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Assessment and Evaluation of Heavy Metals in Fish and Canned-Fish Collected From Local Markets at Ramallah City Using ICP-MS

دراسة تقييمية للمعادن الثقيلة في أسماك ومعلبات أسماك جمعت من أسواق محلية في مدينة رام الله باستخدام جهاز ICP-MS

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Chapter 01

Introduction and Literature Review

1.1 Back ground

Definition of heavy metal

The term" heavy metals" is mostly used as a generic name for metals and metalloids associated with toxicity, adverse effects on living organisms, and environmental pollution.

Scientists differed on the classification of heavy metals, some defined heavy metals as the transition and post transition metal,[1] others defined them as those metals with an elemental density above 7 $g.cm^{-3}$.[2] While other wrote that heavy metals are most often defined as metals having density greater than 5 $g.cm^{-3}$.[3] According to another definition, for a metal to be considered heavy, it must have a density greater than 5 relative to that of water.[4] However, some studies have used the term heavy metals for metals with a density less than 5 $g.cm^{-3}$, [5] such as arsenic (As) which is a non-metal. Some studies have commented on this point and reported that not all "heavy metals" are metals in the first place. They referred to As, which is generally classified as "a heavy metal", although it is a metalloid.[6]

Sources of heavy metals

Heavy metals are natural elements characterized by their high atomic weights. They are released into the aquatic environment through natural process or by human activities including mining, combustion, agriculture, pharmaceuticals and urbanization.

Effect of heavy metals

Trace amounts of some heavy metals, such as cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc are needed by living organisms but excess is usually detrimental. [9] Although many heavy metals are toxic for living organisms under certain conditions. They are toxic in certain forms and in sufficiently high doses, [2] this is why it is important to measure their concentration in representative samples prior to assessing their toxicity.

Heavy metals accumulation

Unlike other contaminants, Heavy metals are nonbiodegradable and hence may persist in the environments, and could bioaccumulate, and bio-magnify up in the food chain due to their ability to bind to short carbon chains. [6,10,11] Eventually, they are transferred to humans through the food chain with a wide-range of potential harmful effects on the health of seafood consumers. [12,13]

Risk assessment of heavy metals in human

The study of heavy metal contamination has received much attention due to their potential toxicity and adverse effects on public health. [14-16] Under certain conditions, these metals could accumulate in the body of various organisms and / or humans to a toxic concentration which could cause serious ecological damage or a pronounced adverse effect on human health. [14-16]

Heavy metals disrupt cellular events including growth, proliferation, differentiation, damage-repairing processes, and apoptosis. Comparison of the mechanisms of action reveals similar pathways for these metals to induce toxicity including reactive oxygen species (ROS) generation, weakening of the antioxidant defense, enzyme inactivation, and oxidative stress. On the other hand, some of them have selective binding to specific macromolecules.[17]

Arsenic, chromium, cadmium, lead, mercury, and antimony are non-essential heavy metals HMs. These can dramatically alter biochemical processes in living organisms. [6,14,18-19] When ingested in excessive amounts, they combine with the body's biomolecules, such as proteins and enzymes to form stable bio-toxic compounds, thereby mutilating their structures and preventing them from bio-reacting their functions.[20]

High-dose mercury and lead, may induce severe complications such as abdominal colic pain, bloody diarrhea, and kidney failure. [21] On the other hand, low-dose exposure is a subtle and hidden threat, unless repeated regularly, which may then be diagnosed by its complications, e.g. neuropsychiatric disorders including fatigue, anxiety, and detrimental impacts on intelligence quotient (IQ) and intellectual function in children.[22]

The toxicity and carcinogenicity of heavy metals are dose dependent. The toxic mechanism of heavy metals functions in similar pathways usually via reactive oxygen species (ROS) generation, enzyme inactivation, and suppression of the antioxidant defense. However, some of them cause toxicities in a particular pattern and bind selectively to specific macromolecules.[17]

Importance of fish

Aquatic products serve as a rich source of healthy vitamins, proteins, omega-3 fatty acids, selenium, calcium and minerals, all of which are important for human health,[23,24] and are increasingly consumed by humans.[25]

Almost all elements that are considered essential and necessary for the maintenance of normal physiological functions are found in seafood.[26,27] The American Heart Association recommends two servings of fish per week as part of a healthy diet.[28]

Description of study area

The West Bank (Figure1.1) is a landlocked territory near the Mediterranean coast of Western Asia. The climate in the West Bank is mostly Mediterranean, slightly cooler at elevated areas compared with the shoreline, west to the area.[29] The total area of the West Bank is estimated at 5760 km^2 .[30] According to The Palestinian Central Bureau of Statistics (PCBS), the number of residents of the west bank in 2009 was about 2281714 inhabitants, 262941 of whom were residents of Ramallah and Al-Bireh governorate, the place in which the study was conducted.[31]

Figure 1.1 Map of the study area in the West Bank – Palestine.

1.1.2 Objectives of the study

The present study aims are: (i) to investigate the presence of heavy metals contaminants in different species of fish, and canned fish; (ii) to examine whether the measured concentrations exceed the maximum permissible limits of these heavy metals that are proposed by Ministry of Agriculture, European Union (EU) and World Health Organization (WHO); and (iii) to assess human health risks associated with consumption of commercial fish and canned fish.

1.1.3 Problem definition

There are four sources of fish at the West Bank, sea fish (wild caught fish from the Mediterranean Sea), river fish (Lake Tiberias), farmed fish, and imported sea fish (fresh or frozen). To our knowledge, there is no available data at the Palestinian Ministry of Health or at the Palestinian Ministry of Agriculture, or at any other governmental institution about the current levels of metals or metalloids such as Cu, Zn, Mg, Mn, Fe, Cr, Co, Ni, Ag, As, Cd, Pb, Hg, or other trace elements in locally-raised or imported fish and canned-fish. In the West Bank, no chemical is regulated due to the absence of a governmental body that could be in charge of monitoring and regulating contaminants levels in all food products.

Fish that are commercially available at Ramallah markets for public consumption were divided into several categories: (i)frozen fish, which is imported from outside the country; (ii) wild fish, which grew naturally without artificially feeding, it might be imported from outside the country, or from coastal water of Gaza Strip, Jafa, or it could be fished from the waters of Lake Tiberias; (iii)farmed fish, from fish farms in Jericho; and (iv) canned fish.

1.1.3.1 Frozen fish

Frozen fish is imported from outside the country, knowing the levels of heavy metals in fish imported from different regions is very important for the consumer health, this is because the environmental situations of imported frozen fish are unknown.

1.1.3.2 Wild-caught fish

The coastal water of Gaza Strip is polluted by effluent from sewage treatment plants and floods from the Gaza Valley which have discharged chemical wastes into the sea. Most domestic wastewater and industrial effluents are transported to treatment plants and ultimately discharged with or without partial treatment into the Mediterranean Sea.[32] Figure 1.2 shows the flow of wastewater in Dier El- Balah in Gaza.

Figure 1.2. The flow of wastewater in Dier El- Balah - Gaza strip.

On the beach of occupied Jafa, large areas appeared polluted with waste water, the reason for the appearance of this spot is the effect of pumping sewage into the sea and the wastes of drilling operations close to the beach. Figure 1.3. shows the pollution spot at Jafa beach.[33]

As Gaza and occupied Jafa, waters of Lake Tiberias are also polluted because of sewage discharge near the lake, catching fish species that purify lake water, such as comb fish, which studies have proven effective in purifying water; [34] and also because of the wrong practices of the residents living nearby, who are continuously dumping pollutants and wastes therein.[34]

Figure 1.3. The beach of occupied Jafa, large areas appeared with waste water.

1.1.3.3 Farmed Fish

Because of the limiting fishing distance, the narrow length of coastal strip, the lack of sea coasts in the West Bank, the Israeli occupation's control over the water resources and their control over quantities and prices as the main sources of fish in the Palestinian markets and the large consumption of fish in the Palestinian society, together these factors led to the establishment of water ponds to raise fish in the West Bank. The first experience of fish farming was in the city of Jericho in 1996. The production rate of the West Bank of fish is at a maximum of 120-150 ton/year,[35] produced by several farms concentrated in the Jordan valley and the North of the West Bank. Figure 1.4. shows fish farming in Palestine.[36]

Figure 1.4. Fish farming activities in Palestine.

1.1.3.4 Canned fish

Canned fish is imported from different countries, knowing the levels of heavy metals in imported canned fish is very important for the consumer health, this is because the environmental situations, the packaging, and storage conditions of canned fish are unknown.

1.1.4 Selection of elements and their toxicity

Basis of selection of elements

Elements as Cd, Hg, As, and Pb are non-essential metals and their toxic effect on human health is well known, while metals as, Cu, Ca, Fe, Mn, and Zn are essential metals, the toxic effect of them on human health begins when they are present in high levels.

1.2 Review of literature

1.2.1 Related works conducted in the region and around the world

Bioaccumulation of heavy metals leads to a diversity of toxic effects on a variety of body tissues and organs, so it is important to determine the levels of heavy metals in commercial fish species in neighboring countries in the region and around the world. This aims to ensuring the security of the food supply and to minimizing potentially dangerous effects on public health.

During the past two decades, only one study was conducted at Gaza Strip, particularly in 2013. This study reported the levels of a number of heavy metals in few commercial fish species (local and imported). Concentrations of Zinc, Lead, Cadmium, Manganese, Copper and Nickel were determined in the muscles of six commercial fish species using atomic absorption spectroscopy (Table 1.1). Three frozen imported fish species (Merluccius hubbsi, Micropogonias furnieri and Pangasius hypothalamus), two cultured species in local farms (Oreochromis niloticus and Sparus aurata) and one marine captured fish species (Mugil cephalus), were studied. The range of mean metals concentrations (in µg/g wet weight) were as follows: Mean cadmium concentrations ranged from < limit of detection (LD) – 0.09 (median not applicable "N.A."), Mean copper concentrations ranged from 0.251-0.907 (median of 0.3722 (n = 6), mean zinc concentrations ranged from 3.705-20.535 (median of 6.667, n = 6), mean manganese concentrations ranged from 0.376-0.834 (median of 0.391, n = 6), nickel concentrations ranged from 0.453-0.978 (median of 0.6705, n = 6), and mean lead concentrations ranged from < limit of detection (LD) – 0.115 (median of 0.172, n = 6).[32]

Table 1.1 The average meal concentrations (µg/g wet weight) ± standard error in muscle of various fish species. Adapted from Elnabris et. al. (2013).[32]

Scientific name	Metals					
	Cd	Cu	Mn	Ni	Pb	Zn
Merluccius hubbsi	< LD	$0.318^{ab} \pm 0.009$	$0.519^{a} \pm 0.069$	$0.707^{ab} \pm 0.044$	< LD	$5.821^{ab} \pm 0.436$
Micropogonias furnieri	0.090 ± 0.039	$0.345^{ab} \pm 0.029$	$0.396^{a} \pm 0.024$	$0.453^{b} \pm 0.041$	$0.552^{a} \pm 0.479$	$20.535^{c} \pm 3.081$
Mugil cephalus	< LD	$0.907^{c} \pm 0.171$	$0.834^{a} \pm 0.414$	$0.978^{a} \pm 0.192$	$0.172^{a} \pm 0.092$	$12.783^{bc} \pm 3.61$
Pangasius hypothalmus	< LD	$0.251^{a} \pm 0.017$	$0.381^{a} \pm 0.04$	$0.511^{b} \pm 0.02$	< LD	$3.705^{a} \pm 0.325$
Oreochromis niloticus	< LD	$0.638^{bc} \pm 0.084$	$0.386^{a} \pm 0.031$	$0.892^{ab} \pm 0.148$	$0.115^{a} \pm 0.07$	$7.522^{ab} \pm 0.963$
Sparus aurata	< LD	$0.399^{ab} \pm 0.031$	$0.376^{a} \pm 0.024$	$0.634^{ab} \pm 0.035$	< LD	$4.946^{ab} \pm 0.904$

Values with different letters in the same column are significantly different ($P < 0.05$).
< LD = values were below the limits of detection by spectrophotometry, 0.001 ppm for Pb and 0.002 ppm for Cd.

Below are some statistical values of the contents of lead, mercury, cadmium, and other trace elements in fish and/or canned-fish as reported in similar studies conducted in neighboring and in some other non-neighboring countries.

In Jordan, Levels of Cd, Cu and Zn in three fish species, Oreochromis aureus, Cyprinus carpio and Clarias lazera, collected from the Northern Jordan Valley were investigated. It was found that levels of these heavy metals in muscles of the three fish species were within the acceptable limits of the Food and Drug Organization (FAO), except for Zn. The mean concentration (in mg/kg dry wt.) of Cd, Cu and Zn in the muscle in the fish species are shown in Table 1.2. The mean cadmium concentrations ranged from 0.02-0.24(median of 0.14), mean copper concentrations ranged from 2.42-3.04 (median of 2.90), and mean zinc concentrations ranged from 30.13-70.76 (median of 30.31). The relatively high Zn concentrations measured in these three fish species were attributed to the increase of agricultural influx and some other anthropogenic activity in that area.[38]

Table 1.2 Concentrations of Cd, Cu, and Zn in µg/g dry wt (mean ± standard deviation) of three fish species collected from Wadi El- Arab, Northern Jordan Valley during March, 2006. Text. Modified from Al-Weher (2008).[38]

Fish species	Cd	Cu	Zn	n*
Oreochromis aureus	0.02± 0.02	2.90± 0.34	70.76±31.21	7
Cyprinus carpio	0.14± 0.07	2.48± 1.00	30.31± 4.16	9
Clarias lazera	0.24± 0.05	3.04± 0.64	30.13± 3.04	9

*: n: number of corresponding fish species used in the analysis.

In China, heavy metal concentrations were measured in 29 marine wild fish species from the South China Sea. Concentrations range (expressed per g wet tissue weight (wwt.)) were as follows: Cd ranged from 0.51–115.81 ng/g, Pb ranged from 0.54–27.31 ng/g, Cr ranged from 0.02–1.26 µg/g, Ni ranged from 8.32–57.48 ng/g, Cu ranged from 0.12–1.13 µg/g, Zn ranged from 2.34–6.88 µg/g, Fe ranged from 2.51–22.99 µg/g, and Mn ranged from 0.04–0.81 µg/g (Mn). Iron concentrations in all and Mn in some fish species were higher than the acceptable daily upper limit (0.8 µg/g Fe, and 0.18 µg/g Mn), suggesting human consumption of these wild fish species may pose a health risk. Human health risk assessment, however, indicated no significant adverse health effects upon consumption any of the studied species.[39]

In Serbia, the concentrations of heavy metals (Cd, Hg and Pb) were determined. Cd concentration ranged from 0.01 to 0.81 mg kg^{-1} in sea fish and from 0.01 to 0.03 mg kg^{-1} in freshwater fish. Hg concentrations were in the range of 0.01-1.47 mg kg^{-1}; the highest Hg value was measured in the predator fish - shark. The highest level of Pb (6.56 mg kg^{-1}) was detected in a blue sea fish (Atlantic mackerel).[40]

In Bosnia and Herzegovina (BiH), heavy metals contents were determined in seven commercial fish species, and other seafood products. Namely, European hake, Atlantic bluefn tuna steak, Atlantic bluefn tuna (canned), Atlantic mackerel, Patagonian squid, Blue mussel, Black tiger shrimp, Indian white prawn. The average concentrations of these heavy metals (expressed per g wet weight ± Standard deviation) were as shown in Table 1.3.[41]

Table1.3 Average heavy metals concentrations in different fish species and other seafood species ± the corresponding standard deviation.[41]

Species	n*	Cd		Hg		Pb	
		Average± SD	Range	Average± SD	Range	Average± SD	Range
European hake	3	0.003±0.001	0.002-0.004	0.023±0.002	0.022–0.02	0.002±0.001	0.001–0.002
Atlantic bluefn tuna steak	3	0.01±0.0	_	0.213±0.096	0.114–0.309	0.003±0.002	0.001-0.004
Atlantic bluefn tuna (canned)	7	0.015±0.003	0.01-0.02	0.06±0.028	0.037-0.116	0.006±0.003	0.001-0.008
Atlantic mackerel	5	0.033±0.009	0.021-.047	0.129±0.247	0.042–0.624	0.007±0.005	ND**-0.01
Patagonian squid	5	0.644±0.252	0.391–0.918	0.02±0.004	0.014–0.024	0.003±0.002	0.001–0.006 0
Blue mussel	5	0.062±0.009	0.049–0.073	0.044±0.011	0.026–0.055	0.161±0.072	0.092–0.278
Black tiger shrimp	4	0.015±0.002	0.013–0.017	0.058±0.023	0.029–0.078	0.014±0.008	<0.001–0.022
Indian white prawn	5	0.002±0.005	0.015–0.027	0.037±0.018	0.008–0.05	0.013±0.008	0.004–0.024

*: n: number of corresponding fish species used in the analysis.
**: ND: Not detected.

1.2.2 Studies concerning heavy metals in Ramallah

To my-knowledge, no one has attempted to measure the contents of lead, arsenic, cadmium, and mercury or other trace elements in fish and canned-fish in Ramallah City or any other Palestinian city, except, the only study conducted at Gaza Strip in 2013 which as previously mentioned, determined the concentrations of Zinc, Lead, Cadmium, Manganese, Copper and Nickel in the muscles of six commercial fish species available in Gaza Strip markets, using atomic absorption spectroscopy.[32] Therefore, the research study described herein is of a crucial importance to the Palestinian consumers in general and to the health of the consumers living in Ramallah city or its rural area, in particular. Knowing the concentrations of such toxic metals/metalloids in fish and canned-fish would ensure public safety and would provide important data to Palestinian officials who should push towards establishing a Palestinian institution to be in charge of food monitoring and regulating chemicals in food products.

1.3 Selected elements and their toxicity

1.3.1 Cadmium

Cadmium (Cd) is a nonessential heavy metal that poses significant risks to human health. Chronic exposure to cadmium results in systemic toxicity and cancer of the lung, breast, prostate, nasopharynx, pancreas and kidneys.[42,43] The liver and kidneys are extremely sensitive to the toxicity of cadmium.[40] Unlike low gastrointestinal absorption, Cd is more efficiently extracted from the lungs by industrial dust. This heavy metal also presents a risk of osteoporosis,[43] testicular neoplasms, renal dysfunction, hypertension, atherosclerosis, growth retardation or neoplasms.[37]

High levels of Cd in water, air and soil can result from industrial activities which could represent significant human exposure to Cd. Exposure to Cd can also occur through smoking, which may lead to increasing Cd concentrations in blood and urine. The presence of Cd in the water of contaminated media could disrupt the necessary mechanisms in the body, causing possible short- or long-term disturbances.[44,45] Cd is classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1).[46] Occupational exposure to Cd may occur in alloy, battery, and glass production and in electroplating industries.[42,43]

1.3.2 Lead

Lead is a harmful environmental pollutant which has high toxic effects to many body organs. It has adverse effects on the neurological, biological, and cognitive functions in the bodies.[47] It could cause loss of appetite, headaches, high blood pressure, abdominal pain, kidney dysfunction, fatigue, insomnia, arthritis, hallucinations and dizziness.[48] Furthermore, Pb could disturb the balance of the oxidant–antioxidant system and induce inflammatory responses in various organs. Exposure to Pb is associated with many diseases.[49,50] Pb may disrupt skeletal hematopoietic function, digestive and male reproductive systems.[51,52,53] Even though Pb could be absorbed through the skin, it is mostly absorbed from respiratory and digestive systems.[54] Pb exposure can induce respiratory, urinary, and cardiovascular disorders due to immune modulation, oxidative, and inflammatory mechanisms.[49,50]

1.3.3 Arsenic

Arsenic is the 20th most abundant element in the earth's crust [55] and it is well known as one of the few metals and metalloids that could have adverse health effects on a large scale.[54] Chronic arsenic toxicity or arsenicosis was previously associated with skin damage and skin cancer, bladder, lungs, kidneys, liver and colon cancers.[57,58] As could cause central and peripheral nervous system damage, cardiovascular disease, birth defects, placental development disorders and other reproductive harm.[59,60]

Arsenic as a dangerous heavy metal, is one of the primary hazard elements for the general public health. Sources of As publicity are occupational or through the tainted meals and water. As has an extended record of use, both as a metalloid substance or as a medicinal product.[54] It is notoriously known as the king of poisons and poison of kings.[61] Arsenic exists in metallic forms (As^0), inorganic (As^{3+} and As^{5+}), natural (from the Earth’s crust), and arsine (AsH_3). The order of decreasing toxicity of As compounds is described as arsine > inorganic species (As^{3+} and As^{5+}) > As^0 > natural arsenicals**.**[62,63]

Primary absorption occurs from the small intestine. Other routes of exposure are skin contact and inhalation. Distribution to many tissues and organs of the body follows, including the lungs, heart, kidneys, liver, muscles and nerve tissue.[64,65]

Acute and chronic As toxicity is associated with the dysfunctions of several important enzymes. The same as other heavy metals, As could inhibit sulfhydryl group containing enzymes, this might cause their dysfunction. In addition, As inhibits the pyruvate dehydrogenase by binding to the lipoic acid moiety of the enzyme. Inactivation of pyruvate dehydrogenase could block the Krebs cycle and inhibits oxidative phosphorylation. Consequently, ATP production decreases, leading to cell damage.[66](redundant keep the ref 66)

1.3.4 Mercury

Mercury (Hg) is existed in three forms: elemental or metallic mercury (Hg^0), inorganic mercury (Hg^+, Hg^{2+}), and organic mercury (commonly methyl or ethyl mercury).[76] The order of increasing toxicity related to different forms of mercury is defined as $Hg^0 < Hg^{2+}$, $Hg^+ < CH_3$-Hg.[77]

Hg^0 could cross the blood-brain barrier (BBB) and the placenta; thus, its neurotoxicity is higher than inorganic Hg which passes through membranes at a slower rate.[16] Hg^0 is oxidized in the body to produce divalent Hg (Hg^{2+}). Hg^0 (liquid) is slightly absorbed from the gastrointestinal (GI) tract and does not appear to be toxic.[78]

Inorganic mercury is concentrated in the kidneys, reabsorbed by proximal tubules or the basolateral membrane by organic anion transporters. Inorganic mercury cannot pass the BBB and the placenta.[78]

Organic Hg is readily absorbed from the gastrointestinal tract (95%) and distributed throughout the body. CH_3-Hg binds to thiol-containing molecules such as cysteine (CH_3-HgCys) so that it can pass BBB. Hair is considered an index of exposure to mercury because CH_3Hg accumulates there. In addition to the hair, mercury is excreted in the urine and stool. CH_3CH_2Hg follows pharmacokinetics similar to CH_3Hg.[78]

Human exposure to mercury occurs through inhalation of elemental (metallic) mercury vapors through industrial practices, dental amalgam, or through ingestion of organic mercury compounds (methyl, dimethyl, or ethyl mercury) primarily through consumption of contaminated fish .[20] Symptoms of mercury poisoning include lung damage and kidney dysfunction.[48] Mercury also affects the functions of the endocrine, immune and nervous systems.[20,79]Children are more sensitive and vulnerable to the harmful effects of mercury than adults.[79] Methylmercury can even cross the placenta and cause damage to the developing fetus in pregnant women.[20, 79]

1.3.5 Chromium

Chromium is the most abundant mineral in Earth's crust,[67] it occurs naturally at high concentration in ultramafic rocks and is a common contaminant in surface and ground water.[68,69] Cr has multiple oxidation states ranging from −2 to + 6, in which the trivalent and hexavalent forms are the most common stable forms.[66] Cr in small amounts is an essential element for the natural metabolism of lipids and proteins, for normal metabolic functions and also as a cofactor for the action of insulin.[70,71]

At high concentrations, Cr especially, in hexavalent form (Cr (VI)). Cr (VI) is related to a number of diseases and pathologies, and is toxic and carcinogenic.[69,72] Chronic inhalation of Cr (VI) affects the respiratory tract[73] and could lead to the development of lung cancer[69,74] in humans. Cr can cause various diseases upon bio-accumulating in the human body. These range from skin, kidney, neurological, and gastrointestinal diseases, to the development of several cancers including, testicles, bone, lungs, larynx, bladder, kidneys, and thyroid.[75]

1.3.6 Nickle

Nickel is a metallic element that is naturally present in the earth's crust. Human exposure to nickel occurs primarily through inhalation and ingestion. Nickel is an immunotoxin and carcinogen, depending on the dose and duration of exposure. Significant amounts of nickel in different forms could be deposited in the human body through occupational exposure and lifelong diet. Chronic exposure to nickel could cause allergic reactions and toxicity in the respiratory tract, pulmonary fibrosis, cardiovascular and kidney diseases.[80,81]

For other heavy metals, such as calcium (Ca), copper (Cu), Iron (Fe), Zinc (Zn), Aluminum (Al) and Manganese (Mn), they are classified as essential or probably essential metals. However, they may cause toxicity effects if the intake exceeds the safe consumption levels (2000 mg per day).[82] High intake of these metals could cause neurotoxicity to human.[83]

Chapter 02

Materials and methods

2.1 Sample collection

2.1.1 Collection of fish specimen

A total of thirteen fish species were purchased between February – April 2021, and a total of eight caned fish were purchased in November 2021. Selection of the fish species and the sampling process was performed following the recommendations of experts at the Palestinian Ministry of Agriculture in Ramallah. Species that are consumed by local Palestinian's residents were selected and purchased. The sampled specimens were placed immediately in poly-ethylene bags, put into isolated container of icebox and, then, brought to the Chemistry laboratory at Bir Zeit University. Table 2.1 and Table 2.2 show the names of fish species and canned fish samples that were selected for this research study.

Table 2.1 List of fish species

Scientific name	Local name	Common name
Epinephelus tauvina	Hamour	grouper fish
Sparus aurata	Danes	Sea bream
Merluccius hubbsi	Bakala	Argentine hake
Coral fish	Murjan	Gyrapidin fish
	Al qarous	sea bass
	Lokus	
Ctenophora	Mesht	Comb
	Salamon	
Pangasius hypothalamus	Filleah	Sutchi catfish fillet
	Sultan Ibrahem	Sultan Ibrahim
Mullidae	Malleta	Red Mullets
	Lavrak	
	Makrel	

Table 2.2 List of canned fish

Canned fish	Production Date	Expiry Date	Batch number
Americana	12-5-2020	12-5-2022	882149000994
Marena	8-2019	8-2023	7290015174480
Henz	25-4-2020	24-4-2024	6290090020062
Al Amed	7-2019	7-2022	8859009600177
Lazeza	9-5-2019	9-5-2022	
Fatafet	3-4-2020	3-4-2024	8852111019332
Arizona	8-2018	8-2022	7290015174770
Al warda Al hamra	4-2019	4-2023	7290011423728

2.1.2 Types and origin of Fish

Types and origin of fish used in the study:

Frozen fish: The Argentine hake (M. hubbsi) which sold as headless and gut, it is originally imported from Argentine, Vietnam and Uruguay respectively. Sutchi catfish fillet (P. hypothalamus), imported from Israeli occupation. These two species were purchased frozen from local markets in Ramallah city.

Cultured fish: Two fish species included in this study, namely, Comb (Ctenophora) and sea bream (S. aurata) are farmed in ponds in a fish farm in Jericho city, they were purchased frozen from local markets in Ramallah city.

Marine fish: Sultan Ibrahim fish also known as threadfin bream, Mackerels, gyrapidin, Sea bass and Mullidae (R Mullets) are five wild fish species usually caught from the shore of the Mediterranean Sea. While, Salmon fish is imported from Norway.

Canned fish: Canned fish were six tuna and two sardine bands. Fatafet, Lazeza and Al warda Al hamra are local industries. The remaining brands are imported from different countries; Henz and Americana from Egypt, Al amed and Arizona from Jordan, Marena from Israeli occupation.

2.1.3 Fish handling and preservation

After taking the identification, fish were washed with deionized water. They were sealed in polyethylene bags and kept in a freezer at -20 C° until chemical analysis.

2.2 Reagents

Deionized water was used to prepare all aqueous solutions. Nitric acid (HNO_3; Meark, supra pure) and Hydrogen peroxide (H_2O_2; Merck, Pro Analysis) were used for digestion.

2.3 Precautions followed to prevent contamination

Several precautions were taken in order to prevent contamination. Contamination was one of the main problems in the sample preparation such as contaminating the samples during sample pre-treatment (weighting, cutting and digestion), to avoid that, an acidic solution 5% (v/v) and deionized water were used to clean all bottles and glassware prior using.

Fish samples were washed by deionized water prior cutting to remove adsorbed salts on skin. Contamination may also occur from acid mixture used for digestion or from atmospheric air of lab. To check whether any error is being introduced into our measurements from any of the mentioned possible sources, method- and reagent- acid blanks were prepared in each set.

2.4 Cleaning procedure

All glassware and a 50 ml polyethylene tubes were soaked in 5% (v/v) nitric acid (8 liters Milli-Q water and 400 ml of 68%M HNO_3) and left for 48 hrs. at least., The 50 ml tubes were then thoroughly rinsed with Milli-Q water, and left to dry for several hrs., then they were closed tightly and stored until needed. The other glassware was kept soaked in the 5% (v/v) HNO_3 until they were needed.

2.5 Digestion procedures

2.5.1 Sample preparation

Fish species were skinned and boned, only the tissue was kept. Fish cans were opened, the soft-tissue-meat was squeezed by hand to eliminate the oil, then stored in plastic bags or in a precleaned polyethylene vials (100 mL) until needed. Each sample was then homogenized using an electric food processor. Finally, all the homogenized samples were stored in freezer at -20˚C.

2.5.2 Freeze drying of samples

All samples (fish and canned fish) were freeze dried using Lyophilizer at An-Najah National University. Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor.

After the samples were freeze dried, they were shipped to Belgium by EPS courier company.

2.5.3 Samples digestion

I. From each fish sample, 3 replicates of approximately 0.2 g (exact weight was recorded) was weighed and directly placed in clean 80-ml microwave digestion vessels.

II. Three replicates of standard reference fish material - ERM-BB422 (European Union Joint Research Centre, JRC-IRMM, Geel, Belium), and three method blanks were subjected to the same the same microwave digestion procedure and analyzed for quality assurance purposes.

III. To each vessel, 4 mL highly purified concentrated HNO_3 (69%, ICP-MS reagent grade) was added. The samples were then allowed to digest at room temperature during 2hours (h) under a fumes-hood.

IV. A stirring bar was placed in each digestion vessel.

V. 400 µl of high-grade hydrogen peroxide was then added to each vessel. The samples were left for 30 minutes (min) before closing them for subsequent digestion.

VI. The sample digestion system used was an automated microwave digester - Discover SP-D (CEM Corporation, Matthews, NC 28104, USA).

VII. Microwaves assisted digestion was accomplished by heating the vessels from room temperature to 200 ^oC at a ramping rate of 5.0 ^oC/min, held isothermal at 200 ^oC for 5 min. Microwave power was set at 300 Watts, and the pressure inside the digestion vessels was maintained constant at 400 psi.

VIII. When the digestion was complete, the samples were diluted to 50 ml with highly purified water obtained from Millipore Milli-Q® Integral 3 Water Purification System (Merck KGaA, Darmstadt, Germany). The system is fitted with Q-POD® Element unit which is a *point-of delivery* purifier designed to deliver ultrapure water, specifically dedicated to ICP-MS analysis.

IX. For elemental analysis, the samples were further diluted to bring the acid concentrations in the solutions to between 2 - 3 %.

2.6 Analysis

I. Elemental analysis was performed with High-Resolution ICP-MS - Element XR (ThermoFisher Scientific, Bremen, Germany).

II. For quantification of metals concentrations, a series of calibration standards were prepared by diluting high purity ICP-MS multi-element standard solution obtained from Agilent Technologies (Santa Clara, CA, USA).

III. For additional quality assurance and quality control (QA/QC) purposes, an external reference standard Material (SRM) 1643f (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) was also analyzed at the *start*, in *middle* and at the *end* of the analysis. The use of a NIST standard in this case was to validate the accuracy and precision of the analysis.

2.7 Determination of the water content

Two to five g of each fish and canned fish sample was weighed in a clean pre-weighed crucible, the sample was dried in an oven at 105 ± 2 C° for two hours until constant mass was attained. The difference in weight and the original sample weight were used to determine the percentage of water in each sample. Samples were analyzed in triplicates and the average water content was then calculated.

2.8 Human Health Risk Assessment of Heavy Metals

Under conditions of regular consumption, it is important to assess the metal pollution index, estimated daily intake (EDI),[32] target hazard quotients (THQ) and hazard index (HI)[14,37] of metals from fish and compare it with the values of the Recommended Daily Dietary Allowance (RDA) established by international food safety organizations[84,85] and with the values of Provisional Tolerable Daily Intake (PTDI) suggested by The Joint Food and Agriculture Organization/ World Health Organization (FAO/WHO) Expert Committee on Food Additives.[86,87]

2.8.1 Metal Pollution Index

Metal pollution index was adopted to assess metal pollution, the following equation was used.[88,89]

$$MPI = (CM_1 \times CM_2 \times CM_3 \times \ldots \times CM_n)^{1/n} \ldots\ldots \text{(Equation 1)}$$

where CM_1 is the concentration of the first metal, Where CM_2 is the concentration of the second metal, Where CM_3 is the concentration of the third metal, Where CM_n is the concentration of the nth metal (µg/g dry wt.) in the tissue sample of particular species.

2.8.2 Estimated Daily Intake (EDI)

Estimated daily intake (EDI) was calculating by the following equation:[90,91]

$$EDI = (Cn \times IGr) / Bwt \ldots\ldots\ldots\ldots \text{(Equation 2)}$$

Where, Cn is the concentration of metal in the selected fish muscles tissue (µg/g dry wt.); IGr is the acceptable ingestion rate, which is 8.22 g/day according to Palestinian Ministry of Agriculture in West Bank, and 11.66 g/day according to directorate General of Fisheries at the Palestinian Ministry of Agriculture in Gaza Strip. Bwt. is the body weight: 70 kg.[92]

2.8.3 Target Hazard Quotient (THQ)

THQ was estimated by the ratio of EDI and oral reference dose (RfD). The ratio value <1 implies non-significant risk effects[93]. The THQ formula is expressed as follows.[94,95]

$$THQs = (Ed \times Ep \times EDI / At \times RfD) \times 10^{-3} \ldots\ldots\ldots\ldots \text{(Equation 3)}$$

Where, Ed is the exposure duration (65) years[92]; Ep is exposure frequency (365 days/year); [96] RfD is the reference dose in µg element per kg per day, in which the values of RfD are set as follows; (Pb = 0.004 mg/kg-d, As = 0.0003 mg/kg-d, Cd = 0.001 mg/kg-d, and Hg = 0.0005 mg/kg-d).[94] At is the average time for the non-carcinogenic element (Ed ×Ep).

2.8.4 Hazard Index (HI)

Hazard index (HI) was calculated for four elements (Pb, As, Cd and Hg) measure in the fish samples, the equation used was as follows:[98]

$$HI = \sum_{i=k}^{n} THQ$$ …………… (Equation 4)

where THQ are the estimated risk value for individual metal.[98] When the HI value is higher than 1, the non-carcinogenic risk effect is considered high for exposed consumers.[99,100]

2.8.5 Carcinogenic Risk

Carcinogenic Risk (CR) was calculated to assess potential cancer cell development in fish or canned-fish consumers' body over a lifetime due to long-term exposure to" toxic" metals. The acceptable range of the CR limit is 10^{-6} to 10^{-4}.[124,125] The following equation was used to assess CR.

$$CR = \frac{Ed \times Ep \times EDI \times CSF}{AT} \times 10^{-3}$$ ………… (Equation 5)

Where, CSF is the oral slope factor of a particular carcinogen (mg/kg-day), CSF values are available only for As (1.5), Pb (0.0085), and Cd (6.3).[126-128]

2.9 Validation of analytical methodology

In this study, the standard reference material ERM-BB422 was used to assess the quality of the whole digestion and analysis procedures. This was achieved by subjecting three replicate samples of ERM-BB422 to the same microwave assisted digestion procedure, and then measuring the concentrations of metals in these samples using ICP-MS. The quality of the analytical method was then evaluated by calculating the percent recovery (%Recovery) of the metals that were reported in the certificate of analysis (COA) as illustrated in the formula shown below:

% Recovery = (Measured Concentration / Certified Concentration) x 100% … (Equation 6)

2.10 Data analysis

Descriptive statistics such as average, range, standard deviation, median, 95% confidence interval (C.I.) were calculated. All statistical calculations and all chart plots were performed using Microsoft excel (Windows 2019).

Chapter 03

Results and Discussion

3.1 Total metal concentrations in fish species and canned fish

3.1.1 Analytical quality assurance

As mentioned earlier in section 2.9, the accuracy of the digestion method was evaluated by using standard reference material (SRM) ERM-BB422 (Joint Research Center of The European Commission) and submitting this material to the same analytical procedure. The measured average concentrations of a number of metals with their percent confidence interval (% CI), and the certified values with their uncertainties are presented in **Table 3.1.1** As shown, the calculated recoveries for all metals reported in the certificate of analysis (COA) were within the acceptable values and ranged between 89 % to 96 %, and were as follows: As 89 %, Zn 90 %, Fe 93 %, Cu 93 %, Mn 93 %, Cd 93 %, and Hg 96 %.

Table 3.1.1 Average experimental values for the ERM-BB422 fish muscle reference standard calculated from three replicates with relative standard deviation, and certified values. (Concentrations are in µg/g dwt.)

ERM-BB422	Measured Average ± %95 CI (n = 3)	Certified Average ± uncertainty	%Recovery
Cd	0.0073 ± 0.0014	0.0075 ± 0.0018	93.3
Hg	0.574 ± 0.0555	0.601 ± 0.030	95.5
Mn	0.342 ± 0.015	0.368 ± 0.028	92.9
Fe	8.710 ± 0.246	9.4 ± 1.4	92.7
Cu	1.549 ± 0.116	1.67 ± 0.16	92.8
Zn	14.457 ± 2.355	16 ± 1.1	90.4
As	11.348 ± 1.957	12.7 ± 0.7	89.4

3.1.2 Total concentrations

The procedure was performed on 13 freeze-dry (i.e., lyophilized) and homogenized fish samples (3-replicates each). The total metal concentrations are presented in **Appendix A.** Average Ag concentrations ranged between 0.005and 0.025 µg/g (mean of 0.013 µg/g dwt.), Average Cd concentrations ranged between 0.002 and 0.109 µg/g (mean of 0.051 µg/g dwt.), average Hg concentrations ranged between 0.002 and 0.178 µg/g (mean of 0.042 µg/g dwt.), average Pb concentrations ranged between 0.017 and 0.072 µg/g (mean of 0.0.041 µg/g dwt.), Mg ranged between 562.587 and1479.003 µg/g (mean of 1041.917 µg/g dwt.), average Al concentrations ranged between 1.63 and 24.52 µg/g (mean of 4.53 µg/g dwt.), average Ca concentrations ranged between 206.599 and 9883.309 µg/g (mean of 738.773 µg/g dwt.), average Cr concentrations ranged between 0.054 and 0.826 µg/g (mean of 0.090 µg/g dwt.), average Mn concentrations ranged between 0.161 and 1.975 µg/g (mean of 0.499 µg/g dwt.), average Fe concentrations ranged between 6.599 and 53.530 µg/g (mean of 11.101 µg/g dwt.), average Co concentrations ranged between 0.020 and 0.094 µg/g (mean of 0.045 µg/g dwt.), average Ni concentrations ranged between 0.005 and 2.903 µg/g (mean of 0.052 µg/g dwt.), average Cu concentrations ranged between 0.768 and 1.729 µg/g (mean of 1.301 µg/g dwt.), average Zn concentrations ranged between 11.088 and 24.293 µg/g (mean of 19.050 µg/g dwt.), and average As ranged between 0.029 and 54.267 µg/g (mean of 0.829 µg/g dwt.).

As a comparison, Text Modified from Al Weher (2008) measured the concentrations (mg/kg dwt.) of Cd, Cu and Zn in three fish species, Oreochromis aureus, Cyprinus carpio and Clarias lazera, collected from the Northern Jordan Valley. It was found the mean cadmium concentrations ranged from 0.02-0.24 (median of 0.14), mean copper concentrations ranged from 2.42-3.04 (median of 2.90), and mean zinc concentrations ranged from 30.13-70.76 (median of 30.31).

For canned fish, average Ag concentrations ranged between 0.002 and 0.064 µg/g (mean of 0.011 µg/g dwt., n=8 for all measured metals), average Cd concentrations ranged between 0.018 and 0.120 µg/g (mean of 0.047 µg/g dwt.), average Hg concentrations ranged between 0.001 and 0.204 µg/g (mean of 0.009 µg/g dwt.), average concentrations Pb ranged between 0.022 and 0.075 µg/g (mean value of 0.029 µg/g dwt.), average Mg concentrations ranged between 660.706 and 1192.722µg/g (mean value of 783.032 µg/g dwt.), average Al concentrations ranged between 3.920 and 25.103 µg/g (mean of 5.086 µg/g dwt.), average Ca concentrations ranged between 163.424 and 11510.431 µg/g (mean of 354.454 µg/g dwt.), average Cr concentrations ranged between 0.050 and 0.101 µg/g (mean value of 0.083 µg/g dwt.), average Mn concentrations ranged between 0.136 and 3.630 µg/g (mean value of 0.259 µg/g dwt.), average Fe concentrations ranged between 23.146 and 60.748 µg/g (mean value 33.681 µg/g dwt.), average Co concentrations ranged between 0.007 and 0.104 µg/g (mean value of 0.046 µg/g dwt.), average Ni concentrations ranged between 0.002 and 0.101 µg/g (mean value of 0.052 µg/g dwt.), average Cu concentrations ranged between 2.264-4.546 µg/g (mean value of 2.721 µg/g dwt.), average Zn concentrations ranged between 19.636- 69.106 µg/g (mean value of 25.868 µg/g dwt.), and average As concentrations ranged between 1.684-5.651µg/g (mean of 3.686 µg/g dwt.).

As shown in **Appendix A**, metal concentrations varied among the different studied fish samples. For essential metals (i.e., Ca, Cu, Fe, and Zn), the concentrations of Ca were the highest ranged between 206.60 and 9883.31 µg/g dwt., followed by those of metal Fe ranged between 6.6and 53.5 µg/g dwt., whereas, the lowest were those of metal Cu ranged between 0.8 and 1.7µg/g dry wt. Results in canned fish revealed that the highest essential metal concentrations were those of Ca ranged between 163.4 and 11510.4 µg/g dwt., followed by those of Zn ranged between 19.6 and 69.1 µg/g dwt., while, the lowest concentrations were those of Cu ranged between 2.3 and 4.5 µg/g dwt. For the non-essential metals (i.e., As, Cd, Pb, and Hg), the highest concentrations in the fish tissue samples were those of As ranged between 0.03 and 54.27 µg/g dwt. followed by those of Hg ranged between 0.0017 and 0.1780 µg/g dwt. while, the lowest concentrations were those of Pb ranged between 0.0171 and 0.0717 µg/g dwt. In the canned fish samples, the highest non-essential metal concentrations were those of As ranged between 1.68 and 5.65 µg/g dwt., followed by those of Hg ranged between 0.0011 and 0.2040 µg/g dwt., whereas, the lowest concentrations were those of Pb as well.

3.2 Metal concentrations in different fish samples

Average concentrations (expressed in µg/g dwt.) of four essential metals (Ca, Cu, Fe, and Zn), three non-essential metals, and one metalloid (Cd, Hg, Pb, and As) in the 13 studied fish species are shown in Table 3.2 (a) and Figure 3.2.1 (a-d) and Figure 3.2.2 (a-d), respectively. The lowest As, and Cd concentrations were those measured in Seabass and Malleta fish samples, respectively (0.029 and 0.002 µg/g dwt.); the lowest levels of Hg and Pb were found in Lokus fish (0.002 and 0.017 µg/g dwt., respectively). The lowest levels of Zn, and Ca were found in Salmon fish (11.1 and 206.6 µg/g dwt., respectively); and the lowest levels of Cu, and Fe were found in Cat fish (0.8 and 6.6 µg/g dwt., respectively). The highest concentrations of As and Cd were detected in Marjan and Mesht fish samples, respectively (54.267 and 0.109µg/g dwt); the highest concentrations of Hg and Pb were found in Marjan and Mesht fish, respectively (0.178 and 0.072 µg/g dwt.); the highest concentrations of Zn, and Ca were found in Bakala and Mesht fish, respectively (24.3 and

9883.3 µg/g dwt) and the highest concentrations of Cu and Fe were measured in Sultan Ibrahem and Mesht fish samples, respectively (1.7 and 53.5 µg/g dwt)

Average concentrations (expressed in µg/g dwt.) of four essential metals (Ca, Cu, Fe, and Zn), three non-essential metals, and one metalloid (Cd, Hg, Pb, and As) in the 8 studied canned-fish samples are shown in Table 3.2 (b) and Figure 3.2.1 (a′-d′) and Figure 3.2.2 (a′-d′) respectively. The lowest levels of As, and Cd were found in Al warda Al hamra, and Americana canned-sardine, respectively (1.684 and 0.018 µg/g dwt.); the lowest levels of Hg, and Pb were found in Americana canned-sardine, and in Fatafet canned-tuna fish, respectively (0.001 and .022 µg/g dwt.); the lowest levels of Zn, and Ca were found in Americana canned-sardine, and in Arizona canned-tuna fish samples, respectively (19.6 and 163.4 µg/g dwt.); and the lowest levels of Cu and Fe were found in Americana canned-fish (2.3 and 23.6 , respectively µg/g dwt.). The highest concentrations of As, and Cd were detected in Lazeza canned-tuna, and Al warda Al hamra canned-sardine samples, respectively (5.651 and 0.120 in µg/g dwt.); the highest concentrations of Hg, and Pb were detected in Arizona canned-tuna and in Americana canned-sardine, respectively (0.204 and 0.748 µg/g dwt.); and the highest concentrations of Zn, Ca, Cu, and Fe were detected in Al warda Al hamra canned-sardine (69.1, 11510.4, 4.5 and 60.7 respectively µg/g dwt.).

3.2.1 Essential Metals: Comparison With Similar Studies

Essential metals, such as Ca, Fe, Cu and Zn, generally accumulate in bio-organisms higher than non-essential metals, such as As, Hg, Cd, and Pb. As mentioned earlier, the average concentrations of Cu in the 13 analyzed fish species are shown in Table 3.2 (a) and Figure 3.2.1 (a) as well. The concentrations of Cu measured in the selected fish species in the study described herein were almost the same compared to the Cu concentrations in fish species collected from the Northern East Mediterranean Sea (Turkey) as reported by Turkmen et al., average Cu concentrations ranged from 1.2 to2.2 mg/kg (n = 3).[101] On the other hand, Our Cu concentrations in the fish species were lower than those reported by Al Weher et al., [38] for fish samples collected from neighboring geographical region, the concentration of Cu was measured in three fish species collected from Wadi El- Arab, Jordan, Cu concentrations ranged from 2.42 to 3.04 mg/kg dwt. Similarly, Aytekin et al. (2019) reported Cu values ranging from 19.35 to 34.23µg/g dwt. for various tissues of Penaeus semiculatus collected from the coast of Iskenderun Gulf (Turkey).[102] The Cu concentrations reported by Aytekin et al. were higher than those measured in this study. Moreover, Cu concentrations in the fish species of our study were lower than the maximum permitted Concentration (MPC) for fish, as suggested by WHO (30 mg/kg).[103]

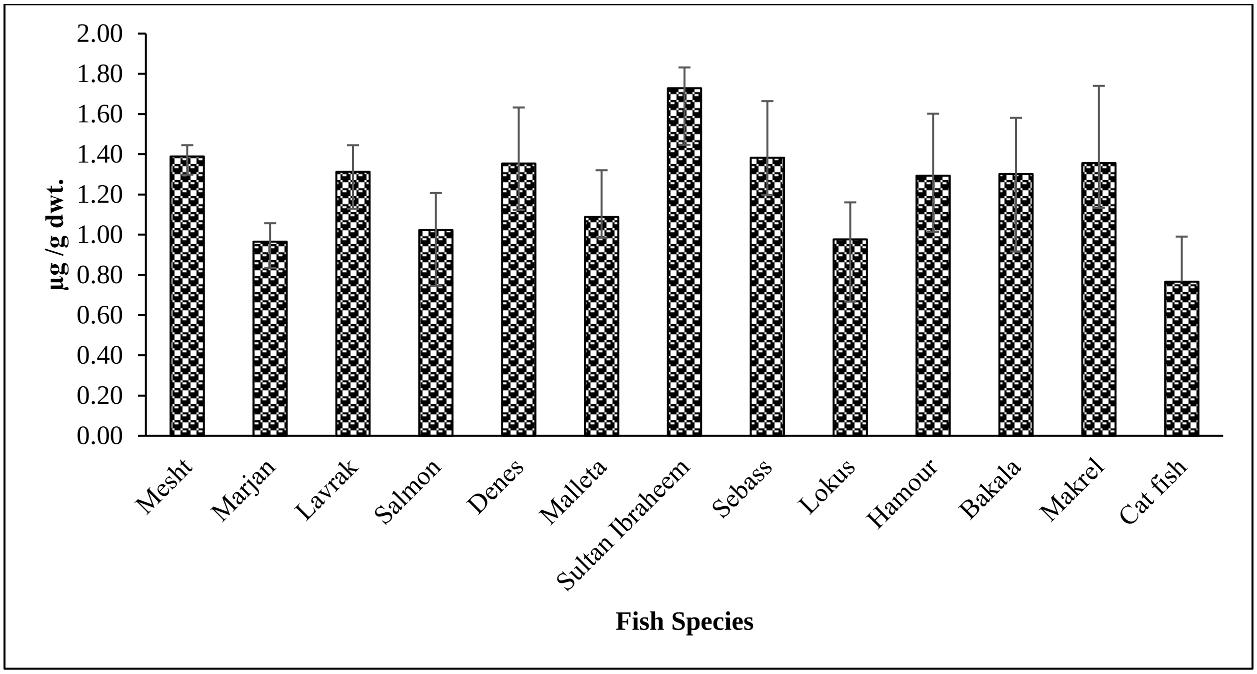

Figure 3.2.1 (a): Cu in Several Fish Species (µg /g dwt.)

Cu concentrations measured in the canned fish samples (average in µg/g dwt. ± %95 CI) are shown in Table 3.2 (b) and Figure 3.2.1 (a′) as mentioned earlier, the concentrations of Cu in our canned-fish samples were much higher than those reported by Tuzen and Soylak (2007) for four canned-fish samples collected from local markets in Turkey, where, Cu concentrations ranged from 1.10 to 2.50 µg/g.[104] , Cu concentrations measured in canned-fish of this study were higher as well compared to those reported by Korfali and Abu Hamdan (2013) for 8 different canned tuna fish, and 6 different sardine canned fish samples collected from local Lebanese markets, where Cu concentrations ranged from 0.20 to 1.60 mg/kg.[105] However, it should be noted that Cu concentrations that we measured in canned fish did not exceed the proposed limit of the WHO (30 mg/kg).[103]

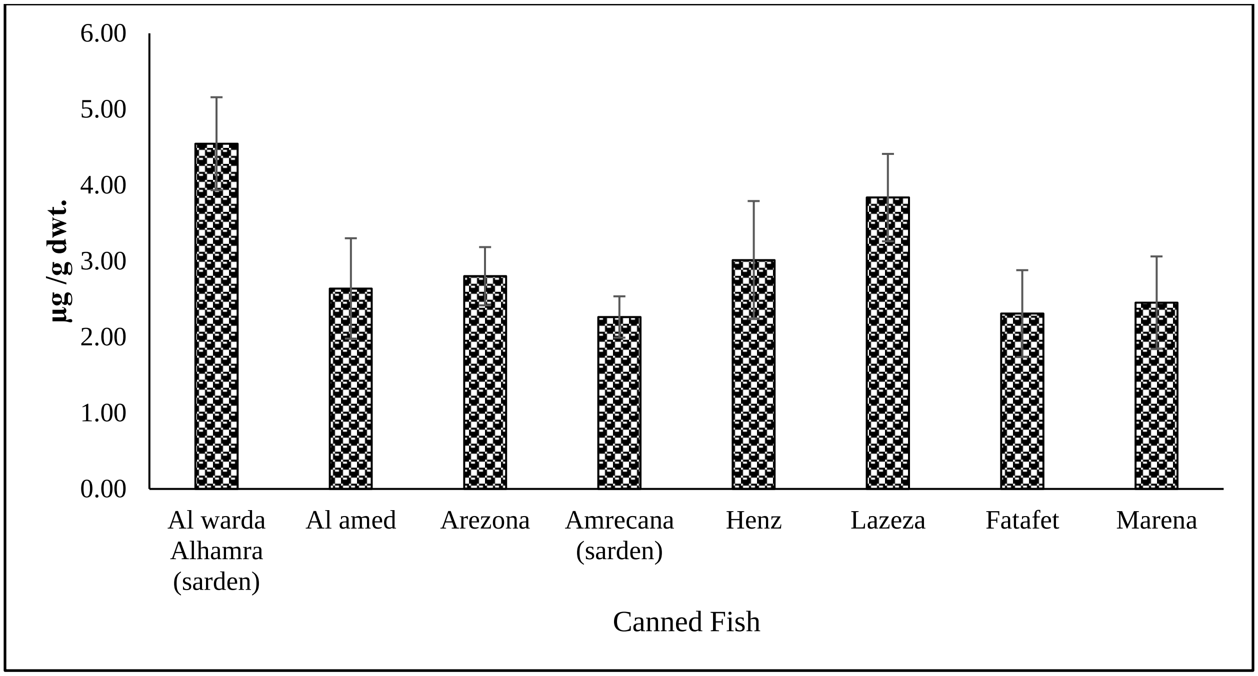

Figure 3.2.1 (a′) Cu in Several Canned Fish (µg /g dwt.)

Zn concentrations in the fish samples of this study are shown in Table 3.2 (a) and Figure 3.2.1 (b). They were slightly lower than those found in similar fish samples collected from Bonny River in Nigeria (ranged from 14.00 to 49.90 µg/g dwt., n = 8) as reported by Abrashi et al. (2017).[106] But, they were lower than those found in in various tissues of Penaeus semiculatus fish marketed in Turkey (ranged from 37.43 to 61.42 µg/g dwt.) as reported by Tuzun Aytekin et al. (2019).[102] Our reported Zn values in fish were also lower than those reported by Amani and Lamia (2012) in a similar study conducted on three different fish species collected from local markets in Saudi Arabia (ranged from 16.79 to 49.43 µg/g dwt.).[107] According to the Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO) regulations, the maximum permissible amount of Zn in fish for human consumption is 30 µg/g dwt.,[108] Zn concentrations found in all our fish tissue samples did not exceed the proposed limit of the FAO, and WHO.

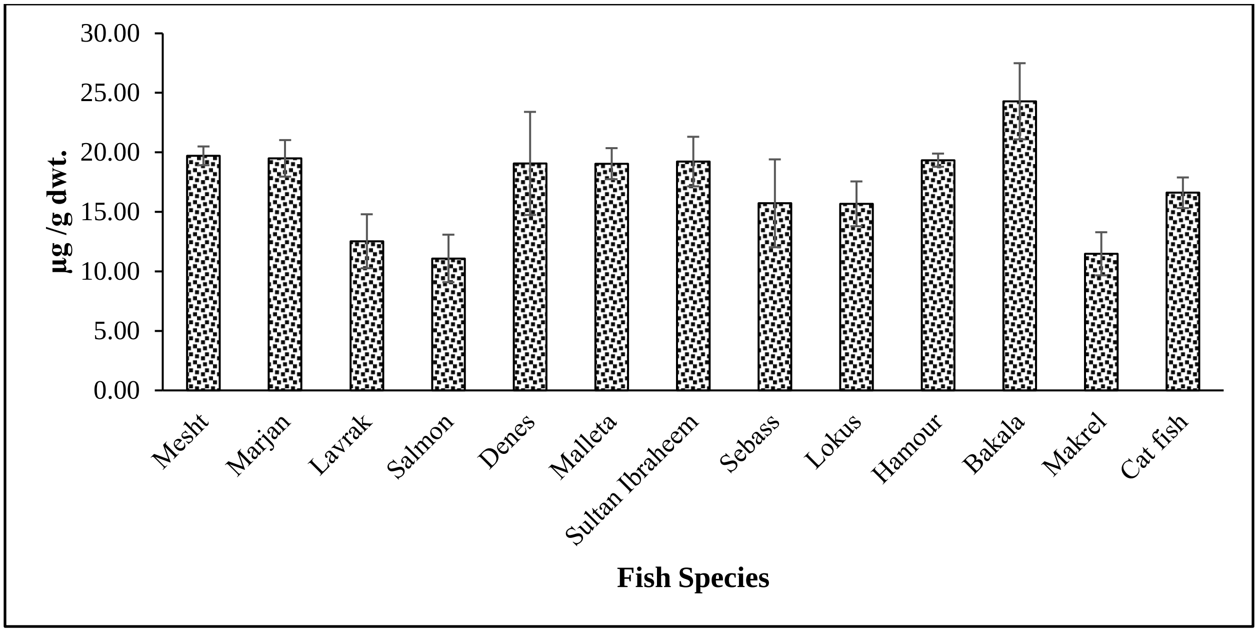

Figure 3.2.1 (b): Zn in Several Fish Species (µg /g dwt.)

Zn concentrations in canned-fish obtained in this study are shown in Table 3.2 (b) and Figure 3.2.1 (b′). They were slightly higher than those found in selected canned-fish samples in Lebanese market (ranged from 2.68- to 11.59 µg/g, n = 102);[109] but, they were higher than those found in 30 canned-fish samples in Iranian markets (Ranged from 4.05 to 36.22 mg/kg, n = 30).[110] Our Zn values in canned-fish samples were higher than those reported by Korfali1 and Abou Hamdan (2013) for 8 different canned-tuna brands and 6 different canned-sardine brands, where Zn concentrations ranged from 4.00 to14.00 µg/g (n = 14).[105] It is important to mention that Zn concentrations in Al warda Al hamra (canned-sardine) and Heinz canned-tuna fish exceeded the guideline value suggested by FAO, and WHO.[108] It should be noted that chronic exposure to Zn might result in Parkinson disease as suggested by Hossain et al.[111]

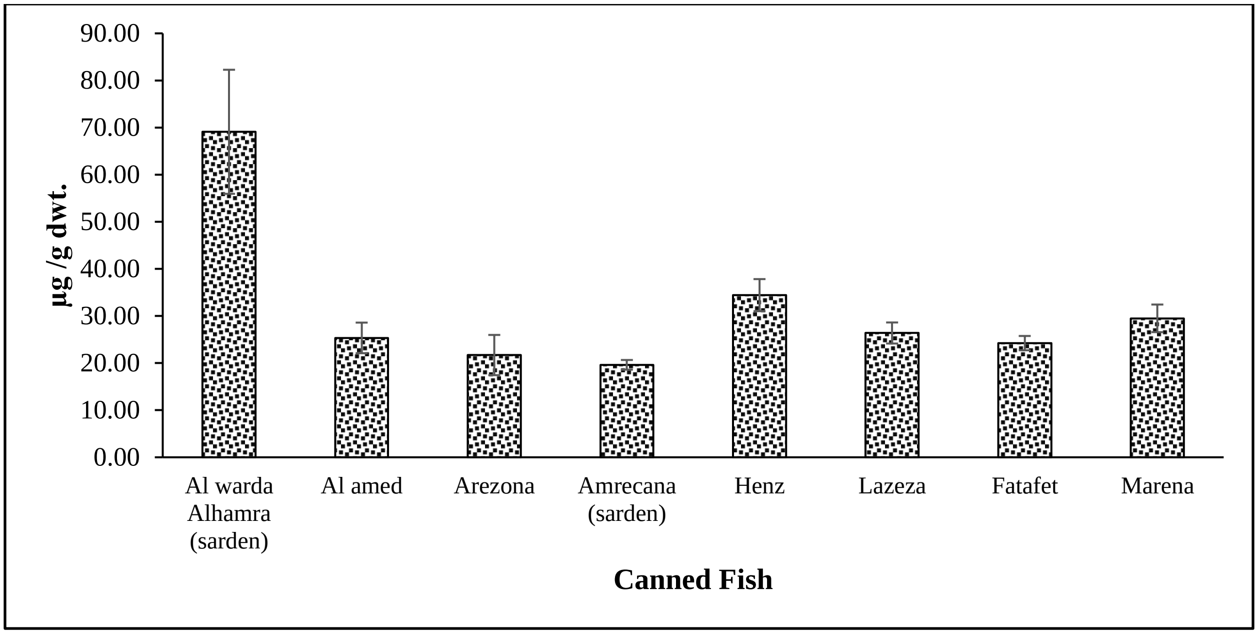

Figure 3.2.1 (b′): Zn in Several Canned Fish (µg /g dwt.)

Concentrations of Fe in the 13 fish species are shown in Table 3.2 (a) and Figure 3.2.1 (c) as stated earlier. They were in the following decreasing order: Hamoure> Sultan Ibrahem> Makrel> Marjan >Salmon> Malleta> Mesht >Seabass>Lokus>Denes>Lavrak>Bakala> Cat fish, with the highest concentration was that measured in Hamour fish (average of 53.530 with a 95% CI of 9.098 µg/g dwt., n = 3), while, the lowest concentration was that measured in Cat fish (average of 6.599 with a 95% CI of 1.589 µg/g dwt., n = 3). It worth mentioning that the maximum permissible concentration (MPC) of Fe is 333.3 µg/g dwt. according to the US FAO/ and the WHO regulations.[112] In the present study, Fe concentrations in all studied fish species, were below the MPC of the US FDA and the WHO.[112] Aytekin et al. (2019)[102] reported Fe concentrations ranging from 16.15 to 24.23 µg/g dwt. in a selected of "highly consumed" locally-farmed fish species in Turkey. Fe concentrations obtained in the 13 fish samples were comparable to those reported by Aytekin et al. (2019).[102] But, they were lower than the Fe concentrations reported by Younis et al. (2021) for a number of fish species collected from Jeddah coast in Saudi Arabia, where Fe concentrations ranged from 81.60 to 188.60 mg/g dwt.[113]

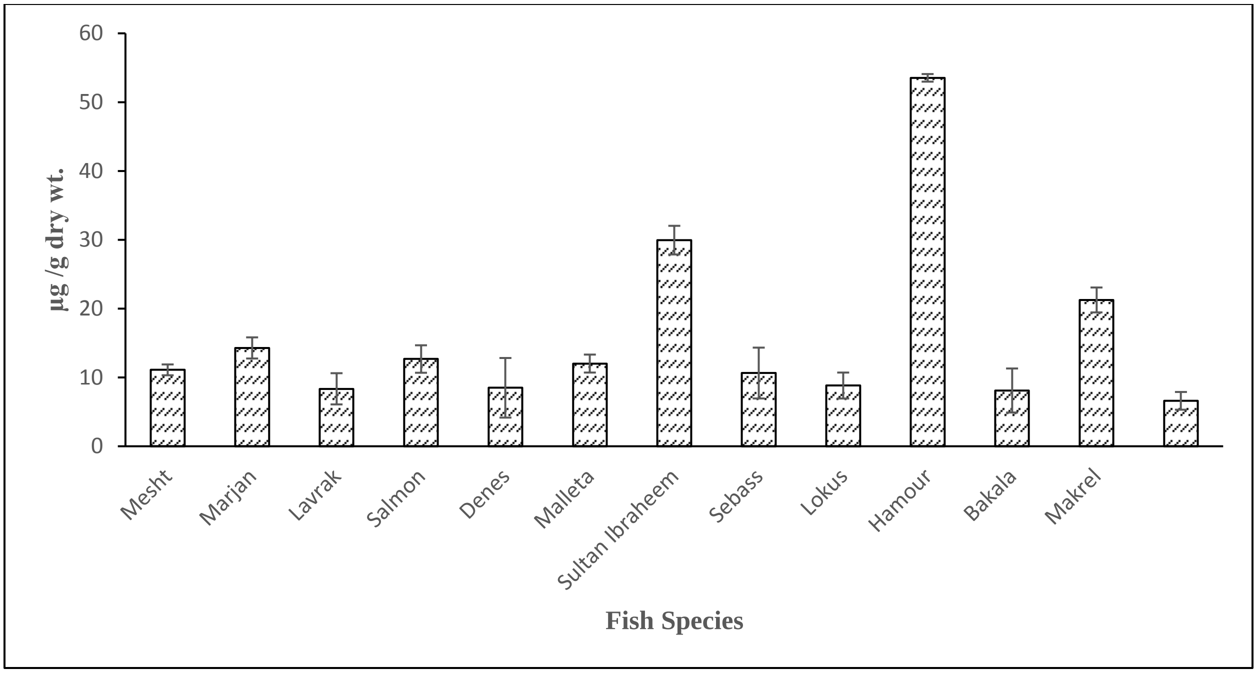

Figure 3.2.1 (c): Fe in Several Fish Species (µg /g dwt.)

Fe concentrations measured in the 8 canned-fish samples Table 3.2 (b) and Figure 3.2.1 (c′) were in the following decreasing order Al warda Al hamra> Marina>Arizona> Heinz> Fatafet> Al Ameed> Lazeza> Americana. Fe concentrations did not exceed the maximum permissible concentration (MPC) of the US FDA and the WHO (333.3 µg/g dwt.). [112] Moreover, Fe concentrations in the canned-fish samples were higher than those reported by Tuzen and Soylak,[104] where Fe concentrations ranged from 10.2 to 30.3 µg/g (n=4) in canned fish samples collected from public markets in Turkey. Fe concentrations in the canned-fish samples under investigation were also higher than those obtained by Korfali and Abou Hamdan (2013), where Fe concentrations measured in 14 "commercially popular" canned-fish samples (manufactured from locally farmed fish) ranged from 3.0 to 21.0 µg/g dwt. in Lebanon.[105]

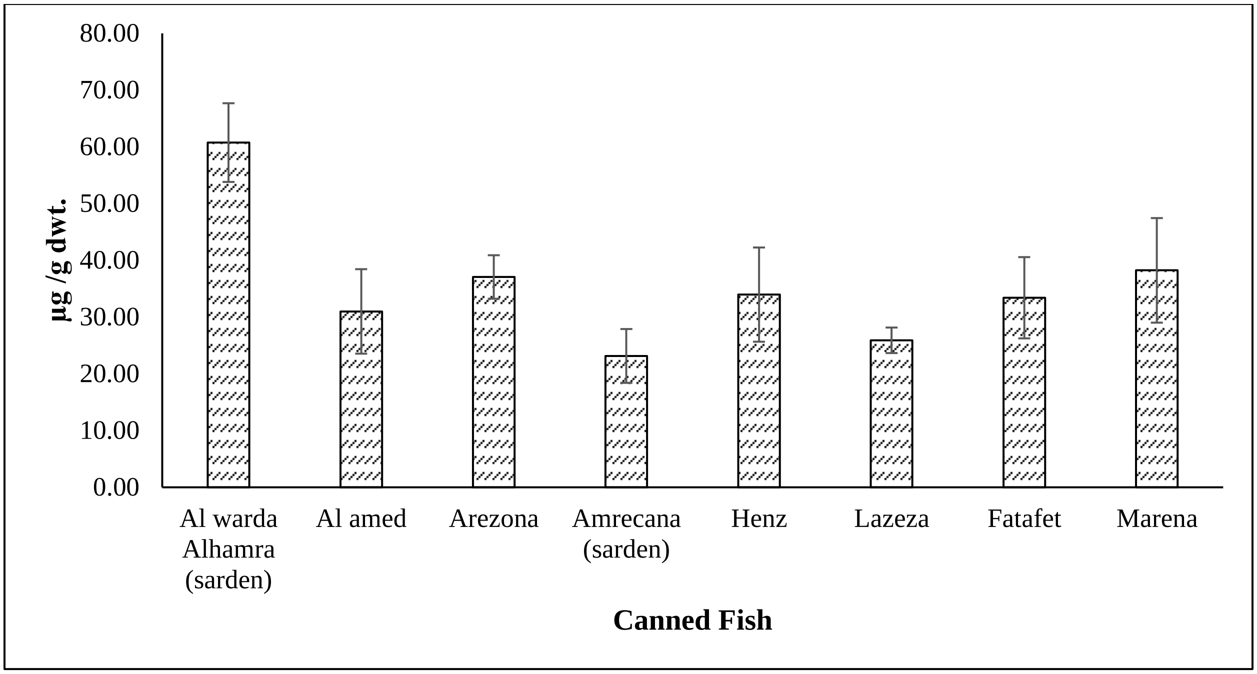

Figure 3.2.1 (c′): Fe in Several Canned Fish (µg /g dwt.)

Ca concentrations in the 13 fish species are shown in Table 3.2 (a) and Figure 3.2.1 (d), as stated earlier, and decreased among the studied species in the following order: Mesht> Bakala> Marjan > Malleta > Sultan Ibrahem > Denes > Makrel > Hamour > Lokus > Seabass > Cat fish > Lavrak > Salmon.

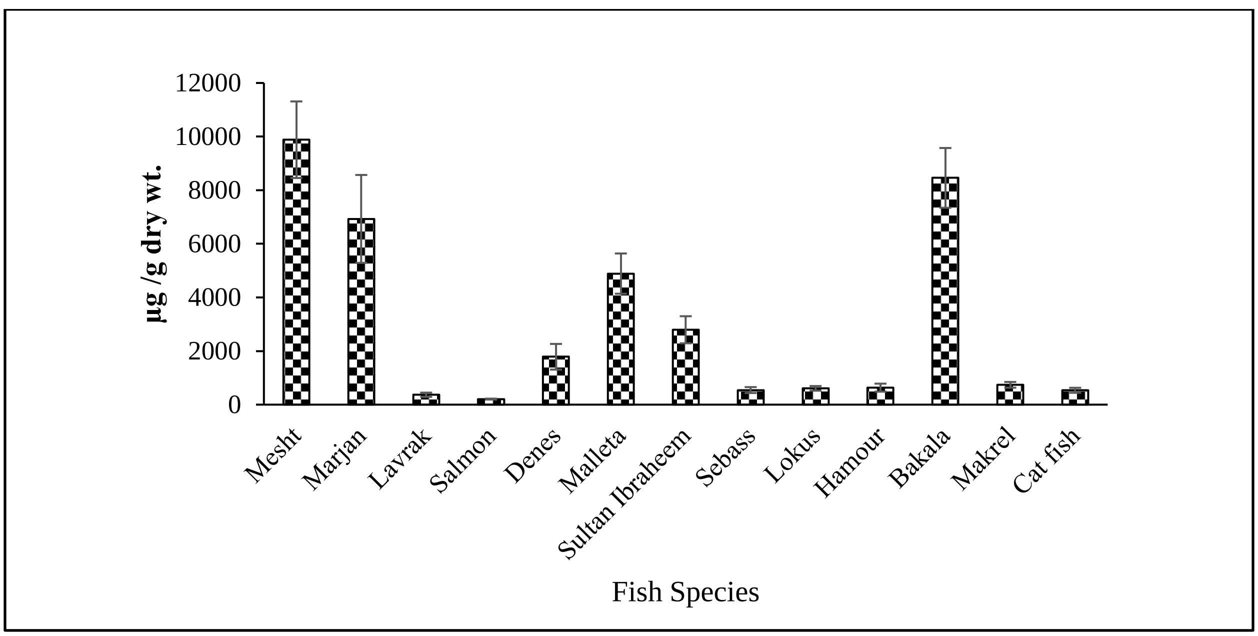

Figure 3.2.1 (d): Ca in Several Fish Species (µg /g dwt.)

In canned fish samples, the trend of Ca concentrations was as follows: Al Warda Al hamra > Americana > Heinz > Marena > Lazeza > Al amed > Fatafet > Arizona.

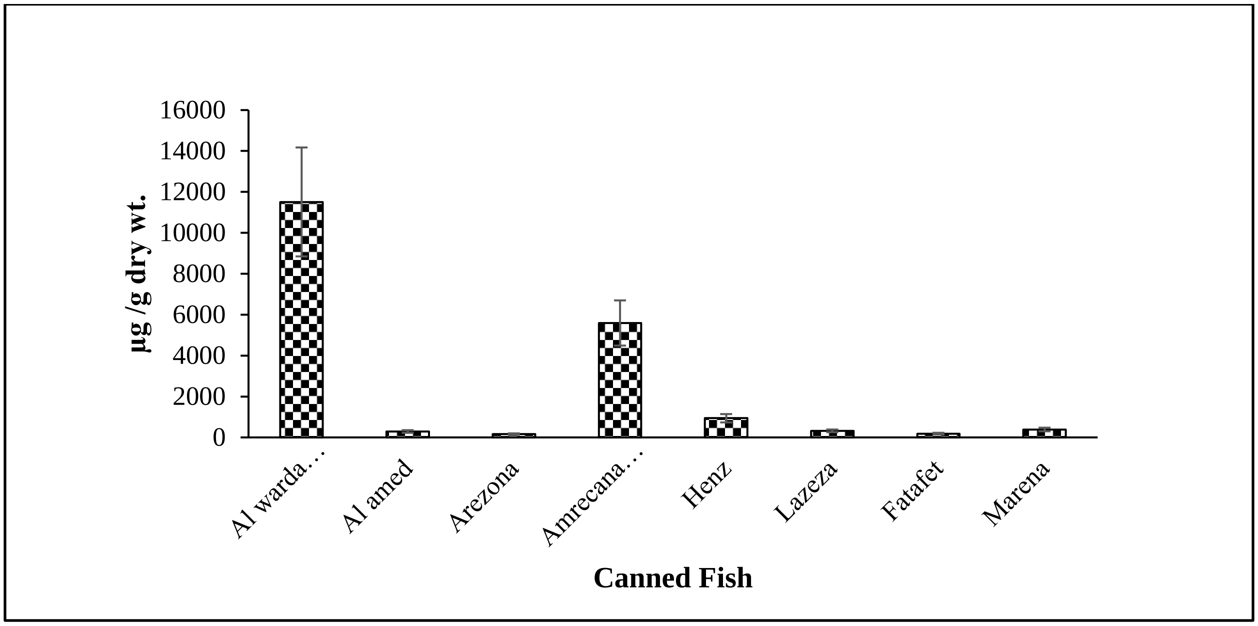

Figure 3.2.1 (d′): Ca in Several Canned Fish (µg /g dwt.)

3.2.2 Non-Essential Metals: Comparison With Similar Studies

The concentrations of lead in the tested fish samples decreased among the studied species in the following order: Mesht> Hamour> Sultan Ibrahem> Cat fish>Bakala> Marjan> Salmon> Malleta> Lavrak> Denese> Seabass> Makrel> Lokus (Table 3.2 (a) and Figure 3.2.2 (a)). The concentrations of Pb in fish samples of this study were lower than those reported by Elsayed et al. (2021)[113] where Pb concentrations ranged from 0.7 to 0.8 mg/kg dry wt.; lower than those reported by Aytekin et al (2019), where Pb concentrations ranged from 22.18 to 62.75 µg/g dwt. in different tissues of Penaeus semiculatus collected from the coast of Iskenderun Gulf (Turkey);[102] and lower than those reported by Abarshi et al. (2017)where Pb concentration ranged from 0.20 to 0.50 µg/g dwt. in some organs of fish sample collected from Bonny River, Nigeria.[106] It is worth noting that our Pb values in fish were lower than the permissible limit of Pb set by European Union (0.3 mg/kg).[114]

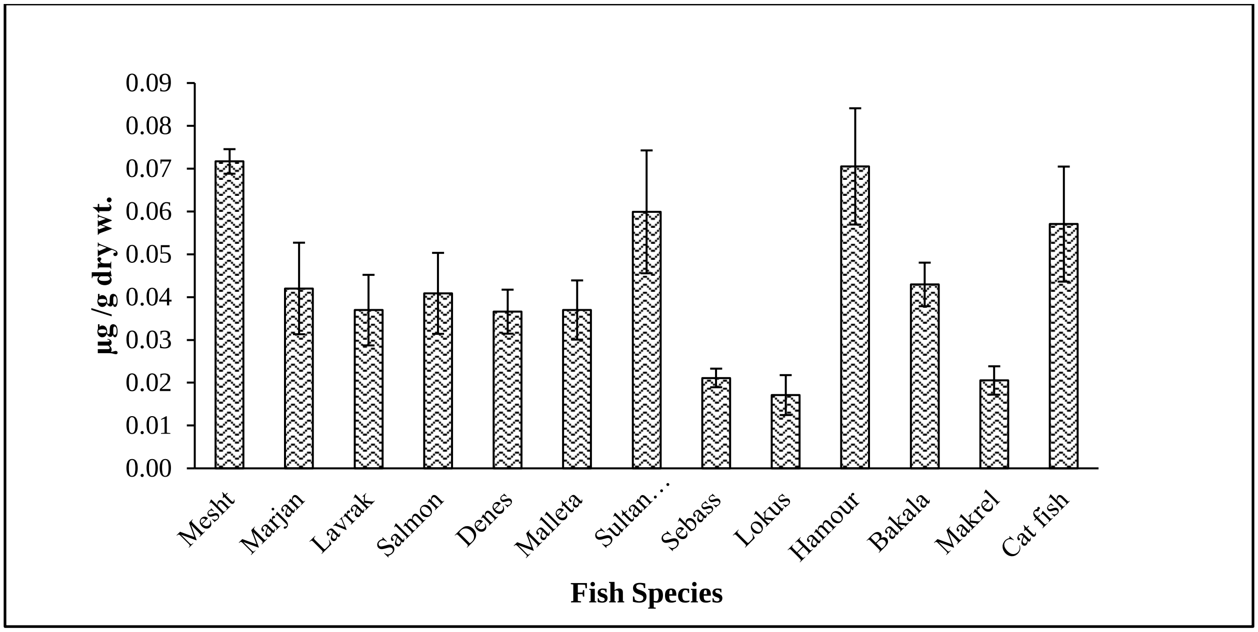

Figure 3.2.2 (a): Pb in Several Fish Species (µg /g dry wt.)

Metals concentrations in canned fish samples are shown in Table 3.2 (b)and Figure 3.2.2 (a′) as stated earlier. Concentrations of Pb in the tested canned-fish samples exhibited the following decreasing order: Americana> Al warda Al hamra> Al amed> Arizona> Heinz> Marena> Lazeza> Fatafet. Upon comparing the concentrations of Pb in canned-fish samples of this study with Pb concentrations reported in similar studies conducted in neighboring countries, the following patterns were observed: (i) the concentration of Pb measured in this study (ranged from 0.022 to 0.075 µg/g dwt.) were relatively lower than those reported by Korfali and Abo Hamdan (2013) for a similar study conducted in Lebanon, where Pb concentrations in canned fish samples ranged from 0.007 to 0.187 µg/g dwt.;[105] (ii) In a another similar study conducted in Iran, Hosseini et al. (2015) reported Pb concentrations ranging from 0.02 to 5.5 mg/kg dwt. for 30 different canned-fish samples,[110] Pb concentrations measured in our study were also lower than those reported by Hosseini et al. (2015); [110] and (iii) Pb concentrations found in the tested canned-fish of this study were below the threshold limit of the European Union (0.3 mg/kg).[114]

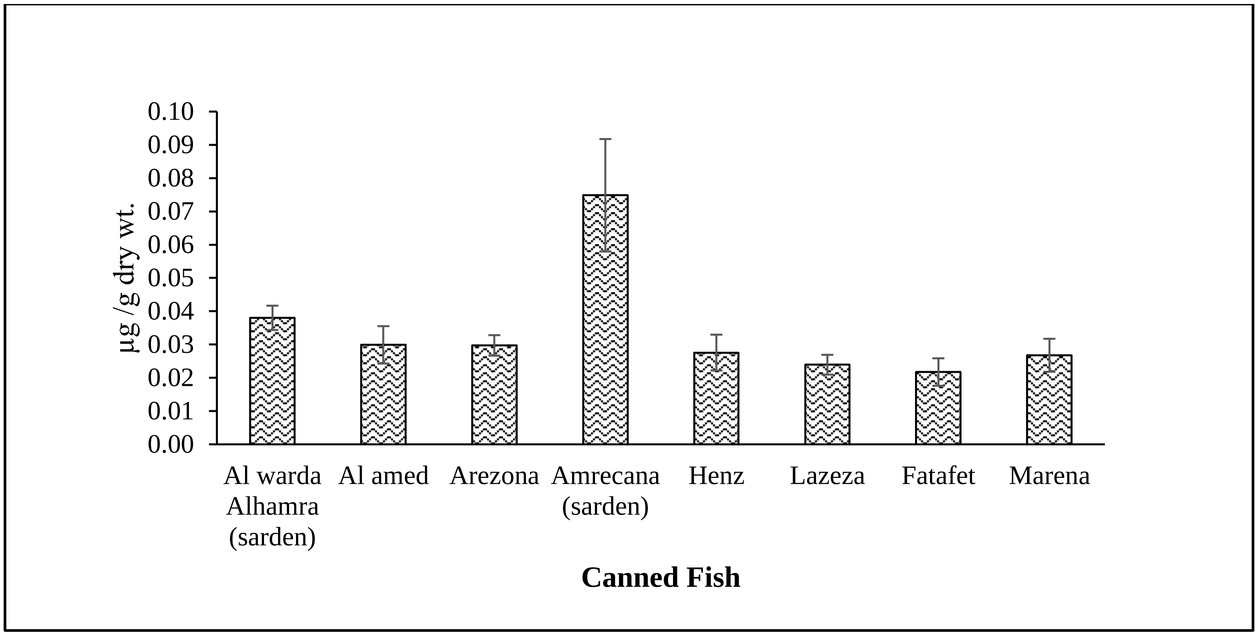

Figure 3.2.2 (a′): Pb in Several Canned Fish (µg /g dry wt.)

As far as As concentrations in the 13 fish tissue samples are concerned, as shown in Table 3.2 (a) Figure 3.2.2(b), As concentrations varied among studied fish species (ranged from 0.03 to 54.27, with a median of 0.8929 µg/g dwt.), and exhibited the following decreasing pattern: Marjan> Sultan Ibraheem> Malleta> Makrel> Bakala> Lavrak> Denese>Salmon> Lokus> Cat fish> Mesht> Hamour> Seabass. Upon comparing our As results measured in fish samples with those of similar studies or with permissible As concentrations described by some international agencies, the following points were observed: (i) As concentrations that was obtained in the fish samples of our study were much higher than those reported by Belivermis et al. (2016) in a study conducted in Turkey, their As concentrations ranged from 5.3 to 18.3 mg/kg dwt. (mean of 9.3 mg/kg dwt., n = 20);[115] (ii) California- Environmental Protection Agency (C-EPA) set up 1.0 mg/kg dwt.as the maximum permissible concentration (MPC) for As in edible fish, thus, As levels in 6 fish out of the 13 tested fish samples exceeded the MPC of California state;[116] and (iii) As contents obtained in 6 fish out of the 13 tested fish samples also exceeded the MPC of As of the US FAO (1.0 mg/kg dwt.).[117] It should also be noted that some workers suggested that long-term exposure to As could cause cancer and skin lesions;[119] or could lead to cardiovascular disease and diabetes.[119] Moreover, some workers also reported that in utero and early childhood exposure to As was linked to negative impacts on cognitive development and increased deaths in young adults.[119]

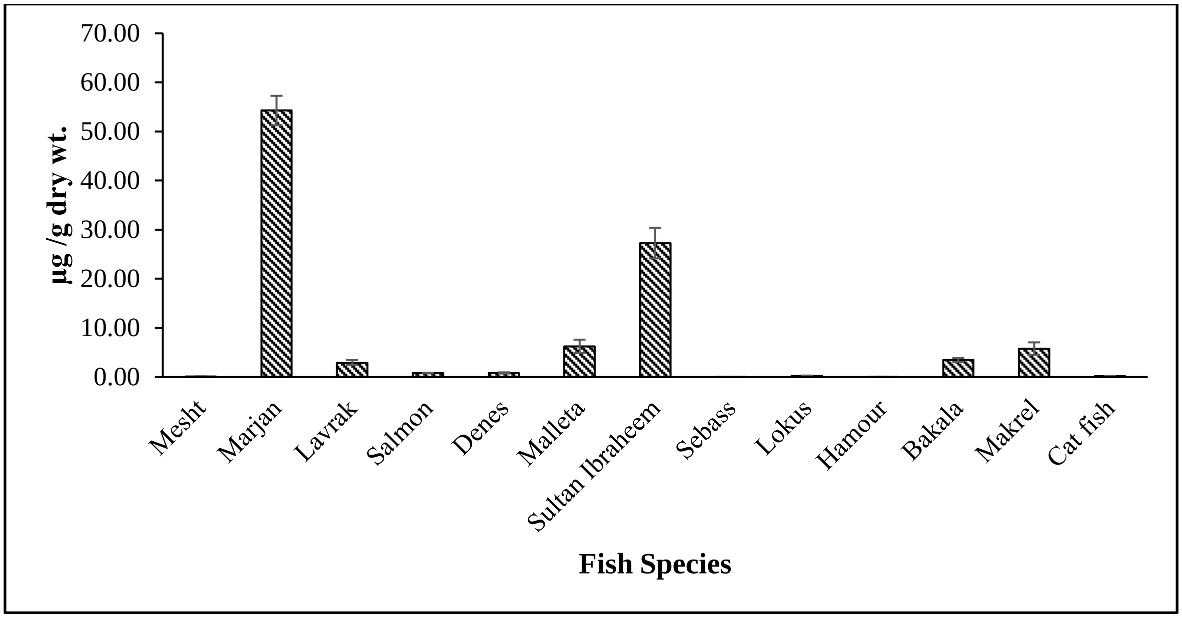

Figure 3.2.2 (b): As in Several Fish Species (µg /g dry wt.)

As concentrations in canned fish samples are shown in Table 3.2 (b) and Figure 3.2.2 (b′), as stated earlier. Concentrations of As in the tested canned-fish samples ranged from 1.68 to 5.65. It should be noted that these concentrations exceeded the MPC of As described by C-EPA and US FAO (1.0 mg/kg dwt.).

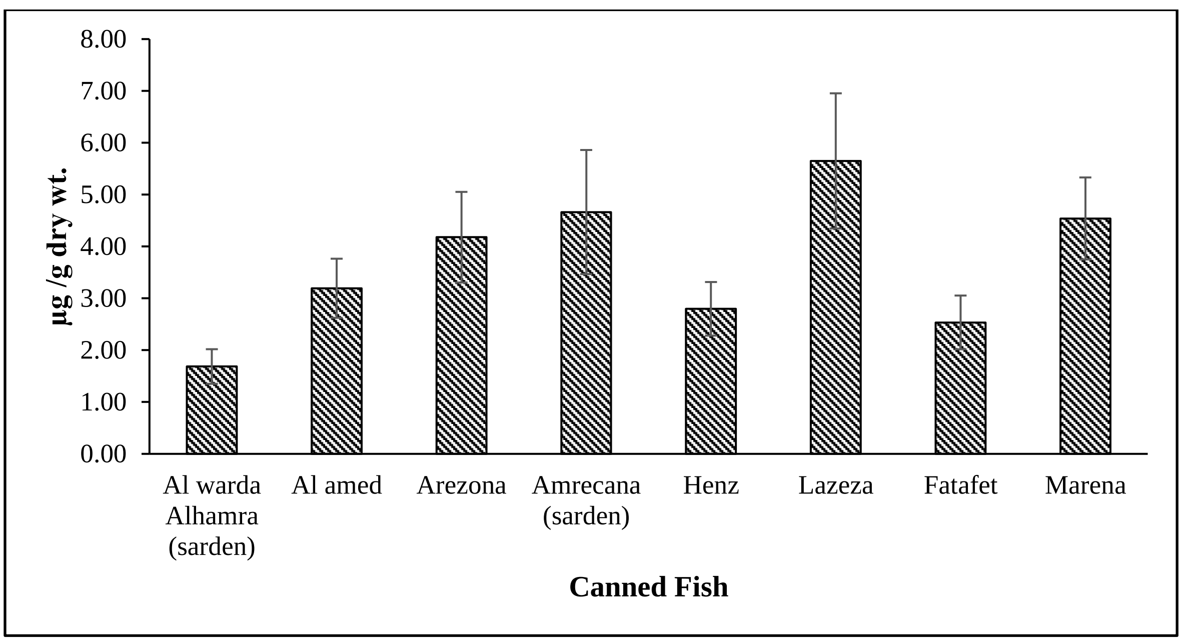

Figure 3.2.2 (b′) As in Several Canned Fish (µg /g dry wt.)

For Cd measured in the studied fish samples, concentrations ranged from 0.002 to 0.109 µg/g dwt. (median of 0.051 µg/g dwt., n = 13, Table 3.2 (a) Figure 3.2.2 (c), and exhibited the following decreasing order among the 13 tested species: Mesht> Makrel> Bakala> Cat fish> Denes> Seabass> Salmon> Marjan> Lavrak> Hamour> Lokus> Sultan Ibrahem> Malleta :, The maximum permissible concentration of Cd is 0.5 mg /kg as described by the EU,[114] and FAO/WHO.[108] In the present study, the concentrations of Cd detected in all 13 fish samples were below the threshold limit of the EU, USFDA, and FAO/WHO. Two similar studies conducted in Saudi Arabi on similar fish species, the first was conducted by Amani and Lamia (2011), their reported Cd concentrations ranged from 1.17 to 4.25 µg/g dwt.;[107] while, the second was conducted by Elsayed et al. (2021), they reported Cd concentrations ranging from 3.00 to 5.10 mg/kg dwt. for five different fish species collected from Jeddah coast.[113] The Cd concentrations obtained for all 13 fish samples under investigation were lower compared to both, those reported by Amani and Lamia, and those reported by Elsayed et al.

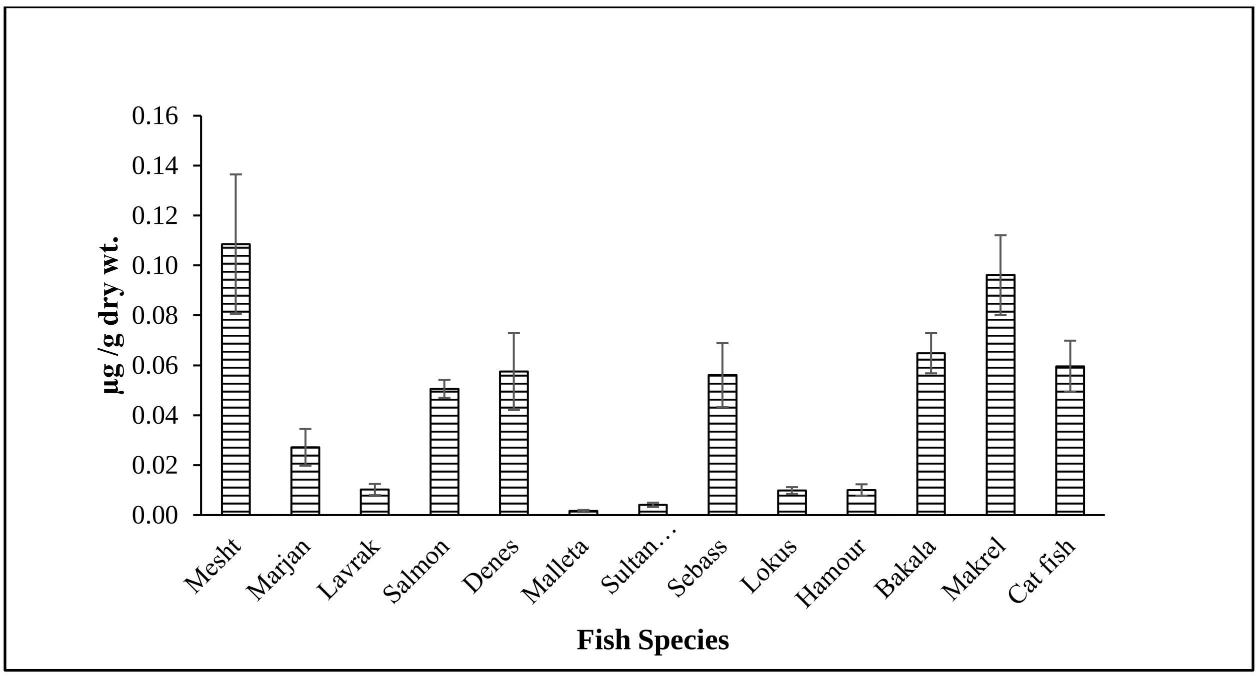

Figure 3.2.2 (c) Cd in Several Fish Species (µg /g dry wt.)

In canned-fish, Cd concentration ranged from 0.0179 to 0.120 µg/g dwt. (with a median of 0.047 µg/g dry wt., n = 8), and exhibited the following decreasing pattern among the tested canned-fish samples: Al warda Al hamra> Lazeza> Heinz> Marina> Fatafet> Al amed> Arizona> Americana (Table 3.2 (b) and Figure 3.2.2 (c′)).Upon comparing Cd concentrations of this study with those obtained by two separate studies conducted on similar canned-fish samples collected from, Lebanese markets (Cd concentrations ranged from 0.021-0.645 µg/g dwt., n = 14),[105] and Iranian markets (Cd concentrations ranged from 0.00-0.37 µg/g dwt., n = 30),[110] we observed that our Cd concentrations were “generally” lower than those reported by both studies. in canned fish.

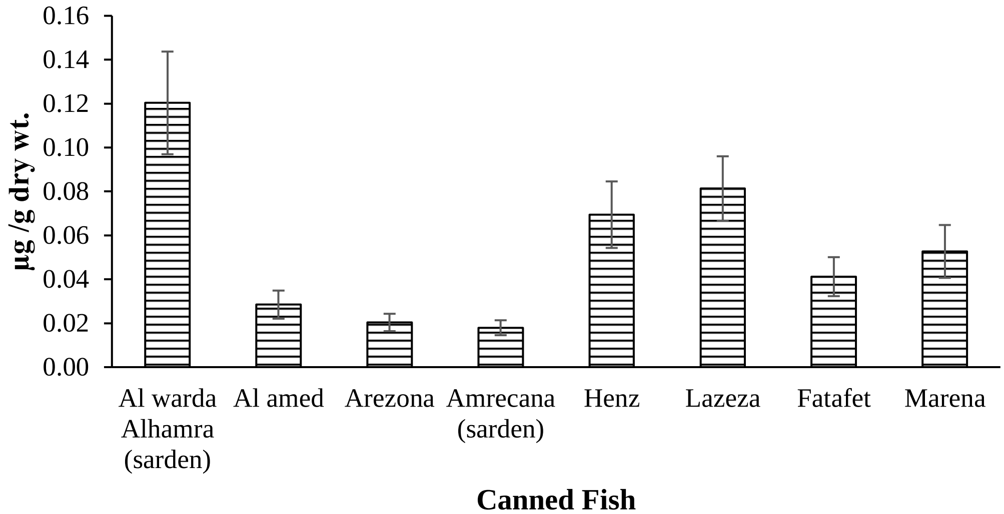

Figure 3.2.2 (c′) Cd in Several Canned Fish (µg /g dry wt.)

As far as Hg concentrations in the 13 fish species under investigation is concerned, it ranged from 0.0017 to 0.178 (with a median of 0.042 µg/g dwt., n = 13, Table 3.2 (a) and Figure 3.2.2 (d)). Moreover, Hg concentrations among all species, exhibited the following decreasing order: Marjan> Mesht> Makrel> Denes> Malleta> Lavrak> Hamour> Sultan Ibrahem> Salmon> Bakala> Seabass> Cat fish> Lokus. These concentrations were comparable to those reported by Amani and Lamia (2011), where Hg concentrations measured in farmed-fish samples collected from Saudi Arabi markets, ranged from 0.014 to 0.055 µg/g dwt.[107] It should be mentioned, however, that Hg concentrations in the 13 tested fish samples were below the maximum permissible concentration of Hg (0.5 mg/kg dwt.) as described by both, the US-FAO, and the WHO.[112]

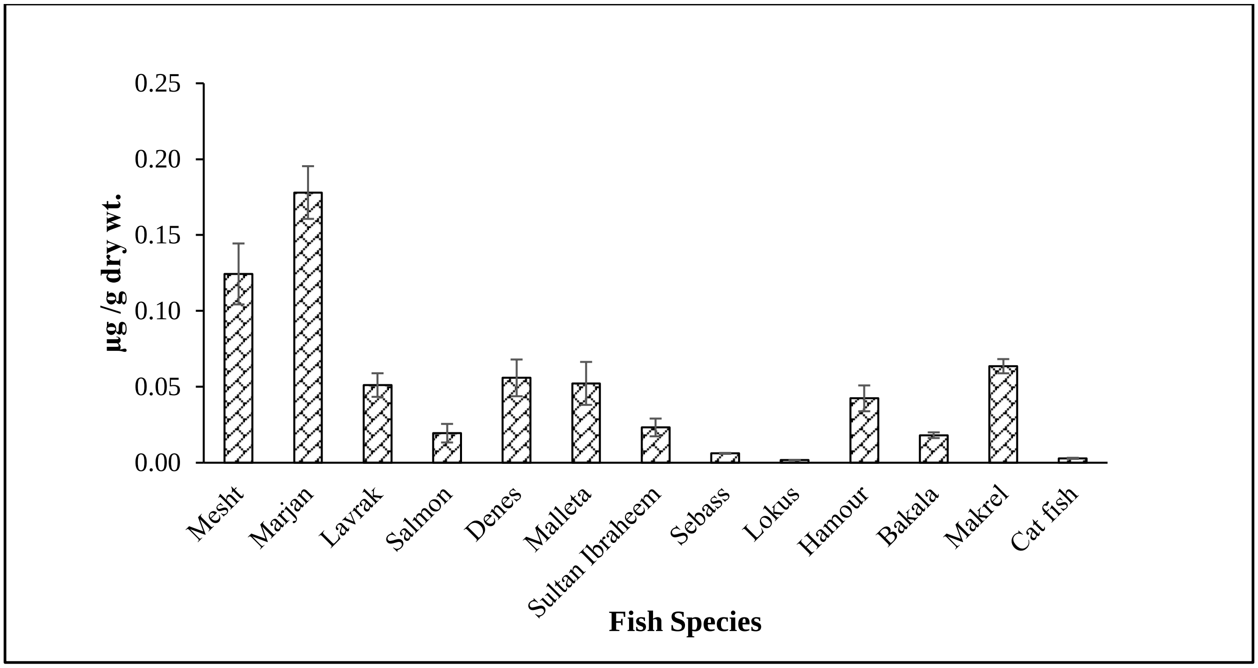

Figure 3.2.2 (d)Hg in Several Fish Species (µg /g dry wt.)

For canned-fish samples, Hg concentrations ranged from 0.001 to 0.204, (with a median of 0.009 µg/g dwt., n = 8, (Table 3.2 (b) and Figure 3.2.2 (d′)), and exhibited the following decreasing order: Arizona> Marina> Heinz> Al amed> Fatafet> Lazeza> Al warda Al hamra> Americana. The concentrations of Hg in our canned-fish samples were relatively lower than those reported in the similar study conducted in Lebanon, which was mentioned earlier (where reported Hg concentrations ranged from 0.025 to 0395 µg/g dwt., n = 14).[105]

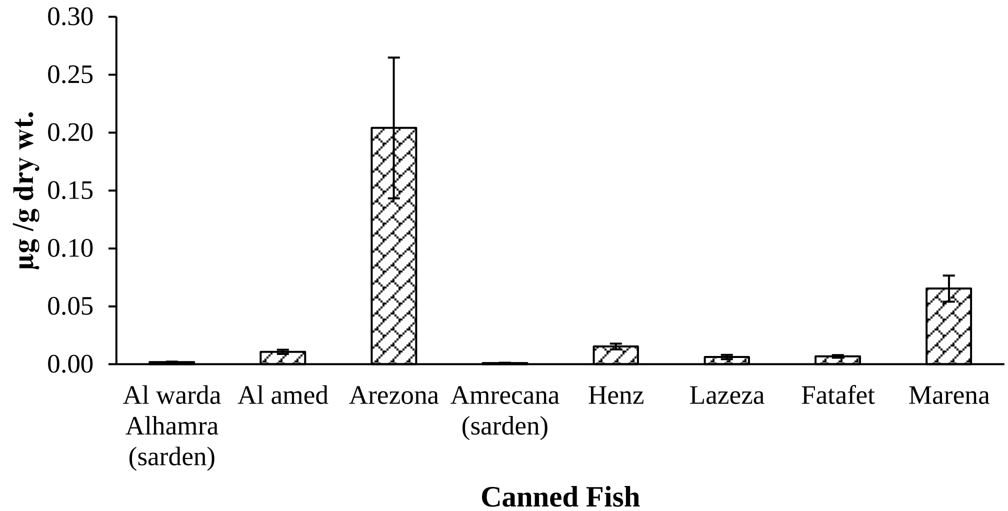

Figure 3.2.2 (d′) Hg in Several Canned Fish (µg /g dry wt.)

Table 3.2 (a) Average concentrations (expressed in µg/g dwt.) of four essential metals (Ca, Cu, Fe, and Zn), three non-essential metals, and one metalloid (Cd, Hg, Pb, and As) in the 13 studied fish species

	Cd	**Hg**	**Pb**	**Ca**	**Fe**	**Cu**	**Zn**	**As**
Mesht	0.11	0.12	0.07	9883.31	11.10	1.39	19.71	0.10
Marjan	0.03	0.18	0.04	6928.59	14.29	0.97	19.50	54.27
Lavrak	0.01	0.05	0.04	368.74	8.35	1.31	12.53	2.92
Salmon	0.05	0.02	0.04	206.60	12.68	1.02	11.09	0.81
Denes	0.06	0.06	0.04	1785.64	8.49	1.35	19.07	0.83
Malleta	0.00	0.05	0.04	4888.35	12.02	1.09	19.05	6.23
Sultan Ibraheem	0.00	0.02	0.06	2793.95	29.95	1.73	19.23	27.27
Sea bass	0.06	0.01	0.02	543.80	10.64	1.38	15.72	0.03
Lokus	0.01	0.00	0.02	606.93	8.82	0.98	15.68	0.23
Hamour	0.01	0.04	0.07	633.58	53.53	1.29	19.35	0.06
Bakala	0.06	0.02	0.04	8459.93	8.10	1.30	24.29	3.48
Makrel	0.10	0.06	0.02	738.77	21.26	1.36	11.48	5.75
Cat fish	0.06	0.00	0.06	534.68	6.60	0.77	16.61	0.19

Table 3.2 (b) Average concentrations (expressed in µg/g dwt.) of four essential metals (Ca, Cu, Fe, and Zn), three non-essential metals, and one metalloid (Cd, Hg, Pb, and As) in the 8 canned fish.

	Cd	Hg	Pb	Ca	Fe	Cu	Zn	As
Al warda Al hamra	0.12	0.00	0.04	11510.43	60.75	4.55	69.11	1.68
Al amed	0.03	0.01	0.03	289.03	30.99	2.64	25.35	3.19
Arizona	0.02	0.20	0.03	163.42	37.06	2.80	21.71	4.18
Americana	0.02	0.00	0.07	5592.89	23.15	2.26	19.64	4.66
Henz	0.07	0.02	0.03	936.99	33.96	3.01	34.42	2.80
Lazeza	0.08	0.01	0.02	321.43	25.90	3.84	26.39	5.65
Fatafet	0.04	0.01	0.02	184.40	33.40	2.31	24.24	2.54
Marena	0.05	0.07	0.03	387.48	38.23	2.45	29.50	4.54

3.2.3 Comparison with Some International Dietary Standards and Guidelines

The mean concentrations of the eight metals measured in the fish samples of the present study, along with maximum permissible concentrations (MCP) described by a number of international organizations such as WHO, EU, and US-FAO are shown in Table 3.2.3.The results revealed that the concentrations of the analyzed metals were lower than the MPC described by all three international agencies except, for As. Estimated daily intake (EDI), target hazard quotient (THQ) and carcinogenic risk (CR) were analyzed to interpret potential effects on public health.

Table 3.2.3 Maximum acceptable levels of heavy metals in fish muscles (mg/kg dwt..) according to international standards.

standards	Cu	Zn	Fe	Pb	As	Cd	Hg	References
This study	1.90	22.55	23.30	0.039	6.26	0.047	0.045	This study
WHO	30		109	0.500			0.5	103
Joint FAO/WHO	30	30	180	2			0.5	108
FAO/WHO limts	30		333.3			0.5	0.5	112
EU				0.3		0.5		114
CEPA					1			116
US FAO					1			117

3.2.4 Comparison With Other Similar Studies

A comparison among the concentrations of metals measured in the fish, and canned-fish samples of the present study, and those reported by similar studies conducted in several countries is shown in Tables 3.2.4 (a) and (b), respectively. The differences in some of the measured metals concentrations upon comparing our results with these studies, as we described and discussed earlier, could be attributed to many reasons, such as, (i) history of contamination of the water, suspended particulate matter (SPM), and sediments, where the tested fish species lived before caught; (ii) possible variations in the age, weight, length, and fat content among fish and canned-fish samples investigated in these studies; (iii) and variations of environmental factors, such as water pH, dissolved oxygen, dissolved carbon, total organic carbon in SPM and sediments, redox potential of sediments, ..etc. Factors that could lead to significant differences in the physical and chemical behaviors of the studied metals, and thus, to their potential uptake, bio-accumulation, and bio-magnification, by the fish species under consideration. It is quite important to state that investigating the variations of these factors to assess their impact on the concentrations of metals in fish, and canned-fish samples were beyond the scope of this study.

Table 3.2.4(a) Comparison of HMs in fish species from Ramallah market with different studies in the world

standards	Cu	Zn	Fe	Pb	As	Cd	Hg	References
West Bank	1.226	17.177	15.832	0.043	7.859	0.043	0.049	This study
Jordan	5.57	43.73				0.13		38
Turkey	27.13	46.88	19.97	39.94		7.22		102
Nigeria	3.5	49.9	216.03	0.2		ND		106
Suadi Arabia	8.72	32.03	114.44	6.99		3.12	0.031	107
Suadi Arabia	12.06		123.54	0.76		3.22		113
Turkey	29.1	172	269	7.9	9.3	1.09		115

Table 3.2.4(b) Comparison of HMs in canned fish from Ramallah market with different studies in the world (in mg/kg dry weight

standards	Cu	Zn	Fe	Pb	As	Cd	Hg	References
West Bank	2.98	31.29	35.43	0.034	3.656	0.054	0.039	This study
Turkey	1.722	16.316	15.12	0.2		0.14		104
Lebanon	0.65	8.43	13.23	0.065		0.21	0.146	105
Iran		11.73		2.33		0.082		110

3.3 Health-Risk Assessment Upon Fish Consumption (Consumption Safety)

3.3.1 Metal Pollution Index (MPI)

Metal Pollution Index (MPI) is used to assess the degree of metals contaminations in fish or canned-fish meat. Equation 1 (Chapter 2) was used to calculate MPI. MPI compares total metals contents of the muscle of the examined fish, or canned fish samples. Calculated MPI values of all tested fish species ranged from 0.022 to 0.324 (median of 0.087, n = 13) as shown in Table 3.3.1 (a), with metals accumulation pattern in the following decreasing order: Marjan> Makrel> Bakala> Sultan Ibrahem> Denes> Mesht> Lavrak> Salmon> Malleta> Cat fish> Hamour> Seabass >Lokus. Although the MPI calculated in Bakala fish was the third highest value compared to those of the other fish species, the potential risk associated with consuming this particular fish species is much higher compared to that associated with consuming any of the other studied species, simply because Bakala fish is the most sold one in the markets of Ramallah city since it is cheap and therefore, all local consumers could afford purchasing it.

Table 3.3.1(a) Metal Pollution Index (MPI) of the examined fish species from local fish market of Ramallah city.

Species	Metal Pollution Index (MPI)
Mesht	0.098
Marjan	0.324
Lavrak	0.087
Salmon	0.075
Denes	0.099
Malleta	0.066
Sultan Ibraheem	0.112
Sea bass	0.022
Lokus	0.016
Hamour	0.037
Bakala	0.115
Makrel	0.164
Cat fish	0.037

For canned-fish samples, the calculated MPI values ranged from 0.051 to 0.151 (median of 0.082, n = 8) as shown in Table 3.3.1 (b), with metals accumulation pattern in the following decreasing order: Arizona> Marina> Heinz> Lazeza> Al amed> Fatafet> Al warda Al hamra> Amricana**.** The lowest MPI values were those calculated in the two sardine canned-fish samples (Al warda Al hamra and Americana), suggesting that sardine canned-fish might be safer than tuna canned-fish. Generally speaking, tuna canned fish (regardless of the brand name) is very popular in the markets of Ramallah (and other Palestinian cities), it is consumed on a regular basis by most families, thus the potential hazard effects on public health that might be observed upon tuna-canned-fish consumption is much greater than what might result from sardine-canned fish consumption.

Table 3.3.1(b) Metal Pollution Index (MPI) of the examined canned fish from local fish market of Ramallah city.

Species	Metal Pollution Index (MPI)
Al warda Al hamra (sarden)	0.060
Al amed	0.073
Arizona	0.151
Americana (sarden)	0.051
Henz	0.095
Lazeza	0.091
Fatafet	0.062
Marena	0.143

3.3.2 Estimated Daily Intake (EDI)

As mentioned earlier in chapter 02 (section 2.8.2), the average quantity of fish consumed for 70 kg person per a day is 8.22 g in the West Bank. But it is 11.66g in Gaza strip. Therefore, the mean of both averages (i.e., that of the West Bank, and that of Gaza Strip) was calculate (9.94 g per day per person) and used to calculate the EDI values using equation 2 (chapter 02). As shown in Table 3.3.2 (a), EDI values were first, estimated for the most commonly consumed fish species in the Ramallah markets. Particularly, for Bakala fish, Denes fish, Cat fish, and Salmon fish. EDI values of Pb ranged from 0.0058 to 0.0081 µg/day.person with highest value found in Cat fish; EDI values of As ranged from 0.0264 to 0.4944 µg/day. person with highest value found in Bakala fish; EDI values of Cd ranged from 0.0072 to 0.0092 µg/day.person with highest value found in Bakala and EDI values of Hg ranged from 0.004 to 0.0079 µg/day.person with highest value found in Denes. The EDI values of Cd, Hg, and Pb were all below the Provisional Tolerable Daily Intake (PTDI) values Table 3.3.2 (a) and for As, we could not find any PTDI value in literature. One should interpret EDI values with caution, it is true that when EDIs values are lower than PTDI it is allowed to use these fish species for edible purposes, however, this does not mean that no negative health effects might be expected upon consuming them.

Table 3.3.2 (a) Estimated daily intake (EDI, µg/day) of metals calculated to asses potential hazard effects in local fish or canned-fish consumers at the city of Ramallah.

Species	Pb	As	Cd	Hg
Denes	0.0052	0.1178	0.0082	0.0079
Bakala	0.0061	0.4944	0.0092	0.0026
Cat fish	0.0081	0.0264	0.0085	0.0004
Salmon	0.0058	0.1146	0.0072	0.0028
PTDI	3.57		1	0.228

As shown in Table 3.3.2(b), EDI values were also calculated, but this time using the average concentration for each of the four selected metals in all studied samples (n = 21). EDI values ranged from 0.0055 for Pb to 0.8885 for As (with a median of 0.0065 $\mu g.kg^{-1}.day^{-1}$, n = 21), and ascended in the following order: As> Hg> Cd> Pb. While, EDI values of Cd, Hg, and Pb did not increase when the data of all 21 samples were pooled, As EDI increased by a factor of almost 2 (0.49 to 0.89). It should be noted that as shown in Table 3.3.2 (c), EDI values of Cd, Hg, and Pb were lower than both RDA and PTDI values of the Joint US-FAO and WHO expert committee for food additives.[86, 87] However, the relatively high EDI of As is quite alarming because arsenic compounds, especially inorganic ones are very toxic. Thus, measuring As concentration in fish, canned-fish, and seafood (in general) on a regular basis is very crucial to ensure public safety. Unfortunately, such regular monitoring of metal and organic contaminants in food products is not available at the Palestinian territories.

Table 3.3.2(b) The EDI and RDA recorded for different non- essential metals detected in fish by human in Ramallah city.

Element	**Avg Concentration µg/g**	**EDI ($\mu g.\ kg^{-1}.day^{-1}$)**	**RDA* ($\mu g\ .kg^{-1}.day^{-1}$)**
Pb	0.039	0.0055	250
As	6.257	0.8885	
Cd	0.047	0.0067	
Hg	0.045	0.0064	30

*RDA: Recommended Daily Dietary Allowance suggested by WHO and joint FAO/WHO

Table 3.3.2(c) The EDI and PTDI recorded for different non- essential metals detected in fish by human in Ramallah city.

Element	Avg Concentration µg/g	EDI ($\mu g.kg^{-1}.day^{-1}$)	PTDI* ($\mu g.kg^{-1}.day^{-1}$)
Pb	0.039	0.006	3.57
As	6.257	0.888	
Cd	0.047	0.007	1
Hg	0.045	0.006	0.228

*PTDI values of Hg, Pb and Cd were based on the data suggested by The Joint FAO/WHO Expert Committee on Food Additives.

3.3.3 Target Hazard Quotient (THQ)

The THQ value is considered one of the reasonable parameters for the risk assessment of metals contaminations and accumulations that might result from consuming contaminated fish.[118] The target hazard quotient (THQ) index is the ratio between measured concentration and oral reference dose. It is weighted by the duration and frequency of exposure, ingested portion size, and body weight.[120] The threshold limit for THQ is 1.0 as suggested by USEPA.[120] While, THQ value less than 1.0 implies non-significant risk effects.[122,123]

The THQ values for the four non-essential elements assessed herein (i.e., As, Cd, Hg, and Pb) were calculated in all 21 samples studied (i.e., fish and canned-fish) using equation 3 (Chapter 02), and are presented in Table 3.3.3. The highest THQ value was that calculated for As in Marjan fish (THQ = 25.7), followed by that calculated for Hg in Arizona canned fish (THQ = 0.058), then by that calculated for Cd in Al warda Al hamra canned-sardine (THQ = 0.017), and lastly, by that calculated for Pb in Americana canned-tuna (THQ = 0.0027).

THQ values were higher than 1 for As in several fishes and canned–fish samples. THQ values of As were, 25.7, 12.9, 3.0, 2.7, 2.7, 2.2, 2.2, 2.0, 1.7, 1.5, 1.4, 1.3, 1.2, 0.8, 0.4, 0.4, 0.1, 0.09, 0.04, 0.03 and 0.01 for Marjan fish, Sultan Ibraheem fish, Malleta fish, Makrel fish, Lazeza canned-tuna, Americana canned-tuna, Marina canned-tuna, Arizona canned-tuna, Bakala fish, Al amed canned-tuna, Lavrak fish, Heinz canned-tuna, Fatafet canned-tuna, Al warda Al hamra canned-sardine, Denes, Salmon, Lokus, Cat, Mesht, Hamour and Sea bass respectively. These THQ values of As suggest that intake of As would be high upon consuming these fish or canned-fish. Such high As intakes might result in a significant bio-accumulation of As in human's body, which could ultimately lead to a wide range of chronic hazard effects.

Table 3.3.3 Non-carcinogenic (THQ) of metals for the targeted species of Ramallah market.

Species	THQ(Pb)	THQ(As)	THQ(Cd)	THQ(Hg)	HI
	RfD=0.004	RfD=0.0003	RfD=0.001	RfD=0.0005	
Mesht	2.54E-03	4.51E-02	1.54E-02	3.53E-02	9.84E-02
Marjan	1.49E-03	2.57E+01	3.86E-03	5.06E-02	2.57E+01
Lavrak	1.31E-03	1.38E+00	1.45E-03	1.45E-02	1.40E+00
Salmon	1.45E-03	3.82E-01	7.18E-03	5.52E-03	3.96E-01
Denes	1.30E-03	3.93E-01	8.17E-03	1.59E-02	4.18E-01
Malleta	1.31E-03	2.95E+00	2.27E-04	1.48E-02	2.97E+00
Sultan Ibraheem	2.13E-03	1.29E+01	5.81E-04	6.60E-03	1.29E+01
Sea bass	7.50E-04	1.39E-02	7.96E-03	1.75E-03	2.44E-02
Lokus	6.08E-04	1.09E-01	1.39E-03	4.82E-04	1.12E-01
Hamour	2.50E-03	2.80E-02	1.43E-03	1.20E-02	4.40E-02
Bakala	1.52E-03	1.65E+00	9.21E-03	5.15E-03	1.66E+00
Makrel	7.29E-04	2.72E+00	1.37E-02	1.81E-02	2.75E+00
Cat fish	2.03E-03	8.79E-02	8.46E-03	8.15E-04	9.92E-02
Al warda Al hamra	1.35E-03	7.97E-01	1.71E-02	4.85E-04	8.16E-01
Al amed	1.06E-03	1.51E+00	4.04E-03	3.02E-03	1.52E+00
Arizona	1.06E-03	1.98E+00	2.89E-03	5.79E-02	2.04E+00
Americana	2.66E-03	2.21E+00	2.55E-03	3.03E-04	2.21E+00
Henz	9.79E-04	1.32E+00	9.86E-03	4.33E-03	1.34E+00
Lazeza	8.50E-04	2.67E+00	1.16E-02	1.75E-03	2.69E+00
Fatafet	7.71E-04	1.20E+00	5.85E-03	1.90E-03	1.21E+00
Marena	9.50E-04	2.15E+00	7.48E-03	1.85E-02	2.18E+00

3.3.4 Hazard Index

For the risk assessment of multiple heavy metals contamination in fish, the total hazard index (HI) was employed, which took summing all calculated THQ values into account. HI values were calculated using equation 4 (Chapter 02) and presented in **Table 3.3.3.** While, THQ is the target hazard quotient calculated for each of the four non-essential elements (As, Cd, Hg, and Pb), separately, HI is the total hazard index calculated for all these four elements altogether. Any HI value that is higher than 1.0 suggests that the corresponding fish species or canned-fish might not be safe for human consumption. HI values were higher than 1.0 for Marjan fish, Lavrak fish, Sultan Ibraheem fish, Malleta fish, Bakala fish, Makrel fish, and for all canned-fish samples except, that calculated for Al warda Al hamra canned-sardine. These HI values suggest that non-carcinogenic health effects, such as cardiovascular diseases[80,81], nervous system damage,[59] malfunctioning of the immune system[79] and genetic mutations, which might alter the development of fetus in pregnant women[79] are all possible chronic toxic effects that might appear at any time in any local Palestinian consumer upon eating any of these “contaminated” fish and canned fish products for years.

3.3.5 Carcinogenic Risk (CR)

Generally, a CR value above 10^{-4} is considered unacceptable, whereas CR ranging from 10^{-4} to 10^{-6} is regarded as an acceptable carcinogenic risk, and a CR value below 10^{-6} is considered negligible. In this study, the CR values of Pb and Cd were below 10^{-6}, suggesting that no major harmful effects might appear as a consequence of Cd, Pb accumulations, while those of As were within the acceptable carcinogenic risk range, for Marjan fish, Sultan Ibraheem fish, Makrel fish, and Lazeza canned-fish **(**Table 3.3.4)

Table 3.3.4 Estimated Carcinogenic Risk (CR) of metals detected in the fish species and canned fish.

Species	Carcinogenic Risk (Pb)	Carcinogenic Risk (As)	Carcinogenic Risk (Cd)
	Csf=0.0085	**Csf=1.5**	**Csf=6.3**
Mesht	8.65E-11	2.03E-08	9.71E-08
Marjan	5.07E-11	1.16E-05	2.43E-08
Lavrak	4.46E-11	6.23E-07	9.15E-09
Salmon	4.93E-11	1.72E-07	4.53E-08
Denes	4.42E-11	1.77E-07	5.15E-08
Malleta	4.46E-11	1.33E-06	1.43E-09
Sultan Ibraheem	7.23E-11	5.81E-06	3.66E-09
Sea bass	2.55E-11	6.26E-09	5.02E-08
Lokus	2.07E-11	4.91E-08	8.78E-09
Hamour	8.51E-11	1.26E-08	8.99E-09
Bakala	5.18E-11	7.42E-07	5.80E-08
Makrel	2.48E-11	1.22E-06	8.60E-08
Cat fish	6.89E-11	3.96E-08	5.33E-08
Al warda Al hamra	4.59E-11	3.59E-07	1.08E-07
Al amed	3.61E-11	6.80E-07	2.55E-08
Arizona	3.59E-11	8.90E-07	1.82E-08
Americana	9.03E-11	9.93E-07	1.61E-08
Henz	3.33E-11	5.96E-07	6.21E-08
Lazeza	2.89E-11	1.20E-06	7.28E-08
Fatafet	2.62E-11	5.40E-07	3.69E-08
Marena	3.23E-11	9.67E-07	4.71E-08

Chapter 04

Conclusions and Recommendations

4.1 Conclusions

Regarding the objectives of this thesis, we can draw the following conclusions:

Generally, essential metals accumulated in fish at higher concentrations than non-essential metals.

All measured metals concentrations were below the maximum permissible concentration (MPC) for fish consumption as proposed by the WHO in 1996 and the EU in 2008, except for As. As concentrations in six fish species (out of 13 studied species), and in all canned-fish samples exceeded the maximum permissible concentrations (MPCs) that were approved by the US-FAO in 1983, and by CEPA between 1995 and 1997.

The highest concentrations of Cd, and Pb were detected in Mesht fish. This was quite surprising because Mesht fish is raised in local farms at Jericho or imported from fish farms of neighboring countries. Careful investigation is needed to determine the source of potential metals contamination of Mesht fish in these farms. While, the highest concentrations of As and Hg were detected in Marjan fish.

As far as the eight studied canned-fish samples, the highest concentrations of Cd and Pb were detected in Al warda Al hamra and Americana canned-sardine. This high Cd and Pb concentration was also quite surprising because sardine fish is supposed to be relatively mush less contaminated with metals or organic pollutants, especially, when compared to canned-tuna fish due to several reasons. First, sardine fish is much smaller than tuna fish and all other fish species investigated; second, the life span of sardine fish is much shorter compared to that of all examined fish species; third, consequently, the weight, and fat content of sardine fish are much less than those of all fish species tested in this study. It should be noted that eight samples of two types of commercially available canned-fish were included in this study, two different brands of canned-sardine, and six different brands of canned-tuna fish. We expected to find higher metals accumulations in the six canned-tuna samples compared to that accumulated in the two canned-sardine samples. Since, the opposite trend was observed; further investigations are needed to identify the sources of metals contaminations in canned-sardine marketed at local markets in Ramallah city. The highest concentration of Hg was detected in Arizona canned fish and the highest concentration of As was detected in Lazeza canned fish. Although, the concentrations of most tested metals were lower than the MPCs of WHO, US-FAO, and EU, this does not mean that all canned-fish products available to the public at Ramallah city are safe for human consumption.

The THQ and HI values for Pb, Hg and Cd did not exceed the threshold limit that equals 1, while those of As, 6 fish species and all canned fish except Al warda Al hamra exceeded the threshold limit of THQ and HI

It should be noted that the calculated CR values of Cd, and Pb were negligible for all tested fish species, while those of As were in the acceptable carcinogenic risk range (between 10^{-4} and 10^{-6}) for Marjan fish, Malleta fish, and Makrel fish, and for Lazeza canned-fish. These findings of As suggested that these fish and canned fish brands cause cancer over a lifetime.

Reviewing the calculated daily intake (DI) values of the tested metals, suggested no harmful effects health are expected to be observed should any of the studied fish species or any of the studied canned-fish samples is used for edible purposes. However, this statement could be quite misleading and the values of other risk assessment parameters should be reviewed with care.

Although the results of EDI showed that the concentration of most studied metals in fish species and canned-fish samples were below the RDA and PTDI that were suggested by the Joint FAO, and WHO Expert Committee on Food Additives, the results of CR revealed that some fish species such as (Marjan, Malleta, Makrel and Lazeza canned fish) are not safe for edible purpose.

Below are some general recommendations that worth consideration:

1- Similar studies are needed to assess contaminations of commercial fish and fish products by other “potentially” toxic metals, such as Ag, Cr, Co, and Ni.
2- Periodical monitoring of potential metals contaminations in commercial fish is needed, especially for Marjan fish, Makrel fish, and Malleta fish, where the highest As concentrations were detected in this study.
3- Establishing of governmental institution at the Palestinian territories to be responsible of setting up the standards and guidelines to be followed in regular monitoring of food and food product safety, in general, and for monitoring levels of important contaminants in commercial fish and canned-fish, in particular, is needed.

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