Toxicology Reports 13 (2024) 101724

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Exposure assessment of pesticide residues, heavy metals, and veterinary drugs through consumption of Egyptian fish samples

Mahmoud M. Ghuniem [*,1], Nermine Gad, Mohamed A. Tahon, Lamia Ryad

Ministry of Agriculture and Land Reclamation, Agricultural Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods (QCAP Egypt), 7-Nadi El-said Street, Dokki, Giza 12311, Egypt

ARTICLE INFO

Handling Editor- Prof. L.H. Lash

Keywords:
Environmental Contaminants
Heavy Metals
Antibiotics
Pesticides
Hazard Quotient, Hazard Index, Risk assessment

ABSTRACT

Environmental contaminants may enter seafood products either through water and sediments or via feed and feed additives or may be introduced during fish processing and storage. The study focused on the nutritional and toxicological significance of heavy metals, antibiotics, and pesticide residues in 48 fish samples collected from the Kafr-ElSheikh governorate in Egypt. Various analytical instruments are used to determine and detect heavy metals, antibiotics, and pesticides. These include Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS), Inductively Coupled Plasma Mass Spectrometer (ICP-MS), and Gas Chromatography-Mass Spectrometer (GC-MS). The following metals were discovered in fish species: arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), and zinc (Zn). Each of these metals was detected 47 times. Chromium (Cr) was detected 40 times, nickel (Ni) was detected 27 times, and lead (Pb) was detected 6 times. The mean concentrations of As, Cd, Cr, Co, Cu, Fe, Ni, Mn, Hg, Pb, and Zn were determined to be 0.025, 0.02, 0.501, 0.50, 0.81, 12.56, 0.5, 0.689, 0.051, 0.031, and 5.78 mg/kg, respectively. All levels of cadmium, mercury, and lead detected in fish samples were significantly lower than the maximum permissible limits set by Egyptian and European standards. Furthermore, in this study, antibiotics and pesticide residues were found to be not detected in all analyzed fish samples. Based on the estimated daily intake and hazard quotient values, the concentration levels of metals found in fish samples seem to pose no significant threat to public health.

1. Introduction

Fish is a valuable protein source for human health, and the amount consumed worldwide has increased dramatically due to its low saturated fat content, high protein, high omega-3 fatty acid, and low fat. It promotes health and avoids chronic diseases; hence it is considered one of the healthiest foods [1]. Environmental contaminants, such as heavy metals, veterinary medications, and pesticides, can accumulate and persist in fish tissues for various reasons. Consequently, humans who ingest contaminated seafood face serious health risks.

Metals are widespread in our food, water, and environment, either naturally or due to human activities such as industrial emissions, agricultural practices, or contamination during manufacturing. Metals have various advantages and are expected to play a significant role in the industry that has dominated human culture. Some metals have essential physiological and biochemical functions in common forms, and an imbalance in their levels can negatively affect the body's ability to cope with them and, consequently, lead to various diseases. [2,3]. Metals have a variety of functions in health and disease, ranging from the need for vital trace elements to the toxicity associated with metal excess. A few metalloids and metals expect enormous parts (biochemical or physiological) in animals as they take part in a vital role in the development of a digestive enzyme that catalyzes chemical reactions in organisms or other critical molecules or substances [4–6]. Metals such as copper (Cu), cobalt (Co), chromium (Cr), magnesium (Mg), iron (Fe), manganese (Mn), nickel (Ni), molybdenum (Mo), selenium (Se), and zinc (Zn) have been identified as key improvements necessary for a variety of physiological and biochemical functions [7–9]. Lacking a stack of these scaled-back supplements result in a collection of insufficiency concerns or conditions. Basic metals are regarded as minor components due to their relatively low concentrations (μg/kg to less than 10 mg/kg) in many typical constructions. Their bioavailability is altered by genuine elements such as temperature, stage connection, adsorption, and sequestration [10–12].

* Corresponding author.
E-mail addresses: Mahmoud.ghuniem@qcap-egypt.com, Mahmoud_ghuniem88@yahoo.com (M.M. Ghuniem).
[1] ORCID ID: 0000-0001-7071-9190

https://doi.org/10.1016/j.toxrep.2024.101724
Received 18 June 2024; Received in revised form 29 August 2024; Accepted 30 August 2024
Available online 6 September 2024

Furthermore, fish can become contaminated with hazardous metals from a range of natural and anthropogenic sources, such as industrial effluent discharge, agricultural runoff, and gasoline from fishing boats [13,14]. The presence of heavy metals in the marine ecosystem, along with the resultant contamination of fish, poses significant risks to both aquatic life and human health due to the consumption of affected fish. The pollution caused by heavy metals, even at minimal concentrations, poses a substantial threat to seafood's consumers [15,16]. Extensive research has been conducted on the prevalence of heavy metal contamination across different marine fish species [17–21].

Drugs (such as antibiotics), whether natural or synthetic, have the power to kill or impede the growth of microorganisms, which could be another source of fish contamination. As a result, antibiotics are in use to treat illnesses, control, and prevent infections, promote growth, and boost productivity [22]. The misuse of medications can lead to the accumulation of drug residues in fish, posing risks such as mutagenicity, carcinogenicity, hypersensitivity, bone marrow suppression, disruption of gut flora, allergic reactions, toxicity, and the emergence of antibiotic-resistant bacteria. This is a significant concern for human health, particularly in the treatment of infections. [23]. The direct toxicity to humans and the emergence of bacterial strains resistant to antibiotics are serious issues associated with antibiotic residues entering the food chain [24].

The European Union has set up Maximum Residue Limits (MRLs) for specific pharmaceuticals in aquaculture, as well as products derived from it. These limits are based on acceptable daily intake levels and the results of toxicological research [25].

Pesticides and other plant protection agents can also be a major cause of pollution for fish. Pesticides are deployed globally to prevent, eradicate, or manage various pests, including disease vectors for humans and animals, as well as undesirable plant or animal species that cause harm or interfere with the production, processing, storage, transport, or marketing of food and agricultural products. Pesticide toxicity to people varies, as does human response and tolerance to a specific pesticide. The pesticide itself may not be toxic to fish, but when it decomposes in water, toxic compounds are produced, such as the pesticide propanil, which is specialized in controlling rice weeds, as it decomposes into a dichloro compound that is harmful to fish, endangering their presence in rice farms [26–28].

There are around 1200 active pesticide chemicals used in agricultural agriculture. The most extensively used are DDT and other pesticides having DDT, chlorine, and phosphorus, where fish are polluted with pesticides that are released into wastewater, and they are concentrated. These pesticides are found in seaweeds and microorganisms, and they are conveyed to fish in addition to what fish consume directly from the water, resulting in humans eating polluted fish [29,30].

It can be said that the high percentage of fat in fish increases the chance that it contains a higher percentage of pesticides, such as eels, as fish can concentrate pesticide insects in their meat until their concentration reaches thousands of times compared to their concentrations in the water surrounding it, as the DDT present at a concentration of 1 ppb in the rivers of Europe reaches 5 ppb, and the same was observed. It is concentrated even in the fish that inhabit these rivers. The phenomena occur in the fish of Clear Lake in California, United States. These insecticides can be classified into the following sections: Insecticides: They are classified into several categories, the most prevalent of which are phosphorous organic compounds, chlorinated hydrocarbons, pyrethroid compounds, carbamate compounds, aquatic herbicides, and snail pesticides [31–33].

Pesticides have numerous negative effects on fish, including weakening their ability to survive growth, and increasing the thickness of their gills, resulting in an obvious lack of organization, osmosis, a sharp drop in blood cells, brain damage, and a decrease in fish resistance to diseases. Lethal doses of pesticides cause immediate death of fish. Because these pesticides are hazardous to fish at low quantities, impairing the effectiveness of their reproductive system and stunting fish, they enter the fish ecosystem and then reach the fish via agricultural drainage or are deposited directly in the water, as with weed killers and snails. These pesticides also have an immunosuppressive effect on fish, causing a lack of appetite and increasing susceptibility to infectious and non-communicable diseases. The most dangerous of these pesticides are chlorinated hydrocarbon insecticides, which are widely used in crops and can survive in the bottoms of rivers and seas for decades, which should not exceed the permissible rate in the water (0.5 micrograms per liter) and should not exceed 0.3 parts per million (ppm) in fish (for example, dieldrin pesticide), as this pesticides have a high ability to accumulate in the bodies of fish and other aquatic long-term consumption of these fish may result in the accumulation of toxicity to humans. [34–36].

Hence, this research aimed to monitor the presence of veterinary drugs, heavy metals, and pesticides in fish collected from Kafr-ELSheikh governorate, Egypt. Forty-eight samples were assessed for the three mentioned contaminants groups, and only positive samples were subjected for the risk assessment and dietary exposure calculations.

2. Materials and methods

2.1. Instruments

In the case of heavy metals, an Ethos Up High-Pressure Microwave system from Milestone – Italy was used. Perkin Elmer inductively coupled plasma mass spectrometer, Model: NexION 2000 in combination with autosampler S10, SV40 BI vacuum pump, copper coil RF, skimmer cone, sampler cone, hyper skimmer cone, Meinhard nebulizer concentric glass C 0.5, ion lens, mist cyclonic spray chamber, quartz torch and chiller – (USA) was used.

In the case of veterinary drugs, an Agilent liquid chromatography-tandem mass spectrometry (LC-MS/MS) model in combination with API 4000 triple quadrupole (Applied Biosystems, Foster City, CA, USA), with electrospray ionization (ESI) interface in both negative ion mode and positive ion mode using Zorbax-C18 column (2.1 mm×50 mm, 1.8 µm) (Merck, Darmstadt, Germany) coupled with Agilent HPLC model 1200 system (Agilent, Santa Clara, USA). The injection volume was 5 µl. The elution flow rate was 0.8 mL/min. N_2 nebulizer gas, curtain gas, and other gas settings were applied according to recommendations made by the manufacturer. The source temperature was 300 °C, the ion spray potential was 5500 V, multiple reaction monitoring (MRM) was applied, and two product ions were selected (for quantification and confirmation transition). Mobile phase solution consisting of (A) 5 mM ammonium format in methanol buffer (1:9) was prepared from 50 mM ammonium hydroxide solution that was previously prepared and formic acid in water adjusted to pH= 2.8±0.1; and (B) methanol.

In the case of pesticides residues, an Agilent LC instrument (1260 Series) coupled to an API 6500 Qtrap tandem mass spectrometer from AB Sciex with an electrospray ionization (ESI) interface was used. For separation a C18 column was used (ZORBAX Eclipse XDB-C18 4.6 × 150 mm, 5µm particle size) (Agilent, USA). An Agilent Gas Chromatograph system 7890 A in combination with tandem mass spectrometer 7000 C series GC.

2.1.1. Apparatus

An Hiedolph rotary evaporator, Sigma centrifuge up to 4500 rpm, Geno/Grinder 2010- SPEX Sample shaker, and calibrated micropipettes in ranges (10–100, 100–1000 µl) from Hirschman Laborgerate, Germany) were in use. The Millipore water purification system in combination with Q-POD element coupled with Merck Millipore – Q® integral 5 (A10®) was used. A solvent dispenser with a 10 mL volume (Hirschman Laborgerate, Germany) was used. Mettler Toledo top bench balance has ranged from 0.1 mg to 210 g in use.

2.2. Chemicals and reagents

For heavy metals: Suprapur® nitric acid (HNO_3), with a concentration of 65 % weight/weight, was obtained from Merck, Germany. Additionally, Emsure® Hydrogen Peroxide (H_2O_2) at 30 % concentration was also obtained from Merck, Germany. The deionization of water was conducted in-house using a Millipore water purification system. A 2 % volume/volume solution of nitric acid was prepared following the method outlined in reference [5].

For both antibiotics and pesticides: acetonitrile and methanol of HPLC grade were bought from Sigma-Aldrich, Germany. Toluene with a purity of ≥ 99.9 % was sourced from Merck, Germany. Additionally, n-hexane with a 97 % was obtained from Sigma Aldrich or equivalent quality. The extraction reagents, including magnesium sulfate, sodium chloride, sodium citrate, and citric acid disodium salt, were obtained as a pre-mixed package from Agilent Technologies.

The deionization of water was conducted in-house using a Millipore water purification system. Sodium hydroxide with a purity of ≥ 99 % is used to create a 10 M solution by dissolving 40 g in 100 mL of deionized water. Citric acid monohydrate, also with a purity of ≥ 99 %, is used to prepare a 1 M citric acid solution by dissolving 21.14 g in 100 mL of deionized water, with the pH adjusted to 4.0 using a 10 M sodium hydroxide solution. Formic acid, with a concentration of 98–100.5, was obtained from Riedel-de Haën. A 30 % ammonium hydroxide solution is diluted to a 10 % solution by mixing 30.3 mL of the 30 % solution with 100 mL of deionized water. Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2-EDTA), of a quality equivalent to or greater than 99 % as provided by Fluka, is used to prepare a 0.5 M Na_2-EDTA solution. This is done by dissolving 18.61 g in 100 mL of deionized water and adjusting the pH to between 8 and 10 with a 10 N NaOH solution. Solid Phase Extraction (SPE) cartridges, specifically Oasis MCX 6 mL with 150 mg of sorbent, are sourced from Waters.

2.3. Certified reference material

For heavy metals, stock standard solutions of reference metals, including As, Pb, Cd, Sb, Hg, Cu, Zn, Fe, Cr, Sn, Co, Mn, and Ni at 1000 mg/L concentration in 2–3 % HNO3, were sourced from Merck, Germany. Additionally, a certified NexION setup standard mixture solution containing Be, Ce, Fe, In, Li, Mg, Pb, and U at 1 μg/L concentration in 1 % HNO_3 was obtained from PerkinElmer, USA. A certified internal standard mixed solution including Bi, Ge, In, ^{6}Li, Sc, Tb, and Y at 10 μg/mL concentration in 5 % HNO_3 was also obtained from PerkinElmer, USA.

In the case of antibiotics, thirty target antibiotics-certified reference materials of different classes (Quinolones, Sulfonamides, Tetracyclines, Macrolide, and Diaminopyrimidine) bought as active ingredients with a high purity (≥ 95 %) procured from Dr. Ehrenstorfer-LGC GmbH, Augsburg, Germany.

In the case of pesticide residues reference standards for approximately 461 pesticides, as listed in Table 1 and sourced from Dr. Ehrenstorfer in Augsburg, Germany, with purities exceeding 95 %, were employed to prepare stock solutions in toluene. These reference standard solutions, with a concentration of 1000 μg/mL, were produced and then stored at - 20 ± 2 ^{0}C. They were used for Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS) and Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) using a solvent mixture of n-hexane and acetone in a 9:1 ratio. The chosen solvents are suitable for the analytes in terms of solubility, stability, and compatibility with the measurement technique, ensuring no adverse impact on the pesticides' integrity.

2.4. Standards preparation

For heavy metals: based on the standard preparation procedures for heavy metals as outlined in [5]. Firstly, eight working standard solutions were formulated for As, Pb, Cd, Sb, and Hg, covering a range from 0.05 to 100 μg/L. Additionally, nine working standard solutions were created for Fe, Sn, Cu, and Zn, with a range of 1–5000 μg/L. Lastly, ten working standard solutions were prepared for Mn, Cr, Co, and Ni, spanning concentrations from 0.05 to 1000 μg/L.

For both antibiotics and pesticide residues: standard solutions of antibiotic and pesticide compounds were prepared in methanol and stored at −18 ^{0}C. Intermediate and working solutions were freshly prepared with each batch of samples. Stock solutions for all antibiotics were adjusted for salt content (when present) to achieve a target analyte concentration of 1000 μg/mL. Calibration mixtures, in a series of 0.25, 0.5, 1.00, 2.00, and 5.00 MRL based on the LOQ of each target antibiotic, were prepared in methanol for LC-MS/MS and stored at −18 ^{0}C.

2.5. Sample collection

In Egypt, fish farms are concentrated in the north of the Delta. Kafr El-Sheikh Governorate, is regarded as one of the most significant areas, owing to Lake El-Burullus and its fame for extensive fish farming. Based on the sampling procedures stated by the Egyptian standards and Codex Alimentarius Commission, forty-eight fish samples were collected from the different farms within Kafr El-Sheikh governorate, depended on agricultural drainage water [36,37]. These areas include farms surrounding drainage No. Seven, farms in the Bridge Alsukna area, farms in the Karkat area, farms surrounding the Nasser drainage, and farms around Talmbat Sabaa. Fig. 1 shows the geographic locations of sampling in Egypt. The collected samples were unprocessed, stored in plastic containers, and labelled with an identification code. The fish samples were kept at −20 0 C until analysis.

2.6. Sample preparation

Samples were analyzed at the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods. Following the validated procedures referenced in [5,38], fish samples for heavy metal analysis were prepared by homogenizing and weighing up to 0.5 g into a microwave digestion vessel. To this, 8 mL of Suprapur nitric acid was added, followed by a gentle shake, and then 2 mL of hydrogen peroxide. The vessel was sealed as per the handbook instructions and placed in its holder in the microwave oven. A thermocouple probe was inserted into the reference vessel before closing the oven door. The microwave program was set to 1800 watts for 15 minutes until the temperature reached 200 ^{0}C, maintained for another 15 minutes, and then allowed to vent until the temperature dropped below 80°C. Post-heating, the thermocouple probe was removed, allowing the vessels to cool before opening. The vessel's lid and walls were rinsed with deionized water, and the solution was transferred to a 50 mL polypropylene tube, adding 0.5 mL of an internal standard mixture containing Bi, Ge, In, ^{6}Li, Sc, Tb, and Y, and diluting to volume with deionized water. A reagent blank was treated identically. Samples were stored in polypropylene tubes until Q-ICP-MS analysis.

The analysis of antibiotics in fish samples used the QuEChERS method, complemented by LC-MS/MS detection as validated and outlined in reference [22]. A 2 ± 0.1 g of fish sample was placed into a 50 mL polypropylene centrifuge tube. The sample was vortexed and homogenized with a mixture of 1 mL of 1 M Na-Citrate buffer at pH 4.0 and 0.5 mL of Na-EDTA at pH 8–10. Subsequently, 10 mL of acetonitrile was added, and the mixture was homogenized for 2–3 minutes using an Ultra-Turrax, then shaken for 1 minute before centrifugation. The supernatant was decanted, evaporated, and subjected to a second extraction with an added volume of acetonitrile, then evaporated to dryness. The residue was reconstituted in 2 mL of dilution solvent and purified using solid-phase extraction (SPE) columns. After sonication, the solution was filtered through a disposable acrodisc syringe filter (0.45 μm) attached to a 5 mL plastic syringe into a vial. A 5 μL aliquot of the final sample was then injected into the LC-MS/MS system. For further

Table 1
Pesticide reference standards for 461 analyzed of different groups analyzed using LC and GC-MS/MS.

#	Compound	#	Compound	#	Compound
1	1-Naphthylacetic acid	41	Brodifacoum	81	Chlorthiophos
2	2-(1-Naphthyl) acetamide	42	Bromacil	82	Chlozolinate
3	Abamectin	43	Bromophos-ethyl	83	Chromafenozide
4	Acephate	44	Bromophos-methyl	84	Cinidon-ethyl
5	Acetamiprid	45	Bromopropylate	85	Cinosulfuron
6	Acrinathrin	46	Bromoxynil-octanate	86	Clethodim
7	Alachlor	47	Bromuconazole	87	Clodinafop free acid
8	Aldicarb	48	Bupirimate	88	Clodinafop-propargyl
9	Aldicarb Sulfone	49	Buprofezin	89	Clofentazine
10	Aldicarb Sulfoxide	50	Butachlor	90	Clomazone
11	Ametoctradin	51	Butocarboxim	91	Cloquintocet-mexyl
12	Ametryn	52	Butocarboxim sulfoxide	92	Clothianidin
13	Amidosulfuron	53	Butralin	93	Coumaphos
14	Aminocarb	54	Butylate	94	Coumatetralyl
15	Amisulbrom	55	Cadusafos	95	Cyanophos
16	Amitraz*	56	Captan*	96	Cyantranilirpole
17	Anilofos	57	Carbaryl	97	Cyazofamid
18	Atraton	58	Carbendazim	98	Cycloheximide
19	Atrazine	59	Carbetamide	99	Cycloxydim
20	Azaconazol	60	Carbofuran	100	Cyflufenamid
21	Azamethiphos	61	Carbofuran−3-hydroxy	101	Cyfluthrin
22	Azimsulfuron	62	Carbosulfan*	102	Cyhalofop-butyl
23	Azinphos-ethyl	63	Carboxin	103	Cyhalothrin - Lambda
24	Azinphos-methyl	64	Chlorantraniliprole	104	Cymiazole
25	Azoxystrobin	65	Chlorbromuron	105	Cymoxanil
26	Barban	66	Chlorbufam	106	Cypermethrin
27	Beflubutamid	67	Chlordane-cis	107	Cyproconazole
28	Benalaxyl	68	Chlordane-trans	108	Cyprodinil
29	Bendiocarb	69	Chlorfenapyr	109	Cyromazine*
30	Benomyl (as Carbendazim)*	70	Chlorfenvinphos	110	Dazomet
31	Bensulfuron-methyl	71	Chlorfluazuron	111	DDD-o,p`*
32	Benthiavalicarb isopropyl	72	Chloridazon	112	DDD-p,p`*
33	Benzoximate	73	Chlorobenzilate	113	DDE-p,p`
34	Bifenazate	74	Chlorothalonil*	114	DDT-o,p`*
35	Bifenthrin	75	Chloroxuron	115	DDT-p,p`*
36	Biphenyl*	76	Chlorpropham	116	DEET
37	Bispyribac-Sodium	77	Chlorpyrifos	117	Deltamethrin
38	Bitertanol	78	Chlorpyrifos-methyl	118	Demeton-S-methyl
39	Bixafen	79	Chlorsulfuron	119	Demeton-S-methyl sulfone
40	Boscalid	80	Chlorthal-dimethyl	120	Desmedipham
#	**Compound**	**#**	**Compound**	**#**	**Compound**
121	Diafenthiuron	161	Ethiofencarb Sulfone	201	Fludioxonil
122	Diazinon	162	Ethiofencarb Sulfoxide	202	Flufenacet
123	Dichlobenil*	163	Ethion	203	Flufenoxuron
124	Dichlofenthion	164	Ethirimol	204	Flumetsulam
125	Dichlofluanid	165	Ethofumesate	205	Flumeturon
126	Dichlorvos	166	Ethoprophos	206	Fluopicolide
127	Diclofop-methyl	167	Ethoxyquin*	207	Fluopyram
128	Dicloran	168	Etofenprox	208	Fluquinconazole
129	Dicofol*	169	Etoxazole	209	Fluroxypyr
130	Dicrotophos	170	Etridiazole*	210	Fluroxypyr-meptyl
131	Dieldrin	171	Etrimfos	211	Flusilazole
132	Diethofencarb	172	Famoxadone	212	Flutolanil
133	Difenoconazole	173	Fenamidone	213	Flutriafol
134	Diflufenican	174	Fenamiphos	214	Fluxapyroxad
135	Dimethachlor	175	Fenamiphos sulfone	215	Foramsulfuron
136	Dimethenamid	176	Fenamiphos sulfoxide	216	Formetanate
137	Dimethoate	177	Fenarimol	217	Formothion*
138	Dimethomorph	178	Fenazaquin	218	Fosthiazate
139	Dimoxystrobin	179	Fenbuconazole	219	Fuberidazole
140	Diniconazole	180	Fenfuram	220	Furathiocarb
141	Dinotefuran	181	Fenhexamid	221	Halosulfuron-methyl
142	Diphacinone*	182	Fenitrothion	222	Haloxyfop
143	Diphenamid	183	Fenoxaprop-p-ethyl	223	Haloxyfop-etotyl
144	Diphenylamine (DPA)	184	Fenoxycarb	224	HCH-alpha
145	Disulfoton sulfone	185	Fenpropathrin	225	HCH-beta
146	Disulfoton sulfoxide	186	Fenpropidin	226	HCH-delta
147	Disulfoton	187	Fenpropimorph	227	HCH-gamma (Lindane)
148	Diuron	188	Fenpyrazamine	228	Heptachlor
149	DMST	189	Fenpyroximate	229	Heptachlor-endo-epoxide
150	Dodemorph	190	Fenthion	230	Heptachlor-exo-epoxide
151	Dodine	191	Fenthion sulfoxide	231	Heptenophos
152	Edifenphos	192	Fenvalerate	232	Hexachlorobenzene (HCB)

(*continued on next page*)

Table 1 (*continued*)

#	Compound	#	Compound	#	Compound
153	Emamectin	193	Fipronil	233	Hexaconazole
154	Endosulfan-alpha	194	Flamprop	234	Hexazinone
155	Endosulfan-beta	195	Flonicamid	235	Hexythiazox
156	Endosulfan-sulfate	196	Florasulam	236	Hymexazol*
157	Endrin	197	Fluazifop-p-butyl	237	Imazalil
158	EPN	198	Flubendiamide	238	Imazamethabenz-methyl
159	Epoxiconazole	199	Flucarbazone Sodium	239	Imibenconazole
160	Ethiofencarb	200	Flucythrinate	240	Imidacloprid

#	Compound	#	Compound	#	Compound
241	Indoxacarb	281	Methoprotryne	321	Parathion-ethyl
242	Iodosulfuron-methyl Sodium	282	Methoxychlor	322	Parathion-methyl
243	Iprobenfos	283	Methoxyfenozide	323	PCB 101
244	Iprodione	284	Metobromuron	324	PCB 118
245	Iprovalicarb	285	Metolachlor	325	PCB 138
246	Isazofos	286	Metosulam	326	PCB 153
247	Isofenphos	287	Metoxuron	327	PCB 180
248	Isofenphos-methyl	288	Metrafenone	328	PCB 28
249	Isofenphos-oxon	289	Metribuzin	329	PCB 52
250	Isoprothiolane	290	Metsulfuron-methyl	330	Penconazole
251	Isoproturon	291	Mevinphos	331	Pencycuron
252	Isoxaben	292	Milbemectin A3	332	Pendimethalin
253	Karbutilate	293	Milbemectin A4	333	Penoxsulam
254	Kresoxim-methyl	294	Mirex	334	Pentachloroanisole (PCA)
255	Lenacil	295	Molloniate	335	Permethrin
256	Linuron	296	Monocrotophos	336	Phenmedipham
257	Lufenuron	297	Monolinuron	337	Phenthoate
258	Malaoxon	298	Monuron	338	Phorate
259	Malathion	299	Myclobutanil	339	Phorate sulfone
260	Mandipropamid	300	Napropamide	340	Phorate sulfoxide
261	Mecarbam	301	Neburon	341	Phosalone
262	Mefenacet	302	Nicosulfuron	342	Phosmet
263	Mefenpyr-diethyl	303	Nitenpyram	343	Phosphamidon
264	Mepanipyrim	304	Novaluron	344	Phoxim
265	Mepronil	305	Nuarimol	345	Picolinafen
266	Mesosulfuron-methyl	306	Ofurace	346	Picoxystrobin
267	Metaflumizone	307	Omethoate	347	Pinoxaden
268	Metalaxyl	308	Ortho Phenylphenol (OPP)	348	Piperonyl butoxide
269	Metaldehyde*	309	Orthosulfamuron	349	Pirimicarb
270	Metamitron	310	Oxadiargyl	350	Pirimicarb desmethyl
271	Metazachlor	311	Oxadiaxyl	351	Pirimiphos-ethyl
272	Metconazole	312	Oxadiazon	352	Pirimiphos-methyl
273	Methabenzthiazuron	313	Oxamyl	353	Prochloraz
274	Methacrifos	314	Oxasulfuron	354	Procymidone
275	Methamidophos	315	Oxycarboxin	355	Prodiamine
276	Methidathion	316	Oxydemeton-methyl	356	Profenofos
277	Methiocarb	317	Oxyfluorfen	357	Profluralin
278	Methiocarb Sulfone	318	Paclobutrazole	358	Profoxydim
279	Methiocarb Sulfoxide	319	Paraoxon-ethyl	359	Promecarb
280	Methomyl	320	Paraoxon-methyl	360	Prometon

#	Compound	#	Compound	#	Compound
361	Prometryn	401	Simetryn	441	Tolylfluanid
362	Propachlor	402	Spinetoram	442	Tralkoxydim
363	Propamocarb	403	Spinosad	443	Triadimefon
364	Propanil	404	Spirodiclofen	444	Triadimenol
365	Propaquizafop	405	Spiromesifen	445	Triallate
366	Propargite	406	Spirotetramate	446	Triasulfuron
367	Propazine	407	Spiroxamine	447	Triazophos
368	Propazine−2-hydroxy	408	Sulcotrione	448	Tribenuron-methyl*
369	Propetamphos	409	Sulfotep	449	Trichlorfon
370	Propiconazol	410	Sulfoxaflor	450	Triclopyr butotyl
371	Propoxur	411	Sulfur*	451	Tricyclazole
372	Propyzamide	412	Tebuconazole	452	Trietazine
373	Proquinazid	413	Tebufenozide	453	Trifloxystrobin
374	Prosulfocarb	414	Tebufenpyrad	454	Triflumizole
375	Prothioconazole*	415	Tebutam	455	Triflumuron
376	Prothiofos	416	Tebuthiuron*	456	Trifluralin
377	Pymetrozine*	417	Tecnazene*	457	Triforine
378	Pyraclostrobin	418	Tefluthrin	458	Triticonazole
379	Pyraflufen-ethyl	419	TEPP- O,S	459	Vamidothion
380	Pyrazophos	420	Tepraloxydim	460	Vinclozolin
381	Pyrazosulfuron Ethyl	421	Terbufos	461	Zoxamide
382	Pyrethrins	422	Terbumeton		
383	Pyridaben	423	Terbuthylazine		

(*continued on next page*)

Table 1 (*continued*)

#	Compound	#	Compound	#	Compound
384	Pyridalyl	424	Terbutryn		
385	Pyridaphenthion	425	Tetrachlorvinphos		
386	Pyrifenox	426	Tetraconazole		
387	Pyrimethanil	427	Tetradifon		
388	Pyriproxyfen	428	Tetramethrin		
389	Pyroxsulam	429	Thiabendazole		
390	Quinalphos	430	Thiacloprid		
391	Quinmerac	431	Thiamethoxam		
392	Quinoxyfen	432	Thifensulfuron-methyl		
393	Quintozene	433	Thiobencarb		
394	Quizalofop-ethyl	434	Thiocyclam Hydrogen Oxalate		
395	Quizalofop-P-ethyl	435	Thiodicarb		
396	Rimsulfuron	436	Thiofanox*		
397	Rotenone	437	Thiometon		
398	Sebuthylazine	438	Thiophanate-methyl*		
399	Sebuthylazine-desethyl	439	Tolclophos-methyl		
400	Simazine	440	Tolfenpyrad		

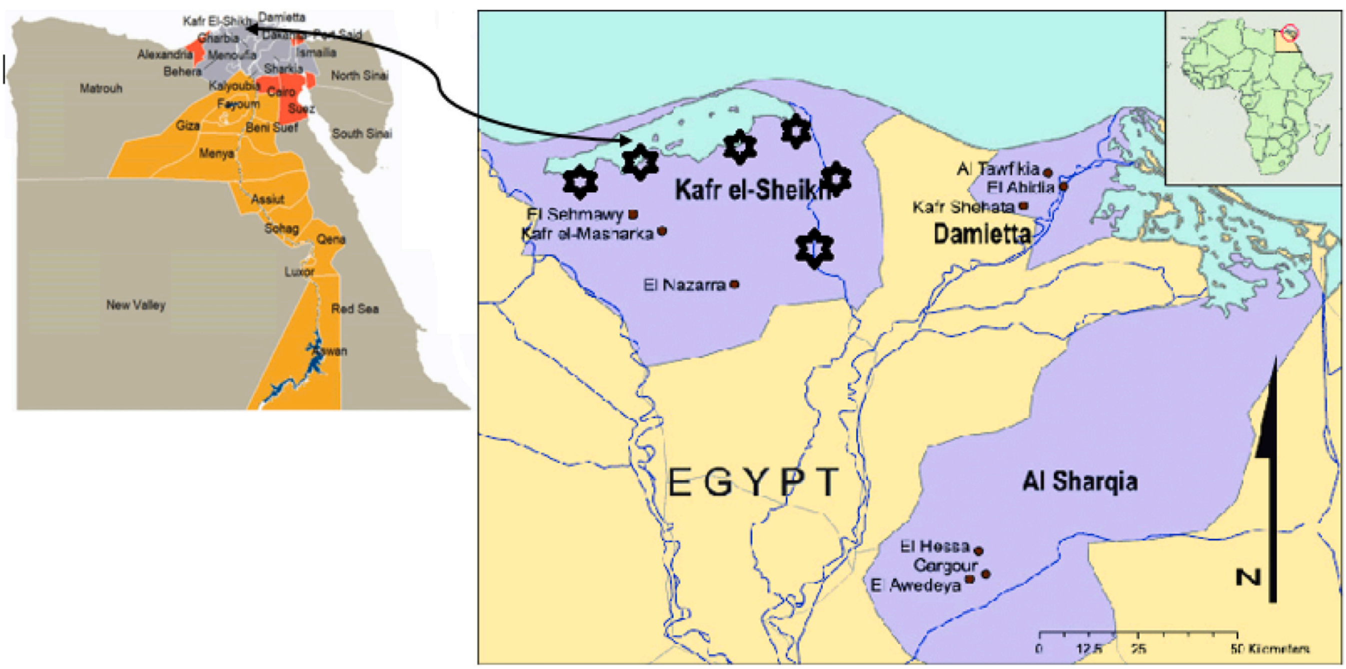

Fig. 1. The geographic locations of sampling in Egypt.

purification, the samples underwent SPE. The SPE cartridge was pre-conditioned with 3 mL of methanol, and the extract, holding the target compounds, was dissolved in 2 mL of methanol and passed through the column. The compounds were eluted using an SPE Manifold at a flow rate of one drop per second, repeated 2 or 3 times, and then concentrated using a rotary evaporator at 35 ± 2 °C.

For the analysis of pesticide residues, the samples underwent a laboratory pre-validated method based on acetonitrile-ethyl acetate extraction for the residue analysis of 461 pesticides in fish, using LC-MS/MS and GC–MS/MS according to the method referenced in [39]. Fish samples weighing 5 g were placed into a 50 mL polypropylene tube, to which 5 mL of deionized water was added. The mixture was vortexed for 5 seconds and allowed to hydrate for 10 minutes. Subsequently, 10 mL of acetonitrile was added, followed by vigorous shaking to mix with the sample. A buffer-salt mixture was then introduced, and the sample was shaken again. Centrifugation was performed at 4000 rpm for 5 minutes, after which an aliquot was filtered through a 0.45 µm syringe filter into clear 2 mL HPLC vials. Approximately 1 µL from each sample was injected directly into the LC-MS/MS and GC-MS/MS systems.

2.7. Determination

2.7.1. Q-ICP-MS analysis

In the case of heavy metals, The Q-ICP–MS should be started by activating the vacuum and water-cooling systems before igniting the plasma. It is crucial to ignite the plasma for at least 30 minutes before beginning the optimization of the instrument. The measurement parameters for the Q-ICP-MS are set up according to the validated method referenced in [5]. Instrumental parameters for the Q-ICP-MS are detailed in Table 2.

The method limits of detection of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, and Zn are 5.98, 5.99, 150.0, 147.9, 147.0, 141.0, 13.98, 148.8, 144.6, 5.97, 5.99, 147.9, and 138.0 µg/kg respectively. While, the practical limits of quantitation that can be determined with acceptable accuracy and precision are 0.02 mg/kg for As, Cd, Pb, and Sb, 0.05 mg/kg for Hg, and 0.5 mg/kg for Co Cr, Cu, Fe, Mn, Ni, Sn, and Zn.

2.7.2. LC-MS/MS analysis

For veterinary, drugs, each compound is quantified and verified

Table 2
Instrumental parameters of Q-ICP-MS.

Parameters	Set Values	Minimum Values	Maximum Values
Nebulizer Gas Flow [NEB]	1.02	0	1.5
Plasma Gas Flow	18	10	20
Auxiliary Gas Flow	1.2	0.6	2
ICP RF Power	1600	500	1600
Analog Stage Voltage	−1800	−3000	0
Pulse Stage Voltage	1000	0	2500
Discriminator Threshold	11	0	1000
Deflector Voltage	−10.25	−100	20
Quadrupole Rod Offset [QRO]	−12	−26	26
Cell Entrance Voltage	−6	−60	20
Cell Exit Voltage	−39	−60	20
Cell Rod Offset [CRO]	−16	−40	10
Axial Field Voltage [AFT]	475	−498	498
Rejection parameters (RPq)	0.25	0.05	0.9
integration time (ms)	2000	0.9	1.62×10^{10}
Sweeps/ reading	20	1	1000
Replicates	3	1	1000

using an Agilent liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. This system is outfitted with an API 4000 triple quadrupole from Applied Biosystems, featuring electrospray ionization (ESI) capable of running in both positive and negative ion modes. A C18 column, as previously mentioned, is used for separation. The mass spectrometer runs in multiple reaction monitoring (MRM) mode, with two distinct MRM transitions employed for the confirmation of each compound. Table (3) details the Instrumental Parameters for the LC-MS/MS analysis.

The method limits of detection of Quinolone compounds, Sulfonamide compounds, Tetracycline compounds, Diaminopyrimidine, Macrolide compounds, and B-Lactam compounds are 1.50, 1.45, 7.46, 0.75, 1.48, and 7.5 μg/kg, respectively. On the other hand, the practical limits of quantitation that can be determined with acceptable accuracy and precision are 5, 5, 25, 2.5, 5, and 25 μg/kg respectively, for Quinolone compounds, Sulfonamide compounds, Tetracycline compounds, Diaminopyrimidine, Macrolide compounds, and B-Lactam compounds.

For pesticide residues: Agilent 1260 Series instrument (LC) was employed for separation, which was connected to an API 6500 Qtrap tandem mass spectrometer from AB Sciex featuring an electrospray ionization (ESI) interface. A ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm particle size) from Agilent, (USA) was used for the separation process. The mobile phase consisted of Solvent A: a 10 mM ammonium formate solution at pH 4 ± 0.1 in a methanol-water mixture (1:9 ratio), and Solvent B: methanol. The linear gradient program commenced at 100 % A, transitioning from 100 % to 5 % A over 0–13 minutes, maintained at 5 % A from 13–21 minutes, then returned from 5 % to 100 % A between 21–28 minutes, and finally held at 100 % A from 28–32 minutes, all at a flow rate of 0.3 mL/min. The source was set to positive mode, with nitrogen nebulizer gas, curtain gas, and other gas parameters adjusted following the manufacturer's guidelines. A consistent source temperature of 400°C and ion spray potential of 5500 V were kept for all chemicals. The declustering potential and collision energy were calibrated through direct infusion of separate pesticide solutions into the MS detector. Multiple reaction monitoring mode was used for both quantification and confirmation purposes.

Table 3
HPLC parameters for LC-MS/MS.

Time	Flow(ul/min)	Buffer(A)	Methanol(B)
0	800	80	20
1.5	800	10	90
6.5	800	10	90
7.5	800	80	20
10	800	80	20

Also, in the analysis of pesticide residues, measurements were conducted using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent Gas Chromatograph system 7890 A, coupled with a 7000 C series GC tandem mass spectrometer. The system includes a triple quadrupole GC/MS EI mainframe, an EI ion source, and an ion gauge controller, achieving routine femtogram-level detection and quantitation limits with ultra-low noise and superior selectivity. The inlet was set to splitless mode, and helium was used as the carrier gas at a flow rate of 1.830 mL/minute. The HP-5 MS capillary column from Agilent Technologies, composed of 5 % biphenyl and 95 % dimethyl siloxane, has an internal diameter of 0.25 mm, a film thickness of 0.52 μm, and a length of 30 m. The temperature program started at 70 °C, held for 2 minutes, then ramped to 150 °C at 25 °C/minute, followed by an increase to 200 °C at 3 °C/minute, and finally to 280 °C at 8 °C/minute, with a 10-minute hold at the final temperature. The total run time was 42 minutes. Pesticide quantification was performed by comparing the peak areas to a calibration curve derived from standards, employing a multiple-point calibration method.

The limits of detection of the multi-residue method (444 pesticides) are about 3 μg/kg, and the practical limits of quantitation that can be determined with acceptable accuracy and precision are 10 μg/kg. On the other hand, The limits of detection of the 17 pesticides including naphthyl acetic acid, captan, carbosulfan, chlorothalonil, dazomet, DDT-o,p, DDT-p,p, endosulfan-alpha, endosulfan-beta, heptachlor, metaldehyde, oxasulfuron, profluralin, prothioconazole, prothiofos, sulfoxaflor, sulfur, thiocyclam hydrogen oxalate, and triforine are about 15 μg/kg, and the practical limits of quantitation that can be determined with acceptable accuracy and precision are 50 μg/kg.

2.8. Health risk estimation

Numerous studies have investigated the pathways of human exposure to heavy metals via the consumption of contaminated foods and beverages. Health risk assessments often rely on certain assumptions. The current study evaluates the health risks associated with the ingestion of heavy metals from dietary sources, examining both their non-carcinogenic and carcinogenic effects.

2.8.1. Estimation of daily and weekly intake

Risk assessment involves the comparison of metal concentration analyses with the estimated provisional tolerable daily intake (EPTDI) and estimated provisional tolerable weekly intake (EPTWI) for detected metals in fish consumed by local consumers. These assessments are based on toxicological concerns and recommended doses set up by the Food Agriculture Organization (FAO)/World Health Organization (WHO). The EPTDI is calculated by multiplying the average metal concentrations found in fish by the consumption rate, which, according to the WHO/Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program (WHO/GEMS/FOOD), is 8.7 g/day for zone "G06" [40]. The average body weight considered is 60 kg, in line with the Food and Nutrition Board guidelines [41,42]. Long-term risk assessments compare the intake levels with toxicological data for metals, calculated by dividing the EPTWI by the acceptable provisional tolerated weekly intake (APTWI) as decided by the Food and Nutrition Board and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The EPTDI is calculated using the following equation:

$$\boldsymbol{EPTDI} = \frac{\boldsymbol{F_C} * \boldsymbol{M_C}}{\boldsymbol{B_W}} * \quad \boldsymbol{10^{-3}} \tag{1}$$

$\boldsymbol{EPTDI}$: ***Estimated provisional tolerable daily intake*** $(\boldsymbol{mg/kg.bw/day})$

F_C : *Food consumption*(g/day) M_C : *Metal concentration*(mg/kg)

B_W : *Average body weight(Kg)* 10^{-3} : *The unit conversion factor*

2.8.2. Hazard quotient

The Target Hazard Quotient (THQ) is a metric developed to quantify non-carcinogenic risk. It is calculated to evaluate the risk associated with the absorption of metal pollutants through fish consumption, highlighting the potential harm from regular intake of contaminants. The THQ values are derived according to the USEPA Region III Risk-Based Concentration Tables [43,44]. The calculation of THQ is based on the equation provided by the US EPA in 2010 and 2018.

$$THQ = \frac{CM * ED * EF * FIR}{RFD * BW * AT_n} * \quad 10^{-3} \tag{2}$$

CM : **Metal concentration(mg/kg)ED**

: **the exposure duration(years)**

EF : **the exposure frequency(day/year)**

FIR : **The food ingestion rate(mL/person/day)**

RFD : **The reference dose of the metal(mg/kg/day)**

BW : **Average body weight(Kg)** 10^{-3} : **The unit conversion factor**

AT_n : **The average exposure time for noncarcinogens(days)**

The Target Hazard Quotient (THQ) is calculated as the ratio of the measured concentration to the oral reference dose, adjusted for exposure duration and frequency, the amount of substance ingested, and body weight. This parameter delineates the duration of exposure and the corresponding risk level. The variables utilized in this computation include Exposure Frequency (EF), representing the number of exposure days per year for non-carcinogenic risk (260 days/year); Exposure Duration (ED), indicating the period for non-cancer risk assessment as adopted by the USEPA (30 years); Food Ingestion Rate (FIR), denoting the daily consumption rate for fish (8.7 mg/day, reflecting the average intake across all fish samples); Concentration of Metals (CM) in fish (mg/kg); Average Body Weight (WB) (60 kg); Average Time of Exposure (ATn), calculated over a period of 30 years (365 days/year); and the Reference Dose (RfD) of the metal (mg/kg/day).

2.8.3. Hazard index

The Hazard Index (HI) is calculated as the cumulative sum of the Target Hazard Quotients (THQs). The (HI) is based on the equation provided by the US EPA in 2010 and 2018:

$$HI = \sum THQ_{contaminant} \tag{3}$$

$THQ_{contaminant}$: **The target hazard quotient of each contaminant**

3. Results and discussion

3.1. Occurrence of antibiotics and pesticide residues in fish samples

For the tested 48 samples, it was seen that there were no residues of antibiotics as well as pesticide residues. In 2020, Fawzy *et al.* reported the presence of both pesticides and 5 antibiotics belonging to other groups except Chloramphenicol which was analyzed in this study in Tilapia collected from the Rosetta Nile branch [45]. This shows that the contamination levels of Tilapia differ depending on the area of collection.

3.2. Cross-contamination of fish samples with different heavy metals

Heavy metals can infiltrate the bodies of fish through various pathways in contaminated water, leading to their accumulation within the organisms. The concentrations of these metals vary across different organs within the fish's body. Fish living in aquatic systems contaminated with heavy metals pose a significant threat due to the accumulation of metals in various vital body tissues such as gills, liver, kidney, skin, and muscle. To adapt to this stressful environment, fish require additional energy derived from essential nutrients like proteins, fats, and carbohydrates. Certain metals like As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, and Zn possess redox potential and can generate reactive oxygen species (ROS) that play a crucial role in regulating fish physiology. ROS serves as an indicator of oxidative stress, which hampers cellular activity by breaking down proteins, lipids, and DNA [46–49]. The bioaccumulation of heavy metals in aquatic organisms through the food chain leads to severe health risks for humans upon consuming contaminated fish. Studies have shown that exposure to As and Pb can result in negative effects on fish, including reduced growth and production, changes in blood parameters, hormonal imbalances, abnormal tissue structures, developmental delays in embryos and larvae, and various diseases [50–52]. Additionally, research has indicated significant contamination of aquatic environments with Cd while, high levels of Cr in fish diets have been linked to decreased growth and feed efficiency in different fish species [53–55]. Furthermore, as soon as mercury is introduced into the water, it is ingested by microorganisms that are then consumed by small fish. These small fish are then preyed upon by larger fish, leading to an accumulation of mercury in the muscle tissue of the fish as it moves up the food chain. This ultimately results in the highest levels of mercury being found in large, long-lived predatory fish like swordfish and sharks [46].

3.2.1. Occurrence of various potentially harmful elements in fish samples

Forty-eight fish samples were analyzed for potentially harmful elements using Quadrupole Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS) to assess the concentration levels of As, Cd, Cr, Co, Cu, Fe, Ni, Mn, Hg, Pb, Sb, Sn, and Zn. The results showed that the most frequently detected metals were As, Cd, Co, Cu, Fe, Hg, Mn, and Zn, each found in 47 instances, followed by Cr detected 40 times, Ni 27 times, and Pb 6 times. The mean concentrations of As, Cd, Cr, Co, Cu, Fe, Ni, Mn, Hg, Pb, and Zn were found to be 0.025, 0.02, 0.501, 0.50, 0.81, 12.56, 0.5, 0.689, 0.051, 0.031, and 5.78 mg/kg, respectively. Furthermore, the measured levels of cadmium, mercury, and lead did not exceed the maximum permissible limits set by Egyptian and European standards for fish [56–58] (See Table 4).

Antimony, a metalloid with no clear biological role, exhibits varying physicochemical and toxicological properties based on its oxidation state and chemical form. It naturally exists in two oxidation states: trivalent and pentavalent. This element is found in the Earth's crust and released into the environment through natural events like volcanic eruptions, dust storms, and wildfires. Trivalent antimony ions are significantly more toxic than their pentavalent counterparts, with a toxicity level tenfold higher, and have been associated with an increased risk of lung cancer [59,60]. Antimony is used in the production of various products, including batteries and pharmaceuticals. Notably, antimony trioxide (Sb_2O_3) is widely used as a catalyst in the synthesis of polyethylene terephthalate (PET), a material commonly used in packaging. However, antimony is a concerning pollutant as it can leach into beverages from PET containers, with the degree of leaching varying with the duration of storage. The substance Sb_2O_3 has been identified as a potential carcinogen and is listed as a priority pollutant by both the European Union and the United States Environmental Protection Agency [61]. In this study, all analyzed fish samples were found to be free from any detectable amount of antimony.

Arsenic, a significant heavy metal, raises concerns for environmental and human health due to its semi-metallic properties, high toxicity, and carcinogenic potential. It is commonly found as oxides, sulfides, or salts of various metals such as sodium, calcium, iron, and copper [62]. In the industrial sector, arsenic is primarily sourced from

Table 4
Element's compositions of the fish samples, number of samples = 48.

Detected elements	Element's concentrations (mg/kg)				**Frequency**		Free samples		Samples Less than LOQs		Samples Above LOQs		**MPLs (mg/kg)** [39,40]	The violated elements		The violated samples	
	Minimum	**Maximum**	**Mean**	**Median**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**		**No**	**%**	**No**	**%**
As	< 0.02	0.056	0.028	0.025	47	100.0 %	0	0.0 %	16	34.0 %	31	66.0 %	-	-	-	0	0.00 %
Cd	< 0.02	< 0.02	0.020	0.020	47	100.0 %	0	0.0 %	47	100.0 %	0	0.0 %	0.05	0	0.0 %		
Co	< 0.5	< 0.5	0.500	0.500	47	100.0 %	0	0.0 %	47	100.0 %	0	0.0 %	-	-	-		
Cr	< 0.5	0.548	0.501	0.500	40	85.1 %	7	14.9 %	38	95.0 %	2	5.0 %	-	-	-		
Cu	< 0.5	4.138	0.808	0.500	47	100.0 %	0	0.0 %	34	72.3 %	13	27.7 %	-	-	-		
Fe	3.56	54.174	12.589	9.985	47	100.0 %	0	0.0 %	0	0.0 %	47	100.0 %	-	-	-		
Hg	< 0.05	0.080	0.051	47.000	47	100.0 %	0	0.0 %	43	91.5 %	4	8.5 %	0.5	0	0.0 %		
Mn	< 0.5	3.970	0.689	0.500	47	100.0 %	0	0.0 %	35	74.5 %	12	25.5 %	-	-	-		
Ni	< 0.5	< 0.5	0.500	0.500	27	57.4 %	20	42.6 %	27	100.0 %	0	0.0 %	-	-	-		
Pb	< 0.02	0.056	0.031	0.020	6	12.8 %	41	87.2 %	3	50.0 %	3	50.0 %	0.3	0	0.0 %		
Sb	N.D	N.D	N.D	N.D	0	0.0 %	47	100.0 %	0	0.0 %	0	0.0 %	-	-	-		
Sn	N.D	N.D	N.D	N.D	0	0.0 %	47	100.0 %	0	0.0 %	0	0.0 %	-	-	-		
Zn	2.61	15.482	5.778	5.320	47	100.0 %	0	0.0 %	0	0.0 %	47	100.0 %	-	-	-		

LOQs: Limit of quantifications.
N.D: Not detected.
MPL: Maximum permissible limit.

phosphate fertilizers, metal hardening processes, paints, and textiles. It is also present in certain foods and beverages, including grape juice, rice, Indian mustard, carrots, tomatoes, flour, spinach, shellfish, chicken, and shrimp. Exposure to soluble inorganic arsenic can lead to acute poisoning. High arsenic intake may result in severe gastrointestinal distress, disruptions in the blood and circulatory systems, neurological damage, liver enlargement, anemia, hemolysis, skin discoloration, peripheral neuropathy, cerebral damage, and can be fatal [63,64]. In this study, the concentrations of arsenic were ranged between < 0.02 and 0.056 mg/kg in the analyzed fish samples. 100 % of the analyzed samples had detectable amounts of arsenic, of which 34 % have arsenic levels that were found to be less than the quantification limit. On the other hand, 66 % of the analyzed samples had detectable amounts of arsenic above the quantification limit.

Cadmium ranks as the seventh most hazardous heavy metal on the Agency for Toxic Substances and Disease Registry (ATSDR) list [65]. The primary environmental source of cadmium is the combustion of coal. Additionally, cadmium can be found as a contaminant in a variety of products, such as fertilizers, pesticides, detergents, and refined petroleum products. Foods known to have cadmium include peanuts, soybeans, rice, medicinal herbs, lettuce, corn, oats, wheat, spinach, fish, shrimp, and mushrooms. The predominant routes of cadmium intake in humans are through the consumption of food and the smoking of tobacco. Exposure to exceedingly high levels of cadmium can lead to grave health issues in both humans and animals, manifesting as bone disorders, liver, and kidney damage, and in extreme cases, death [63,66]. In this study, 100 % of the analyzed samples had detectable amounts of cadmium, but all levels were found to be less than the quantification limit. All these cadmium levels did not exceed the maximum permissible limit of cadmium stated by Egyptian and European standards in fish (0.05 mg/kg).

Chromium ranks as the 17th most abundant element in the Earth's mantle and naturally occurs as chromite in serpentine and ultramafic rocks, or it forms complexes with other metals such as bentorite ($Ca_6(CrAl)_2(SO_4)_3$), crocoite ($PbCrO_4$), tarapacaite (K_2CrO_4), and vauquelinite (CuPb2CrO4PO4OH). It is used in various industrial processes, including water cooling, electroplating, leather tanning, paper pulp production, and petroleum refining. As a result, the presence of hexavalent chromium in groundwater is often considered a sign of anthropogenic contamination [67,68]. The most prevalent oxidation states of chromium in the environment are Cr (III) and Cr (VI), which have markedly different properties. Cr (III) is essential for its role in the metabolism of proteins and sugars, while Cr (VI) is potentially toxic and carcinogenic, adversely affecting metabolic processes [69]. In this study, the concentrations of chromium were ranged between < 0.5 and 0.548 mg/kg in the analyzed fish samples. 85.1 % of the analyzed samples had detectable amounts of chromium, of which 95 % had chromium levels that were found to be less than the quantification limit. On the other hand, 5 % of the analyzed samples had detectable amounts of chromium above quantification.

Cobalt, along with its compounds, is prevalent in nature and plays a vital role in various human endeavors. As a crucial element found at the active site of vitamin B12, cobalt is instrumental in numerous biological processes. The sources of cobalt exposure are categorized into four groups: dietary, environmental, occupational, and pharmaceutical [70,71]. The highest systemic concentrations of cobalt in the human body are typically achieved through oral supplementation and internal exposure. However, overexposure to cobalt has been linked to several adverse health effects. Toxicological impacts of cobalt include vasodilation, skin flushing, and cardiomyopathy, affecting both animals and humans [72]. In this study, cobalt was detectable in 100 % of the analyzed samples; however, the concentrations were below the quantification limit.

Copper, a vital heavy metal, is ubiquitous in all living organisms, the food chain, and environmental elements such as soil and water. It is a necessary nutrient for humans and animals, needed in small quantities.

Copper plays key roles in biological processes, including the synthesis of hemoglobin, regulation of iron metabolism, cellular metabolism, the formation of connective tissue, and bone development, as referenced in studies [73,74]. The primary sources of environmental copper are smelting, mining, and refining activities. Foods's rich in copper include organ meats, nuts, shellfish, beans, and cocoa. A copper deficiency can lead to a reduced white blood cell count, anemia, osteoporosis in infants and children, and connective tissue disorders leading to skeletal issues. Conversely, excessive intake of copper may cause acute poisoning, manifesting as vomiting and temporary gastrointestinal upset, with symptoms such as nausea and abdominal pain. Prolonged exposure to high levels of copper can result in liver damage and can be fatal [64]. In this study, the concentrations of copper were ranged between < 0.5 and 4.14 mg/kg in the analyzed fish samples. 100 % of the analyzed samples had detectable amounts of copper, of which 72.3 % have copper levels that were found to be less than the quantification limit. On the other hand, 27.7 % of the analyzed samples had detectable amounts of copper above the quantification limit.

Iron, the fourth most abundant element on Earth, forms the majority of the planet's crust. It is vital for almost all living organisms, to engage in numerous metabolic processes. In the cells of microorganisms, plants, and animals, iron participates in various functions such as electron transfer, oxidation-reduction of substrates, hormone production, oxygen transport and storage, DNA synthesis, repair, cell cycle regulation, nitrogen fixation, and defense against reactive oxygen species [75]. Additionally, iron is crucial for neuronal communication, playing a key role in the myelination of white matter in the central nervous system, which encompasses the brain and spinal cord. Iron enters the food chain through various pathways, including environmental pollution from dust, soil, and water, leaching from iron cookware, and contamination during food processing [76]. Iron deficiency commonly leads to anemia in humans, while excessive iron consumption is associated with a spectrum of health issues, including heightened risks of cardiovascular disease, cancer, hormonal imbalances, arthritis, diabetes, and liver conditions [77]. In this study, the concentrations of iron ranged between 3.56 and 54.17 mg/kg in the analyzed fish samples.

Lead is a highly toxic metal that has contaminated the environment and caused health problems globally. Primary sources of lead exposure are industrial processes, contaminated drinking water, certain foods, and tobacco use [78]. Industrial products including gasoline, corrosion-resistant paints, tin can solder, water pipes, and batteries have historically had lead. Foods that may have lead include lettuce, carrots, rice, seafood, wine, beer, beetroots, potatoes, calcium supplements, cocoa powder, eggs, mineral salt, wheat, and paprika powder. The biological impact of lead exposure varies with the level and length of exposure. It can severely damage the brain, nervous system, red blood cells, and kidneys. Moreover, lead can cross the blood-brain barrier, and disrupt the normal development of the brain in infants [63,66]**.** In this study, the concentrations of lead were ranged between < 0.02 and 0.056 mg/kg in the analyzed fish samples. 12.8 % of the analyzed samples had detectable amounts of lead, of which 50 % have lead levels that were found to be less than the quantification limit. On the other hand, 50 % of the analyzed samples had detectable amounts of lead above quantification but did not exceed the maximum permissible limit of lead stated by Egyptian and European standards in fish (0.3 mg/kg).

Manganese is the twelfth most abundant element in the Earth's crust and is naturally present in various type of foods, water, soil, and rocks. This essential mineral plays a crucial role in the growth, development, and maintenance of health for humans, plants, and animals [79]. Diets rich in manganese, particularly those including wheat and rice, contribute significantly to its intake. However, increased consumption of manganese leads to diminished gastrointestinal absorption and heightened biliary elimination. Excessive exposure to manganese can be toxic, especially affecting the central nervous, cardiac, respiratory, and reproductive systems. The central nervous system is particularly susceptible, with toxicity manifesting at lower concentrations compared to other systems. Notably, manganese presence in drinking water has been linked to significant neurodevelopmental risks in children. [80,81]. In this study, the concentrations of manganese were ranged between < 0.5 and 3.97 mg/kg in the analyzed fish samples. 100 % of the analyzed samples had detectable amounts of manganese, of which 74.5 % have manganese levels that were found to be less than the quantification limit. On the other hand, 25.5 % of the analyzed samples had detectable amounts of manganese above the quantification limit.

Mercury is recognized as a highly toxic heavy metal, primarily introduced into the environment through human activities such as agriculture, municipal and industrial wastewater discharge, mining, and cremation [82,83]. It is emitted as vapor by large-scale industrial operations and is used in various products including electrical switches, batteries, thermometers, fluorescent bulbs, and mercury lamps. Foods that may have mercury include seafood, fish oil, eggs, products from cetaceans, and mushrooms. Mercury exposure can lead to nose irritation, skin burns, and damage to the brain, central nervous system, kidneys, and lungs [63,66]. In this study, the concentrations of mercury were ranged between < 0.05 and 0.08 mg/kg in the analyzed fish samples. 100 % of the analyzed samples had detectable amounts of mercury, of which 91.5 % have mercury levels that were found to be less than the quantification limit. On the other hand, 8.5 % of the analyzed samples had detectable amounts of mercury above the quantification but did not exceed the maximum permissible limit of mercury stated by Egyptian and European standards in fish (0.5 mg/kg).

Nickel is an essential micronutrient for various organisms, including certain microorganisms, plants, and mammals. It is present in numerous staple foods, animal products, and other dietary items. In addition to its biological importance, nickel has several industrial uses, notably in the production of stainless steel. The primary source of nickel intake in humans is through food consumption, with fresh fruits, vegetables, meats, poultry, fish, oils, whole grains, oats, dried fruits, fats, eggs, lentils, red kidney beans, legumes, canned foods, beverages, chocolates, milk, dietary supplements, and dairy products all being potential sources [84–86]. Occupational exposure to nickel has been linked to elevated levels of the metal in urine, blood, and body tissues. Skin contact with soluble nickel salts or metallic nickel can lead to allergic dermatitis. Inhalation of nickel sub- sulfide (Ni_3S_2) is recognized as a significant respiratory carcinogen that can deeply penetrate the lungs and strongly adhere to epithelial cells. Water-soluble nickel compounds may be inhaled and then eliminated by the kidneys. Chronic exposure to nickel is associated with the development of respiratory conditions such as bronchitis, asthma, and other related disorders [87]. In this study, nickel was detectable in 100 % of the analyzed samples; however, the concentrations were below the quantification limit.

Tin is recognized as a potentially toxic metal accumulating in the tissues of humans and animals. It can be released into the environment through various means such as road wear, agricultural activities, volcanic activity, wind erosion, and forest fires. The most common oxidation states of inorganic tin found in environmental samples are Sn (II) and Sn (IV). Both forms can form numerous stable inorganic compounds, with stannic tin also able to produce a volatile hydride (SnH_4) and several organometallic compounds of toxicological significance [88,89]. Tin contamination is detectable in wastewater and natural water bodies, including rivers, estuaries, and oceans. Additionally, tin is commonly used in the lining of steel cans for food processing and preservation. Chronic consumption of canned foods may lead to serious health issues such as anemia, gastrointestinal disturbances, and liver and kidney damage [90]. In this study, all analyzed fish samples were found to be free from any detectable amount of tin.

Zinc is a crucial element for various species, playing a pivotal role in the cellular functions of living organisms. Insufficient levels of zinc are associated with various adverse health conditions, including weakened immune systems. Recognized as an essential yet potentially harmful metal, excessive amounts of zinc can negatively affect both environmental and human health. Foods such as meats, fish, poultry, grains, and dairy products are rich sources of zinc [91,92]. Notably, clinical evidence suggests that intranasal application of zinc gluconate gels can lead to anosmia, the loss of smell, in individuals. However, oral zinc supplementation has been shown to mitigate the effects of the common cold. The toxicity of zinc is influenced by its speciation and concentration. Labile zinc species, for example, are more dangerous than their tightly bound counterparts due to their easier assimilation by humans, microorganisms, and plants, affecting the food chain, soil, and sediment. Excessive zinc exposure in olfactory neurons can induce pyroptosis, a form of cell death mediated by inflammasomes [93,94]. In this study, the concentrations of zinc ranged between 2.61 and 15.48 mg/kg in the analyzed fish samples.

The study revealed that the levels of Zn, Fe, Cr, Co, Mn, As, Pb, Cd, Cu, and Ni in fish samples from markets in Saudi Arabia, Jordan, Bangladesh, Nigeria, Turkey, Pakistan, and India exceeded those reported in our research. [95–101], (See Table 5).

3.3. *Estimating the Health Risk*

In this study, for health risk estimation estimated daily and weekly intake (EPTDI, EPTWI), the target hazard quotient (THQ), and the hazard index (HI), were calculated.

3.3.1. *Estimating the daily and weekly intake*

The study selected eleven elements—As, Cd, Mn, Ni, Pb, Co, Cr, Cu, Fe, Hg, and Zn—for the estimation of daily and weekly intake levels in fish samples. The findings showed that the consumption of these elements did not exceed the Acceptable Provisional Tolerable Weekly Intake (APTWI) for any of the tested fish samples, even at their highest concentrations. The estimated provisional tolerable weekly intake for As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn varied between $2.46 * 10^{-5}$ – $4.87 * 10^{-5,}$ $1.74 * 10^{-5}$, $4.35 * 10^{-4}$, $4.36 * 10^{-4}$ – $4.77 * 10^{-4}$, $7.03 * 10^{-4}$ – $3.6 * 10^{-3}$, $1.1 * 10^{-2}$ – $4.71 * 10^{-2}$, $4.43 * 10^{-5}$ – $6.97 * 10^{-5}$, $5.99 * 10^{-4}$ – $3.45 * 10^{-3}$, $4.35 * 10^{-4}$, $2.73 * 10^{-5}$ – $4.91 * 10^{-5}$, $5.03 * 10^{-3}$ – $1.35 * 10^{-2}$ mg/kg bw/day, respectively. However, it is not possible to establish acceptable upper intake levels for Co, as current human data do not provide a clear dose-response relationship, according to sources such as JECFA, the Food and Nutrition Board, EFSA, and the UK Expert Group on Vitamins and Minerals See Table 6.

3.3.2. *Estimating the hazard quotient*

Table 7 presents the estimated Target Hazard Quotients (THQ) for individual elements resulting from the consumption of various fish. The United States Environmental Protection Agency (USEPA) references [43, 44] state that a THQ value of 1 is the acceptable guideline limit. The THQ values for all elements in the fish were found to be below 1, showing that there is no significant non-carcinogenic health risk associated with the ingestion of a single heavy metal through the dietary consumption of these fish. The THQ values of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn were found to be $1.93 * 10^{-02}$,$2.07 * 10^{-03}$,$1.72 * 10^{-01}$,$1.89 * 10^{-02}$,$1.07 * 10^{-02}$,$7.99 * 10^{-03}$,$8.28 * 10^{-02}$, $2.93 * 10^{-03}$, $2.58 * 10^{-03}$,$1.46 * 10^{-03}$,$5.33 * 10^{-03,}$ respectively.

3.3.3. *Estimating the hazard index*

The study's findings show that the cumulative impact of all tested elements was below the allowable threshold of 1. Furthermore, Cobalt (Co) and Mercury (Hg) were identified as the primary contributors to the Hazard Index (HI) in fish, as detailed in Table 7. Consequently, this implies that there is no significant non-carcinogenic health risk associated with the consumption of these elements through the ingestion of the fish, as the obtained results were all below 1.

4. Conclusion

The potential health effects of heavy metals and agricultural chemicals from consuming contaminated fish are a significant concern. This study aimed to assess the presence of heavy metals, antibiotics, and pesticides in 48 fish samples from Kafr-ELSheikh Governorate, Egypt. The predominant elemental metals found were As, Cd, Co, Cu, Fe, Hg, Mn, and Zn, detected 47 times each, followed by Cr (40 detections), Ni (27 detections), and Pb (6 detections). Hazard Quotient (HQ) values were below 1 for all elements, showing no non-carcinogenic health risk from consuming these fish. Additionally, the cumulative impact of all elements was within safe levels. The fish samples were also free from antibiotic residues across six therapeutic classes and devoid of pesticides. Thus, the study concludes that consuming fish from this region poses no associated health risks. However, ongoing evaluation and monitoring of aquaculture zones are essential to guarantee the safety of these products for Egyptian consumers.

CRediT authorship contribution statement

Nermine Gad: Writing – original draft, Formal analysis. **Mahmoud Mustafa Ghuniem:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Lamia Ryad:** Writing – review & editing, Writing – original draft. **Mohamed A. Tahon:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 5
Ranges of elemental concentrations mg/kg in fish samples from present study and from other countries.

Elements	This study (Wet wt)	Saudi Arabia (Dry wt) [95]	Jordan (Dry wt) [96]	Bangladesh (Dry wt) [97]	Nigeria (Dry wt) [98]	Turkey (Wet wt) [99]	Pakistan (Wet wt) [100]	India (Dry wt) [101]
As	< 0.02–0.06	NA	NA	1.97 – 6.24	NA	NA	NA	NA
Cd	< 0.02 - < 0.02	1.7 – 5.1	0.5 – 2.0	0.09 – 0.87	ND	NA	53.3 – 71.7	0.02 – 0.57
Co	< 0.5 - < 0.5	NA	1.5 – 7.1	NA	NA	NA	NA	NA
Cr	< 0.5–0.55	38.6 – 113.3	1.0 – 10.3	0.47 – 2.07	NA	1.03–1.79	489.0 – 703.0	0.1 – 1.6
Cu	< 0.5–4.14	7.7 – 18.9	0.5 – 2.0	8.33 – 43.18	3.5 – 15.75	0.66–1.98	46.3 – 303.0	0.9 – 6.5
Fe	3.56–54.17	81.6 – 188.6	2.5 – 20.5	NA	102 – 565.6	29.1–93.61	NA	32.1 – 240.5
Hg	< 0.05–0.08	NA	NA	NA	NA	NA	NA	0.01–0.23
Mn	< 0.5–3.97	3.1 – 5.4	1.0 – 3.3	9.43 – 51.17	9.34 – 43.72	NA	NA	1.4 – 7.8
Ni	< 0.5 - < 0.5	17.3 – 92.1	1.0 – 5.0	0.69 – 4.36	5.33 – 30	0.32–1.72	74.7 – 135.0	NA
Pb	< 0.02–0.056	0.7 – 0.8	1.5 – 8.3	1.76 – 10.27	0.2 – 5	0.071–10.87	226.3 – 599.3	0.01 – 0.26
Sb	N.D	NA	NA	NA	NA	NA	NA	NA
Sn	N.D	NA	NA	NA	NA	NA	NA	NA
Zn	2.61–15.48	NA	1.1 – 35	42.83 – 418	14 – 124.5	8.99–51.13	NA	NA

ND: Not detectable.
NA: Not analyzed.

Table 6
Estimated daily intakes of detected elements (mg/kg b.w/day).

Assessed elements	APTWI (mg/kg bw/ week)	Mean concentrations			Maximum concentrations		
		Concentrations (mg/ kg)	**EPTWI (mg/kg bw/ week)**	**EPTWI As % of APTWI**	**Concentrations (mg/ kg)**	**EPTWI (mg/kg bw/ week)**	**EPTWI As % of APTWI**
As	0.015	0.03	0.000025	0.164 %	0.06	0.000049	0.325 %
Cd	0.007	0.02	0.000017	0.249 %	0.02	0.000017	0.249 %
Co	-	0.50	0.000435	-	0.50	0.000435	-
Cr	0.029	0.50	0.000436	1.504 %	0.55	0.000477	1.644 %
Cu	1.17	0.81	0.000703	0.060 %	4.14	0.003600	0.308 %
Fe	5.25	12.59	0.010953	0.209 %	54.17	0.047132	0.898 %
Hg	0.005	0.05	0.000044	0.887 %	0.08	0.000070	1.394 %
Mn	1.28	0.69	0.000599	0.047 %	3.97	0.003454	0.270 %
Ni	0.12	0.50	0.000435	0.363 %	0.50	0.000435	0.363 %
Pb	0.025	0.03	0.000027	0.109 %	0.06	0.000049	0.196 %
Zn	4.65	5.78	0.005026	0.108 %	15.48	0.013469	0.290 %

EPTWI: Estimated provisional tolerable weekly intake.
APTWI: Accepted provisional tolerable weekly intake.

Table 7
Target hazard quotient (THQ), hazard index (HI) for the intake of analyzed elements.

Assessed elements	RfD (mg/kg/ day)	CM (mg/ kg)	THQ	HI	HI %
As	3.00E−04	0.06	1.93E−02	3.26E−01	5.91 %
Cd	1.00E−03	0.02	2.07E−03		0.63 %
Co	3.00E−04	0.50	1.72E−01		52.79 %
Cr	3.00E−03	0.55	1.89E−02		5.79 %
Cu	4.00E−02	4.14	1.07E−02		3.28 %
Fe	7.00E−01	54.17	7.99E−03		2.45 %
Hg	1.00E−04	0.08	8.28E−02		25.38 %
Mn	1.40E−01	3.97	2.93E−03		0.90 %
Ni	2.00E−02	0.50	2.58E−03		0.79 %
Pb	4.00E−03	0.06	1.46E−03		0.45 %
Zn	3.00E−01	15.48	5.33E−03		1.63 %

RfD: Reference dose.
CM: Maximum metal concentration.
THQ: Target hazard quotient.
HI: Hazard index.

Data Availability

No data was used for the research described in the article.

Acknowledgment

The authors wish to express their sincere gratitude to the Director and the entire team at the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Agricultural Research Center, Egypt, for their generous support throughout this research project.

Author contribution statements

Mahmoud M. Ghuniem, Nermine Gad, and Mohamed A. Tahon conceived of the presented idea. Mahmoud M. Ghuniem, and Nermine Gad developed the theory and performed the computations. Mahmoud M. Ghuniem and Nermine Gad, and Mohamed A Tahon carried out the experiment. Mahmoud M. Ghuniem and Nermine Gad wrote the manuscript and designed the figures with support from Mohamed A. Tahon and Lamia Ryad. Lamia Ryad supervised the project. All authors discussed the results and contributed to the final manuscript.

References

[1] F. Fayet-Moore, K. Baghurst, B.J. Meyer, Four models including fish, seafood, red meat and enriched foods to achieve Australian dietary recommendations for n-3 lcpufa for all life-stages, Nutrients 7 (2015) 8602–8614.

[2] M.M. Ghuniem, M.A. Khorshed, E.R. Souaya, Method validation for direct determination of some trace and toxic elements in soft drinks by inductively coupled plasma mass spectrometry, Int. J. Environ. Anal. Chem. 99 (6) (2019) 515–540, https://doi.org/10.1080/03067319.2019.1599878.

[3] M.M. Ghuniem, M.A. Khorshed, M.M.H. Khalil, Determination of some essential and toxic elements composition of commercial infant formula in the Egyptian market and their contribution to dietary intake of infants, Int. J. Environ. Anal. Chem. 100 (5) (2020) 525–548, https://doi.org/10.1080/03067319.2019.1637426.

[4] M. Sarwar, N. Ahmad, A.A. Rajput, M. Tofique, Search for Varietal Resistance within Stored Wheat Genotypes against the Infestation of Red Flour Beetle, Tribolium castaneum Herbst (Coleoptera: Tenebrionidae). Proce. 4th Inter. Congress of Entomological, Sci., Univ. Agric., Faisalabad, Sept. 22-23 (2020) 23–27.

[5] M.M. Ghuniem, M.A. Khorshed, E.R. Souaya, Optimization and validation of an analytical method for the determination of some trace and toxic elements in canned fruit juices using quadrupole inductively coupled plasma mass spectrometer, J. AOAC Int. 102 (1) (2019) 262–270, https://doi.org/10.5740/jaoacint.18-0022.

[6] S.M. El-Safty, M.A. Khorshed, M.M. Ghuniem, Rapid determination of mercury in dust emission using Cold Vapour Inductively Coupled Plasma Optical Emission Spectrometer (CV ICP OES), Int. J. Environ. Anal. Chem. *102* (1) (2022) 270–292, https://doi.org/10.1080/03067319.2020.1720012.

[7] L. Zheng, C.C. Kuo, J. Fadrowski, J. Agnew, V.M. Weaver, A. Navas-Acien, Arsenic and Chronic Kidney Disease: A Systematic Review (Springer), Curr. Environ. Health Rep. Vol. 1 (Issue 3) (2014) 192–207, https://doi.org/10.1007/s40572-014-0024-x.

[8] M. Ghuniem, M.A. Khorshed, S.M. El- Safty, E.R. Souaya, M. Khalil, Potential human health risk assessment of potentially toxic elements intake via consumption of soft drinks purchased from different Egyptian markets, Int. J. Environ. Anal. Chem. 102 (15) (2020) 3485–3507, https://doi.org/10.1080/03067319.2020.1770742.

[9] M.M.H. Khalil, M.A. Khorshed, M.M. Ghuniem, Res. J. Chem. Environ. Sci. 4 (2016) 15–21.

[10] M.M. Ghuniem, M.A. Khorshed, M. Reda, S.M. Mahmoud, G. Hammad, Assessment of the Potential Health Risk of Heavy Metal Exposure from the Consumption of Herbal, Black and Green Tea, Biomed. J. Sci. Tech. Res. 16 (1) (2019) 11810–11817, https://doi.org/10.26717/bjstr.2019.16.002806.

[11] N. Johri, G. Jacquillet, R. Unwin, Heavy metal poisoning: The effects of cadmium on the kidney, BioMetals Vol. 23 (Issue 5) (2010) 783–792, https://doi.org/10.1007/s10534-010-9328-y.

[12] M.M. Ghuniem, M.A. Khorshed, S.M. El-safty, E.R. Souaya, M.M.H. Khalil, Assessment of human health risk due to potentially toxic elements intake via consumption of Egyptian rice-based and wheat-based baby cereals, Int. J. Environ. Anal. Chem. 102 (18) (2020) 6936–6954, https://doi.org/10.1080/03067319.2020.1817911.

[13] A.C. Bosch, B. O'Neill, G.O. Sigge, S.E. Kerwath, L.C. Hoffman, Heavy metals in marine fish meat and consumer health: a review, J. Sci. Food Agric. 96 (2016) 32–48, https://doi.org/10.1002/jsfa.7360.

[14] A. Arulkumar, S. Paramasivam, R. Rajaram, Toxic heavy metals in commercially important food fishes collected from Palk Bay, Southeastern India, Mar. Poll. Bull. 119 (2017) 454–459, https://doi.org/10.1016/j.marpolbul.2017.03.045.

[15] Griboff, J., Wunderlin, D.A., Monferran, M.V., 2017. Metals, As and Se determination by inductively coupled plasma-mass spectrometry ICP-MS in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health?.

[16] L. Makedonski, K. Peycheva, M. Stancheva, Determination of heavy metals in selected black sea fish species, Food Cont. 72 (2017) 313–318, https://doi.org/10.1016/ j.foodcont.2015.08.024.

[17] L.N. Murthy, C.O. Mohan, C.N. Ravishankar, R. Badonia, Biochemical quality and heavy metal content of fish meal and squid meal produced in veraval, Gujarat. Indian J. Fish. 60 (2013) 113–117. ⟨http://hdl.handle.net/123456789/1718⟩.
[18] M. Javed, N. Usmani, Assessment of heavy metal Cu, Ni, Fe Co, Mn, Cr, Zn pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of Mastacembelus armatus, SpringerPlus 2 (2013) 1–13, https://doi.org/10.1186/2193-1801-2-390.
[19] K.J. Elnabris, S.K. Muzyed, N.M. El-Ashgar, Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip Palestine, J. Assoc. Arab Univ. Bas. Appl. Sci. 13 (2013) 44–51, https://doi.org/10.1016/j.jaubas.2012.06.001.
[20] K.M. El-Moselhy, A.I. Othman, H.A. El-Azem, M.E.A. El-Metwally, Bioaccumulation of heavy metals in some tissues of fish in the Red Sea. Egy. J. Bas. Appl. Sci. 1 (2014) 97–105, https://doi.org/10.1016/j.ejbas.2014.06.001.
[21] P. Kulawik, W. Migdał, F. Gambuś, E. Cieślik, F. Özogul, J. Tkaczewska, K. Szczurowska, I. Wałkowska, Microbiological and chemical safety concerns regarding frozen fillets obtained from Pangasius sutchi and Nile tilapia exported to European countries, J. Sci. Food Agric. 96 (2016) 1373–1379, https://doi. org/10.1002/jsfa.7233.
[22] Dalia Nabil, Saad Gadallah, Lamia Ryad, Nermine Gad, Determination and validation of Sulfonamide Antibiotics in liver tissue of buffalo using QuEChERS method and LC MS/MS analysis, N. Y Sci. J. 2017 10 (6) (2017) 115–120.]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 16. doi:10.7537/marsnys100617.16.
[23] F. Cañada-Cañada, a Muñoz de la Peña, a Espinosa-Mansilla, Analysis of antibiotics in fish samples, Anal. Bioanal. Chem. 395 (2009) 987–1008, https://doi.org/10.1007/s00216-009-2872-z.
[24] SHAHID, A.– ALI, M.A.– MUZAMMIL, S.– ASLAM, B. – SHAHID, M.– SAQALEIN, M.– AKASH, M.S.H.– ALMATROUDI, A.– ALLEMAILEM, K.S. – KHURSHID, M (2021). Antibiotic residues in food chains; Impact on the environment and human health: A Review – 3959.
[25] European Commision (1990). European Commission, Council Regulation 2377/90/EC, Off. J. Eur. Union L224 (1990), Consolidated version of the Annexes I to IV updated up to 20.01.2008obtained from www.emea.eu.int (18.08.90).
[26] G. Darko, O. Akoto, Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana, Food Chem. Toxicol. 46 (12) (2008), https://doi.org/10.1016/j.fct.2008.09.049.
[27] EU Pesticides Database (v.2.2). (2021). EU Pesticides MRLs Database (v.2.2). ⟨https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr⟩.
[28] M.M. Issa, M. Taha, S. El- Marsafy, A.M. Khalil, M. M. H, E.H. Ismail, Acetonitrile-Ethyl acetate based method for the residue analysis of 373 pesticides in beeswax using LC-MS/MS and GC–MS/MS, J. Chromatogr. B 1145 (2020) 122106, https://doi.org/10.1016/j.jchromb.2020.122106.
[29] H.M. Refai, A.M. Helmy, M.M. Ghuniem, Exposure and cancer risk assessment of polycyclic aromatic hydrocarbons (PAHs) in River Nile of Egypt, Int. J. Environ. Anal. Chem. 104 (3) (2022) 552–565, https://doi.org/10.1080/03067319.2021.2022656.
[30] Pihlström, T., Fernández-Alba, A.R., Ferrer Amate, C., Erecius Poulsen, M., Lippold, R., Carrasco Cabrera, L., Pelosi, P., Valverde, A., Mol, H., Jezussek, M., Malato, O., & Štěpán, R. (2022). SANTE 11312/2021. https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf.
[31] E.D. Prudnikov, Theoretical calculation of the standard deviation in atomic emission spectroscopy, Spectrochim. Acta Part B: At. Spectrosc. 36 (4) (1981) 385–392, https://doi.org/10.1016/0584-8547(81)80039-0.
[32] RASSF Window. (2022). EU Rapid Alert System for Food and Feed (RASFF) portal. ⟨https://food.ec.europa.eu/safety/rasff-food-and-feed-safety-alerts_en⟩.
[33] T. van der Velde-Koerts, A. Rietveld, P.E. Boon, Use of food consumption data of food balance sheets and national food consumption surveys in deterministic long-term dietary exposure assessments of pesticides, Food Chem. Toxicol. 151 (2021), https://doi.org/10.1016/j.fct.2021.112104.
[34] S. Walorczyk, D. Drożdżyński, Improvement and extension to new analytes of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography–tandem quadrupole mass spectrometry revisited, J. Chromatogr. A 1251 (2012), https://doi.org/10.1016/j.chroma.2012.06.055.
[35] WHO. (1997). Guidelines for predicting dietary intake of pesticide residues. 2nd revised edition, GEMS/Food Document WHO/FSF/FOS/97.7, Geneva.
[36] Egyptian Organization for Standardization and Quality (2020), guidelines for recommended methods for sampling methods to estimate pesticide residues for compliance with maximum limits, part (1) ES No. 1465/2020, Cairo, Egypt.
[37] Codex Guideline, (1999). Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs, no. CAC/GL 33/1999.
[38] A. Sepe, L. Ciaralli, M. Ciprotti, R. Giordano, E. Funari, S. Costantini, Determination of cadmium, chromium, lead and vanadium in six fish species from the Adriatic Sea, Food Addit. Contam. 20 (6) (2003) 543–552, https://doi.org/10.1080/026520303100006979.
[39] M.M. Issa, M.S. Taha, A.M. El- Marsafy, M.M.H. Khalil, E.H. Ismail, AcetonitrileEthyl acetate based method for the residue analysis of 373 pesticides in beeswax using LCMS/MS and GC–MS/MS. J. Chromatogr. B 1145 (2020) 122106.
[40] Global Environment Monitoring System-Food Contamination, Monitoring and Assessment Program (GEMS/Foods) Program of food safety and food aid, Geneva, Switzerland (2012).
[41] Food and Nutrition Board, Recommended Dietary Allowances/RDA (National Academy of Sciences, Washington, 1989).
[42] Food and Nutrition Board, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (National Academy of Sciences, Washington, 2001).
[43] US EPA (2010), Integrated Risk Information System (IRIS), United States Environmental Protection. Available online: http://www.epa.gov/iris/index.html.
[44] US EPA, 2018. ⟨https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables-november-2018⟩.
[45] Fawzy Eissaa, Khaled Ghanema, Mahmoud Al-Sisi (2020). Occurrence and human health risks of pesticides and antibiotics in Nile tilapia along the Rosetta Nile branch, Egypt, Elsevier, Toxicology Reports Volume 7, 2020, Pages 1640-1646, journal homepage: ⟨www.elsevier.com/locate/toxrep⟩.
[46] P. Govind, S. Madhuri, A.B. Shrivastav, Contamination of Mercury in Fish and Its Toxicity to Both Fish and Humans: An Overview, Int. Res. J. Pharm. 3 (11) (2012).
[47] K.M. El-Moselhy, A.I. Othman, H. Abd El-Azem, M.E.A. El-Metwally, Bioaccumulation of Heavy Metals in Some Tissues of Fish in the Red Sea, Egypt, Egypt. J. Basic Appl. Sci. 1 (2014) 97–105, https://doi.org/10.1016/j.ejbas.2014.06.001.
[48] M. Mokarram, A. Saber, V. Sheykhi, Effects of Heavy Metal Contamination on River Water Quality Due to Release of Industrial Effluents, J. Clean. Prod. 277 (2020) 123380, https://doi.org/10.1016/j.jclepro.2020.123380.
[49] B. Yu, X. Wang, K.F. Dong, G. Xiao, D. Ma, Heavy Metal Concentrations in Aquatic Organisms (Fishes, Shrimp and Crabs) and Health Risk Assessment in China, Mar. Pollut. Bull. 159 (2020) 111505, https://doi.org/10.1016/j.marpolbul.2020.111505.
[50] R.J. Erickson, D.R. Mount, T.L. Highland, J. Russell Hockett, C.T. Jenson, The Relative Importance of Waterborne and Dietborne Arsenic Exposure on Survival and Growth of Juvenile Rainbow Trout, Aquat. Toxicol. 104 (2011) 108–115, https://doi.org/10.1016/j.aquatox.2011.04.003.
[51] A.E.D.H. Sayed, H.A.M. Elbaghdady, E. Zahran, Arsenic-Induced Genotoxicity in Nile Tilapia (*Orechromis niloticus*); The Role of Spirulina Platensis Extract, Environ. Monit. Assess. 187 (2015) 1–10, https://doi.org/10.1007/s10661-015-4983-7.
[52] B. Kumari, V. Kumar, A.K. Sinha, J. Ahsan, A.K. Ghosh, H. Wang, G. DeBoeck, Toxicology of Arsenic in Fish and Aquatic Systems, Environ. Chem. Lett. 15 (2017) 43–64, https://doi.org/10.1007/s10311-016-05889.
[53] A. Taweel, M. Shuhaimi-Othman, A.K. Ahmad, Assessment of Heavy Metals in Tilapia Fish (Oreochromis niloticus) from the Langat River and Engineering Lake in Bangi, Malaysia, and Evaluation of the Health Risk from Tilapia Consumption, Ecotoxicol. Environ. Saf. 93 (2013) 45–51, https://doi.org/10.1016/j.ecoenv.2013.03.031.
[54] A. Chahid, M. Hilali, A. Benlhachimi, T. Bouzid, Contents of Cadmium, Mercury and Lead in Fish from the Atlantic Sea (Morocco) Determined by Atomic Absorption Spectrometry, Food Chem. 147 (2014) 357–360, https://doi.org/10.1016/j.foodchem.2013.10.008.
[55] Z. Selcuk, S.U. Tiril, F. Alagil, V. Belen, M. Salman, S. Cenesiz, O.H. Muglali, F. B. Yagci, Effects of Dietary L-Carnitine and Chromium Picolinate Supplementations on Performance and Some Serum Parameters in Rainbow Trout (Oncorhynchus mykiss), Aquac. Int. ;18 (2010) 213–221, https://doi.org/10.1007/s10499-008-9237-z.
[56] Egyptian Organization for Standardization and Quality. (2008). Maximum levels for metals (Copper- Iron- Zinc) in food. ES: 2360, ICS: 67.040, Cairo, Egypt.
[57] Egyptian Organization for Standardization and Quality. (2010). Setting maximum levels for certain contaminants in foodstuffs. ES: 7136, Cairo, Egypt.
[58] Commission of the European Communities. (2023) Setting Maximum Levels for Certain Contaminants in Foodstuffs. Commission Regulation 2023/9015.
59] S. Franco, C.B. Melanie, G.F. Aliya, A. Roberta, Antimony Sleep. -Relat. Disord.: NHANES 2005–2008. Environ. Res. 156 (2017) 247–252.
[60] J. Xiuming, W. Shengping, X. Guoqiang, Cloud point extraction combined with electrothermal atomic absorption spectrometry for the speciation of antimony (III) and antimony (V) in food packaging materials, J. Hazard. Mater. 175 (2010) 146–150.
[61] L. Ya-An, J. Shiuh-Jen, A.C. Sahayam, Determination of antimony compounds in waters and juices using ion chromatography inductively coupled plasma mass spectrometry, Food Chem. 230 (2017) 76–81.
[62] N. Singh, D. Kumar, A. Sahu, Arsenic in the environment: effects on human health and possible prevention, ournal Environ. Biol. 28 (2) (2007) 359–365.
[63] L.O.-S. Andre, R.G.B. Paulo, J. Silvana do Couto, C.M. Josino, Dietary intake and health effects of selected toxic elements, Braz. J. Plant Physiol. 17 (1) (2005) 79–93.
[64] M. Manju, Effects of heavy metals on human health, Int. J. Res. -GRANTHAALAYAH, Soc. Issues Environ. Probl. (2015) 1–7.
[65] J. Monisha, T. Tenzin, A. Naresh, B.M. Blessy, N.B. Krishnamurthy, Toxicity, mechanism and health effects of some heavy metals, Interdiscip. Toxicol. 7 (2) (2014) 60–72.
[66] R.T. Raja, S. Namburu, Impact of heavy metals on environmental pollution, J. Chem. Pharm. Sci. 3 (2014) 175–181.
[67] S. Mandina, M. Tawanda, Chromium, an essential nutrient and pollutant: A review, Afr. J. Pure Appl. Chem. 7 (9) (2013) 310–317.
[68] S. Rumpa, N. Rumki, S. Bidyut, Sources and toxicity of hexavalent chromium, J. Coord. Chem. 64 (10) (2011) 1782–1806.

[69] T. Swapnil, K.D. Manas, K.S. Bhupendra, Cloud point extraction and diffuse reflectance-Fourier transform infrared spectroscopic determination of chromium (VI): a probe to adulteration in food stuffs, Food Chem. 221 (2017) 47–53.

[70] H.W. Claudia, S.C.M. Adnivia, S.J.G. Erik, S.L. Vivian, B. Carolina de Castro, T. K. Nirmal, F. Renata, H.R. Andre, Toxicity assessment of arsenic and cobalt in the presence of aquatic humic substances of different molecular sizes, Ecotoxicol. Environ. Saf. 139 (2017) 1–8.

[71] L. Laura, V. Bart, V.D.S. Catherine, W. Floris, M. Leen, Cobalt toxicity in humans. A review of the potential sources and systemic health effects, Toxicology 387 (2017) 43–56.

[72] B.O. Catalina, S.R. Fuensanta, M.C.P. Jose, Determination of cobalt in food, environmental and water samples with preconcentration by Dispersive Liquid-Liquid Microextraction, Am. J. Anal. Chem. 3 (2012) 125–130.

[73] S.C. Izah, N. Chakrabarty, A.L. Srivastav, A review on heavy metal concentration in potable water sources in Nigeria: Human health effects and mitigating measures, Exp. Health 8 (2016) 285–304.

[74] M. Sevcikova, H. Modra, A. Slaninova, Z. Svobodova, Metals as a cause of oxidative stress in fish: A review, Vet. Med. 56 (2011) 537–546.

[75] K.S. Ramesh, H.N. Shivraj, K. Young-Soo, Food science and technology for management of iron deficiency in humans: A review, Trends Food Sci. Technol. 53 (2016) 13–22.

[76] S.G. Rosalind, A.W. Anna, J.F.-T. Susan, H. Rachel, D.Y. Scott, R.B. Martin, D.C. C. Allan, A.E. Louise, J.W. Michael, K. Alexander, B.B. Karl, W.P.S. Edwin, Dietary iron intakes based on food composition data may underestimate the contribution of potentially exchangeable contaminant iron from soil. J. Food Compos. Anal. 40 (2015) 19–23.

[77] V. Chitra, T. Kavita, B.S. Anupam, Determination of iron (III) in food, biological and environmental samples, Food Chem. 221 (2017) 1415–1420.

[78] P. Sharma, R.S. Dubey, Lead toxicity in plants, Braz. J. Plant Physiol. 17 (1) (2005) 35–52.

[79] A.B. Santamaria, Manganese exposure, essentiality and toxicity, Indian J. Med. Res. 128 (2008) 484–500.

[80] F.B. Maryse, S. Céline, C. Pierre, F. Delphine, Low level exposure to manganese from drinking water and cognition in school-age children, Neurotoxicology 64 (2018) 110–117.

[81] A.L. Valfredo, G.N. Cleber, A.B. Marcos, An automated preconcentration system for the determination of manganese in food samples, J. Food Compos. Anal. 22 (2009) 337–342.

[82] C.W. Chen, C.F. Chen, C.D. Dong, Distribution and accumulation of mercury in sediments of Kaohsiung River Mouth, Taiwan, APCBEE Procedia 1 (2012) 153–158.

[83] A.T. Jan, I. Murtaza, A. Ali, Q.M.R. Haq, Mercury pollution: An emerging problem and potential bacterial remediation strategies, World J. Microbiol. Biotechnol. 25 (2009) 1529–1537.

[84] P. Francesco, B. Beatrice, F. Giovanni, C. Stefano, C. Antonio, Role of diet in nickel dermatitis, Open Chem. Biomed. Methods J. 2 (2009) 55–57.

[85] T.H. Lynne, K.B. Hudson, C.A. Bruce, J.V. Melissa, R.O. Adriana, Derivation of an oral toxicity reference value for nickel, Regul. Toxicol. Pharmacol. 87 (2007) 1–18.

[86] S. Pizzutelli, Systemic nickel hypersensitivity and diet: myth or reality? Eur. Ann. Allergy Clin. Immunol. 43 (1) (2011) 5–18.

[87] D.-C. Aleksandra, B. Urszula, The impact of nickel on human health, J. Elem. 13 (4) (2008) 685–696.

[88] F. Cima, Tin: environmental pollution and health effects, Environ. Pollut. Health Eff. (2011) 351–359.

[89] A. Hamid, E. Homeira, Imprinted polymer-based extraction for speciation analysis of inorganic tin in food and water samples, React. Funct. Polym. 73 (2013) 634–640.

[90] L.W. Kailas, K. Shiv, B. Prasad, Adsorption of tin using granular activated carbon, J. Environ. Prot. Sci. 3 (2009) 41–52.

[91] M.R. Anna, S. Samir, Zinc intake and its dietary sources: results of the 2007 Australian national children's nutrition and physical activity survey, Nutrients 4 (2012) 611–624.

[92] L. Huan, S. Aijaz, Z. Yajun, Q. Xianjin, C. Kai, Z. Tianqing, Y. Longwei, X. Danying, X. Jianlong, QTL underlying iron and zinc toxicity tolerances at seedling stage revealed by two sets of reciprocal introgression populations of rice (Oryza sativa L.), Crop J. 4 (2016) 280–289.

[93] H. Heidi, S.V. Kavitha, S.D.J. George, C. Divaker, G.S. Howard, B.G. Mary, Mechanistic studies of the toxicity of zinc gluconate in the olfactory neuronal cell line Odora, Toxicol. Vitr. 35 (2016) 24–30.

[94] J.K. Man, I.B. Maxim, Y. Jung-Seok, L. Seunghak, H.H. Yun, Y.L. Ju, M. Bhoopesh, M.K. Kenneth, Transformation of zinc-concentrate in surface and subsurface environments: Implications for assessing zinc mobility/toxicity and choosing an optimal remediation strategy, Environ. Pollut. 226 (2017) 346–355.

[95] M.Y. Elsayed, A.A. Abdel-Wahab, A.A. Nasser, A.E. Soltan, R. Mostafizur, Nutritional value and bioaccumulation of heavy metals in muscle tissues of five commercially important marine fish species from the Red Sea, Saudi J. Biol. Sci. 28 (2021) 1860–1866.

[96] H.A.H. Ahmed, S.I. Naim, Heavy metals in eleven common species of fish from the Gulf of Aqaba, Red Sea, Jordan J. Biol. Sci. 1 (2008) 13–18.

[97] M.S. Rahman, A.H. Molla, N. Saha, A. Rahman, Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Dhaka, Bangladesh, Food Chem. 134 (2012) 1847–1854, https://doi.org/10.1016/j.foodchem.2012.03.099.

[98] M.M. Abarshi, E.O. Dantala, S.B. Mada, Bioaccumulation of heavy metals in some tissues of croaker fish from oil spilled rivers of Niger Delta region, Nigeria, Asian Pac. J. Trop. Biomed. 7 (2017) 563–568, https://doi.org/10.1016/j.apjtb.2017.05.008.

[99] A.B. Yilmaz, Levels of heavy metals Fe, Cu, Ni, Cr, Pb, and Zn in tissue of Mugil cephalus and Trachurus mediterraneus from Iskenderun Bay, Turkey, Environ. Res. 92 (2003) 277–281, https://doi.org/10.1016/s0013-93510200082-8.

[100] H. Ahmad, A.M. Yousafzai, M. Siraj, R. Ahmad, I. Ahmad, M.S. Nadeem, W. Ahmad, N. Akbar, K. Muhammad, Pollution problem in river Kabul: accumulation estimates of heavy metals in native fish species, BioMed. Res. Intern. (2015) 1–7, https://doi.org/10.1155/2015/537368.

[101] A. Velusamy, P. Satheeshkumar, A. Ram, S. Chinnadurai, Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbour, India. Mar. Poll. Bull. 81 (2014) 218–224, https://doi.org/10.1016/j.marpolbul.2014.01.049.".