentration to some extent after which the bioaccumulation will occur. Therefore, the total concentration of metals will control the regulation and bioaccumulation capacity of each tissue[70]. Metal selectivity index (MSI) measures the affinity of the species to accumulate a metal in that tissue/organ of the body. MSI is calculated for all the metals except mercury and calcium as the percentage of absolute concentration of a metal in a tissue to the total concentration of all metals in that tissue[71]. MSI (%) values are represented as **Fig. (3a – j)**, based on the MSI values, the affinity for bone of *Argyrols spinifer* can be ranked in the order Fe>Co>Zn>Cr>Al>Ni>Mn>Cu>Pb=V. However, *Nemipterus japonicus* follow the order Fe>Co>Mn> Cr>Zn>Al>Cu>Ni>Pb=V. Brain of *Scomber japonicus* in decreasing order of Zn>Fe>Cr>Co>Cu> Ni>Al>Mn>Pb**>**V. Whereas, *Pomadasys stridens* follows the order of Fe>Zn>Cu>Co>Cr>Al>Mn>Ni>Pb**>**V. Significant differences in metal affinity was noticed between fishes as well as tissues. However, a significant difference in Al, Pd, Mn, Co affinity was observed between tissues and species. These differences might be due to the site difference, availability of metals and feeding habit. These results suggest that both pelagic and demersal fish has almost same kind of metal affinity. All the fishes are reported to be shown with high affinity towards Zn, Fe, Cu, Mn, Cr, and Co, irrespective of their feeding habit. Relative tissue-occupying capacity of a metal in a particular tissue is known as tissue selectivity index (TSI). It is the percentage of ratio between absolute concentration of a metal in a tissue and total concentration of that metal in all tissues[71]. TSI (%) values were calculated for all the metals and are plotted **Fig. (4a – j)**, it is very difficult to conclude which metal has the maximum tissue occupying capacity in a particular tissue of selected fishes, in *Sauridaundo squamis* **Fig. (4a),** Zn, Pb, Cu, Fe, and Cr showed maximum TSI in brain, and Al, Pb, Cu, Fe, and Co in bone. High TSI values in bone indicated the metals high occupying capacity in this part, in *Euthynnus affinis* **Fig. (4b),** Pb, Cr, Zn & Ni showed high TSI values in brain beside, high TSI values for all metals in bone and maximum accumulation capacity was with Pb**,** whereas in *Peneus japonicas* **Fig. (4h),** Al, Mn, Co showed the maximum tissue-occupying capacity in bone followed by V in brain.

The tissue-occupying capacity of mercury was high for brain followed by bone **Fig. (5).** the analysis showed that there is no significant difference between the fishes for TSI of all metals, whereas significant variation in tissue-occupying capacity was observed for Hg. Unlike MSI, no unique ranking could be obtained with TSI, which is a multi dependent factor. During the ranking of the TSI, there is high species-metal specificity; species–tissue interaction and metal-tissue selectivity were observed.

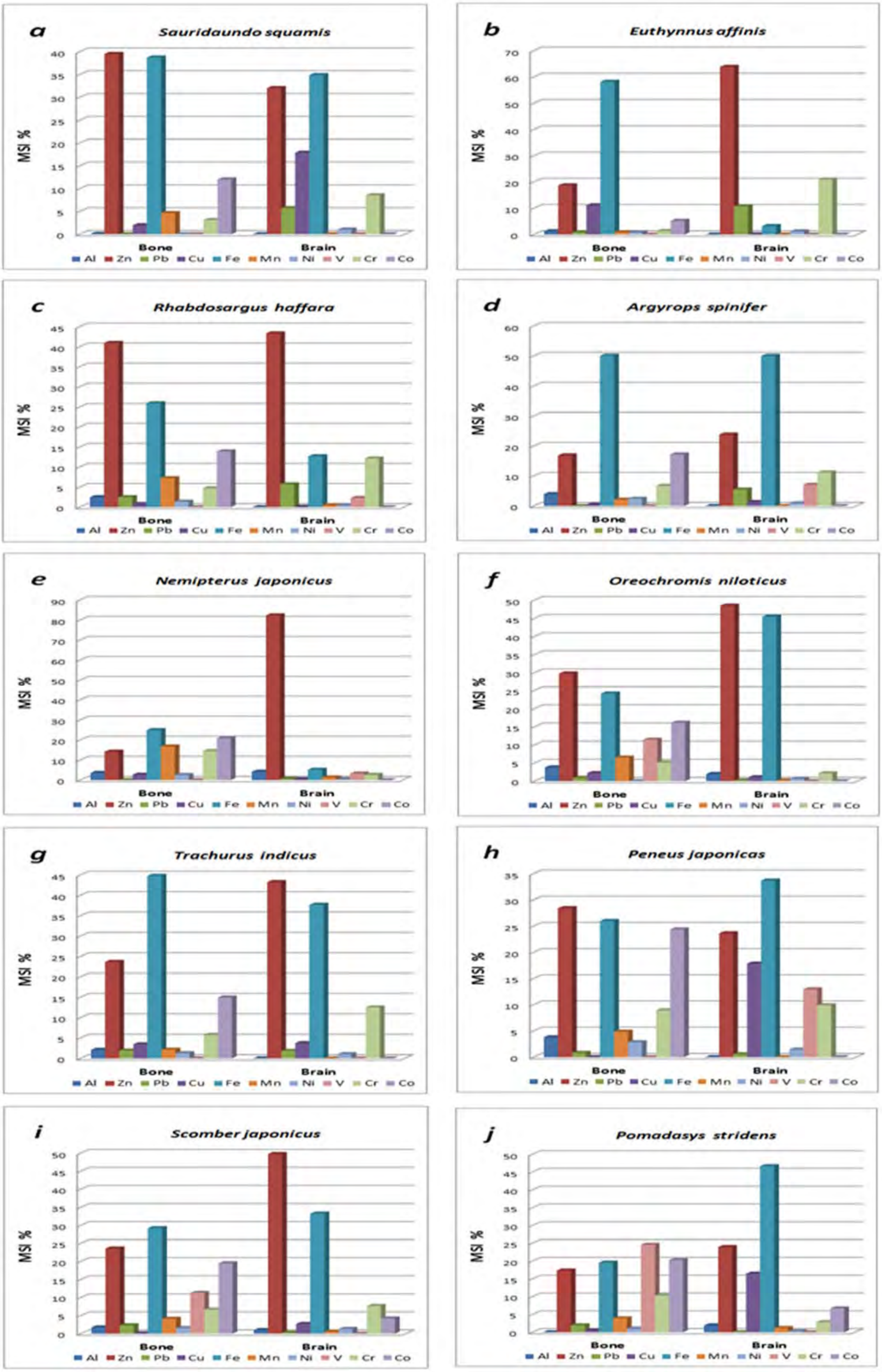

Figure 3: MSI for bone and brain of fishes.

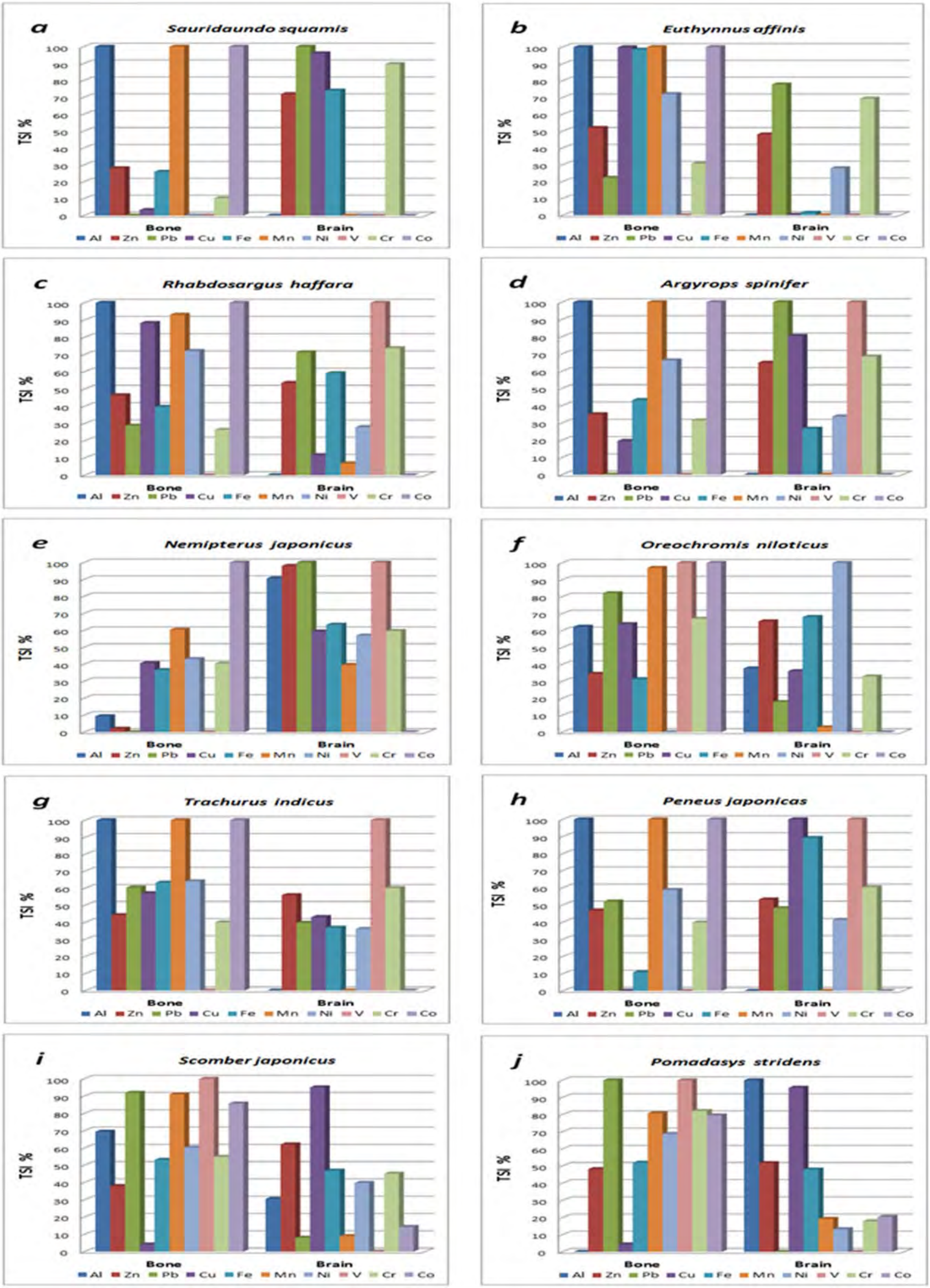

Figure 4: TSI for bone and brain of fishes.

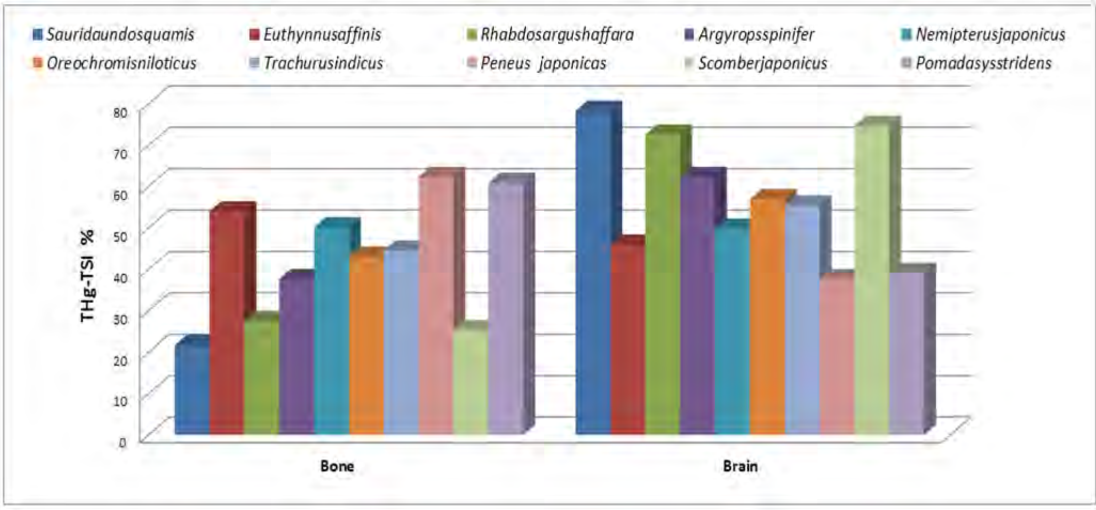

Figure 5: TSI of T Hg in bone and brain of fishes.

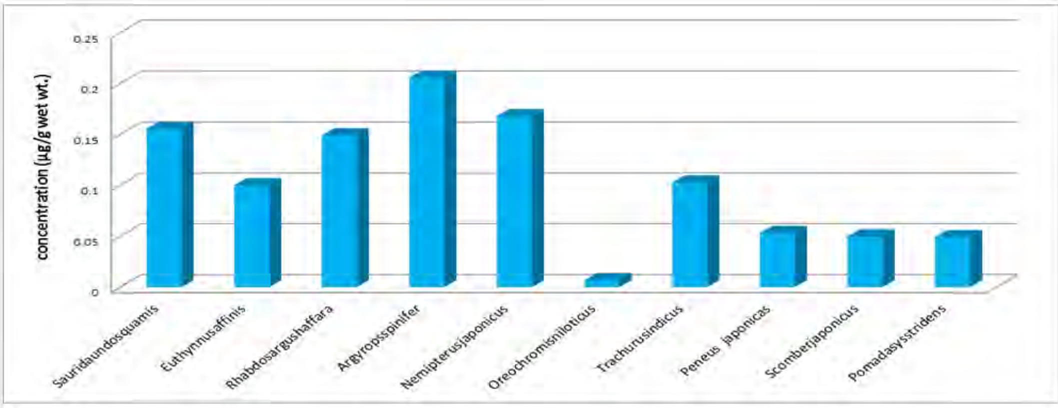

Figure 6: Total mercury concentration (µg/g wet wt.) in bone and brain of different fish species.

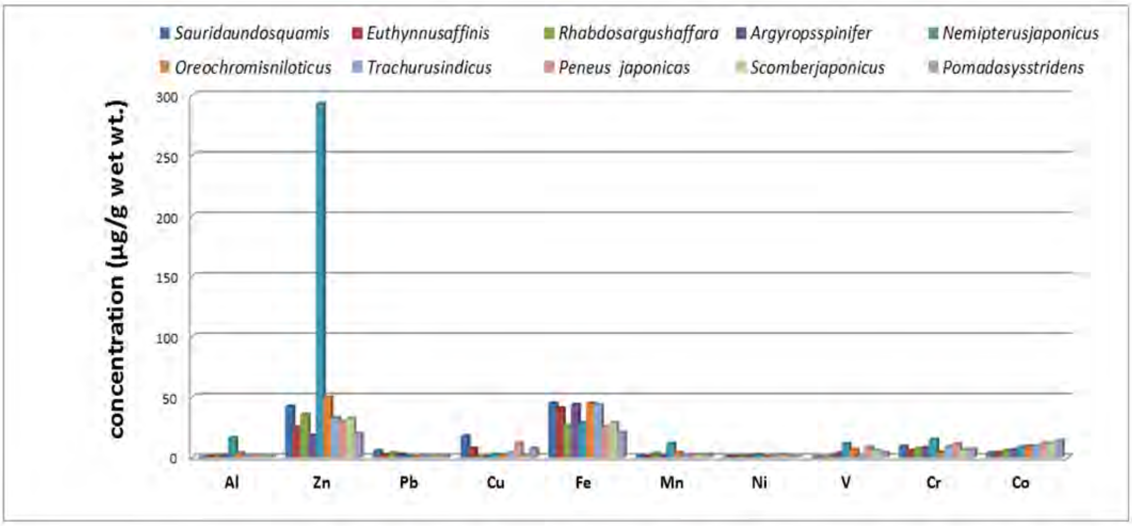

Figure 7: Total metals in fishes of Suez Gulf.

Total metal concentration in Aquatic species

Total content of Hg in each fish was presented in **Fig. (6).** Maximum concentration was obtained for *Argyrops spinifer* 0.2059 µg/g wet wt., followed by *Nemipterus japonicus* 0.1680 µg/g wet wt. and *Sauridaundo squamis* 0.1554 µg/g wet wt. The highest concentration of T Hg was obtained for Predator *Argyrops spinifer* 0.2059 µg/g wet wt. followed by Carnivores 0.1680 µg/g wet wt. However, the lowest concentration was given by another Omnivore *Oreochromis niloticus* 0.0069 µg/g wet wt. The *Argyrops spinifer* (Predator) collected mainly from just downstream of industrial outlets (location 4) where the presence of high sediment Hg concentration. Inhabits shallow waters and over sandy or mud-sandy bottoms and it feeds on benthic invertebrates, mainly mollusks, important food fish. Because of these factors, *Argyrops spinifer* has shown higher concentrations of mercury. The mercury content in the fish is mainly influenced by various factors such as size and age, trophic position (feeding habits) as well as the high background concentration in water and sediment[71]. All the samples analyzed in the present study showed lower concentrations than the permissible limit 0.4 µg/g wet wt.

The total accumulations of individual metals in different fishes have been calculated without Ca and represented in **Fig. (7)**. the total Al accumulation in the fishes were in the order of, *Nemipterus japonicus>Oreochromis niloticus>Peneus japonicas> Argyrops spinifer>Scomber japonicus> Trachurus indicus***>** *Rhabdosargus haffara> Euthynnus affinis> Pomadasys stridens> Sauridaundo squamis.* In the case of Zn, the order is *Nemipterus japonicus> Oreochromis niloticus> Sauridaundo squamis> Rhabdosargus haffara> Trachurus indicus***>** *Scomber japonicus> Peneus japonicas> Euthynnus affinis> Pomadasys stridens> Argyrops spinifer.* In addition to that, *Nemipterus japonicus* and *Oreochromis niloticus* showed higher concentrations of Mn. For Pb, *Sauridaundo squamis* and *Rhabdosargus haffara* showed higher concentrations than the *Peneus japonicas* and *Oreochromis niloticus*. Cu accumulation was maximum in *Sauridaundo squamis* followed by *Peneus japonicas* and *Euthynnus affinis.* Moreover, *Nemipterus japonicus* exhibit higher concentrations of Al, Zn, Cu, Ni, V, and Cr than others. Beside, the accumulation was maximums in *Pomadasys stridens* followed by *Peneus japonicas* and *Scomber japonicus* for Co. It was found that the carnivore's fishes have accumulated the maximum total concentration of all metals in its tissues, which was much higher than the metal accumulated by the omnivore. In the present study, different fishes contained variable levels of different metals. The concentration of all metals studied was added for particular fish. Then, the total metal accumulation in the fish body with Ca was in the order of *Nemipterus japonicus>Peneus japonicas> Sauridaundo squamis> Trachurus indicus> Argyrops spinifer> Scomber japonicus***>** *Rhabdosargus haffara> Oreochromis niloticus> Pomadasys stridens***>** *Euthynnus affinis*. The results were supported by earlier studies, which reported that the metals accumulation in fish species was in the order of carnivorous> Predator>phytoplankton>zooplankton[72]. In short, the intertaxon variability in metal accumulation can be explained by species-specific differences in bioaccumulation dynamics as well as differences in metal exposure, such as those induced by habitat characteristics (temperature, metal speciation and partition) or dictated by dietary preferences (feeding habits), foraging behaviors, food web structure[73]. Maximum accumulation in *Nemipterus japonicus* may be due to the fact that they are bottom dwellers and Carnivore and feed up on a lot of detritus matter. The accumulation of maximum amount of metals in the internal organs like brain is attributed to fat solubility of heavy metals in the diet digested by the fishes might have reached the brain through blood.

CONCLUSION

The present study revealed that the major sources of metals in the fish species are food and feeding habit. The variation in of accumulated metals in different organs of fishes may be attributed to the proximity of the tissues to the availability of metals, i.e. the quantity present in the surrounding environment, age and type of the fish and role of the tissue in the detoxification process. The total metal accumulation in the fish body was in the order of *Nemipterus japonicus>Peneus japonicas>Sauridaundo squamis>Trachurus indicus>Argyrops spinifer>Scomber japonicus***>***Rhabdosargus haffara>Oreochromis niloticus> Pomadasys stridens***>***Euthynnus affinis*. The relatively greater level of these metals in brain indicates the greater solubility and retention of the metals in fats. The greater occurrence of these metals in bone may be associated with calcification process and competition with enzymes for active sites. The observed concentration of total mercury in bone and brain was 0.0069 to 0.2095 µg/g wet wt. (mean value 0.1040 µg/g wet wt.), which is lower than the prescribed limits (0.4 µg/g wet wt.). The calculated MSI and TSI values anticipate a high concentration of metals in the sediments and water of Suez Gulf which can induce an increased accumulation in fishes in future. High metal accumulation was noticed in brain than bone which suggested the importance of considering the fish's organs as indicators of metallic pollution in Gulf. As fishes are staple food for human being, the accumulation of metals exceeding the permissible limits is a serious health concern. The study thus highlighted the heavy metal bioaccumulation potential in fishes which constitute an important group of animals in this brackish water ecosystem. As the high metal availability in the backwaters are attributed to anthropogenic origin, adequate strategies are to be adopted in order to control their presence so that the possible health hazards to different life forms including man can be prevented.

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