Heavy Metal Concentrations in Commercially Available Fishes in Gaza Strip Markets

Submitted by
Shareef Khalil Muzyed

Supervised by
Dr. Nizam M. El-Ashgar
Dr. Kamal J. Elnabris

A Thesis Submitted in Partial Fulfillment of The Requirements for the Degree of Master of Science in Chemistry

Gaza-Palestine
1432هـ - 2011م
DECLARATION

"I hereby declare that this manuscript is my own work and that, to the best of my knowledge and belief, it contains no material previously published, or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text".

Signature :
Name: Shareef Khalil Muzyed
Date: July, 2011

Copy Rights
All Rights Reserved : No part of this work may be reproduced, translated or stored in any kind of a retrieval system, without a prior permission of the author.
Abstract

Heavy Metal Concentrations in Commercially Available Fishes in Gaza Strip Markets

Concentrations of Zinc, Lead, Cadmium, Manganese, Copper and Nickel were determined in the muscles of six commercial fish species available in Gaza Strip markets, using atomic absorption spectroscopy after a modified wet digestion process. 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. Levels of metals in µg/g were as follows: Cu: 0.251-0.907, Zn: 3.705-20.535, Mn: 0.376-0.834, Ni: 0.453-0.978, Pd: Nd-0.552.

All results were below the limits for fish consumption proposed by World Health Organization (1989, 1996) and Ministry of Agriculture, Fisheries and Food (1995). Lead and cadmium concentrations in Micropogonias furnieri fish exceeded the limits in fish proposed by EC (2005). Mugil Cephalus accumulated the highest levels of Cu, Mn and Ni, while the highest levels of Zn, Cd and Pb were detected in Micropogonias furnieri. Transfer factor indicated that local fishes (Sparus aurata, Oreochromis niloticus and Mugil cephalus), accumulated heavy metals from water. Diet and sediment were considered as additional sources for heavy metals.

The estimated maximum total dietary intakes of all metals from the studied fishes were below the maximum acceptable daily intake values set by WHO (1993) and USA National Research Council (1989). Results showed that according to metals levels, consumption of studied fish had no threat to consumers health.

Keywords: Gaza Strip, heavy metals, commercial fish, accumulation factor, consumption safety, wet digestion.
مستخلص

تم تعيين تركيز الزنك والرصاص والمنجنيز والنحاس والكادميوم والنكل في عضلات ستة أنواع من الأسماك المتاحة تجارياً في قطاع غزة باستخدام جهاز مقياس الامتصاص الذي بعد عملية الهضم الرطب. عينات السمك التي درست كانت عبارة عن ثلاثة أنواع من الأسماك المستخدمة كانت من الأسماك المجمدة المستوردة من الخارج (بكلاء و جربع و فيليه) و نوعين من الأسماك هي أسماك منتجة في مزارع محلية (بلطي و دنيس) و نوع من الأسماك المصطادة من البحر (بوري). تبين من الدراسة أن مستويات المعادن المقاسة بالميزكوجرام لكل جرام من العضلات كانت كما يلي: بالنسبة للنحاس تراوحت بين 0.25-0.90 وفي الزنك 3.70-20.53 وفي المنجنيز 0.38-0.83 و في النكل 0.45-0.95. بالنسبة الكادميوم لم يتم اكتشافه في بعض الأسماك و وصل أعلى متوسط للتركيز له في الأسماك الأخرى إلى 0.09 أما الرصاص فلم يتم اكتشافه في بعض الأسماك و وصل أعلى متوسط للتركيز له في الأسماك الأخرى إلى 0.55 ميكروغرام لكل جرام من العضلات. جميع النتائج كانت أقل من الحد الأقصى المسموح به وفقاً لمعايير منظمة الصحة العالمية (1989، 1996) ومعايير وزارة الزراعة والصيد السمكي والأغذية البريطانية (1995). تراكيز الرصاص والكادميوم في أسماك الجرع كانت أعلى من الحد المسموح به وفقاً لوثيقة الاتحاد الأوروبي (2005). أسماك البوري ركزت أعلى تركيز من النحاس والمنجنيز والنكل بينما تجاوزت أعلى تركيز للزنك والكادميوم والرصاص في أسماك الجرع. معامل الانتقال أشار إلى أن الأسماك المحلية (بلطي و بوري) ركزت المعادن الثقيلة من الماء.
I dedicate my research to my lovely mother, sisters, wife and son
Acknowledgment

First of all, I thank God for helping me in every moment.

Thanks to my supervisor Dr. Nizam M. El-Ashgar for his guidance, help, encouragement and advice during work.

I would like to express my deep gratitude to my second supervisor Dr. Kamal J. Elnabris who had been a good source of motivation, inspiration and challenge through this research, who taught me how to perform my work in a better way even if it under stress and to feel comfortable and confident while dealing with any problem.

Thanks to Prof. Dr. Adel Awadallah in the Department of Chemistry at the Faculty of Science, the Islamic University of Gaza for proposing to conduct the present study.

I would like to express my full appreciation and gratitude to Prof. Dr. Nabila Bakry the previous Chief of the Pesticides Department at the University of Alexandria, and Dr. Khalid Yasseen Alexandria, Egypt for their great help in completing the analysis process of samples of the study within the Department's laboratories.

I extend thanks also to Dr. Yasser El- Nahhal for great support and significant guidance throughout the period of the research.

Thanks to engineer Jihad Salah in the Directorate General of Fisheries in the Ministry of Agriculture for providing me with information about types and amounts of fishes consumed by humans in Gaza Strip.

Thanks to people in health department at Gaza Municipality for their helping in the collection process of frozen fish samples.

And finally, special thanks to the Islamic University of Gaza which was my second home and it will have this value in my heart for ever.

Special Thanks to my wife for her kind and support and to my son.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1 Introduction and Literature Review</td>
<td></td>
</tr>
<tr>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1 Description of study area</td>
<td>3</td>
</tr>
<tr>
<td>1.1.2 Problem definition</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2.1 Frozen fish</td>
<td>5</td>
</tr>
<tr>
<td>1.1.2.2 Wild-caught fish</td>
<td>6</td>
</tr>
<tr>
<td>1.1.2.3 Farmed Fish</td>
<td>7</td>
</tr>
<tr>
<td>1.1.3 Accumulation factor (Transfer factor)</td>
<td>8</td>
</tr>
<tr>
<td>1.1.4 Investigation of the public health hazard</td>
<td>9</td>
</tr>
<tr>
<td>1.2 Major objectives of the present study</td>
<td>10</td>
</tr>
<tr>
<td>1.3 Review of literature</td>
<td>11</td>
</tr>
<tr>
<td>1.3.1 Related works done in the region and around the world</td>
<td>11</td>
</tr>
<tr>
<td>1.3.2 Studies concerning heavy metals in Gaza Strip</td>
<td>17</td>
</tr>
<tr>
<td>1.4 Selection of elements and their toxicity</td>
<td>18</td>
</tr>
<tr>
<td>1.4.1 Basis of selection of elements</td>
<td>18</td>
</tr>
<tr>
<td>1.4.2 Selected elements and their toxicity</td>
<td>19</td>
</tr>
<tr>
<td>1.4.2.1 Cadmium</td>
<td>19</td>
</tr>
<tr>
<td>1.4.2.2 Lead</td>
<td>19</td>
</tr>
<tr>
<td>1.4.2.3 Copper</td>
<td>20</td>
</tr>
<tr>
<td>1.4.2.4 Zinc</td>
<td>20</td>
</tr>
<tr>
<td>1.4.2.5 Manganese</td>
<td>21</td>
</tr>
<tr>
<td>1.4.2.6 Nickel</td>
<td>21</td>
</tr>
<tr>
<td>Chapter 2 Materials and methods</td>
<td></td>
</tr>
</tbody>
</table>
2.1 Sample collection .............................................................................................................. 23
  2.1.1 Collection of fish specimen ......................................................................................... 23
  2.1.2 Sediment, water and feed sampling ........................................................................... 24
2.2 Reagents ............................................................................................................................ 26
2.3 Digestion procedures ........................................................................................................ 26
  2.3.1 Digestion of fish Samples ........................................................................................... 26
  2.3.2 Digestion of sediment samples .................................................................................. 26
  2.3.3 Digestion of water samples ....................................................................................... 26
  2.3.4 Digestion of fish diet samples .................................................................................... 27
2.4 Blank preparation ............................................................................................................. 27
2.5 Analytical Technique ....................................................................................................... 27
2.6 Validation of analytical methodology (Recovery test) ..................................................... 28
2.7 Data analysis .................................................................................................................... 30

Chapter 3 Results

3.1 Recovery test ................................................................................................................... 31
3.2 Metal concentrations in different fish species ................................................................. 31
3.3 Correlation Analysis of heavy Metals ............................................................................... 37
3.4 Heavy metal concentrations in water, sediment and fish diet samples ......................... 39
3.5 The accumulation factor ............................................................................................... 39
3.6 Health-Risk Assessment for fish consumption ............................................................... 40

Chapter 4 Discussion

4.1 Accumulation of metals by fish species .......................................................................... 41
4.2 Correlation between metals among fish species ............................................................. 44
4.3 Accumulation factor (Transfer factor) ............................................................................ 45
4.4 Consumption safety........................................................................................................ 47
  4.4.1 Concentrations of trace elements in fishes and comparison with international
dietary standards and guidelines................................................................. 47
  4.4.2 Daily consumption safety........................................................................... 48
4.5 Comparison of metals concentrations with other studies.............................. 51
Conclusion............................................................................................................. 53
Recommendations................................................................................................. 54
References
Appendices
Appendix 1 :Amounts (in tones) of fish consumed by people in Gaza strip in 2010.
LIST OF TABLES

Table 1.1 Distribution and amounts (in tones) of fish consumed by people in Gaza strip in 2010................................................................. 5

Table 2.1 List of fish species, number and size of fishes used in this study........... 23

Table 3.1 Recovery of various metals from fish muscles ........................................ 31

Table 3.2 Range of metal contents (µg/g wet weight) and mean±standard error in various fishes........................................................................................................ 33

Table 3.3 Kendall tau correlation coefficients τ for various metals in the six commercial fish species ................................................................. 38

Table 3.4 The average concentrations of metals in water, sediment and diet samples.... 39

Table 3.5 Accumulation factor from water, sediment and food for the three local fish types.................................................................................................................. 40

Table 3.6 The estimated daily intake (µg/day) of metals by humans in Gaza Strip from fish muscles................................................................. 40

Table 4.1 The heavy metals concentrations (mg /L) in freshwater used in cultured pond and comparison with international standards................................................................. 46

Table 4.2 Maximum acceptable levels of heavy metals in fish muscles (µg/g) according to international standards................................................................................. 47

Table 4.3 Heavy metal concentration (µg/g wet wt) in muscles of different fishes from various countries ................................................................. 52
LIST OF FIGURES

Figure 1.1 Map shows the study area in Gaza strip –Palestine.............................. 4
Figure 1.2 Wastewater discharged directly into the sea in Wadi Gaza region............... 6
Figure 1.3 Discharging wastewater into the sea in Dier El- Balah city at the middle region of Gaza strip.............................................................................................................. 6
Figure 3.1 Difference of Cu average concentration (µg/g) among the six fish species....... 34
Figure 3.2 Difference of Zn average concentration (µg/g) among the six fish species ...... 35
Figure 3.3 Difference of Cd average concentration (µg/g) among the six fish species ..... 35
Figure 3.4 Difference of Mn average concentration (µg/g) among the six fish species.... 36
Figure 3.5 Difference of Pb average concentration (µg/g) among the six fish species...... 36
Figure 3.6 Difference of Ni average concentration (µg/g) among the six fish species...... 37
ABBREVIATIONS

ADI  Acceptable daily intake
EPA  Environmental Protection Agency
FAO  Food and Agriculture Organization
PTWI Provisional Tolerable Weekly Intakes
PCBS Palestinian Central Bureau of Statistics
US EPA United States Environmental Protection Agency
Nwrg New Weapons Research Group
NHMRC National Health and Medical Research Council
EU European Union
MAFF Ministry of Agriculture, Fisheries and Food
WHO World Health Organization
USFDA United States Food and Drug Administration
UNEP United Nations Environment Programme
MRL Maximum residue limits
EFSA European Food Safety Authority
APHA American Public Health Association
EC European Commission
TDI Tolerable daily intake
# LIST OF ELEMENTS NAMES AND THEIR SYMBOLS

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>Pb</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cd</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
</tr>
<tr>
<td>Chromium</td>
<td>Cr</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
</tr>
<tr>
<td>Strontium</td>
<td>Sr</td>
</tr>
<tr>
<td>Vanadium</td>
<td>V</td>
</tr>
<tr>
<td>Arsenic</td>
<td>As</td>
</tr>
<tr>
<td>Mercury</td>
<td>Hg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Mg</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Al</td>
</tr>
<tr>
<td>Selenium</td>
<td>Se</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
</tr>
<tr>
<td>Silver</td>
<td>Ag</td>
</tr>
</tbody>
</table>
Chapter One
Introduction and Literature Review
1.1 Background

Definition of heavy metals
The term heavy metals is a general collective term which applies to group of metals and metalloids with atomic density greater than 4g/cm$^3$ or 5 times or more greater than water [1], they are also known as trace elements because they occur in minute concentrations in biological systems.

Effect of metals
Depending upon their concentration they may exert beneficial or harmful effects on plant, animal and human life [2]. Some of these metals are toxic to living organisms even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. When ingested in excess amounts heavy metals combine with body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thereby mutilating their structures and hindering them from the bioreactions of their functions [1].

Heavy metal contamination of aquatic system
In the last decades, contamination of aquatic systems by heavy metals has become a global problem. Heavy metals may enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition [3].

Fate of heavy metals in aquatic system (heavy metal accumulation)
In natural aquatic ecosystems, metals occur in low concentrations. As they cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals and thus, causing heavy metal pollution in water bodies [4]. Metals entering the aquatic ecosystem can be deposited in aquatic organisms through the effects of bioconcentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level [5]. In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues [6].
Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in the blood stream to either a storage point (i.e. bone) or to the liver for transformation and/or storage [7].

**Risk assessment of heavy metals in human.**

Like in other organisms, heavy metals are not destroyed by humans [8]. Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans. Therefore, the heavy metals are among most of the pollutants, which received attention in various countries and considered the most dangerous category of pollutants in the sea [9].

An early example of an environmental problem due to heavy metal occurred in 1952, in the vicinity of the Japanese fishing harbour of Minimata. This disease (Minimata disease) was a result of consuming fish and shrimps contaminated by methyl mercury and non-organic mercury from the wastewaters discharged by Chlor-alkali factories. Another example is the Ita-Ita Disease in Fugawa, Japan in 1955 [10]. It was the result of consuming rice, fish and bivalves that were Cd-contaminated from wastewaters discharged by nearby mining [10, 11].

**Importance of fish**

The growing human population has increased the need for food supply. Because they are good protein sources, the demand for fish and shellfish products has increased. Worldwide, people obtain about 25% of their animal protein from fish and shellfish [12]. In 2004, about 75% (105.6 million tones) of estimated world fish production was used for direct human consumption [13]. It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 [14].

The real importance of fish in human diet is not only in its content of high-quality protein, but also to the two kinds of omega-3 polyunsaturated fatty acids: eicosapentenoic acid (EPA) and docosahexenoic acid (DHA). Omega-3 (n-3) fatty acids are very important for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery [15, 16]. Fish also contain vitamins and minerals which play essential role in human health.
Since diet is the main route of exposure to heavy metals, and fish represent a part of human diet, it is not surprising that polluted fish could be a dangerous dietary source of certain toxic heavy metals [17].

In the last two decades there has been a growing interest in assessing the levels of heavy metals in food including fish (mainly concerned with commercial species). Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazardous effect on human health.

1.1.1 Description of the study area (Geography and population)

Gaza Strip (Figure1.1) is located on the southeastern shore of the Mediterranean Sea, bordered by the Occupied Palestinian territories to the east and north, and Egypt to south. The total area is estimated at 365km$^2$. Its length along the coast is 45km and width ranges from 5 to 12 Km. Gaza strip has a semi-arid Mediterranean climate, with mean temperature varying from 12-14 $^\circ$C in January, to 26-28 $^\circ$C in August.

Gaza Strip is one of the most density populated areas of the world. According to The Palestinian Central Bureau of Statistics (PCBS), the number of residents of the Gaza Strip in 2010 was about 1,530,000 inhabitant [18]. The population in Gaza is expected to reach 3.1 millions in 2025 [19].

This population expansion will lead to an increase in demand for basic food items including fish. As a result, fish is now brought in from many places to fill this increased demand.
1.1.2 Problem definition

Gaza Strip have a beach with about 40 Km long, that could be a significant source for fish for people in the strip. However, because of the Israeli occupation procedures which controls the fishing in Gaza sea people in Gaza strip were deprived from this vital source of food. Therefore people and government in Gaza tend to arise many pools for breeding fish, these pools use sea and ground water. Also, Because of is relatively cheap prices, a large sector of people in Gaza Strip depends on frozen fish which are imported from different geographical regions around the world. According to information obtained from the Directorate General of Fisheries in Palestinian Ministry
of Agriculture, the total amount of fish consumed by people in Gaza Strip was about 6382.7 tones in 2010. Table 1.1 summarizes types, amounts and sources of fish used in Gaza Strip for human consumption. Each fish species according to its type or source can be considered as a source of heavy metals for the residents of Gaza Strip, so levels of heavy metals in these fishes should be detected.

Table 1.1

Distribution and amounts (in tones) of fish consumed by people in Gaza strip in 2010

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Local fish</th>
<th></th>
<th>Imported fish</th>
<th></th>
<th>Salted fish</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>Fresh source</td>
<td></td>
<td>Frozen source</td>
<td></td>
<td>Salted source</td>
<td></td>
</tr>
<tr>
<td>1724</td>
<td>1000</td>
<td></td>
<td>46.5</td>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>478</td>
<td>O.P.T.</td>
<td></td>
<td>83.2</td>
<td></td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>2581</td>
<td>Foreign countries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultured</td>
<td>300</td>
<td></td>
<td>1478</td>
<td></td>
<td>170</td>
<td></td>
</tr>
</tbody>
</table>

Total = 6382.7 tones

O.P.T. = Occupied Palestinian Territories

1.1.2.1 Frozen fish

The environmental situations imported frozen fish are unknown; accordingly, it is necessary to know about the potential accumulation of heavy metals in these imported fish.

Another important issue that should be addressed concerning imported fish is the potential substituting species by others of a different price or origin through the commercial chain. Such illegal issue of seafood substitution reaches high levels in many developing countries, including Italy, Spain, Ireland, and the USA. In hake markets for example, several cases of substitution have been documented. In Spanish market, the cheap African hake species were found to substitute the American and European species which are more expensive [21].

Since heavy metals have been found in different fish species caught from African marine waters [22], thus the potential of substituting a fish species by others from polluted geographical region such as African waters through the commercial chain is of
concern. Therefore, the knowledge of levels of heavy metals in fish imported from different geographical regions is very important for the health of the consumers.

1.1.2.2 Wild-caught fish

The coastal water of Gaza Strip became polluted from the effluents of wastewater treatment plants and from the flooding of Gaza valley presenting chemical wastes to the sea. Most of the amounts of domestic wastewater and industrial effluents are transported by the sewer system to the wastewater treatment plants and finally discharged with or without partial treatment into the Mediterranean sea, contaminating the coastal waters. Figure 1.2, 1.3 shows the flow of wastewater in tow reagions of gaza strip (Wadi Gaza , Dier El- Balah, respectively).

![Figure 1.2 Wastewater discharged directly into the sea in Wadi Gaza region.](image1)

![Figure 1.3: Discharging wastewater into the sea in Dier El- Balah city at the middle region of Gaza Strip](image2)
1.1.2.3 Farmed fish

Fish farms in Gaza Strip mainly include two species, the fresh-water species Tilapia (*Oreochromis niloticus*) and the marine species sea bream (*Sparus aurata*). Fish farms use ground water and seawater for cultivation of fresh-water and marine species, respectively. They also use artificial pelleted feed.

Water quality is the major factor in aquaculture sustainability. However, little is known about water quality in these land-base fish rearing ponds. Cultured fishes may absorb dissolved elements and trace metals from its feeding diets and surrounding water leading to their accumulation in various tissues insignificant amounts and exhibit eliciting toxicological effects at target criteria [23].

In addition, metal contamination is one of the collateral effects of war [24], therefore, after the last war on Gaza on December 27, 2008 to January 18, 2009, medium-high levels of heavy metals contaminations in certain areas of the land soil of Gaza were documented, which was attributed mainly to the effects of enemy missiles [25]. In a study conducted by the New Weapons Research Group (Nwrg; is an independent committee of scientists and experts based in Italy) after El Forgan War, results showed that many Palestinian children have unusually high concentrations of metals in the hair caused by enemy bombing during the war. Results indicated to environmental contamination with heavy metals, which can cause health and growth damages due to chronic exposure [26]. Therefore, the potential for seawater, ground water contamination is a legitimate concern [24].

Although it is well known that fish muscle is not an active tissue in accumulating heavy metals, the present study is concerned in heavy metal concentrations in the fish muscles because they are the most consumed portion by the people. Furthermore it was documented that fish accumulates substantial amounts of metals in their tissues and thus, represent a major dietary source of these metals for humans [27-29], these amounts could exceed acceptable levels in the muscle of some fish in polluted regions. For this reason, determination of heavy metals levels of fish was done on muscles.
1.1.3 Accumulation factor (Transfer factor)

The accumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals in organs and/or tissues of organisms, such as fish [7]. The accumulation factor consists of ratios of the concentration of a given contaminant in biota (a particular metal concentration in fish muscle) to that in an abiotic media (water, sediment and food). Having a good understanding of the accumulation factor is important in predicting the relative contributions of abiotic media as a source of heavy metals accumulation in fish and the accumulation efficiency for any particular pollutant in any fish organ. In addition, such information is crucial in making accurate risk assessment for seafood safety purposes and its possible health consequences to humans. Inorganic contaminants such as heavy metals entering coastal waters may be concentrated by edible marine organisms to varying degrees from either water, food or sediment. The presence of high levels in fish environment does not indicate a direct toxic risk to fish, if there is no significant accumulation of metals by fish tissues [30].

Accumulation factor shows how many times a fish concentrates a metal above a certain environmental level. Unlul and Gumgum, [31] described that levels of metals in upper members of the web like fish can reach levels many times higher than those found in aquatic environment or in sediment.

Fish accumulate different amounts of metals depending on many factors such as physiological needs, feeding habits and genetic composition, sex of each fish species and the biochemical significant role of each metal [5, 30].

The greater transfer factor for any environmental source indicates that metals transferred to fish tissues from that source more than other sources [32].

Being one of the first investigations concerning heavy metals concentrations in commercial fish in this country, it was important to clarify the accumulation of heavy metals by determining the transfer factors of all metals in locally collected fish species from water, sediment and diet.
1.1.4 Investigation of the public health hazard

Under regular consumption habits, it is important to assess the daily intake of metals from fish and compare it with the total acceptable daily intake (ADI) values set by international organizations for health safety.

This can be obtained by multiplying the average quantity of fish consumed per Palestinian individual per day by the concentration of each element in studied fish muscles [16].

Daily intake of heavy metals from fish = \( A \times B \)

Where

- \( A \) is the average quantity of fish consumed per Palestinian individual per day
- \( B \) is the concentration of metal in studied fish species
1.2 Objectives of the present study are:

I. To investigate the presence of heavy metals contaminants like cadmium (Cd), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) in the muscles of some important commercial fish available in Gaza strip market.

II. To find out whether the fish from the local market have elevated concentrations of these heavy metals in their tissues that could render them dangerous for human consumption, by comparing the results generated in the present study with the maximum permissible limits of these heavy metals proposed by Ministry of Agriculture, Fisheries and Food (MAFF, England), European Union (EU) and World Health Organization (WHO).

III. To address the deficiency in the data on heavy metal levels in commonly available commercial fish.

IV. To assess fish accumulation with heavy metals from water, sediment and diet.
1.3 Review of literature

1.3.1 Related works done in the region and around the world

Due to their toxicity and accumulation in biota, determination the levels of heavy metals in commercial fish species have received considerable attention in different countries in the region and around the world. Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazard effect on human health. Some of the important documented contributions relevant to the present study are as follows:

In Egypt concentrations of Zn, Cu, Pb and Cd were determined in gills, skin and muscles of two fish species (*M. cephalus* and *Liza ramada*) from five locations in Lake Manzala by Bahnasawy, et al., [12]. They indicated that, the values of the metals detected in the fish muscles (the edible part) were within the permissible levels according to According to National Health and Medical Research Council (NHMRC,1987) limits.

Abdallah, [33], studied the concentrations of five trace metals namely, Cd, Pb, Cu, Cr and Zn in muscles of some commercially fish species collected from two coastal areas of the Egyptian coast of the Mediterranean Sea west of Alexandria (El-Mex Bay and Eastern Harbour). For all trace element examined, in all fishes zinc was the highest (up to 57 µg/g) followed by Cr, Cu, Pb and Cd. The levels of Cr surpassed the maximum permissible concentration in most fish tissues, followed by Pb and Cd in some species. Cu and Zn concentrations were found to be below the maximum permissible levels proposed by Food and Agriculture Organization.

Saeed and Shaker [34] presented a report about concentrations of Fe, Zn, Cu, Mn, Cd and Pb in *O. niloticus* (Tilapia) fish tissues, water and sediments in northern Delta Lakes. They found that The edible part of *O. niloticus* from Lake Edku and Manzala contained the highest levels of Cd while fish from Manzala Lake contained the highest level of Pb. They reported that Nile tilapia caught from these two Lakes may pose health hazards for consumers.

In their study to determine the accumulation and the distribution of Cu and Zn in *Tilapia zillii* and *M. cephalus*. Authman and Abbas, [35] suggested that the consumption of fishes of the lake could pose the health damage to the local population whose diet consist mainly of fish, and they recommended to rescue lake Qarun from
these serious ecological problems. In their study to determine the accumulation and the distribution of Cu and Zn in the two fish species, bioaccumulation factor showed that the trend of accumulation of metals in fish organs was apparent in liver, gills and muscles, respectively. Moreover they reported that *T. zillii* seemed to be more contaminated with Zn and Cu than *M. Cephalus*. As they mentioned their result was in disagreement with many previous findings which pointed that *Mugil* species seemed to be more accumulated with heavy metals than Tilapia species.

The levels of Fe, Mn, Zn, Cu, Pb and Cd were studied by Ali and Abdel-Satar, [23], in water, sediment, fish and fish diets in some fish farms in El–Fayoum province in Egypt. They showed that with exception of zinc the values of heavy metals exceeded that of the permissible limits in water. Heavy metals in the fish flesh showed that *Mugil* species tended to accumulate more concentration of Cu, Zn, Pb, and Cd than Tilapia species. They were the diets considered as additional ambient heavy metals sources. So, results revealed that the studied fish farms suffered from serious environmental problems such as poor water quality, improper management and absence of scientific monitoring, therefore by time the heavy metals problems cause toxicological effects for the end users and costumers.

The distribution of eight heavy metals, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in muscles, gills, livers and bones of five fish species namely, *Sargus sargus, Siganus rivulatus, Mugil cephalus, Caranx crysos* and *Scomberomorus commerson* collected from El-Mex Bay Alexandria, Egypt were studied by Khalid, [36]. Results for weekly intake demonstrated that the concentrations of Cd, Cu, and Pb in muscles of the five fishes were lower than the Provisional tolerable weekly intake (PTWI) values set by international organization and accordingly there is no risk for human consumption of flesh of these fishes. In a study performed by Rashed, [32], results indicated that *T. niloticus* is one of the aquatic organisms affected by heavy metals. He showed that according to the Egyptian Organization for Standardization, concentrations of Cr, Cu, Fe, Mn, Ni, Co, Zn and Sr in the edible parts of the investigated fish were in the safety permissible level for human uses.
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 by Al-Weher, [37] . He found that levels of these heavy metals in muscles of the three fish species were within acceptable limits by FAO standards, except for the Zn concentration in muscles of Oreochromis aureus (70.76±31.21 µg/g dry wt.) which were attributed to the increase of agricultural influx and some other anthropogenic activity in that area.

In Turkey, concentrations of Zn, Cu, As, Cd, Hg and Pb in seven popular fish species of Ataturk Dam Lake (Euphrates, Turkey) were investigated by Mol, et. al [39]. They found that the fish from Ataturk Dam Lake are not heavily burdened with metals, but they should be controlled periodically to avoid excessive intake of trace metals by human, and to monitoring the pollution of aquatic environment.
In a study performed by Yilmaz, [3], to compare concentrations of Cd, Cu, Mn, Pb, and Zn in tissues of three economically important fish (Anguilla anguilla, M. cephalus and O. niloticus) inhabiting Koycegiz Lake-Mugla (Turkey), the highest concentration of trace metals in the tissues of M. cephalus in lake was attributed to the trophic characteristics of this species, that M. cephalus reflects the metal concentrations in surface and suspended particulate matter, showing high metal concentrations. He considered M. cephalus as an adequate and most suitable species for use as biomonitors of trace metals pollution in the Koycegiz Lake. Consequently, he recommended the use of these species as biological indicators as a tool for future monitoring programs, to evaluate the evolution of heavy metal pollution in that area. Results indicated that, concentrations of Pb and Zn for O. niloticus; Pb, Zn and Cd for A. anguilla; and Cd and Zn for M. cephalus were found higher than the Turkish Food Codex, European Units and WHO limits for human consumption in edible parts of the fish samples and posed a risk for human health.

In another search, Yilmaz, et al., [40] measured the concentrations of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn in muscle, gill and liver of two fish species (Leuciscus cephalus and Lepomis gibbosus) caught from Saricay, South-West Anatolia (Turkey). Results indicated that the concentrations were below the limits for fish proposed by FAO/WHO, EU and Turkish Food Codes and safe within the limits for human consumption in the edible parts of fish species in that region.

A comparison of Fe, Cu, Ni, Cr, Pb and Zn levels of Grey Mullet (M. cephalus) and Sea bream (S. aurata) caught in Iskenderun Bay (Turkey) was made by Yilmaz, [41]. Generally, Results indicated that M. cephalus showed higher levels of metal contamination than S. aurata which attributed to the difference in foraging habits of the two species.

Levels of Fe, Cu, Ni, Cr, Pb, and Zn were determined in the muscle, skin and gonads in M. cephalus and Trachurus mediterraneus species from Iskenderun Bay in Turkey by Yilmaz, [42]. The results of this study showed that metals seem to be more concentrated in M. cephalus than in that of T. mediterraneus. He reported that concentrations of some metals in some tissues exceeded the acceptable levels for a food source for human consumption designated by various health organizations.
In France, Shinn, et al., [43] assessed heavy metals contamination in River Lot between 1987 and 2007. The concentration of Cu, Zn, Cd and Pb were quantified in muscle and liver of fish species from the river as well as in water, sediment and moss. The results showed that the average concentrations of Cd in fish muscle in 2007 were above the maximum safe level for human consumption defined by the EC.

In Nigeria, Alinnor and Obiji [44] performed a study to examine trace metal (Pb, Fe, Cd, Mn, Hg, Cu and Zn composition in fish samples from Nworie River and in frozen fish samples purchased from Ekeonunwa. They pointed out that untreated waste products discharged into Nworie river contaminated the biota in the aquatic system with these elements which it's toxicants will be transferred to man by consumption of fish obtained from the river. Also they found that frozen fish samples purchased from Ekeonunwa market were contaminated with heavy metals.

Furthermore, Olowu, et al., [45], determined the concentrations of Zn, Ni and Fe in tissues of two fish species, Tilapia and Cat fish from two stations in Lagos, Nigeria. They concluded that both fish species may be considered safe for consumption, but the need for continuous monitoring to prevent bioaccumulation is necessary.

In another study in Nigeria Christopher, et al, (2009) [46], studied the Distribution of Pb, Zn, Cd, As and Hg in Bones, Gills, Livers and Muscles of Tilapia (O. niloticus) from Henshaw town beach market in Calabar. The results showed that the muscle of Tilapia contained the least concentrations of the heavy metals determined.

Obasohan and Eguavoen [47] investigated accumulation levels of Cu, Mn, Zn, Cd, Ni and Pb in a freshwater fish (Erpetoichthys calabaricus) from Ogba river, Nigeria, during dry and rainy seasons. Findings showed that the accumulation levels in fish exceeded the levels of the metals in water and indicated bioaccumulation in fish and no significant differences of metal levels between the dry and rainy season. Findings also showed that both dry and rainy season mean levels of Cu, Mn and Ni in fish exceeded WHO recommended limits in food, suggested that the fishes of the river are not suitable for human consumption. They recommended that a close monitoring of metal pollution
of Ogba river is strongly advocated, in view of the possible risks to health of consumers of fish from the river.

In India, Raja, et al. [48], determined the concentrations of Cr, Cd, Cu, Fe, Mg, Mn, Ni, Co, Zn and Al in four commercially available marine edible fish species from Parangipettai coast. They reported that metal levels in edible parts of the investigated fish was in the permissible safety levels for human uses according to FAO, 1983; EC, 2001; Food and Drug Administration (FDA, 2001) standards.

Lakshmanan, et al, [49], investigated the concentrations of Zn, Pb, Cr, Co and Cd in five of the most commercially important fishes in the Parangipettai coast, India. The results revealed that the muscle concentrations of Cr, Zn, Pb, Cd and Co ranged from 0.415±0.27-1.168±1.49; 0.103±0.14- 0.807±0.13; 0.062±0.00-1.569±1.41; 0.004±0.00-0.114±0.14 and 0.006±0.00-0.014±0.00 ppm respectively. Another study performed by Sivaperumal, et al., [50], evaluated concentrations of Cd, Pb, Hg, Cr, As, Zn, Cu, Co, Mn, Ni, and Se in commercially important species of fish, shellfish and fish products from fish markets in and around the Cochin area, India. Results showed that different metals were present in the samples at different levels but within the maximum residual levels prescribed by the European Union (EU) and USFDA and the fish and shellfish from that areas, in general, were safe for human consumption.

In USA, an investigation of As, Cd, Mn, Pb, Hg, and Se levels in commercial fish in New Jersey were performed by Burger and Gochfeld, [15]. They reported that the levels of most metals were below those known to cause adverse effects in the fish themselves. However, the levels of As, Pb, Hg, and Se in some fish were in the range known to cause some sub-lethal effects in sensitive predatory birds and mammals and in some fish exceeded health-based standards set by FAO, Environmental Protection Agency (EPA), WHO, FDA.

In Argentina, Marcovecchio, [51] considered M. furnieri fish as good bioindicator of heavy metal pollution in ecosystem. A marked relationship between metal contents of the studied species and their trophic and ecological habits was observed. The results
indicated that levels of Hg, Cd and Zn found in edible muscle tissue were lower than the international standards for human consumption.

In Uruguay *M. furnieri*, is considered as one of fish species which local population commonly uses for consumption. Viana, et al., [52] determined concentrations of Cu, Hg and Zn in muscle tissue of seven fish species including *M. furnieri*, from coastal waters of Uruguay. Results showed that the fish studied were acceptable for human consumption.

### 1.3.2 Studies concerning heavy metals in Gaza Strip

Generally, studies concerning heavy metals concentrations in Gaza Strip are rather little. In 2009 an assessment were performed by United Nations Environment Programme (UNEP) [53], to evaluate heavy metal concentrations in soil, drinking water and sewage water following the last war in December 2008. Results showed that untreated (and even partly treated) sewage sludge contains large quantities of pathogen as well as elevated levels of heavy metals.

Nejem, et al., [54], performed a study to detect some heavy metals (Zn, Cu, Ag, Pb, Hg and Cd) levels in some fruit and leaves grown in the polluted soil due to sewage water diffusion into planted land of Um Al Nasser village. Results showed a significant pollution of plants with some of the studied metals, which exceeded in some cases the allowed values approved by WHO and FAO.

Mourtaja, [55], determined concentrations of Zn, Cr, Cd, Pb and Cu in three marine fishes namely, Grey mullet, Barracuda and Sigan species. The average concentrations of these heavy metals in muscles of Grey mullet were for Zn: 4.675; Cr: 0.120; Cd: 0.096; Pb: 2.606 and Cu: 0.3743 (µg/g dry wt.). In Barracuda the average concentrations were 6.030, 0.151, 0.092, 2.618 and 0.247(µg/g dry wt.), respectively. In Sigan fish the averages were 6.258, 0.141, 0.123, 2.389 and 0.570 (µg/g dry wt.), respectively.

Concentrations of Cu, Mo, Pb, Cd, Cr, Hg, Co, Zn and Fe were determined in some groundwater wells in Gaza strip by El-Nahhal, [56]. Results in some wells showed that concentration of lead, cadmium, iron, and chromium were above EPA standards.
In 2001 Palestinian ministry of environmental affairs [20] assessed pollution sources in Gaza strip, it reported that the seawater quality of Gaza Strip has been highly polluted by sewage, sediments, nutrients, pesticides, litter and marine debris, and toxic wastes during thirty years of Israeli occupation. And this has left the coastal area in a bad and neglected state. Also it reported that lab analyses of the wastewater of some polluting industries in Gaza Strip showed that it contains some heavy metals with concentrations exceeding the permissible limits. This wastewater contains quantities of water used by the human population, whether for domestic or industrial purposes which are transported by the sewer system to the wastewater treatment plants and finally discharged with or without partial treatment into the Mediterranean Sea, contaminating the coastal waters.

Twelve elements included Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analyzed by Shomar, et al. [57] in 120 wastewater samples. The results revealed that domestic wastewater influent contains considerable amounts of metals.

1.4 Selection of elements and their toxicity

1.4.1 Basis of selection of elements

Elements as Cd and Pb are non essential metals and their toxic effect on human health is well known, while metals as, Cu, Mn, Ni and Zn are essential metals, the toxic effect of them on human health begins when they are present in high levels. These elements may be added on the ecosystem through human activities or from natural sources as it was explained previously. Many studies assessed concentration of these heavy metals in fish species all over the world. In Gaza Strip which represents the study area, concentrations of these metals were determined in soil, groundwater, sewage water and plant [20, 53, 54, 56, 57], and in several cases concentrations of some metals were exceeding the permission limits allowed by international organizations such as EPA, WHO and FAO [20].
1.4.2 Selected elements and their toxicity

1.4.2.1 Cadmium:

Cadmium is a metal from group II B that has an atomic weight of 112.41 with specific gravity of 8.65; the ionic form of cadmium (Cd^{2+}) is usually combined with ionic forms of oxygen (cadmium oxide, CdO), chlorine (cadmium chloride, CdCl_2), or sulfur (cadmium sulfate, CdSO_4).

Cadmium is a natural element in the earth’s crust. It is usually found as a mineral with other elements. All soils and rocks, including coal and mineral fertilizer, have some cadmium in them. In industry and consumer products, it is used for batteries (Ni-Cd batteries of mobile phones), pigments, metal coatings and plastics. It is also a constituent in many other things such as alloys. Cd enters air from mining, industry, and burning coal and household wastes. Its particles can travel long distance in air before falling to ground or water [58].

Cadmium is widely distributed at low levels in the environment and is not an essential element for humans, animals and plants. The WHO/FAO has determined a maximum tolerable weekly intake of 7µg Cd/kg of body weight [59]. The EU maximum residue limit (MRL) permitted in fish is 0.1–0.3 µg/g for Cd [60].

Toxicity symptoms induced by cadmium include gastrointestinal disorders, kidney failure and hypertension. It is also reported that, intoxication with Cd in pregnant women has been related to reduced pregnancy length and newborn weight and, recently, to disorders of the endocrine and/or immune system in children [61].

1.4.2.2 Lead

Lead is a naturally occurring element; it is a member of Group 14 (IVA) of the periodic table, has an atomic weight of 207.2; with specific gravity of 11.34 and exists in three states: Pb (0), the metal; Pb (II); and Pb (IV). Lead is a blush-gray heavy metal and it is usually found combined with two or more other elements to form lead compounds [62].

Lead is found in small amount in the earth’s crust. It can be found in all parts of our environment. Most of it came from human activities, like mining, manufacturing and the burning of fossil fuels. The principal source of Pb in the marine environment appears to be the exhaust of vehicles run with leaded fuels that reaches the sea water by a way of rain and wind blown dust [63]. Lead is found at high concentration in muscles and organs of fish. When accumulates in the human body, it replaces calcium in bones [64].
Lead exposure has been mainly related to retardation of neurobehavioral development [65, 66]. The EU maximum residue limits permitted in fish is 0.3 µg/g for Pb, 0.1–0.3 µg/g for Cd [60].

Data from European Food Safety Authority (EFSA) have related exposure to Cd and Pb to effects like neurotoxicity, nephrotoxicity, carcinogenicity and endocrine and reproductive failures in adults [60].

Moderate exposure to Pb and Cd can also significantly reduce human semen quality and is related to many diseases in adults and children (e.g., damage to DNA or impairment of the reproductive function) [67].

### 1.4.2.3 Copper

Copper belongs to group I-B of the periodic table, it has an atomic weight of 63.55 with a specific gravity of 8.96 with oxidation states of +2, +1. The important ores of Cu are Chalcocite (CuFeS₂), Cuprite (Cu₂O) and Malachite [CuCO₃, Cu(OH)₂]. Copper is widely used for wire production and in the electrical industry. Its main alloys are brass (with zinc) and bronze (with tin). Other applications are kitchenware, water delivery systems, and copper fertilizers [68].

Copper is considered as an essential constituent of metalloenzymes of living organisms and is required in hemoglobin synthesis and in catalysis of metabolic reactions[10]. It plays a crucial role in many biological enzyme systems that catalyze oxidation/reduction reactions. However, if present at relatively high concentrations in the environment, toxicity to aquatic organisms may occur. Copper under ionic forms Cu²⁺, Cu₂OH⁺ and CuOH⁺ is toxic to fish [69].

High copper levels lead to an increase in the rate of free radical formation [70] teratogenicity [71], and chromosomal aberrations [72, 73].

### 1.4.2.4 Zinc

Zinc is a bluish white soft metal, belongs to group II-B of the periodic table, it has atom weight of 65.38, and density of 7.13. The oxidation state of zinc in nature is II [68].

The most common minerals of zinc are zinc sulphide (ZnS), zincite (ZnO), and smithsonite (ZnCO₃) [74]. Zinc is fourth among metals of the world in annual consumption (behind Fe, Al, and Cu) [75]. Zinc is used in many industries as manufacture of dry cell batteries, production of alloys such as brass or bronze, producing a galvanized coating [74]. The main sources of Zn pollution in the environment are zinc fertilizers, sewage sludges, and mining [68].
Zinc is an essential element for the life of animal and human beings [74]. It present in many enzymes involved in important physiological functions like protein synthesis. Also it is essential for male reproductive activity. Zinc has been reported to cause the same signs of illness as does Pb, and can easily be mistakenly diagnosed as lead poisoning, excess amount of Zn can cause system dysfunctions, cause impairment of growth and reproduction, The clinical signs of Zn toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anemia [1].

1.4.2.5 Manganese
Manganese is a whitish-grey metal, very brittle, and oxidizes superficially in air. It is a member of group VIIA of the periodic table with an atomic weight of 54.94, and a specific gravity of 7.2 g cm\(^{-3}\). It resembles Fe in chemical behavior. Mn has oxidation states of I, II, III, IV, VI, and VII [68]. In natural environment Mn rarely occurs in free state, but mostly in combined form [74]. Mn is frequently found in metamorphic, sedimentary, and igneous rocks. Its average content in the lithosphere is about 1000 ppm. As its ionic size is similar to Ca, the two elements can replace each other in silicate minerals. Mn also replaces Fe in magnetite. Although there are more than 100 Mn minerals such as sulfides, oxides, carbonates, silicates, phosphates, arsenates, tungstates, and borates, the most important Mn mineral is the native black manganese oxide, pyrolusite (MnO\(_2\)). Other main ores are rhodochrosite (MnCO\(_3\)), manganite (Mn\(_2\)O\(_3\)-H\(_2\)O), hausmannite (Mn\(_3\)O\(_4\)), braunite (3Mn\(_2\)O\(_3\)-MnSiO\(_3\)), and rhodonite (MnSiO\(_3\)) [68]. Manganese has many applications in industry, it is used for production of ferromanganese steels, electrolytic manganese dioxide for use in batteries, alloys, catalysts, antiknock agents, pigments, Dryers, wood preservatives and coating welding rods. Manganese is an essential element and present in all living organisms. The excess amount of manganese affects central nervous system, causes liver cirrhosis and a higher concentration of it produces a poisoning called Manganese [Parkinson disease] [74].

1.4.2.6 Nickel
Nickel is a silvery white, hard and malleable metal. It belongs to the so-called iron-cobalt group (group VIII) of the periodic table, Ni has atomic weight of 58.71, with specific gravity of 8.9. It is very abundant element. It is found in all soils and is emitted from volcanoes. It normally occurs in oxidation states 0 and II. Nickel is used as an
alloy in the steel industry, electroplating, Ni/Cd batteries, arc-welding, rods, pigments for paints and ceramics, surgical and dental prosthesis, molds for ceramic and glass containers, computer components, and catalysts [68].

At very trace levels, Ni is considered as an essential trace element [50, 76]. It acts as an activator of some enzyme systems but its toxicity at higher levels is more prominent. High levels of Ni can cause respiratory problems and it is carcinogenic [50, 77, 78].
Chapter Tow
Materials and Methods
2.1 Sample collection

2.1.1 Collection of fish specimens

A total of 59 of six fish species were purchased in August, 2010. The sampling process was performed with helping of expert from health department at the Gaza municipality. The specimens were placed immediately in polyethylene bags, put into isolated container of polystyrene icebox and, then, brought to the Chemistry laboratory at the Islamic University - Gaza.

Measurements of the Basic Biological Parameters

Fishes were first identified by species, then the total length (cm) and the body wet weight (g) of each specimen were measured. The detailed information is listed in Table 2.1.

Table 2.1
List of fish species, number and size of fishes used in this study.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local Name</th>
<th>Common Name</th>
<th>No</th>
<th>TL (cm) mean±SD</th>
<th>BW (g) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Merluccius hubbsi</em></td>
<td>&quot;پکلاء&quot; Pakala</td>
<td>Argentine hake</td>
<td>10</td>
<td>18.36±1.63</td>
<td>194.35±27.10</td>
</tr>
<tr>
<td><em>Micropogonias furnieri</em></td>
<td>&quot;جرع&quot;Jaraa</td>
<td>Croaker fish</td>
<td>9</td>
<td>29.84±1.40</td>
<td>314.49±37.31</td>
</tr>
<tr>
<td><em>Pangasius hypothalamus</em></td>
<td>&quot;فیله&quot;Filleah</td>
<td>Sutchi catfish fillet</td>
<td>10</td>
<td>25.31±1.39</td>
<td>357.6±55.64</td>
</tr>
<tr>
<td><em>Sparus aurata</em></td>
<td>&quot;دنئس&quot;Danese</td>
<td>Sea bream</td>
<td>10</td>
<td>27.05±0.70</td>
<td>360.02±23.41</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>&quot;بلطي&quot;Bolty</td>
<td>Nile tilapia</td>
<td>10</td>
<td>25.11±0.90</td>
<td>311.45±14.28</td>
</tr>
<tr>
<td><em>Mugil cephalus</em></td>
<td>&quot;بوري&quot;Bory</td>
<td>Grey mullet</td>
<td>10</td>
<td>35.04±2.03</td>
<td>381.58±71.56</td>
</tr>
</tbody>
</table>

TL = Total length, BW = Body weight

*Merluccius hubbsi* : sold headless and gutted.
*Pangasius hypothalamus* : sold in the form of slides and not whole fish.
Types and origin of Fish

Fish used in the present study included:

**Frozen fish**: The Argentine hake (*M. hubbsi*) which sold as headless and gutted, the croaker fish (*M. furnieri*) and sutchi catfish fillet (*P. hypothalamus* also known as *P. Pangasius*) are originally imported from Argentina, Uruguay and Vietnam, respectively. They were purchased frozen from local markets in Gaza city.

**Cultured fish**: The two fish species; sea bream (*S. aurata*) and Nile tilapia (*O. niloticus*) were purchased from a fish farm in Gaza Strip.

**Marine fish**: The grey mullet (*M. cephalus*) was bought from local fishermen who caught them from the coastal waters of Mediterranean sea at Wadi Gaza, south of Gaza City and north of the Middle Governorate (Fig.1.1).

Fish handling and preservation

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

2.1.2 Sediment, water and fish diet sampling

Sediment, water and fish diet samples were collected for the purpose of determining the accumulation factor in local fish species. The same elements that were analyzed in fish samples were also measured in the food pellets, water and sediment samples described below.

In present study, the term “local fish” was referred to three fish species; the marine fish species *S. aurata*, the freshwater fish species *O. niloticus* and the wild marine fish species *M. cephalus*.

**Water samples**: Three types of water samples were collected for analysis. The first is the municipal tap water which is used for raising the freshwater fish species *O. niloticus*. The second is the salt ground water which is pumped from a coastal well dug near the farm and used for cultivation the marine fish species *S. aurata*. A total of 4 liters of water samples were collected from the three sampling sites as follows: 2 samples (500ml each) were collected from *O. niloticus* farms, 2 samples just like them were collected from *S. aurata* farms. Four natural seawater samples (500ml
each) were collected from four locations along the coast of the Mediterranean sea at Wadi Gaza region where the depth of water is about 1.5 m, which represent the depth where *M. cephalus* is frequently caught by local fishermen throw fishing net. All samples were collected in 1.5 liter, polyethylene bottles, which were pre-washed with 10% nitric acid and de-ionized water. Before sampling, the bottles were rinsed at least three times with water from the sampling site. The bottles were immersed to about 20 cm below the water surface to prevent contamination of heavy metals from air. All water samples were immediately brought to the laboratory where they filtered through Whatman No.41 (0.45 µm pore size) filter paper. The samples were acidified with 2 ml nitric acid to prevent precipitation of metals, reduce adsorption of the analytes onto the walls of containers and to avoid microbial activity, then water samples were stored at 4°C until the analyses.

**Sediment samples:** Bottom sediment samples were obtained from the same point where seawater samples were collected. At each point, three sediment samples were taken superficially by using pre cleaned 100 ml, wide mouthed, disposable plastic containers and packed separately in pre cleaned polyethylene bags. They were brought to the laboratory, dried in oven (200 °C) to constant weight and then ground into fine powder using pestle and mortar.

**Fish diet:** A total of 100g of different types of commercial fish diet which is used in the farm for feeding of *S. aurata* and *O. niloticus* were collected and then ground into fine powder using pestle and mortar.

The accumulation factors of six heavy metals from fish raising water and diet for *S. aurata* and *O. niloticus* species were calculated, while it was calculated from seawater and sea sediment for *M. cephalus*.

The accumulation factor was calculated in several studies [4, 32, 35, 79-81] according to the formula 1:

\[
\text{Accumulation Factor} = \frac{\text{concentration of metal in fish muscle}}{\text{concentration of metal in abiotic media}} \tag{1}
\]

Where the abiotic media represents the water, sediment and food samples

If the transfer factor is greater than 1.0 then bioaccumulation for metals occurs by fish species.
2.2 Reagents

De-ionized water was used to prepare all aqueous solutions. All plastic and glassware used were rinsed and soaked in 10% (v/v) HNO₃ overnight. They were rinsed with de-ionized water and dried prior using [82]. All acids (Nitric, HNO₃; Sulfuric, H₂SO₄; Hydrochloric, HCl and Perchloric acid, HClO₄) and oxidants (Hydrogen peroxide, H₂O₂) were of highest quality from Merck, Germany.

2.3 Digestion procedures

2.3.1 Digestion of fish Samples

In the present study fish digestion procedure was modified from Manutsewee, et al. [83], who used the same mixture for digestion but they followed ultrasound-assisted acid leaching procedure after fish drying for twenty hours. The present study followed wet digestion process with different concentrations and volumes of acids. kjeldahl digestion plate was used for heating.

Before analysis, the fish were thawed and dissected. The muscular tissues on the dorsal surface of each fish were taken out and homogenized. About 4 grams of the homogenized muscles (without skin) of each specimen were taken and placed in a 300 ml digestion tube. A digestion mixture containing 6.0 ml of high purity HNO₃ (Merck) plus 2 ml of HCl (10 M) and 4 ml of H₂O₂ (35%) was added to each tube. The samples were then heated at 130 °C by kjeldahl heating digester with air condenser until a clear solution was obtained. After cooling, the samples were filtered through Whatman filter paper. The digested portion was diluted to a final volume of 50 ml using de-ionized water. Blank reagents without fish samples were also digested using the same method.

2.3.2 Digestion of sediment samples

For extraction of heavy metal from sediments the standard method described by American Public Health Association [84] was followed. Three representative subsamples each of about 1g of dried soil was digested with 15 ml of a 5:1:1 mixture of HNO₃, H₂SO₄ and HClO₄ in water bath maintained at 80 °C until a transparent solution was obtained. After cooling, the solutions were filtered through Whatman filter paper and diluted to 100 ml with deionized water.

2.3.3 Digestion of water samples

Five ml of concentrated HCl (10 M) was added to 250 ml of each water sample placed in 600 ml beaker, and evaporated to 25 ml. The concentrate was transferred to a 50 ml
volumetric flask and diluted to mark with deionized water. Prior analysis, the solutions were filtered through Whatman number 42 filter paper [45].

2.3.4 Digestion of fish diet samples
Six subsamples of 0.500±0.001 g from the homogenized mixture of fish diet were placed in 100 ml test tubes. The mixture was digested with 12 ml of aqua regia solution comprising a combination of hydrochloric acid and nitric acid in a ratio of 3:1. Samples were heated in water bath at 90 °C for 3 days until a clear solution was obtained. The resulting solutions obtained at the end were transferred to a 50 ml volumetric flask and diluted to mark with deionized water, then filtered through Whatman filter paper. The filtrate was stored until metal determination.

2.4 Blank preparation
At each step of the digestion processes of the samples acid blanks (laboratory blank) were done using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contain the same digestion reagents as the real samples with the same acid ratios but without fish sample. After digestion, acid blanks were treated as samples and diluted with the same factor. They were analyzed by atomic absorption spectrophotometry before real samples and their values were subtracted to check the equipment to read only the exact values of heavy metals in real samples. Each set of digested samples had its own acid blank and was corrected by using the it's blank sample.

2.5 Analytical Technique
Trace elements relate to the very small amounts of the analyte found in the sample which requires special instrumental techniques to be determined. Not long ago, trace levels were around µg/g levels, nowadays concentration levels are ranging from µg/g to ng/g or lower. On the other hand, one element at a high concentration in a sample can be considered as a trace in another.

The analytical technique used to determine heavy metal levels in all samples was thermoelement Solaar S4 Atomic Absorption Spectroscopy (International Equipment Trading Ltd, USA). It is a standard laboratory analytical tool for metal analysis and is based on the absorption of electromagnetic radiation by atoms. The absorption wavelengths and detections limits for the heavy metals were 217.0 nm and 0.001 ppm.
for Pb, 228.8 nm and 0.002 ppm for Cd, 279.5 nm and 0.01 ppm for Mn, 213.9 nm and 0.001 ppm for Zn, 324.7 nm and 0.02 ppm for Cu and 232.0 nm and 0.01 ppm for Ni.

The key feature is the production of free, ground state atoms from the sample, which pass through the light beam from the hallow cathode lamp. For many conditions the absorption of radiation follows Beer’s law:

\[ A = abc \]

Where, \( A \) is the absorbance, \( a \) is the absorptivity, \( b \) is the bath-length of absorption and \( c \) is the concentration of the absorbing species.

Beer’s law shows a relation between absorption and concentration of analyte, so calibration of the instrument is needed.

**Calibration of instrument**

Calibration requires the establishment of a relationship between signal response and known set of standards. The standards in atomic absorption spectrometry refer to the production of a series of aqueous solutions of varying concentrations (working standards) of the analyte of interest. By measuring the signals for a series of working solutions of known concentrations it is possible to construct a suitable graph. Then, by presenting a solution of unknown concentration to the instrument, a signal is obtained which can be interpreted from the graph, thereby determining the concentrations of the element in the unknown.

The actual concentration of each metal was calculated using the formula:

Actual concentration of metal in sample = (µg/g)R × dilution factor

Where:

(µg/g)R = AAS Reading of digest

Dilution Factor = Volume of digest used/Weight of digested sample

**2.6 Validation of analytical methodology (Recovery test)**

In the present study a modified method was employed for digestion process. To check the accuracy of the digestion method and the subsequent validity of analytical technique, homogeneous mixtures of six samples of fish muscles were spiked with standard solutions of all metals considered in present study. The concentration of each metal in it's standard solution was 1000 µg/g. The first three samples were spiked
with 0.1 ml of the standard solution of each metal. The other three samples were spiked with 0.3 ml. A mixture with fish muscles, but without any metal spiking was used as a control. All mixtures were then subjected to the digestion procedure used in present study, then digested samples were transferred to a 100 ml volumetric flask and diluted to mark with deionized water. The resulting solutions were analyzed five times for metal concentration. The error percentage between the five readings was less than 3%. The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery as follows:

\[
\% \text{ Recovery} = \frac{T - C}{T} \times 100
\]

Where:

\( T \) = Concentration of a metal in treatment sample.

\( C \) = Concentration of a metal in control sample

**Precautions followed to prevent contamination**

One of the main problems in the sample preparation is the contamination of the sample during sample pre-treatment (weighting, cutting and digestion). Therefore, several precautions should be taken in order to prevent contamination, such as using acidic solution 10% (v/v) and deionized water to clean all bottles and glasswares prior using. Water samples were acidified at time of collection in order to reduce adsorption of the analytes onto the walls of containers, to prevent the precipitation of metals and to avoid microbial activity.

Fish samples were washed by deionized water prior cutting to remove adsorbed metals on skin. Soil samples were dried before digestion to prevent contamination of water. 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 acid blank were prepared in each set.
2.7 Data analysis

Descriptive statistics such as average, range, standard deviation and standard error values were calculated. Kruskal–Wallis nonparametric one-way analysis of variance was used to examine differences among fish types.

One-way analysis of variance (ANOVA) was used after the logarithmic transformation was done on the data to improve normality followed by Duncan multiple range test to assess whether the means of metal concentrations were varied significantly among fish species. Possibilities less than 0.05 were considered statistically significant (p < 0.05).

The nonparametric Kendall tau correlations were used to examine relationships among metals in the same fish species.

The Kendall tau rank correlation coefficient (or simply the Kendall tau coefficient, Kendall’s $\tau$ or Tau test(s)) is used to measure the strength of the relationship (or the degree of correlation) between two variables. It is feasible to carry out the hypothesis tests (assessing the significance) with the help of Kendall’s tau.

**Interpretation of output of Kendall’s tau ($\tau$)**

The interpretation of the strength of correlation of Kendall’s tau was based on the following:

- 0.00 correlation indicates no correlation i.e. there is no relationship between the two variables
- 0.00 - .20 – Very Weak
- 0.21 - .40 – Weak
- 0.41 - .60 – Moderate
- 0.61 - .80 – Strong
- 0.81 – 1.00 - Very Strong

All statistical calculations were performed with SPSS 11.0 for Windows. Microsoft Excel (2003) was used to calculate mean and standard deviation, also Microsoft Excel was used to plot graphs.
Chapter Three

Results
3.1 Recovery test

The results of recovery of digestion method and analytical technique used in present investigation are reported in Table 3.1. The table shows the following percentage recovery: on average Pb, 95.5%; Mn, 92%; Cu, 90.5%; Cd, 90.5%; Ni 97%; Fe, 96.5% and Zn, 96.5%.

Table 3.1

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration of metal added (μg/g)</th>
<th>Concentration of metal recovered (μg/g)</th>
<th>Average percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>1.00</td>
<td>0.99</td>
<td>95.5 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.78</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>1.00</td>
<td>0.96</td>
<td>92.0 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>1.00</td>
<td>0.94</td>
<td>90.5 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.62</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>1.00</td>
<td>0.90</td>
<td>90.5 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>1.00</td>
<td>1.03</td>
<td>97.0 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>1.00</td>
<td>0.89</td>
<td>96.5 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>1.00</td>
<td>0.96</td>
<td>96.5 %</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.90</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Metal concentrations in different fish species

The concentrations of Cu, Zn, Mn, Ni, Cd and Pb in the muscles of the analyzed six commercial fish species are presented in Table 3.2 by range values, averages and standard errors.

The average values of the same heavy metals concentrations in the muscles of the different fish species are compared in Figures 3.1-3.6. All results are expressed on a wet weight basis as µg/g wet wt.

There were vast differences among the heavy metal concentrations in the muscles of different fish species. The highest concentrations were for Zn, and the lowest were for Cd (Table 3.2).

Based on the average of the concentrations of metals in the different fish species (Table 3.2), the highest levels of Zn, Cd and Pb were found in M. furnieri, while the highest levels of Ni, Cu and Mn were found in M. cephalus. On the other hand, the lowest concentrations of Cu and Zn were detected in P. hypothalamus. S. aurata showed the
lowest average concentration of Mn, while *M. furnieri* gave the lowest concentration of Ni. *M. furnieri* is the only fish species which contained the six metals under study.

Calculation of the average concentrations of Zn, Ni, Cu, Mn, Pb, and Cd in the muscles of the six fish species in µg/g±SD gave the following results: Zn: 9.05±8.32, Ni: 0.69±0.35, Cu: 0.48±0.33, Mn: 0.48±0.56, Pb: 0.13±0.59, Cd: 0.01±0.05. This leads to the following ranking: Zn > Ni > Cu > Mn > Pb > Cd

The metal levels in the fish muscles of each of the six species yield a similar ranking, except for *M. furnieri*, in which metal concentrations followed the order Zn > Pb > Ni > Mn > Cu > Cd.

Statistical analysis using the one-way ANOVA showed a significant difference in Cu (*p* = 0.001), Zn (*p* = 0.001), Cd (*p* =0.001) and Ni (*p* =0.003) levels for the fish species analyzed. There were no significant differences in Pb and Mn levels among the fish species.
Table 3.2
Range of metal contents (µg/g wet weight) and mean±standard error in various fishes

<table>
<thead>
<tr>
<th>Fish name</th>
<th>No. of samples</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Ni</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean±SE</td>
<td>Range</td>
<td>Mean±SE</td>
<td>Range</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>M. hubbsi</td>
<td>10</td>
<td>0.28-0.35</td>
<td>0.318±0.01</td>
<td>4.50-8.55</td>
<td>5.821±0.4</td>
<td>0.24-0.98</td>
<td>0.519±0.07</td>
</tr>
<tr>
<td>M. furnieri</td>
<td>9</td>
<td>0.25-0.51</td>
<td>0.345±0.03</td>
<td>9.47-32.85</td>
<td>20.535±3.1</td>
<td>0.26-0.50</td>
<td>0.396±0.02</td>
</tr>
<tr>
<td>P. hypothalmus</td>
<td>10</td>
<td>0.18-0.35</td>
<td>0.251±0.02</td>
<td>2.44-5.97</td>
<td>3.705±0.32</td>
<td>0.26-0.70</td>
<td>0.381±0.04</td>
</tr>
<tr>
<td>S. aurata</td>
<td>10</td>
<td>0.29-0.57</td>
<td>0.399±0.03</td>
<td>3.42-12.93</td>
<td>4.946±0.9</td>
<td>0.26-0.50</td>
<td>0.376±0.02</td>
</tr>
<tr>
<td>O. niloticus</td>
<td>10</td>
<td>0.37-1.20</td>
<td>0.638±0.08</td>
<td>4.25-12.40</td>
<td>7.522±0.96</td>
<td>0.28-0.45</td>
<td>0.386±0.03</td>
</tr>
<tr>
<td>M. cephalus</td>
<td>10</td>
<td>0.41-2.27</td>
<td>0.907±0.17</td>
<td>3.09-33.31</td>
<td>12.783±3.61</td>
<td>0.30-4.54</td>
<td>0.834±0.31</td>
</tr>
</tbody>
</table>
Table 3.2 shows that the lowest and highest values of Cu in fish species were 0.18 µg/g in *P. hypothalmus* and 2.27 µg/g in *M. cephalus*. Cu average concentration in *M. cephalus* was significantly higher (P ≤ 0.033) than all other fish types (Figure 3.1) with average value of 0.907±0.171 µg/g followed by *O. niloticus* with average of 0.638±0.084 µg/g. The pattern of the average Cu concentration in the muscles of the remaining fish types in order of decreasing contents were *S. aurata > M. furnieri > M. hubbsi > P. hypothalamus* with average values of 0.399±0.031, 0.345±0.029, 0.318±0.009 and 0.251±0.017 µg/g, respectively.

![Figure 3.1](image.png)

**Figure 3.1** Difference of Cu average concentration (µg/g) among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

The lowest and highest values of Zn in fish species were 2.44 µg/g in *P. hypothalmus* and 33.31 µg/g *M. Cephalus*. The average concentration of Zn in *M. furnieri* was significantly higher (P≤0.006) than all other species with value of 20.35±3.08 followed by *M. cephalus* with average concentration of 12.78±3.61 (Figure 3.2). Average concentration of Zn was in the order *O. niloticus > M. hubbsi > S. aurata > P. hypothalamus* with values of 7.522±0.963, 5.821±0.436, 4.946±0.904, 3.705±0.325 µg/g, respectively.
Figure 3.2  Difference of Zn average concentration (µg/g) among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

Except in *M. furnieri*, the Cd concentration was below the detection limit in all fish species. Concentration of Cd in *M. furnieri* ranged from ND to 0.30 µg/g with an average concentration of 0.09±0.04 µg/g which was significantly higher (P≤0.001) than all other species.

Figure 3.3 Difference of Cd average concentration (µg/g) concentration among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

The highest concentration of Mn was in *M. cephalus* with value of 4.54 µg/g, while *M. hubbsi* gave the lowest value with 0.24 µg/g. There were no significant differences (P > 0.05) in Mn average concentrations among the species (Figures3.4). Average concentration of Mn was in *M. cephalus* > *M. hubbsi* > *M. furnieri* > *O. niloticus* > *P. hypothalamus* > *S. aurata* with values of 0.834±0.414, 0.519±0.069, 0.396±0.024, 0.386±0.031, 0.381±0.04 and 0.376±0.024 µg/g, respectively.
Figure 3.4 Difference of Mn concentration among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

Lead (ranging from ND to 4.34 µg/g) was detected only in three fish species; *M. furnieri*, *M. cephalus* and *O. niloticus*. The highest value of Pb concentration was in *M. furnieri* fish which gave an average concentration of 0.552±0.479 µg/g followed by *M. cephalus* and *O. niloticus* with average concentrations of 0.172±0.092 and 0.115±0.07 µg/g, respectively (Figure 3.5). There were no significant difference of Pb concentration was detected among all fish species (P > 0.05).

Figure 3.5 Difference of Pb average concentration (µg/g) among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

Ni concentrations among the fish species ranged from 0.22µg/g in *M. furnieri* to 2.35 µg/g in *M. cephalus*. The average concentration of Ni can be ordered as follows: *M. cephalus* > *O. niloticus* > *M. hubbsi* > *S. aurata* > *P. hypothalmus* > *M. furnieri*, with average values of 0.978±0.19, 0.892±0.15, 0.707±0.04, 0.634±0.03, 0.511±0.02 and 0.453±0.04 respectively. Figure 3.6, shows that the average concentration of Ni in *M.
*cephalus* was significantly higher (p ≤ 0.028) than *M. furnieri, P. hypothalamus* and *S. aurata*.

![Graph showing nickel concentration among six fish species](image)

**Figure 3.6** Difference of Ni average concentration (µg/g) among the six fish species. The different letters beside the vertical bars indicate that the values are significantly different (p < 0.05).

### 3.3 Correlation Analysis of heavy Metals

Nonparametric kendall tau test correlation was used to examine the relationships among the different metals in each fish species (Table 3.5). In general, the Pair wise correlation showed fairly high positive correlations between some metals and others, but negative or no correlations with others. The majority of Mn concentrations were positively correlated; exceptions were with Cu, and Ni in *M. hubbsi* and with Zn in *O. niloticus*. Strong, significant (p < 0.05) and positive correlations were observed between Cu-Ni (τ = 0.618), Mn-Cu (τ = 0.719), Mn-Ni (τ = 0.750) and Zn-Ni (τ = 0.790) in *M. furnieri, M. cephalus, P. hypothalamus* and *O. niloticus*, respectively. On the other hand, moderate correlations were observed between Mn-Cu (τ = 0.467), Mn-Zn (τ = 0.511) and Pb-Zn (τ = 0.548) in *P. hypothalamus, S. aurata* and *M. cephalus*, respectively. On the other hand, the remaining relations were ranged from weak to very weak positive correlations (τ < 0.40.) between the different metals in the different fish species and with low levels of confidence (p > 0.05).

Despite the negative correlations were found between Mn-Cu, Mn-Ni, Cu-Zn (*M. hubbsi*) Pb-Cu, Pb-Zn (*M. furnieri* and *O. niloticus*), Mn-Zn, Pb-Ni (*O. niloticus*) and Cu-Ni (*M. cephalus*), no significance confidence were detected between them (p > 0.05).
Table 3.3

Kendall tau correlation coefficients $\tau$ for various metals in the six commercial fish species

<table>
<thead>
<tr>
<th></th>
<th>$M.\ hubbsi$</th>
<th>$M.\ furnieri$</th>
<th>$P.\ hypothalmus$</th>
<th>$S.\ aurata$</th>
<th>$O.\ niloticus$</th>
<th>$M.\ cephalus$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manganese with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>NS</td>
<td>0.215</td>
<td>NS</td>
<td>NS</td>
<td>0.150</td>
<td>0.277</td>
</tr>
<tr>
<td>Copper</td>
<td>-0.315</td>
<td>0.389</td>
<td>0.467*</td>
<td>0.045</td>
<td>0.302</td>
<td>0.719**</td>
</tr>
<tr>
<td>Cadmium</td>
<td>NS</td>
<td>0.131</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>0.023</td>
<td>0.222</td>
<td>0.200</td>
<td>0.511*</td>
<td>-0.345</td>
<td>0.36</td>
</tr>
<tr>
<td>Nickel</td>
<td>-0.067</td>
<td>0.255</td>
<td>0.750**</td>
<td>0.289</td>
<td>0.029</td>
<td>0.229</td>
</tr>
<tr>
<td><strong>Lead with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>NS</td>
<td>-0.043</td>
<td>NS</td>
<td>NS</td>
<td>-0.483</td>
<td>0.304</td>
</tr>
<tr>
<td>Cadmium</td>
<td>NS</td>
<td>0.152</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Zinc</td>
<td>NS</td>
<td>-0.129</td>
<td>NS</td>
<td>NS</td>
<td>-0.026</td>
<td>0.548*</td>
</tr>
<tr>
<td>Nickel</td>
<td>NS</td>
<td>0.214</td>
<td>NS</td>
<td>NS</td>
<td>-0.028</td>
<td>0.111</td>
</tr>
<tr>
<td><strong>Copper with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>NS</td>
<td>0.196</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Zinc</td>
<td>-0.180</td>
<td>0.278</td>
<td>0.111</td>
<td>0.090</td>
<td>0.073</td>
<td>0.200</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.180</td>
<td>0.618*</td>
<td>0.386</td>
<td>0.270</td>
<td>0.150</td>
<td>-0.085</td>
</tr>
<tr>
<td><strong>Cadmium with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>NS</td>
<td>0.065</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Nickel</td>
<td>NS</td>
<td>0.227</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Zinc with</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>-0.244</td>
<td>0.109</td>
<td>0.023</td>
<td>0.067</td>
<td>0.790**</td>
<td>0.423</td>
</tr>
</tbody>
</table>

* $p \leq 0.05$ (correlation is significant); ** $p \leq 0.01$ (correlation is highly significant)
3.4 Heavy metal concentrations in water, sediment and fish diet samples

The average values of heavy metals concentrations in water, sediment and fish diet samples are given in Table 3.4.

Table 3.4
The average concentrations of metals in water, sediment and diet samples

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Heavy metal concentrations (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
</tr>
<tr>
<td>Seawater(^a)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sediment(^a)</td>
<td>3.13</td>
</tr>
<tr>
<td>Salt water(^b)</td>
<td>0.28</td>
</tr>
<tr>
<td>Fish diet(^b)</td>
<td>15.67</td>
</tr>
<tr>
<td>Fresh water(^c)</td>
<td>0.30</td>
</tr>
<tr>
<td>Fish diet(^c)</td>
<td>10.23</td>
</tr>
</tbody>
</table>

\(^a\): Mugil cephalus, \(^b\): Sparus aurata, \(^c\): O. niloticus

3.5 The accumulation factor

The accumulation factor of heavy metals from water, sediments and diet in fish muscles is shown in Table 3.5. The results showed that accumulation factor of water were greater than those of sediments and diet. All accumulation factors of water were greater than 1 except for Pb in *M. cephalus*. On the other hand, the accumulation factor of sediment and diet of all metals were found to be less than 1.

The accumulation factor from water to fish in case of *M. cephalus* was in the order of Zn (65.623) > Cu (22.525) > Mn (9.151) > Ni (2.649) > Pb (0.809). Copper was the greatest metal accumulated by *M. cephalus* from sediment, while the accumulation factor of Pb was the lowest one.

In *S. aurata*, the highest value of the accumulation factor from water was for Zn followed by Mn, Ni, and Cu. Accumulation factor for Zn was the highest from diet followed by Ni, Cu and Mn.

A different order of Zn (29.125) > Ni (23.766) > Mn (9.492) > Pb (8.214) > Cu (2.11) were observed from water within *O. niloticus*. The accumulation factor from diet followed the order Zn > Cu > Ni > Mn > Pb.

Since the concentrations of Cd were below the detection limit in all fish species, the accumulation factor for this metal was not determined.
Table 3.5

Accumulation factor from water, sediment and food for the three local fish types.

<table>
<thead>
<tr>
<th>Fish name</th>
<th>Element</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Ni</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mugil cephalus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sediment /fish</td>
<td>0.289</td>
<td>0.06</td>
<td>0.02</td>
<td>0.049</td>
<td>Nd</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Sea water/ fish</td>
<td>22.525</td>
<td>65.623</td>
<td>9.151</td>
<td>2.649</td>
<td>Nd</td>
<td>0.809</td>
<td></td>
</tr>
<tr>
<td>Sparus aurata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food/fish</td>
<td>0.025</td>
<td>0.04</td>
<td>0.007</td>
<td>0.031</td>
<td>Nd</td>
<td>Nd</td>
<td></td>
</tr>
<tr>
<td>Salt water/ fish</td>
<td>1.408</td>
<td>23.496</td>
<td>4.682</td>
<td>1.915</td>
<td>Nd</td>
<td>Nd</td>
<td></td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food/fish</td>
<td>0.062</td>
<td>0.218</td>
<td>0.014</td>
<td>0.047</td>
<td>Nd</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Fresh water/ fish</td>
<td>2.11</td>
<td>29.125</td>
<td>9.492</td>
<td>23.766</td>
<td>Nd</td>
<td>8.214</td>
<td></td>
</tr>
</tbody>
</table>

3.6 Health-Risk Assessment for Fish Consumption (Consumption Safety)

According to the directorate General of Fisheries in Palestinian Ministry of Agriculture, the average quantity of fish consumed per person (assuming a 70-kg person) per a day in Gaza strip is 11.66g. Multiplying this value by the average concentration of each metal (Cu, Zn, Mn, Ni, Cd and Pb) in analyzed fish, the average daily intake (consumption) of metals from fish can be estimated. The daily consumption of Cu, Zn, Mn, Ni, and Pb in the fishes of this study was ranged from 2.93-10.58, 43.2-239.44, 4.38-9.72, 5.28-11.40 and 1.34-6.44, respectively (Table3.6). The average daily intake of metals through fish consumption can be ordered as following: Zn>Ni> Cu ≈ Mn >Pb>Cd.

Table 3.6

The estimated daily intake (µg/day) of metals by humans in Gaza Strip from fish muscles

<table>
<thead>
<tr>
<th>Fish name</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Ni</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merluccius hubsi</td>
<td>3.71</td>
<td>67.87</td>
<td>6.05</td>
<td>8.24</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Micropogonias furnieri</td>
<td>4.02</td>
<td>239.44</td>
<td>4.62</td>
<td>5.28</td>
<td>1.05</td>
<td>6.44</td>
</tr>
<tr>
<td>Pangasius hypothalamus</td>
<td>2.93</td>
<td>43.20</td>
<td>4.44</td>
<td>5.96</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Sparus aurata</td>
<td>4.654</td>
<td>57.67</td>
<td>4.38</td>
<td>7.39</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td>7.44</td>
<td>87.71</td>
<td>4.50</td>
<td>10.40</td>
<td>Nd</td>
<td>1.34</td>
</tr>
<tr>
<td>Mugil cephalus</td>
<td>10.58</td>
<td>149.05</td>
<td>9.72</td>
<td>11.40</td>
<td>Nd</td>
<td>2.01</td>
</tr>
</tbody>
</table>
Chapter Four
Discussion
As a modified method of digestion was used in the present study, a validation was necessary. Tables 3.1 showed the following percentage recovery: on average Pb:95.5%; Mn:92%; Cu:90.5%; Cd: 90.5%; Ni: 97%; Fe: 96.5% and Zn: 96.5%. These results show good recoveries of spiked samples with error percentage less than 15%, that indicates an accuracy of analytical methodology.

In some cases, the percentage of recovery was higher than 100%, as in Ni and Fe this suggested some contamination with the two metals from lab air, glasswares, Metal tools, an error in weighing process or an error in measurement process.

**4.1 Accumulation of metals by fish species**

This study was undertaken to investigate heavy metal concentrations in edible parts (muscles) of six commercially important fish species in Gaza strip market, and to detect whether their levels are potentially harmful for human health if included in the diet. *M. hubbsi, M. furnieri, P. hypothalamus, S. aurata, M. cephalus* and *O. niloticus* were selected because they are the most commonly consumed fish in Gaza Strip.

The levels of heavy metals were determined in the muscles in each species because of it's importance for human consumption.

It is well known that its very difficult to compare the metal concentrations even between the same tissue in different species because of the difference in many factors as, the aquatic environments, concerning the type and the level of water pollution, feeding habits whether omnivorous or carnivorous, and level of fish presence in water, whether pelagic or benthic fish etc. Kamaruzzaman, et al., [30], indicated that there were a relation between metal concentration and several intrinsic factors of fish such as organism size, genetic composition and age of fish. Taking all these factors into account it was very difficult to compare metal concentrations between the six fish species in present study, because of the difference in environmental medium and habits, so the interested was in metals levels in fish muscles regardless of fish type or fish environment.

This investigation showed that different fish species contained different concentrations of a certain metal in their muscles. Kalay, et al., [29], reported that different fish species accumulate metals in their tissue in significantly different values. Moreover, Canli and Atli, [85], reported that levels of heavy metals in fish vary in various species and different aquatic environments.
On the other hand, Farkas, et al. [86], attributed the differences of concentrations of metals between fishes to feeding habits, the bio-concentration capacity of each species and to the biochemical characteristics of the metal. In addition Romeo, et al.[87], described that the ability of fish to accumulate heavy metals depends on ecological needs, metabolism, degree of pollution in sediment, water and food, as well as salinity and temperature of water.

Zinc concentration was the highest of all metals in all fish species (Table 3.2). This agrees with several studies performed in many countries. Huang, [5] found the order of concentrations of four heavy metals in common benthic fishes decreasing as: Zn > Cu > Cd = Pb, in addition Chen and Chen [11], ordered the concentrations of some metal in fish as the following: Zn = Fe > Cu = Mn > Cd. Bahnasawy et al., [12], summarized that the average concentrations of the metals in fish tissues from Lake Manzala, Egypt exhibited the following order: Zn>Cu>Pb>Cd.

These results were in agreement with results from present study in which concentrations of the metals followed the order Zn > Ni > Cu > Mn > Pb > Cd.

When a comparison between Zn, Cu and Cd concentrations in some fishes was performed by Chen and Chen [88], results indicated that only Zn significantly showed both gastrointestinal absorption and gill uptake, while Cd and Cu were significantly absorbed by gill rather than digestive tract absorption. The importance of Zn for biota that it serves as a co-factor for dehydrogenating enzymes and in carbonic anhydrase also, Zn is essential for male reproductive activity [1].

Mn, Cu and Ni are essential elements for biota, their average concentrations in fish muscles in the present study were between: 0.376-0.834 for Mn; 0.251-0.907 for Cu and 0.453-0.978µg/g for Ni.

Qiao, et al., [89], found the range of Cu in muscles of some fish species from Taihu Lake, China between ND and 1.890±0.301 µg/g. Uysal, et al., [90] detected Cu in muscles of some migratory fish with a range from 1.03±0.1 to 5.11±0.4 µg/g, while Mn concentration ranged from 0.09-0.28 µg/g. Concentrations of Ni in M. cephalus fish ranged from 3.63±0.28 to 4.55±0.15 µg/g, while Cu concentrations ranged from 1.73±0.09 to 2.72 ±0.17 µg/g. These were the results of a study performed by Laxmi, et al., [91], who detect the bioaccumulation of heavy metals in two fish species from south east coast of India.

Generally, in present study, concentrations of non-essential elements (Cd and Pb) in fish muscles were lower than those of essential metals (Zn, Mn, Ni and Cu) (Table 3.2).
This result is consistent with what Huang et al. [92] reported, that the accumulation levels of the essential metals in fish are generally higher and more homeostatic than the non-essential metals.

Table 3.2 demonstrated that *M. cephalus* gave the highest concentration of Mn and it contained a considerable amount of Zn comparing with other fishes. Also *M. cephalus* had concentrations of Cu and Ni significantly higher than some other fishes (Fig. 3.1 and 3.6).

This result may be interpreted by feeding habits of *M. cephalus* which is considered as a filter and detritus-mud feeder [88], which means that it can accumulate metals from both water and sediment. Kilgour, [93] pointed out that animals which live with relationship with sediment, show relatively high levels of metals in their tissues. Moreover, as *M. cephalus* lives in shallow water near the coast, so it can accumulate metals from polluted water and sediment in higher amounts than other fishes which live far away from the coast [42].

Yilmaz, [41] compared concentrations of Fe, Cu, Ni, Cr, Pb and Zn in *M. cephalus* with those concentrations in *S. aurata* from Iskenderun Bay in Turkey, Yilmaz described that according to its foraging habits *M. cephalus* showed higher levels of metals than *S. aurata*.

Also *M. cephalus* accumulated more concentrations of heavy metals than *O. niloticus*, according to its trophic characteristic, that was the result of a study performed by Yilmaz, [3]. In addition, Ali and Abdel-Satar, [23] results mentioned that *Mugil* species tended to accumulate more concentration of Cu, Zn, Pb, and Cd than Tilapia species. *M. furnieri* gave the highest concentrations of Pb, Zn, and Cd by 0.552, 20.535 and 0.090 µg/g, respectively, also *M. furnieri* accumulated highest concentrations of Cd and Zn than other species in present study (Fig. 3.2 and 3.3), this may interpreted by living and feeding habits of *M. furnieri* (croaker) which is a bottom-dwelling marine species, found in muddy and sandy bottoms in coastal waters. It is considered as a benthic feeders, it feeds on benthic migratory crustaceans and sessile mollusks and occasionally preying on fish [94, 95].

Romeo et al. [87], reported that levels of metals found in tissues of benthic fish were always higher than those found in pelagic fish.

In Argentine, Marcovecchio, [51], determined Zn concentration of *M. furnieri* by 20.5 µg/g, a value related to our average value of Zn (20.53 µg/g) in *M. furnieri*. 

43
High concentration of both Zn and Cd in *M. furnieri* may be attributed to the relation between them which has been studied in terrestrial and marine mammals. The increase in Zn concentration was attributed to compensate the increase in Cd concentration due to pollution processes, and this mechanism probably includes the synthesis of metallothioneins (or metallothionein-like proteins), which would bind both Cd and Zn in a molar ratio of 1:1 [51].

*P. hypothalmus* gave the lowest concentrations of Cu and Zn. It also accumulated low levels of Mn and Ni with no significant difference than the lowest values obtained from *S. aurata* and *M. furnieri* in the present study (Figures 6.4, 6.6, respectively). Pb and Cd concentrations in *P. hypothalmus* were below the detection limits (Table 3.2).

These low levels of metals accumulation in this species may be attributed to fish environment or fish feeding habits. *P. hypothalmus* is a freshwater fish present in the main water basins of South-East Asia rivers and aquaculture ponds. As an omnivorous species, Pangasius is fed agricultural by-products, mainly rice bran and soy [96].

### 4.2 Correlation between metals among fish species

The correlation coefficient has been calculated in order to understand the overall relationships among the various elements.

Table 3.3 showed a strong significant relationship between Mn and Cu in two fish species (*P. hypothalamus, M. cephalus*), this agrees with the result found by Yilmaz et al., [40] who indicated a highly positive relationship between Mn and Cu in two fish species (*Leuciscus cephalus, Lepomis gibbosus*). Correlation between Pb and Mn was positive in all fish species that contained amounts of the two metals (*O. niloticus, M. cephalus* and *M. furnieri*). Yilmaz et al., [40], pointed out highly positive relationship between the two metals in some fish species. Moreover Burger, [15], found that Mn and Pb were the only metals that were positively correlated for all fish species under study. Saeed and Shaker, [34], attributed the increasing of Pb and Mn in Delta lakes to engine boats which distributed them in water.

Relation between Cu and Zn was a positively week correlation in five fish species, and negative in the sixth one. Yilmaz, et al., [40], mentioned low relationship between Cu and Zn in fish. Correlation between Cu and Ni in *M. cephalus* species was a very low negative relationship (Table 6.3), this correlation may deceive us to predict that as one metal is
high the other should be low, but *M. cephalus* showed concentrations of Cu and Ni that significantly higher than other fishes (Figures 3.1 and 3.6).

Burger, [15], reported that from correlation between metals we can not predict the concentration of a metal will be high or low depending on concentration of other metal. On the contrary, correlation between Pb and Zn in *M. cephalus* was a moderate positive correlation (\( \tau = 0.548 \)) but transfer factors for Pb and Zn in *M. cephalus* from water were 0.809 for Pb and 65.623 for Zn which means that fish did not accumulate Pb from water, although, Zn was accumulated in fish muscles from water, this may attributed to biochemical characteristics of the metal or biological needs of fish, that fish accumulates essential metals in their tissues more than non essential metals [92].

### 4.3 Accumulation factor (Transfer factor)

The presence of metals in high levels in fish environment does not indicate a direct toxic risk to fish, if there is no significant accumulation of metals by fish tissues [30].

In the present study, transfer factors (tfs) of six metals for sediment and food and water in local fishes (Table 3.5) were computed. The results indicated that transfer factors from sediment (for *M. cephalus*) and diet (artificial food only for *O. niloticus* and *S. aurata*) were all below 1.00 which means that no bioaccumulation of any metal was occurred from sediment or artificial food by fishes. On the other hand, all transfer factors (tfs) from water were higher than 1.00 - except lead in *M. cephalus* which means that the three local fish species accumulated metals from water. This result agrees with many previous studies. Rashed, [32] determined transfer factors for Co, Cr, Cu, Fe, Mn, Ni, Sr and Zn from water, sediment and plant in *Tilapia nilotica* fish in Nasser lake, results indicated that only transfer factors from water for all metals were >1.00, which means that fish accumulated metals from water.

Also Abdel-Baki, et al., [4] calculated transfer factors of five heavy metals from water and sediment in Tilapia fish, results indicated that fish accumulated all metals in its tissues from water. Transfer factors of metals from water in fish muscles were 41.789, 8.621, 11.923 24.714, 35.938 for Pb, Cd, Hg, Cu, Cr, respectively.

In *M. cephalus* the transfer factor of Pb from water was 0.809 (< 1.00) which means that no bio-accumulation of Pb occurred from water, on the other hand transfer factor from water was higher than transfer factor of Pb from sediment (Table 3.5), this
indicated that water was the major source of Pb for *M. cephalus*, but fish did not face high concentration of Pb to be accumulated, that accumulation of metals only begins when organisms are faced with high concentration in the surrounding medium [4].

*O. niloticus* showed bioaccumulation of all metals except Cd from water. Since water quality is the major factor in aquaculture sustainability [4], concentrations of heavy metals presence in freshwater used in *O. niloticus* ponds were compared with international guidelines for freshwater quality. Table 4.1 showed that levels of heavy metals in fresh water used in aquaculture pond was in the permissible levels set by international organizations.

*Sparus aurata* fish which is not a filter feeder fish, showed the lowest transfer factors from water comparing with *O. niloticus* and *Mugil cephalus* for all metals by values of 1.41 (Cu), 23.5 (Zn), 4.69 (Mn), 1.9 (Ni) (Table 3.5).

**Table 4.1**
The heavy metal concentrations (mg/L) in freshwater used in cultured pond and it’s comparison with international standards.

<table>
<thead>
<tr>
<th>Element</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Mn</td>
</tr>
<tr>
<td>WHO 1993</td>
<td>0.04</td>
</tr>
<tr>
<td>EPA 2002</td>
<td>-</td>
</tr>
<tr>
<td>EC, 1998</td>
<td>-</td>
</tr>
<tr>
<td>EPA 1985</td>
<td>-</td>
</tr>
</tbody>
</table>
4.4 Consumption safety

4.4.1 Concentrations of trace elements in fishes and comparison with international dietary standards and guidelines

Table 4.2 shows the maximum acceptable concentrations in fish muscles of the six metals described in the present study set by many international organizations as WHO, EC and MAFF.

Table 4.2

<table>
<thead>
<tr>
<th>Organization</th>
<th>Pb</th>
<th>Cu</th>
<th>Ni</th>
<th>Mn</th>
<th>Cd</th>
<th>Zn</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 1996</td>
<td>2.00</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[77]</td>
</tr>
<tr>
<td>WHO 1989</td>
<td>2.00</td>
<td>30</td>
<td>0.5-1.0</td>
<td>1.00</td>
<td>1.00</td>
<td>100</td>
<td>[99]</td>
</tr>
<tr>
<td>EC 2005</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>[97, 100]</td>
</tr>
<tr>
<td>MAFF 1995</td>
<td>2.00</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>[77]</td>
</tr>
</tbody>
</table>

The maximum average concentration of Cu in the present study was 0.907±0.171 µg/g in *M. cephalus* (Table 3.2). This value was far away from the maximum acceptable limit of WHO which limited the maximum concentration of Cu in fish muscles by 30 µg/g [77, 99]. MAFF in 1995, proposed the maximum acceptable concentration of Cu in fish muscles by 20 µg/g [77].

The maximum average concentration of Pb in the present study was 0.552±0.479 µg/g in *M. furnieri* (Table 3.2). This value exceeded the maximum acceptable limit of Pb in fish muscles proposed by EC, which set MRL of Pb in fish muscles by 0.2 µg/g [97, 100], but the highest average concentration of Pb observed in the present study was lower than the maximum limits identified by WHO and MAFF which were 2.00 µg/g [77, 99].
The maximum average concentration of Ni in all analyzed samples was 0.978 ±0.192 µg/g wet wt. in *M. cephalus* (Table 3.2). This value was close to WHO guidelines which marked the maximum acceptable level of Ni in fish muscles by 1.0 µg/g wet wt. [99].

Cadmium is considered as non essential metal for biota. In the present Cd was detected only in one fish species (*M. furnieri*), with average concentration of 0.090±0.039 µg/g (Table 3.2). This value is nearly 2 times higher than the maximum acceptable limit of Cd in fish set by EC which detected it by 0.05 µg/g [97, 100], but the highest value of Cd in the present study was 11 times lower than the maximum permissible value set by WHO which limited it by 1.00 µg/g [99].

The maximum average concentration of Mn in the present study was 0.834 ±0.414 µg/g in *M. cephalus*. WHO identified the maximum tolerable limit of Mn in fish muscles by 1.00 µg/g [99].

Zn is an essential trace element in our diet that is required for the synthesis of DNA, RNA, and protein and thus for cell division. In the present study, Zn represented the highest concentration between metals in all fish species. It's maximum average concentration was 20.535±3.081 µg/g in *M. furnieri*. This value was far away from the maximum acceptable limit of Zn in fish muscles set by WHO in 1989 which limited it by 100 µg/g [99]. MAFF in 1995 limited the maximum acceptable value of Zn in fish muscles by 50 µg/g [77].

### 4.4.2 Daily consumption safety

The estimated maximum total dietary intakes of the six heavy metals in fish from present study were 10.58, 239.44, 9.72, 11.40, 1.05 and 6.44 µg per day for Cu, Zn, Mn, Ni, Cd and Pb, respectively (Table 3.6).

For Cu, the maximum value of consumption from fish per day obtained from the present study was 0.0105 mg which was far below the maximum acceptable daily intake value proposed by USA National Research Council in 1989, which limited it by 2-3 mg per day [5].
Maximum daily intake of Zn, in the present study was 0.239 mg. Comparing this value with international standards from USA and WHO which limited the maximum tolerable daily intake (TDI) of Zn by 15 and 19 mg per day, respectively [5, 11], it can be concluded that there was no health risk by consumption of Zn from fishes in the present study.

For Pb and Cd, the maximum daily intake values obtained from the present study were 0.006 and 0.001 mg per day, respectively. These values were far below the maximum TDI values set by WHO which identified them by 0.21 and 0.06 mg per day, respectively [16].

It is worth mentioning that Palestinian customers who consumed amounts of fish with an average of 410 g or more per day may be exposed to amounts of Pb exceed the maximum allowable standards identified by international organizations. On the other hand, people consumed amounts of fish with an average of 700 g or more per day may be exposed to amounts of Cd exceed the maximum allowable standards proposed by international organizations, which makes their health at risk.

WHO in 1993 set TDI of Ni of 0.3 mg per day [16]. This value is a 30 times higher than the maximum value obtained for Ni in the present study which was 0.011 mg per day. But Palestinian People who consumed amounts of fish with an average of 350 g or more per day may be exposed to amounts of Ni exceed the maximum allowable standards identified by international organizations.

Maximum daily intake of Mn in the present study was 0.009 mg. Comparing this value with the international standards from USA and WHO which proposed the maximum acceptable level of Mn consumption by 2-5 mg per day [11]. It was obvious that consumption of Mn from fish species in the present study was not harmful for human health.

Therefore, the daily intake of Cu, Zn, Mn, Ni, Cd and Pb for regular consumption (less than 350 g per day) of the six fish species in the present study did not has any hazardous effect on human health.
Moreover, daily intake values of heavy metals from fish were determined in many previous studies in several countries as Taiwan, Egypt and Saudi Arabia.

In a study performed by Huang, [5], the maximum daily intake of Zn, Cd, Cu and Pb from consumption fishes from eastern Taiwan were 241, 32, 9.6 and 11.9 μg per day, respectively. In addition Chen and Chen, [11], determined the daily intake of Zn, Cd and Mn from consumption of nine species of fishes from Taiwan as 459, 284 and 522 μg per day, respectively.

In Egypt Khalid, [101], examined the maximum daily intake of Cd, Cu and Pb to be 4.73, 38.66 and 18.77 μg per day, respectively.

In Saudi Arabia several studies were performed to investigate the maximum daily intake of heavy metals in fish. Al Bader, [16], indicated that the maximum total dietary intakes of Cd, Zn, Pb and Ni in fish muscles were 0.013, 11.61, 0.05, .012 μg/day, respectively. Also Al Saleh and Shinwari, [102], reported that the maximum total dietary intakes of Cd, Pb and Ni in fish were 0.07, 0.13 and 0.73 μg/day, respectively.
4.5 Comparison of metals concentrations with other studies

In order to have a clear judgment about the level of pollution in tissue of certain types of fish consumed by people in Gaza strip, the obtained data should be compared with some other literature data belongs to comparable studies in our country. Because of absence of literature about commercial fish in Gaza Strip market, we compared our results with other studies in the world.

Table 4.3 shows a comparison between the concentrations of metals in the present study with concentrations in other studies conducted on the same fish species in several countries. The variations of heavy metal concentrations in fish from different areas of the world may be due to differences in metal concentrations and chemical, physical characteristics of water from which fish were sampled [12].

For *O. niloticus*, Table 4.3 clearly shows that concentrations of Zn, Cu and Mn in Tilapia fish muscles in the present study gave a reasonable level comparing with other results. Generally, concentrations of non essential metals (Cd, Pb) in *O. niloticus* showed lower levels than other open literature in Table 4.3.

Concentrations of all metals in *M. cephalus* fish in the present study were clearly lower than other studies from Turkey and Egypt.

Moreover, concentrations of all metals in *S. aurata* were lower than values obtained from Turkey in several studies. In addition concentrations of metals were close to concentrations of those in wild and cultured *S. aurata* fish from Italy.

Cadmium and Zn concentrations in *M. furnieri* from present study were close to values obtained by Marcovecchio, [51] in Argentine.
### Table 4.3
Heavy metal concentration (µg/g wet wt) in muscles of different fishes from various countries

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Location</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Ni</th>
<th>Cd</th>
<th>Pb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O. niloticus</strong></td>
<td>Gaza strip</td>
<td>0.638</td>
<td>7.522</td>
<td>0.386</td>
<td>0.892</td>
<td>Nd</td>
<td>0.115</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>2.39</td>
<td>14.66</td>
<td>7.66</td>
<td>0.51</td>
<td>1.51</td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>1.94</td>
<td>-</td>
<td>-</td>
<td>0.77</td>
<td>1.74</td>
<td></td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Saudia</td>
<td>1.08</td>
<td>-</td>
<td>-</td>
<td>0.0075</td>
<td>0.397</td>
<td></td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>0.313</td>
<td>1.915</td>
<td>0.108</td>
<td>0.053</td>
<td>0.015</td>
<td>0.418</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>0.323</td>
<td>2.364</td>
<td>0.203</td>
<td>0.113</td>
<td>0.006</td>
<td>0.395</td>
<td>[99]</td>
</tr>
<tr>
<td><strong>M. cephalus</strong></td>
<td>Gaza strip</td>
<td>0.907</td>
<td>12.783</td>
<td>0.834</td>
<td>0.978</td>
<td>ND</td>
<td>0.172</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Gaza strip</td>
<td>0.09</td>
<td>1.08</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>0.60</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>1.76</td>
<td>-</td>
<td>-</td>
<td>0.60</td>
<td>1.51</td>
<td></td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>4.610</td>
<td>22.240</td>
<td>-</td>
<td>-</td>
<td>1.320</td>
<td>2.130</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>1.457</td>
<td>38.237</td>
<td>-</td>
<td>1.227</td>
<td>-</td>
<td>7.457</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>1.26</td>
<td>40.2</td>
<td>4.21</td>
<td>5.68</td>
<td>0.45</td>
<td>0.61</td>
<td>[104]</td>
</tr>
<tr>
<td><strong>S. aurata</strong></td>
<td>Turkey</td>
<td>1.39</td>
<td>47.25</td>
<td>-</td>
<td>1.34</td>
<td>-</td>
<td>6.42</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Gaza strip</td>
<td>0.399</td>
<td>4.946</td>
<td>0.376</td>
<td>0.634</td>
<td>Nd</td>
<td>Nd</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>0.86</td>
<td>56.3</td>
<td>3.98</td>
<td>3.19</td>
<td>0.50</td>
<td>0.62</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>0.51</td>
<td>31.23</td>
<td>-</td>
<td>0.86</td>
<td>-</td>
<td>7.33</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>0.32</td>
<td>6.22</td>
<td>0.90</td>
<td>3.97</td>
<td>0.37</td>
<td>0.51</td>
<td>[105]</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>1.36</td>
<td>6.34</td>
<td>0.49</td>
<td>2.03</td>
<td>16</td>
<td>0.72</td>
<td>[105]</td>
</tr>
<tr>
<td></td>
<td>Cultured Italy</td>
<td>0.30</td>
<td>3.7</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[106]</td>
</tr>
<tr>
<td></td>
<td>Wild Italy</td>
<td>0.37</td>
<td>4.2</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
<td></td>
<td>[106]</td>
</tr>
<tr>
<td><strong>M. furnieri</strong></td>
<td>Uruguay</td>
<td>0.345</td>
<td>20.535</td>
<td>0.396</td>
<td>0.453</td>
<td>0.09</td>
<td>0.552</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>-</td>
<td>20.5</td>
<td>-</td>
<td>-</td>
<td>Nd</td>
<td>-</td>
<td>[51]</td>
</tr>
</tbody>
</table>

*a* Concentrations converted to wet. wt. dividing by 4.31 as factor [106]
Conclusion

Generally, fish in present study accumulated essential metals in their muscles in higher levels than non essential metals.

All results were bellow the maximum limits for fish consumption proposed by WHO (1989, 1996) and MAFF (1995).

Lead and cadmium concentrations in *Micropogonias furnieri* fish exceeded the maximum limits approved by EC (2005).

Among the six fish species, higher concentrations of Zn, Cu, Mn, Ni, Cd and Pb were found in *Mugil cephalus* and *Micropogonias furnieri*.

Daily intake of all metals showed that in case of regular consumption there was no harmful effect on general public health from the consumption of the studied fish.

Transfer factor (accumulation factor) indicated that local fish (*Sparus aurata, Oreochromis niloticus and Mugil cephalus*) accumulated heavy metals from water. Diet and sediment were considered as additional sources for heavy metals.
**Recommendations**

Although all results of heavy metal concentrations studied showed that regular consumption of the six fish species did not cause any harm effect on human health, recommendations should be taken into consideration:

1- Similar studies may be performed to check contamination with other toxic heavy metals such as mercury, chromium, cobalt and arsenic in commercial fish.

2- Periodical monitoring of heavy metals level in commercial fish is needful, especially for *Mugil cephalus* and *Micropogonias furnieri*, which showed the highest concentrations of studied metals.

3- Since *Mugil Cephalus* from Wadi Gaza gave the highest levels of Mn, Cu and Ni, the environment quality of that region must be under control and discharging of raw wastewater into the sea should be prevented.

4- Establishing of suitable Palestinian standards for fish quality included both fresh and frozen types according to international guidelines is required.
References


22- Sodoumou, Z., Gnassia-Barelli, M., Siau, Y., Morton, V. and Romeo, M. - *Distribution and concentration of trace metals in tissues of different fish species*


35- Authman, M. and Abbas, H.- Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (tilapia zili and Mugil cephalus) of lake Qarun, Fayoum province, Egypt. Pakistan journal of biological science, 2007. 10(13).


39- Mol, S., Ozden, O. and Oymak S.- Trace Metal Contents in Fish Species from Ataturk Dam Lake (Euphrates, Turkey). Turkish Journal of Fisheries and Aquatic Sciences, 2010. 10: p. 209-213.


64- *Lead and health*, in working party on lead in the environment. 1980, Department of Health and Social Security DHSS. London.


77- Ikema, A. and Egieborb, N. - *Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and...*


85- Canl, M. and Atli, G. - The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, 2003. 121: p. 129-136


كميات الأسماك المستهلكة في قطاع غزة للعام 2010

<table>
<thead>
<tr>
<th>الكميات (طن)</th>
<th>المصدر</th>
</tr>
</thead>
<tbody>
<tr>
<td>1724</td>
<td>1 - النتاج من الصيد البحري</td>
</tr>
<tr>
<td>300</td>
<td>2 - الاستزراع السمكي</td>
</tr>
<tr>
<td>1000</td>
<td>3 - أسماك طازجة من مصر</td>
</tr>
<tr>
<td>478</td>
<td>4 - أسماك طازجة مستوردة من داخل الخط الأخضر</td>
</tr>
<tr>
<td>46.5</td>
<td>5 - أسماك مجمدة من داخل الخط الأخضر</td>
</tr>
<tr>
<td>83.2</td>
<td>6 - أسماك مجمدة من الضفة الغربية</td>
</tr>
<tr>
<td>2581</td>
<td>7 - أسماك مجمدة من الخارج</td>
</tr>
<tr>
<td>67</td>
<td>8 - أسماك مملحة من داخل الخط الأخضر</td>
</tr>
<tr>
<td>103</td>
<td>9 - أسماك مملحة من الخارج</td>
</tr>
<tr>
<td>6382.7</td>
<td>المجموع</td>
</tr>
</tbody>
</table>

متوسط نصيب الفرد في قطاع غزة من الأسماك في العام 2010 وفقاً للتعداد السكاني للقطاع، والذي بلغ 1.5 مليون نسمة هو 0.25 كيلوغرام إياً بمعدل 11.65 غرام في اليوم.

الإدارة العامة للثروة السمكية