HEAVY METALS SAFETY ASSESSMENT OF *TRACHURUS TRACHURUS, SCOMBER SCOMBRUS*AND *TRACHURUS MURPHYII*IMPORTED FROZEN FISH SOLD IN ZARIA METROPOLIS, NIGERIA

BY

Abdullahi ABUBAKAR, B.Sc. (ED) CHEMISTRY ABU (2009), PGDAC ABU (2011)
MSc/SCI/963/2011-2012

DISSERTATIONSUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

DECEMBER, 2015

Declaration

I hereby declare that the work in this Dissertation entitled "Heavy Metals Safety Assessment of *Trachurus trachurus, Scomber scombrus, Trachurus murphyi* Frozen Fish Imported and Sold in Zaria Metropolis, Nigeria" has been carried out by me in the Department of Chemistry under the supervision of Prof. A. Uzairu, Dr. P. A. Ekwumemgbo and Dr. O. J. Okunola. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other institution.

Abdullahi Abubakar _____ _____

Signature Date

Certification

This Dissertation entitled "Heavy Metals Safety Assessment of *Trachurus trachurus, Scomber scombrus, Trachurus murphyi* Frozen Fish Imported and Sold in Zaria Metropolis, Nigeria"by Abubakar Abdullahi meets the regulations governing the award of the degree of Masters of Science in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. A. Uzairu Chairman, Supervisory Committee	_____ Signiture	_____ Date
Dr. P. A. Ekwumemgbo Member, Supervisory Committee	_____ Signature	_____ Date
Dr. O. J. Okunola Member, Supervisory Committee	_____ Signature	_____ Date
Prof. V.O. Ajibola Head of Department	_____ Signature	_____ Date
Prof. K. Bala Dean, School of Postgraduate Studies	_____ Signature	_____ Date

Dedication

This Dissertation is dedicated to Almighty Allah and to my family who has supported me throughout the programme.

Acknowledgements

All praises and thanks to Almighty Allah Who in His Infinite mercy, granted me protection and ability to do this work.

I would like to express my deepest gratitude to my supervisors Prof. A. Uzairu, Dr. P. A. Ekwumemgbo and Dr. O. J. Okunola, whose constructive criticisms and encouragement made it possible for the successful completion of this Dissertation. Your patience and valuable contribution guided me throughout the research period for which I am grateful.

My gratitude goes to Head of Department Prof. V. O. Ajibola, and other members of staff who worked hard at facilitating the program. My sincere appreciation goes to Dr. Sani Uba, Dr. Gideon Shallangwa, Dr. Okibe Friday, Mal. Abdulkadir Tanimu, Ogwuche Edwin, Mallam Bashir Musa and Mal. Mohammed Lawal Abubakar for their contribution and support.

My profound gratitude goes to my family, especially my mum Haj. Amina A. Galadima, for their continuous love, prayers and supports. I sincerely appreciate the support, kindness and encouragement of my cousins; Ibrahim Mu'azzmu Aliyu, Hauwa Aliyu Imam, Shettima Mohammed Aliyu and Abubakar Aliyu Imam.

Abstract

This study aimed at assessment of safety levels of cadmium, lead, mercury, iron and nickel in frozen fish*Trachurus trachurus, Scomber scombrus* and *Trachurus murphyi* imported into Nigeria and sold in Zaria metropolis. The assessment involves variation of heavy metals levels in fishthat are supplied from Russia (RS) and Europe (EU) fishing origins (zones). The concentrations ofCd, Pb, Hg, Fe and Niwith respects to each species were assessed on the bases of fish import batches and variation of metals localization in different fish organs/tissues (skin, muscles, gills, liver, intestine, kidneys, brain and bone). Accumulation levels of metals concentrations in different fish tissues were determined with flame Atomic Absorption Spectrophotometer (FAAS). The highest concentration of the metals determined across RS and EU fishing origins were given as follows: for *Trachurus trachurus* species, the cadmium concentrations are 5.142mgkg^{-1} (RS) and 6.517mgkg^{-1} (EU), lead concentrations are 15.742mgkg^{-1} (RS) and 18.058mgkg^{-1} (EU), mercury concentrations are 78.208mgkg^{-1} (RS) and 72.592mgkg^{-1} (EU), Iron concentrations are 230.14mgkg^{-1} (RS) and 836.26mgkg^{-1} (EU), and nickel concentrations are 10.308mgkg^{-1} (RS) and 18.383mgkg^{-1} (EU). For *Scomber scombrus* fish species, the cadmium concentrations are 19.758mgkg^{-1}(RS) and 10.658mgkg^{-1}(EU), lead concentrations are 17.558mgkg^{-1} (RS) and 8.725mgkg^{-1} (EU), mercury concentrations are 76.000mgkg^{-1} (RS) and 80.358mgkg^{-1} (EU), iron concentrations are 375.93mgkg^{-1} (RS) and 225.25mgkg^{-1} (EU) and nickel concentrations are 7.017mgkg^{-1} (RS) and 9.067mgkg^{-1} (EU). For *Trachurus murphyi* fish species, cadmium concentrations are 36.650mgkg^{-1} RS and 37.867mgkg^{-1} (EU), lead concentrations are 10.825mgkg^{-1} (RS) and 20.225mgkg^{-1} (EU), mercury concentrations are 115.21mgkg^{-1} RS and 86.483mgkg^{-1} (EU), iron concentrations are 818.05mgkg^{-1} (RS) and 431.84mgkg^{-1} (EU) and nickel concentrations are 3.775mgkg^{-1} (RS) and 4.208mgkg^{-1} (EU). The trend in metals accumulation across the entire tissues were found to show similarities for *Trachurus trachurus* and *Scomber scombrus* which were in this other: Fe > Hg > Pb > Ni > Cd. While, the species *Trachurus murphyi* was found to showed accumulations in the order: Fe > Hg > Pb > Cd > Ni. The entire tissues showed significant variation (at $p<0.05$) in the accumulation levels of the studied heavy metals across the entire batches; majorly liver, intestine and kidneys accumulates highly than the corresponding skin, muscles, gills, brain and bones.The concentrations of heavy metals obtained in all the tissues of *Trachurus trachurusScomber scombrus* and *Trachurus murphyi* fish wereabove the safety limits recommended by FAO/WHO. The risk of human health for heavy metals with the entire fish tissues are considerably high and individuals consuming fish livers, intestine and kidneys might be at higher risk from ingestion of toxic metals at unacceptable concentrations. The results of the research work shows that the studied imported fish species are unfit for human consumption.

Table of Contents

Tittle pagei

Declarationi

Certificationiii

Dedicationiv

Acknowledgementsv

Abstractvi

Table of Contentsvii

List of Tablesxi

List of figuresxiv

1.0 INTRODUCTION..... 1

1.1 Research Problem..... 2

1.2 Research Questions..... 4

1.3 Justification of the Research..... 4

1.4 Aim and Objectives..... 5

1.5 Research Hypothesis..... 6

2.0 LITERATURE REVIEW 7

2.1 Distribution of Fish Species..... 7

2.2 Pollution of Aquatic System..... 8

2.2.1 Sources of aquatic system pollution 9

2.2.2 Problem associated with aquatic system pollution 12

2.3 Heavy Metals Studies on Imported Fish..... 13

2.4 Health Implication of Heavy Metals in Fish..... 14

2.5 Choice of Analytical Techniques 17

2.5.1 Digestion method of analysis..... 17

2.5.2 Microwave heating source 18

2.5.3 Method validation 20

2.6 Risk Assessment Studies of Fish Species..... 21

2.7 Heavy Metals Limits 22

3.0 MATERIALS AND METHODS **23**

3.1 Materials 23

3.1.1 Apparatus/equipment/reagents 23

3.1.2 Preparation of reagents 24

3.2 Methods 29

3.2.1 Determination of sample population 29

3.2.2 Sample collection 31

3.2.3 Fish pre-treatment and dissection 31

3.2.4 Digestion 32

3.2.5 Samples analysis by atomic absorption spectroscopy 33

3.2.6 Data analysis and presentation 33

3.3 Quality Assurance Experiment 35

3.3.1 Spiking (recovery) experiment 35

3.3.2 Limit of detection of the metals 36

3.3.3 Limit of quantification of the metals 36

3.3.4 Relative standard deviation of the metals 37

3.4 Risk Assessment Index 37

3.4.1 Hazard quotient (HQ) 37

3.4.2 Daily intakes of metals 38

3.4.3 Health risk index 39

4.0 RESULTS **40**

4.1 Quality Assurance Results 40

4.2 Cadmium Concentrations in Tissues of *Trachurustrachurus* Species 45

4.3 Cadmium Concentrations in Tissues of *Scomber scombrus* Species 48

4.4 Cadmium Concentrations in Tissues of *Trachurus murphyi* Species 55

4.5 Lead Concentrations in Tissues of *Trachurus trachurus* Species 60

4.6 Lead Concentrations in Tissues of *Scomber scombrus* Species 65
4.7 Lead Concentrations in Tissues of *Trachurus murphyi* Species 70
4.8 Mercury Concentrations in Tissues of *Trachurus trachurus* Species 74
4.9 Mercury Concentrations in Tissues of *Scomber scombrus* Species 79
4.10 Mercury Concentrations in Tissues of *Trachurus murphyi* Species 82
4.11 Iron Concentrations in Tissues of *Trachurus trachurus* Species 86
4.12 Iron Concentrations in Tissues of *Scomber scombrus* Species 91
4.13 Iron Concentrations in Tissues of *Trachurus murphyi* Species 96
4.14 Nickel Concentrations in Tissues of *Trachurus trachurus* Species 101
4.15 Nickel Concentrations in Tissues of *Scomber scombrus* Species 106
4.16 Nickel Concentrations in Tissues of *Trachurus murphyi* Species 110
4.17 Profiles of Heavy Metal in Tissues of Fish Species 114
4.18 Comparative Mean Concentration of Cd in Fish Species 121
4.19 Comparative Mean Concentration of Pb in Fish Species 121
4.20 Comparative Mean Concentration of Hg in Fish Species 121
4.21 Comparative Mean Concentration of Fe in Fish Species 125
4.22 Comparative Mean Concentration of Ni Fish Species 125

4.23 Risk Assessment Index 128
4.23.1 Risk assessment Index for *Trachurus trachurus* species 128
4.23.2 Risk assessment index for *Scomber scombrus* species 132
4.23.3 Risk assessment index for *Trachurus murphyi* species 133

5.0 DISCUSSIONS 135
5.1 Quality Assurance 135
5.2 Cadmium Concentrations in Tissues of *Trachurus trachurus* Species 135
5.3 Lead Concentrations in Tissues of *Trachurus trachurus* Species 138
5.4 Mercury Concentrations in Tissues of *Trachurus trachurus* Species 139
5.5 Iron Concentrations in Tissues of *Trachurus trachurus* Species 141
5.6 Nickel Concentrations in Tissues of *Trachurus trachurus* Species 143
5.7 Cadmium Concentrations in Tissues of *Scomber scombrus* Species 144

5.8 Lead Concentrations in Tissues of *Scomber scombrus* Species 146
5.9 Mercury Concentrations in Tissues of *Scomber scombrus* Species 147
5.10 Iron Concentrations in Tissues of *Scomber scombrus* Species 148
5.11 Nickel Concentrations in Tissues of *Scomber scombrus* Species 149
5.12 Cadmium Concentrations in Tissues of *Trachurus murphyi* Species 151
5.13 Lead Concentrations in Tissues of *Trachurus murphyi* Species 153
5.14 Merury Concentrations in Tissues of *Trachurus murphyi* Species 154
5.15 Iron Concentrations in Tissues of *Trachurus murphyi* Species 156
5.16 Nickel Concentrations in Tissues of *Trachurus murphyi* Species 157
5.17 Profile of Metals Concentration in Tissues of Fish Species 158
5.18 Comparative Mean Concentrations of Cd in Tissues of Fish Species 161
5.19 Comparative Mean Concentrations of Pb in Tissues of Fish Species 162
5.20 Comparative Mean Concentrations of Hg in Tissues of Fish Species 162
5.21 Comparative Mean Concentrations of Fe in Tissues of Fish Species 163
5.22 Comparative Mean Concentrations of Ni in Tissues of Fish Species 163

5.23 Risk Assessment Index 164
5.23.1 Risk assessment index for *Trachurus trachurus* species 164
5.23.2 Risk assessment index for *Scomber scombrus* species 166
5.23.3 Risk assessment index for *Trachurus murphyi* species 168

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS 170
6.1 Summary 170
6.2 Conclusion 171
6.3 Recommendations 171
REFERENCES 172
APPENDICES 193

List of Tables

Table 2.1Heavy metals limits……..……22

Table 3.1 Microwave operation……...…..…33

Table 4.1Quality assurance of metals in tissues of*Trachurus trachurus* species…….....40

Table 4.2. Quality assurance of metals in tissues of*Scomber scombrus* species.……....41

Table 4.3 Quality assurance of metals in tissues of*Trachurus murphyi* species……...…..42

Table 4.4 Cd concentrations in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight…….....44

Table 4.5 Correlation of Cd in tissues of *Trachurus tracurus* from RS fishing zone.…..……...46

Table 4.6 Correlation of Cd in tissues of *Trachurus trachurus* from EU fishing zone.…….....47

Table 4.7 Cdconcentrations in tissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight…….....49

Table 4.8 Correlation of Cd in tissues of *Scomber scombrus* from RS fishing zone...……...50

Table 4.9 Correlation of Cd in tissues of *Scomber scombrus* from EU fishing zone.……..…51

Table 4.10 Cd concentrations in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight……...53

Table 4.11 Correlation of Cdin tissues of *Trachurus murphyi* from RS fishing zone…….54

Table 4.12 Correlation of Cd in tissues of *Trachurus murphyi* from EU fishing zone……...….55

Table 4.13 Pb concentrations in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weigh…….…….57

Table 4.14 Correlation of Pb in tissues of *Trachurus trachurus* from RS fishing zone…….58

Table 4.15 Correlation of Pbin tissues of *Trachurus trachurus* from EU fishing zone…….....59

Table 4.16 Pb concentrations in tissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight..…….…61

Table 4.17 Correlation of Pb in tissues of *Scomber scombrus* from RS fishing zone……..……62

Table 4.18 Correlation of Pb in tissues of *Scomber scombrus*from EU fishing zone…….....63

Table 4.19 Pb concentrations in tissues of *Trachurus murphyi* (mgkg^{-1}) wet weight…….....…..65

Table 4.20 Correlation of Pb in tissues of *Trachurus murphyi* from RS fishing zone……..…66

Table 4.21 Correlation of Pb in tissues of *Trachurus murphyi* from EU fishing zone…….....68

Table 4.22 Hg concentrations in tissues of *Trachurus trachurus* (mgkg^{-1}) wet weight……..…69

Table 4.23 Correlation of Hg in tissues of *Trachurus trachurus* from RS fishing zone…….…70

Table 4.24 Correlation of Hg in tissues of *Trachurus trachurus* from EU fishing zone…..…..…72

Table 4.25 Hg concentrations in tissues of *Scomber scombrus* (mgkg^{-1}) wet weight……..…..73

Table 4.26 Correlation of Hg in tissues of *Scomber scombrus* from RS fishing zone……...75

Table 4.27 Correlation of Hg in tissues of *Scomber scombrus* from EU fishing zone….....76

Table 4.28 Hg concentrations in tissues of *Trachurus murphyi* (mgkg^{-1}) wet weight……77

Table 4.29 Correlation of Hgin tissues of *Trachurus murphyi* from RS fishing zone…….....79

Table 4.30 Correlation of Hg in tissues of *Trachurus murphyi* from EU fishing zone…..….....80

Table 4.31 Fe concentration in tissues of *Trachurus trachurus* (mgkg^{-1}) wet weight……...81

Table 4.32 Correlation of Fe in tissues of *Trachurus trachurus* from RS fishing zone.....83

Table 4.33 Correlation of Fein tissues of *Trachurus trachurus* from EU fishing zone…….....84

Table 4.34 Fe concentration in tissues of *Scomber scombrus* (mgkg^{-1}) wet weight……...…85

Table 4.35 Correlation of Fe in tissues of *Scomber scombrus* from RS fishing zone……...87

Table 4.36 Correlation of Fe in tissues of *Scomber scombrus* from EU fishing zone……...88

Table 4.37 Fe concentration in tissues of *Trachurus murphyi* (mgkg^{-1}) wet weight…….…....…..89

Table 4.38 Correlation of Fein tissues of *Trachurus murphyi* from RS fishing zone…….…..91

Table 4.39 Correlation of Fe in tissues of *Trachurus murphyi* from EU fishing zone…….....92

Table 4.40 Ni concentration in tissues of *Trachurus trachurus* (mgkg^{-1}) wet weight…….…....93

Table 4.41 Correlation of Niin tissues of *Trachurus trachurus* from RS fishing zone……...95

Table 4.42 Correlation of Niin tissues of *Trachurus trachurus* from EU fishing zone….....96

Table 4.43 Ni concentration in tissues of *Scomber scombrus* (mgkg^{-1}) wet weight…..…….....97

Table 4.44 Correlation of Niin tissues of *Scomber Scombrus* from RS fishing zone…….…....99

Table 4.45 Correlation of Ni tissues of *Scomber Scombrus* from EU fishing zone……...100

Table 4.46 Ni concentration in tissues of *Trachurus murphyi* (mgkg^{-1}) wet weight……...101

Table 4.47 Correlation of Ni in tissues of *Trachurus murphyi* from RS fishing zone…….....103

Table 4.48Correlation of Niin tissues of *Trachurus murphyi*from EU fishing zone.....104

Table 4.49 HQ, DIM and HRI of metals in muscles tissues of *Trachurus trachurus* species…..116

Table 4.50 HQ, DIM and HRI of metals in muscles tissues of *Scomber scombrus*species..…117

Table 4.51 HQ, DIM and HRI of metals in muscles tissues of *Trachurus murphyi*species.….118

List of Figures

Figure 4.1 Mean concentrations of metals in tissues of *Trachurus trachurus* species…….....105

Figure 4.2 Mean concentrations of metals in tissues of *Scomber scombrus* species……..……...106

Figure 4.3 Mean concentrations of metals in tissues of *Trachurus murphyi* species.……..……107

Figure 4.4Comparative mean concentrations for Cd in the tissues of fish species…….....109

Figure 4.5Comparative mean concentrations for Pb in the tissues of fish species……..110

Figure 4.6 Comparative mean concentrations for Hg in the tissues of fish species…….....111

Figure 4.7 Comparative mean concentrations for Fe in the tissues of fish species…….....113

Figure 4.8 Comparative mean concentrations for Ni in the tissues fish species……...……...…114

Figure 3.2 Cadmium calibration graph…….....179

Figure 3.2 Lead calibration graph……...…….…...….180

Figure 3.2 Mercury calibration graph…….….181

Figure 3.2 Iron calibration graph…….....182

Figure 3.2 Nickel calibration graph…….……....183

CHAPTER ONE

1.0 INTRODUCTION

Fish is a member of *paraphyletic* group of organisms that consist of gills bearing aquatic craniate animals that lack limbs and digits. They include the living hagfish, lampreys, cartilaginous and bony fish as well as various extinct related groups. Fish are abundantly found in nearly all aquatic environments, from high mountain streams to the abyssal deepest oceans. They exhibit greater diversity, than any other class of vertebrates and consist of about 32800 species (Fish base, 2014).Fish has played a historically important role in early globalization which was not adequately recognized. Although in the year 1500 A.D., the value for the consumption of fish as source of animal protein was realized by the *Ichthyological* mentor "Pierre Belon" (Nellen and Dulcic, 2008). It has been considered the most important animal food that have helped to foster the early links in trade between nations (Kurien, 2006).

In 2010, the livelihoods of an estimated 54.8 million people wereengaged in the primary sector of fish production and another estimate of 7 million were fishers and fish farmers (FAO, 2012). These were found concentrated more in Asia (about 97%), followed by Latin America and the Caribbean (1.5%), and Africa (about 1%). Extension in numerous jobs of fisheries sector also leads to ancillary activities such as processing, packaging, marketing and distribution, manufacturing of fish-processing equipment, net and gear making, ice production and supply, boat construction and maintenance, research and administration.Employment in the fisheries sector presented growth with about 2.1% per year faster than even employment in traditional agricultural sector that has growth of only 0.5% per year (FAO, 2012). All of these employment,together with their dependents, was estimated to support the livelihoods of about 10 – 12% (660–820 million people) of the world's population as at 2010 (FAO, 2012).

Heavy metal pollutantion of water have become a major environmental concern, almost since the advent of agricultural and industrial revolution (Aderinola *et al*., 2009). The threat of toxic metals in the environment was more serious, due to their non-biodegradable nature and their long biological half-lives when accumulated (Oluyemi and Olabanji, 2011). They are difficult to rid out of environment completely from once they are introduced(Aderinola *et al*., 2009). Many of these metals tend to remain in the ecosystem and eventually move from one compartment to the other posing a treat within the food chain (Hayat and Javed, 2008). Fish are often representing the apex of food chain and hence are widely used to evaluate the health of ecosystem (Canli and Atli, 2003). Fish bio-accumulates some metals in various tissues from their surrounding water and this can lead to death in the aquatic systems and humans (Mieiro *et al*., 2012; Simon *et al*., 2013). In contrast, heavy metals tend to accumulate in the marine ecosystemby natural processes, latter accumulate in fish tissues, and subsequently transferred to higher trophic levels via food chain. Since fish was considered as the apex form of food chain, once affected, the entire food chain would also be at high risk (Aderinola *et al*., 2009).

1.1 Research Problem

Fish may present consequences to human health especially when their habitat is contaminated with toxic substances of natural or anthropogenic sources (Katherine, 2001). Continuous lodgment of materials into water bodies from natural process, agricultural practice, industrial/mining activities and domestic sewages may render high concentration of harmful substances, usually effluents such as polychlorobiphenyls (PCBs), dioxins, pesticides and heavy metals in a given environment. Water habitat remains the major terminal body of the environment receiving pollutant from different sources. Heavy metal pollutants have received

considerable attention compared to other, forms of pollutants , due to their toxicity, bioaccumulation and non-boidegradability in different organs of aquatic animals (Vinodhiniand Narayanan, 2008; Eneji *et al.*, 2011; Babatunde *et al*., 2012). The present world trades of sea foods including fish are supplied in freezing form, this only preserved it from decomposition by slowing down some biochemical activities, but do not have any impact on the presence of heavy metals contaminants.

Consumption of frozen fish was found to supersede any other source of animal protein such as beef, mutton and chicken among others in Nigeria. Massive importation of frozen fish in the country has ranked Nigeria the largest importer of frozen fish in Africa (Akintunde, 2012). Consequently, different varieties of frozen fish species are imported, although the species *Trachurus trachurus* (Shawa), *Trachurus murphyi* (mackerel) and *Scomber scombrus* (Scumbiasaidene), are the most common and more consumed in Zaria metropolis. These fish are imported by companies in a packed form, frozen and in batches. They are reported to be oceanic harvest and they are either *epipelagic* or *benthopelagic* schooling species. Fish bio-accumulates environmental pollutants, like heavy metals and persistent organic compound to a much higher degree compared to water where they lived. The reason is that fish are known to be mobile, travelling over a long distance in different waters where they may carry with them contaminants like heavy metals and persist in them for a long period. Several studies has show that, wild captured marine fish accumulates heavy metals to a greater extend that can be of health concern (Domingo *et al*., 2006; Noik*et al*., 2011; Lillian and Lorraine, 2012; Mieiro *et al*., 2012).The concern about the high levels of heavy metals in seafoodsparticularly frozen fish has prompted several researches and are of greater concern. Comparative assessment on heavy metals accumulation in fish reveals that, *Trachurus trachurus* in particular was found to store high

concentration of metals (Storelli, 2008). The studies of heavy metals bio-indicator of marine life, recorded fish among others represent the major aquatic indicator of metals (Lasheen *et al*., 2012; Aftab *et al*., 2013; Tanee *et al*., 2013). Thus, there is a need for continuous assessment of heavy metals safety on fish supply especially in the developing nations. This is especially important because globalization and free-trade agreements, generally affect the dietary patterns of food supply worldwide.

1.2 Research Questions

In West African countries, Nigeria was rated the major seafood importer (Essuman, 1992; Akintunde, 2012) and particularly Zaria metropolis shows a significant high growth in consumption of imported frozen fish. With the observed high impact of global pollution on aquatics lives, the following questions are needed to be considered.

1. Frozen fish are imported from different countries (fishing zones) into Nigeria. Are there variations in heavy metals contaminations in fish imported from these countries?
2. Imported frozen fish are supplied in batches. Are there variations in the heavy metals concentration between different batches of fish supplied?
3. Is there variation in the heavy metals distribution in different fish organs?
4. How safe are such fish from toxic metals as compared to the limits recommended by the joint FAO/WHO?

1.3 Justification of the Research

Fish constitutes the most consumable and valuable seafood, due to high source of animal protein apart from minerals, lipid and vitamins (FAO, 2012; Ekpenyong *et al*., 2013). However,

the nutritional values of most fish nowadays have decreased with replacement to high concentration of contaminants, due to environmental pollution (Abowei and Ekubo,2011).Literature revealed that human exposure and accumulation of heavy metals is at its rapid rate due to increase in natural and anthropogenic activities (Li *et al*., 2005). The exposure pathways include: the inhalation of contaminated air, ingestion of contaminated products such as water, medicinal herbs and food in which the aquatic sea food is the major source (Li *et al*., 2005; Damodhar and Vikram, 2012).With the observed growth in fish consumption and low level of consumer safety control on import of food commodities and frozen fish, in Nigeria, this study focussed at investigating the safety levels of heavy metals in certain imported fish species against possible health hazards and provide moredata on the concentration level of heavy metals in imported frozen fish sold in Zaria metropolis and Nigeria.

The study would alert the people of Zaria metropolis and Nigerians on the possibility of heavy metals accumulation in frozen fish species that may be of health consequences. This research would notify Kaduna state government to establish regular food management and safety control for monitoringlevels of heavy metal in imported frozen fish in the state and at the local levels. This research study may also bring proper attention to the Nigerian government food agencies and the suppliers of seafood on the safety control of hazardous heavy metals in frozen fish species.

1.4 Aim and Objectives

The aim of this research work, is to assess the safety levels of heavy metals in some imported frozen fish species sold in Zaria metropolis, Nigeria with the following objectives:

1. To determine the concentration of heavy metals in frozen fish *Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi* species and variation of its localization in various organs/tissues(skin, muscles, gills, liver, intestine, kidney, brain and bone).
2. To determine the variation in heavy metals concentrationsin the fish of one batch relative to the others that have been supplied from different fishing zones (areas),
3. To compare the levels of heavy metals in different fish species with standard limits,
4. Todetermine the human health risk in consumption of some organs of the fish using standard indices.

1.5 Research Hypothesis

The research study was built on the heavy metal independent variable and the dependent monitoring and assessment of safety guided by the following hypotheses.

1. Null hypothesis (H_o): The safety of heavy metals concentrations in frozen fish imported are within the stipulated safety limits stated by FAO/WHO (2003) which can represent a valuable source of animal protein and essential micronutrients for required nutrition and good health.
2. Alternative hypothesis (H_1): The fish contain high concentration of heavy metals above the safety limits and the products can not represent a valuable food and may require urgent control and regular monitoring.

CHAPTER TWO

2.0 LITERATUREREVIEW

2.1 Distribution of Fish Species

Demands of fish in Nigeria have led to the availability of various fish species (Akintunde 2012). The horse mackerel (*Trachurus trachurus*) among others is a member of a large carangid family, which includes many important schooling species that resides close to the sea upper layer (Fish base, 2014). *Trachurus trachurus* is commonly found from the waters off West Africa/Cape Verde Islands to the Norwegian Sea, including Iceland, as well as in the Mediterranean and Black Sea. It is the representative of the *Trachurid* sub-family, which is widely distributed in the major seas of the world and often supports important fisheries as reported by the International Council for the Exploration of the Sea (ICES, 2001).

Atlantic Mackerel, (*Scomber scombrus*), is wide-ranging mesopelagic species distributed from North-west Africa to Iceland and northern Norway in the Eastern North Atlantic ocean, including the Black Sea, Mediterranean and Western Baltic and from North Carolina to Labrador in the Western North Atlantic (Collette, 1986).While, Jack Mackerel (*Trachurus murphyi)* is distributed throughout the south-eastern Pacific, both inside the exclusive economic zones (EEZs) and on the high sea, ranging from the Galapagos Islands and south of Ecuador in the north to southern Chile. Following the strong increase in its abundance, *Trachurus murphyi* expanded its distribution toward the west and crossed the Pacific Ocean along the West Wind

Drift, reaching New Zealand watersand in south-eastern waters of the Australian EEZ in the early to mid-1980s (Elizarov *et al*., 1993; Taylor, 2002). Cordova *et al*. (2008) described *Trachurus murphyi* as a benthopelagic species but it also has diurnal migratory behaviour, as being found deeper during the day (50 – 180m) than at night (10 – 40m).

2.2 Pollution of Aquatic System

In the last decades, contamination of aquatic systems by heavy metals has become a global problem. Heavy metals have entered aquatic systems from different channels of natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbor activities, geological weathering of the earth crust and atmospheric deposits (USEPA, 2014). The contamination of freshwaters with a wide range of heavy metal pollutants has become a matter of great concern (Nadeem *et al.*, 2014). Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to anthropogenic activities (Kalay and Canli, 2000). Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior (Bat*et al*., 2009).

Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment (Olaifa *et al*., 2004; Uzairu *et al.*, 2009). They can take up metals concentrated at different levels in their different body organs (Khaled 2004; Yilmaz, 2005). The concern about the high levels of trace metals in foods has prompted several statutory bodies such as the WHO to establish maximum allowable concentrations for some of the metals in food (WHO, 2005). Thus the World Health Organization (WHO) as well as the Food and Agriculture Organization (FAO) of the United Nations stated that "monitoring eight

elements in fish Hg, Cd, Pb, As, Cu, Zn, Fe, Sn is obligatory", while the monitoring of others though are not obligatory but may be useful in some application (Staniskiene *et al*., 2006).

2.2.1 Sources of aquatic system pollution

There are many of forms of aquatic pollutants that endanger marine life. Some of them are more obvious than others, but all contribute to an unhealthy marineflora and fauna and many times, the death of its creatures. This may include the effects of oil spills, runoffs, air pollution, illegal dumpingand routine maintenance of large ships on the ocean. Oil is perhaps the most publicly recognized toxic pollutant of rivers and marine bodies. Recently, in April, 2010, one of the biggest oil spill in recorded history happened for British Petroleum (B.P.) oil along the Gulf of Mexico which was found to spills about 205.8million gallons(776 million liters) into the water body,endangers the entire marine life (Maureen, 2010). Similarly in 1991,during the Persian Gulf War, about 240 million gallons spilled from oil terminals and tankers off the coast of Saudi Arabia (Picot and Gill, 1996). Largest tanker accidents like the Exxon Valdez that has been grounded on Bligh Reef, spilling nearly 11million gallons of oil into the Prince William which Sound in March of 1989, are dramatic and devastating to the entire surrounding marine ecosystem for many years(Picot and Gill, 1996).Sources of aquatic pollution are basically from organic and inorganic subtances.European guide on water pollution (2014) outlined the major factorsthat contributes to source of metal and pollution of aquaticenvironment;

i. *Industrial waste*:Industrial waste products usually contains huge amount of toxic chemicals that when dump in any water body, may serve as pollutants which can cause damage to us and our environment. Many industries do not have proper waste management system, their waste areusually drained into fresh water, rivers, canals and later in to sea/oceans. Most of these

waste contain substances such as Pb, Hg, Cd, S, asbestos, nitrates and many other harmful chemicals which has potentials forserious hazard to our ecosystem.

ii. *Sewage and waste water*: The sewage and waste water which are produced by household are mostly released into sea water via urban drainages. The sewage water carries harmful bacteria and chemicals that can cause serious health problems. Micro-organisms inconjuction with metal substances in water are known to causes some of very deadly diseases and become the breeding grounds for other creatures that act like carriers (Shesterin, 1996). These carriers inflict these diseases via various forms of contact onto an individual.

iii. *Mining activities*: Mining is the process that involves crushing of rocks, extracting coal and other minerals from underground deposits. These deposits (minerals) contain elements that when extracted in the raw form contains harmful substances. These substances are usually washed into water system and increase the amount of toxic elements and hence causing damage to aquatic life and the environment.

iv. *Marine dumping*: The garbage produce by household in the form of rubbish if collected and deposited into the sea some of this items can take from 2 weeks to 200 years to decompose. Such this items in aquatic system causesserious harm toplans and animals and latter end in human.

v. *Oil spill*:Accidental Oil leakage pose a huge concern as large amount of insoluble oil are are settle on surface of water body causingproblem to aquatic organisms.Ships carrying large quantity of oil may spill oil which can cause varying damage to species in the ocean depending on quantity of oil spill, size of ocean, toxicity of the pollutants.

vi. *Burning of fossil fuels*:fossil fuel like coal and oil when burnt produce substantial amount of ash in the atmosphere. The particles which contain toxic chemicals when mixed with water

vapor result in acid rain. Also, carbon dioxide is released from burning of fossil fuels which result in global warming.

vii. *Chemical fertilizers and pesticides*: Chemical fertilizers and pesticides that are use for agricultural practices, usually got mixed up with water latter became harmful to both plants and animals. Also, when it rains, the chemicals got mixed with rainwater and flow down into rivers and canals which may later pose serious damages to aquatic animals.

viii. *Leakage from sewer lines*: A small leakage from the sewer lines can contaminate the underground water and make it unfit for the people to drink. Also, when not repaired on time, the leaking water can come on to the surface and become a breeding ground for insects and mosquitoes.

ix. *Global warming*: An increase in earth's temperature due to greenhouse effect results in global warming. It increases the water temperature and result in death of aquatic animals and marine species which later results in water pollution.

x. *Radioactive waste*:Nuclear energy is produced using nuclear fission or fusion. The radiant elements that is used in production of nuclear energy is mostly Uranium which is highly toxic chemical. The nuclear waste that is produced by radioactive material needs to be disposed off to prevent any nuclear accident. Nuclear waste can have serious environmental hazards if not disposed off properly. Few major accidents from natural disaster and military testing facilities have already taken place in Ukrain, Russia and Japan.

xi. *Urban development*: As population has grown, so has the demand for housing, food and cloth. As more cities and towns are developed, they have resulted in increase use of fertilizers to produce more food, soil erosion due to deforestation, increase in construction activities,

inadequate sewer collection and treatment, landfills as more garbage is produced, increase in chemicals from industries to produce more materials.

xii. *Leakage from the landfills*: Landfills are nothing but huge pile of garbage that produces awful smell and can be seen across most cities. When it rains, the landfills may leak and the leaking can pollute the underground water into aquatic system with large variety of contaminants.

xiii. *Underground storage leakage*: Transportation of coal and other petroleum products through underground pipes is well known. Accidentals leakage had happened recently with B.P. oil damage to aquatic environment and adding concentration to hazardous metals in water system.

2.2.2 Problem associated with aquatic system pollution

Toxic materials are the major problem concerning the aquatic system and are often refers to the materials that are capable of causing side effects of modern living (Aynur, 2009). Chemical residues from agricultural practices and industrial processes such as heavy metals, dioxins, and other pollutants find their way into the ocean and sink to the bottom. Bottom feeding organisms ingest these chemicals and contaminate the food chain (Aynur, 2009). The smaller fish is eaten by the larger fish, which is then eaten by a human. Toxins build up in the tissues of the people who eat the contaminated fish and this can lead to illnesses like cancer, as well as reproductive disorders, birth defects and other long term health problems (UNEP, 2003). The article states that "Unless urgent action is taken to tackle the sources of the problem, it is likely to escalate rapidly."

2.3 Heavy Metals Studies onImported Fish

The term ‘heavy metals’ refer to chemical elements with a specific gravity that is at least five (5) times the specific gravity of water. The specific gravity of water is one (1) at 4°C. Simply stated, specific gravity is a measure of the density of a given amount of a solid substance when it is compared to an equal amount of water (Glanze, 1996).There are about twenty three (23) elements which constitute the term “heavy metals”; Sb, As, Bi, Cd, Ce, Cr, Co, Cu, Ga, Au, Fe, Pb, Mn, Hg, Ni, pt, Ag, Te, Tl, tin, U, V and Zn(Glanze, 1996). Hence, the following Cd, Hg, As and Pb, which are often the most hazardous substances and found in the list of twenty (20) Priority Pollutants compiled by Agency for Toxic Substances and Disease Registry in cooperation with U.S. Environmental Protection Agency(ATSDR Mission Statement, 2001). The location of these metals in the systems of organism, may not have any biological role (Ahmed *et al*., 2010).

Iwegbue *et al*. (2009) determine the concentrations of heavy metals in tissues of somebrands of imported canned sardinesthat are mostly consumedin Nigeria.However, the concentrations of Cd, Pb and Ni in some brands was found to accumulate highly above the permissible limits recommended by WHO (1995). Similarly, Beetseh and Abraham (2013) found concentrations of Pb, Fe and Cd of certain imported frozen fish species are safe for human comsumption but concluded in the later time consuming suchfish species can lead to bioaccumulation and build uphigh metals concentration in human body exposing it to danger. While in another studies on imported canned fish shows the level of Cd and Hg are likely to constitute a significant health hazard (Babalola*et al*., 2014). The heavy metals; Pb, Ni and Cu in some frozen and canned fish from Thailand and Malesia which are commonly cosumed in Pakistan was found with concentrations greater than the tolerable limits by USEPA (2002)

(Sidra*et al*., 2014).Also Eze and Ogbuehi (2015) found imported *Gadus morhua* and *Molva molva*species contain high level of heavy metals as such that exceeded the maximum permissible limits by FAO (2003) and WHO (1995).

2.4 Health Implication of Heavy Metals in Fish

Fish are often representing the apex of food chain and as a results they are widely use to evaluate the health of aquatic ecosystems for the presence of pollutants. Fish may concentrate large amounts of metals from the water and therefore are responsible for adverse effects and death in the aquatic systems and human (Mansour and Sidky, 2002; Farkas *etal.*, 2002).The contamination of freshwaters with a wide range of heavy metal has become a matter of great concern. Discharge of heavy metals into river or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior (Allen, 1995). Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment (Olaifa *et al*., 2004). They can take up metals concentrated at different levels in their different body organs (Khaled, 2004).

Cadmium is one of the metal that has not been identified with any beneficial properties to support aquatic life(Eisler, 1985; Eisler 2000; Nordberg *et al*., 2007). At high concentration, Cd is toxic to all life, including plants, fish, birds, mammals (including humans), and microorganisms (Eisler, 2000; Nordberg *et al*., 2007; ATSDR, 2008). It has properties that are responsible for causing cancer, birth defects and genetic mutations in humans (Eisler, 2000; Nordberg *et al*., 2007; Borgmann *et al*., 2005). Particularly fishand human among other forms of live are often responded greatlyto Cd exposure (Hansen *et al*., 2002). Human accumulations of Cdare mainlydue to fish consuption.

Lead is classified among the most hazardous heavy metals which have no known biochemical benefits to animals and humans (Reena *et al.*, 2011) and this has continued to pose a serious threat to the health of many children as well as adults. It is capable of inducing oxidative damage to brain, heart, kidneys and reproductive organs (El-Ghasham *et al.*, 2008). The effects of lead-induced oxidative stress are due to the effects of Pb on membranes, DNA, and antioxidant defense systems of cells (Daniel *et al.*, 1997). Recent epidemiological and toxicological studies have reported that Pb exposure can causes several diseases including hypertension, kidney disease, neurodegenerative disease and cognitive impairment (FAO, 2012).The study by Omima and Aboud (2010) shows that consumption of Pb contaminated fish is another possible way of Pb exposure. Fish bio-concentrate large amount metals in their various tissues.

Mercury occurs naturally in a form of mineral substance and widely distributed throughout the environment as a result of natural and human activities. Inorganic Hg is the most common form of metal that is released into the environment mostly by industrial and mining activity. Global method of gold mining has been assign as the most responsible activities that led to Hg contamination in aquatic ecosystems (Malm *et al.*, 1995). Report of annual investigation showed 1350 tons of Hg are widely released into aquatic environment as a results of gold mining (Johnson, 2015). Mercury in the aquatic environment, appears partly as inorganic and latter converted to methylmercury by bacterial activities which is rapidly absorbed by fish through their gills and skin. Fish is the main route of environmental exposure to Hg and thus, the main source of methylmercury in human diets (Shimshack*et al.*, 2007).Report of several studies revealed 95% of Hg exposures are as a results of fish consumption (Clakson, 1997; Mahaffey and Rice, 1998).In human system, methylmercury accumulateat higher rate than it is excreted.

Because it has a strong affinity for sulfhydryl groups in tissues and accumulates to a greater concentration in brain, muscle, and kidney (NAS, 2000).The study by Jane *et al*. (2003) revealed that health risks of Hg was identified as a results of fish consumption when 1year survey of an internal medicine practice was conducted and found substantial fraction of patients who diets high in fish consumption have high blood Hg levels exceeding the maximum level recommended by the U.S. Environmental Protection Agency and National Academy of Sciences.

Iron, an essential mineral found in every cell of the human body, assists in the creation of hemoglobin in red blood cells (Lofts *et al*., 2008). Iron is highly reactive in freshwater and has an oxidation-reductionchemistry that has a number of significant roles in the cyclingand bioavailability of other elements (Lofts *et al*., 2008). High concentration of Fe in an aquatic environment can as well leads to its higher level in aquatic organisms and fish because of tissues accumulation (Lofts *et al*., 2008).The U.S. Food and Drug Administration defines an intake of up to 45mg of Fe per day as safe. A severe overdose of Fe occurs when the amount of Fe is 50 to 100 times more than the recommended dietary dose. Such high levels of Fe destroy cells in the gastrointestinal tract, causing vomiting, bloody diarrhea and at times death (CCFAC, 2011).

Nickel is a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Ni is taken up (Cempel and Nikel, 2006).Nickel and Ni compounds have many industrial and commercial uses, and the progress of industrialization has led to increased emission of pollutants into aquatic ecosystems.Fish represent is the major aquatic organism that accumulates Ni in various tissues (Obasohan,2008). Although Ni is omnipresent and is vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms. The

absorption of Ni is dependent on its physicochemical form, with water-soluble forms (chloride, nitrate, sulphate) being more readily absorbed (Cempel and Nikel, 2006).Nickel is a ubiquitous metal frequently responsible for allergic skin reactions and has been reported to be one of the most common causes of allergic contact dermatitis, as reflected by positive dermal patch tests (Cempel and Nikel, 2006).Nickel compounds have been well established as carcinogenic in many animal species and by many modes of human exposure but their underlying mechanisms are still not fully understood (Diagomanolin *et al.*, 2004). The bioavailability of Ni and the presence of constituents that promote oxygen-free radical reactions evidently influence the carcinogenicity of Ni oxides and related compounds (Jensen*et al.*, 2003).

2.5 Choice of Analytical Techniques

2.5.1 Digestion method of analysis

Solid samples such as fish tissue must be transformed to liquid phase which will involve digestion whereby the organic components are destroyed by acid releasing the metals that are dissolve into solution so that it can be analyze spectroscopically (Ghanthimathi *et al.*, 2012). The former classic digestion method called dry ashing, which involves solid sample is placed in furnace at 500°C with $MgO/Mg(NO_3)_2$ (Vilano and Rubio, 2001). This method provides total decomposition of organic matter, however, it is tedious, time consuming and causes loss of some or even total analyte on the process (Ghanthimathi *et al.*, 2012). Another method that is currently adopted is wet acidic digestion such as the method by the United State Environment Protection Agency (USEPA, 1998), method 3050B using HNO_3 or mixture of several concentrated acids such as HNO_3/H_2O_2 are used and the mixture is heated up to 95^0C.Concentrated Nitric acid, HNO_3 (boiling point 122^0C) makes an oxidizing attack on many samples not dissolved by HCl.

Hydrogen peroxide, H_2O_2 (boiling point 150°C) is a strong oxidizer which used together with HNO_3 to dissolve matters that is not fully decomposed by HNO_3. H_2O_2 is added to the samples with nitric acids so as decrease nitrous vapors and speeds up the digestion of organic substances by elevating the reaction temperature (Ashoka*et al.*, 2009).

This system of open digestion was reported as slow (Nam *et al*., 2010), requires more acid to aids full digestion and gives out dangerous nitrogen oxides (NO_x) fumes, with poor recovery due to volatility of metallic analyte at the required temperature. Therefore, the use of simple, fast and suitable extraction method which overcomes all the above setbacks in monitoring total metallic content in fish tissue is critical and has a great importance among the environmental studies (Ghanthimathi *et al.*, 2012). This is achived through the use of closed system and the closed vessel that can achieve higher temperature and pressure (Ghanthimathi *et al.*, 2012).

2.5.2 Microwave heating source

A microwave oven is an appliance that heats by bombardment with electromagnetic radiation in the microwave region causing polarized molecules to rotate and build up thermal energy in the process known as dielectric heating(Richard*et al.*, 1986). Although this technique makes use of microwave radiation, the direct effects of this radiation are of minor importance, at most. Microwaves cannot rupture molecular bonds directly because the corresponding energy is too low to excite electronic or vibrational states. Rotational excitation of dipoles and molecular motion associated with the migration of ions are the only processes that are observed in this microwave field (Nadkarmi, 1984). For this reason, an expression such as “microwave-assisted digestion” is preferable and recommended.

Microwave assisted (wet) acid digestion procedures are gaining popularity recently due to the speed of the digestion process as well as less possibility of contamination during the process (Soylak *et al.,* 2004). The critical parameters in this procedure are the digestion temperature, time program and the type of chemical used (Souza *et al*., 2007). Temperature is a critical factor in acid digestion that will ensure complete decomposition of organic matter and provide clear solution. Microwave digestion system allows real time temperature specification (usually around 200°C) that is required to increase the boiling temperature of the polarized acid mixtures, which will ensure the digestion of the sample. The microwave offers advantages of short digestion time, less acid consumption and high extraction efficiencies in digestion procedures that make them preferable when compared with the conventional methods (Ghanthimathi *et al.,* 2012). The earliest attempts at microwave-assisted digestion were performed using home appliance microwave ovens. This was necessary because commercial devices were not available at the time (Ghanthimathi *et al.*, 2012). Although the use of domestic microwave ovens in laboratory experiments has disadvantage over the modern laboratory design, in terms of safety and performance (Ghanthimathi *et al.,* 2012). All relevant parameters, such as reagent volume, digestion time, applied/input power and addition of reagent composition lend themselves to straightforward control. Although there is no specific heating temperature, the digestion temperature depends on the polarization strengths of the acid mixture towards microwave radiation and the operating power of the microwave oven (Wladiana *et al.*, 2009). However, non-pressurized microwave systems are limited by a low maximum digestion temperature, which cannot exceed the ambient pressure boiling point of the acid (or the acid mixture), they provide the best option with regard to the safety of personnel, because no over

pressure can occur. Moreover, non-pressurized microwave-assisted digestion is suitable for on-line digestions in continuous-flow systems (Ghanthimathi *et al.*, 2012).

2.5.3 Method validation

Method validation is a vital component to ascertain the reliability of analytical data (ISO 17025, 2005). By applying methods validated according to common procedures and performance criteria, the quality and comparability of the analytical results can be ensured (D'Ilio *et al*., 2008).All methods used in analytical chemistry are subject to error. Therefore, the methods should be evaluated as well as tested to confirm that it is suitable for the intended purpose. The main task of method validation is measurement of specific method validation characteristics. Other essential tasks of method validation include using the internal quality control procedures, participation in suitable proficiency schemes and accreditation of the laboratory according to international standards like ISO/IEC 17025 (Boque *et al*., 2002).

Jinadasa and Kolita (2012) stated that, "method validation is a confirmation by examination and the provision of objective evidence in which a particular requirements for a specific intended procedure are fulfilled, a process of evaluating method performance and demonstrating that it meets a particular requirement" (ISO/IEC:17025, 2005). The method validation is the process of acquiring the necessary information to assess the ability of method to perform its intended task. It give an information on the reliability of the results at hand, determine the condition of such results and the limitation of the method used. Validation of analytical methods employ in analytical laboratories is a requirement to meet the ISO/IEC 17025 accreditation, standard and to ensure that the test method gives correct and reliable results. Method validation is an essential part of analytical method development procedures (Taverniers *et al*., 2004).

2.6 Risk Assessment Studies of Fish Species

Anthropogenic activities of mining and industrial waste are the major sources of heavy metal contamination in the environment (Lee and Stuebing, 1990). Under certain conditions, thesemetals are accumulated in human system to a toxic level which can lead to damages (Jefferies and Freestone, 1984). Several methods have been proposed to estimate the potential health risks of metals pollutants, divided mainly into carcinogenic and noncarcinogenic effects. For carcinogenic metal contaminants, the observed or predicted exposure concentrations are compared with thresholds for adverse effects or the toxicant reference value (TRV) as determined by dose–effect relationships (Solomon *et al*., 1996). The probability risk assessment technique has been adopted by a number of researchers to fully utilize available exposure and toxicity data (Solomon *et al*., 1996; Giesy*et al*., 1999; Cardwell *et al*., 1999; Hall *et al*., 1999, 2000; Wang *et al*., 2002; Sajjad *et al*., 2009; Okunola *et al*., 2011). However, these methods are only applied to quantifythe magnitude of health risks of carcinogenic pollutants,but not for quantifying non-cancer risks. Non-cancer risk assessment methods do not provide quantitative estimates on the probability of experiencing non-cancer effects from contaminant exposures. These are typically based on the hazard quotient (HQ), which is a ratio of determined dose of a pollutant to the dose level (Reference Dose or R_fD). If the ratio is less than one (1), there will be no any obvious risk. Conversely, an exposed population of concern will experience health risks if the dose is equal to or greater than the R_fD. Also, the health risk index (HRI) for the individuals of populationscan be assessed based on daily intake of metals (DIM) relative to reference oral dose (R_fD) for heavy metal contaminants (Sajjad *et al*., 2009; Okunola *et al*., 2011).

Several study have been proposed for estimation of the potential risks of heavy metals to human health in fish. The report by Afiza*et al.* (2013) found that investigated heavy metals Pb and Cd have concentration below the recommended daily intake but risk assessments shows the fish species are unsafe for consumption because it may has effect in the latter time due accumulation.

2.7 Heavy Metals Limits

Table 2.1 indicates the heavy metals average daily intake limits sets by theCodex Committee on Contaminants in Foods(2011): working documwnt for Food and Agriculture Organization (FAO) and World Health Organization (WHO).Food and Agricultural Organization of the United Nation (2003) in the document "Heavy Metals Regulations Legal Notice No. 66" specifythemean of heavy metal that should not be exceeded, in any part of the edible fish products. The metals reference oral dose (R_fD) given byUSEPA, 2009.The limits/maximum daily allowance for Ni intake given by the standards of WHO documents (2005).

Table 2.1 Heavy metals limits

Heavy metals (contaminant)	Limits/Maximum Levels	Mean total content by FAO (2003)	Reference Oral Dose (R_fD) by USEPA (2009)
Cadmium	0.8µg/kg body weight per day(CCFAC, 2011)	0.1ppm (0.1mgkg^{-1}of fresh weight).	1.0x10^{-3} mgkg^{-1}day^{-1}
Lead	0.03 – 9µgkg^{-1} body weight per day for Children and from 0.02 - 3µgkg^{-1} body weight per day for adults (CCFAC, 2011)	0.4ppm(0.4mgkg^{-1} of fresh weight).	3.5 x 10^{-3}mgkg^{-1}day^{-1}
Mercury	0.3µgkg^{-1} body weight per day (CCFAC, 2011)	0.5ppm (0.5mgkg^{-1} of fresh weight).	1.0x10^{-4}mgkg^{-1}day^{-1}
Iron	0.8mgkg^{-1} (CCFAC, 2011)	-	7.0 x 10^{-1}mgkg^{-1}day^{-1}

Nickel	0.2mgkg^{-1} (WHO, 2005)	-	2.0 x 10^{-2}mgkg^{-1}day^{-1}

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus/equipment/reagents

The apparatus used at the course of laboratory work include One-mark pipettes of 1cm^3, 2cm^3, 5cm^3 and 10cm^3 capacities (class B), One-mark volumetric flasks of capacities, 100cm^3, and 1000cm^3 (class B), Graduated measuring cylinders of 10cm^3, Watch glass, Desiccators, Plastic Petri dish, Plastic trays, Surgical dissecting kit, Whatman filter paper, Polytetraflouroethylene bottles, Surgical gloves,Masking tape, Wash bottlesand Glass rod stirrer.

While th equipment include Thermostat domestic Microwave ovum(Model MW028A-MG720),Varian 240 FS Atomic Absorption Spectrophotometer (FAAS) and Sartorius analytical balance (Model ED224-S).

All reagents used were of recognized analytical grade. The reagents involves during the course of laboratory work includes;Cadmium(II)nitrate($Cd(NO_3)_2$),Lead(II)nitrate ($Pb(NO_3)_2$)Mercury(II)chloride ($HgCl_2$),Iron(III)chloride ($FeCl_3.6H_2O$), Nickel(II)nitrate($Ni(NO_3)_2.6H_2O$), Conc. Nitric acid (65%, density = 1.4gcm^{-1}at 20^0C), and Conc. Hydrogen peroxide (35%, density = 1.13 gcm^{-1} at 25^0C).

3.1.2 Preparation of reagents

i. *Acid solution*

The procedure for preparing 10% nitric acidper dm^3(v/v), fromthe concentrated nitric acid (65%) was done by adding 150cm^3 of the concentrated acid into 500$cm^3$of distilled water in 1 dm^3volumetric flask and filled-up to the mark.The volume of conc. NHO_3 required to produce 10% diluted acid was calculated by algebraic rearrangement. 10% x (1 dm^3 /65%) = 154 cm^3

ii. *Cadmium standard solution*

Analytical stock solution of the Cadmium was prepared such that it has a concentration of 1000$mgdm^{-3}$. The mass of $Cd(NO_3)_2$ required to produced 1000 $mgdm^{-3}$ of Cd was computed by algebraic rearrangement. The molar mass of $Cd(NO_3)_2$, was calculated by combining the relative atomic masses as;

$$112.412+(2\times(14.0067+[3\times15.9994]))=236.4218 gmol^{-1}$$

Therefore, 236.428$gmol^{-1}$ mass of $Cd(NO_3)_2$ contained 112.412$gmol^{-1}$ mass of Cd, and thenthe mass of $Cd(NO_3)_2$ required to produced1.0g of Cd was givenby algebraic rearrangement as follow; 236.4218/112.412 = 2.1032g.

Stoichiometrically 2.1032g of matrix $Cd(NO_3)_2$ was weighed to prepared stock solution of 1g of Cd per 1 dm^3. 2.1032g of anhydrous $Cd(NO_3)_2$ was dissolve in 250$cm^3$of de-ionized water in a beaker and then quantitatively transferred to a 1 dm^3 one mark volumetric flask and filled up to the mark with de-ionized water. By means of pipette, 10cm^3 of the Cd stock solution was transferred to a 100cm^3 one mark volumetric flask and make up to the mark with de-ionized water to produce anintermediate standard solution with concentration of 100 $mgdm^{-3}$.

Cadmiumstandard solutions for AAS calibration was prepared by pipetting, 1cm^3, 2cm^3, 3cm^3, 4cm^3 and 5cm^3, aliquot portions of the intermediate solution into 100cm^3 volumetric flask respectively and diluted to the mark with de-ionized water. These standards consisting ofCd in a concentrations of 1mg, 2mg, 3mg, 4mg and 5mg per liter corresponding to 1ppm, 2ppm, 3ppm, 4ppm and 5ppm respectively.

iii. *Lead standard solution*

Analytical stock solution of the Pb was prepared such that it contains a concentration of 1000$mgdm^{-3}$. The computational calculation was given by algebraic rearramgement.Molar mass of $Pb(NO_3)_2$, was calculated by combining the relative atomic masses as follows.

$207.2 + (2\times(14.0067 + [3 \times 15.9994])) = 331.2gmol^{-1}$

Therefore, 331.2$gmol^{-1}$of $Pb(NO_3)_2$ contained 207.2$gmol^{-1}$ of Pb and then the mass of $Pb(NO_3)_2$required to give 1.0g of Pb, was computed by algebraic rearramgement as follows; 331.2/207.2 = 1.5985g.

Stoichiometrically 1.5985g of $Pb(NO_3)_2$ was weighed to produced Pb stock solution with concentration of 1000 $mgdm^{-3}$. 1.5980g of anhydrous$Pb(NO_3)_2$ was dissolve in 250cm^3 of de-ionized water in a beaker and quantitatively transferred to 1 dm^3 one mark volumetric flask and make up to the mark with de-ionized water. Lead intermediate solution corresponding to

concentration of 100mgdm^{-3} was prepared by pipetting, 10cm^3 of the stock solution into 100cm^3 one mark volumetric flask and make up to the mark with de-ionized.

Lead standard solutions for AAS calibration was produced by pipetting 1cm^3, 2cm^3, 3cm^3, 4cm^3 and 5cm^3, aliquot portions of Pb intermidiate solution into 100cm^3 one mark volumetric flask separately, and filled up to the mark with de-ionized water. These standards consist of Pb in a concentrations of 1mg, 2mg, 3mg, 4mg and 5mg per liter corresponding to 1ppm, 2ppm, 3ppm, 4ppm and 5ppm respectively.

iv. *Mercury standard solutions*

From algebraic rearrangement, it was calculated that1.3536g of $HgCl_2$was required to produce1000mgL^{-1}of Hg stock solution. 1.3536g anhydrous salts of $HgCl_2$was weighed and dissolve in 250cm^3 of de-ionized water in a beaker and quantitatively transferred to1 dm^3 volumetric flasks and filled-up to the mark.Mercury intermediate solution corresponding to concentration of 100 mgdm^{-3} was prepared by pipetting 10cm^3 aliquot of theHg stock solution and transferred to 100cm^3 one mark volumetric flask and make up to the mark with de-ionized.

Mercury standard solutions for AAS calibrations was prepared by pipetting, 2cm^3, 4cm^3, 6cm^3, 8cm^3 and 10cm^3, aliquot portions of the intermidiate solutioninto 100cm^3 one mark volumetric flask separately, and each were diluted to the mark with de-ionized water. These standard solutions contain Hg in a concentration of 2mg, 4mg, 6mg, 8mg and 10mg per liter corresponding to 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively.

v. *Iron standard solutions*

From algebraic rearrangement, it was calculated that 4.8400g of $Fe_2Cl_3.6H_2O$ was required to produce 1000 mgdm^{-3} of Fe stock solution.4.8400g of hydrated $Fe_2Cl_3.6H_2O$ was dissolved in 250cm^3 de-ionized water in a beaker and quantitatively transferred to 1000cm^3

volumetric flasks and make up to the mark. An intermediate standard solution of iron that has a concentration corresponding to 100 $mgdm^{-3}$ (100ppm) was prepared. By means of pipette, 100cm^3 of Fe stock solution was transferred to 1000cm^3 one mark volumetric flask and make up to the mark.

Iron standard solutions for AAS calibrations was prepared by transffering, 50cm^3, 100cm^3, 150cm^3, 200cm^3 and 250cm^3 aliquot portions of the intermediate solution 1 dm^3 one mark volumetric flask separately, and each were diluted to the mark with de-ionized water. These standard solutions contain Fe in a concentrations of 10mg, 20mg, 30mg, 40mg and 50mg per liter corresponding to 10ppm, 20ppm, 30ppm, 40ppm and 50ppm respectively.

vi. *Nickel standrad solutions*

From algebraic rearrangement, it was calculated that 4.9544g of $Ni(NO_3)_2$.6H2O was required to produce 1000$mgdm^{-3}$ of Ni stock solution 4.9544g of hydrated $Ni(NO_3)_2$.6H2O was dissolve in 250$cm^3$of de-ionized water a in a beaker and quantitatively transferred to1 dm^3volumetric flasks and filled-up to the mark.An intermediate solution of Ni in a concentration corresponds to 100$mgdm^{-3}$ was prepared by pipetting 10cm^3 of Ni stock solution and transferred to 100cm^3 one mark volumetric flask and make up to the mark with de-ionized water.

Nickel standard solutions for AAS calibration was prepared by pipetting, 1cm^3, 2cm^3, 3cm^3, 4cm^3, and 5cm^3, aliquot portions of the intermediate Ni solution and transferred to 100cm^3 one mark volumetric flask separately, and each was dilute to the mark with de-ionized water. These solutions contain Ni in a concentration of 1mg, 2mg, 3mg, 4mg, and 5mg per liter corresponding to 1ppm, 2ppm, 3ppm, 4ppm and 5ppm respectively.

viii *Multi-element standard solution*

Multi-element standard solution (MESS) containing the respective metals; Cd, Pb, Hg, Fe and Ni was produced from the stock solution. The stock solutionwas prepared from the matrix compound of the respective metals by weighing2.1032g, 1.5980g, 1.3536g, 4.8401g and 4.9544gof$Cd(NO_3)_2$,$Pb(NO_3)_2$, $HgCl_2$, $FeCl_3.6H_2O$ and $Ni(NO_3)_2.6H_2O$respectively anddissolved in 100cm^3 de-ionized water and quantitatively transferred to 1 dm^3 volumetric flask and filled up to the mark.The stock solution contain a concentration of 1000 $mgdm^{-3}$ with respects to each of the metal involved. Then MESS prepared from the stock solution by transferring 10cm^3 by means of pipette to 100cm^3 one mark volumetric flask and make up to the mark with de-ionized water and mixed well. TheMESS solution contain a concentration of100$mgdm^{-3}$ (100ppm)with regards toeach of the respective metals; Cd, Pb, Hg, Fe and Ni.The choice of MESS for determing method performance depends on two major conditions;it is recommended to prepare and analyze one matrix spike sample for each batch of 10 or 20 samples,the concentration of the analyte in the matrix spiking solution is usually 50 - 100 times higher than the concentration found in the unspiked sample, and the MESS volume chosen should be ensure that it does not significantly change the sample volume by more than 5% (otherwise, it must be taken into account).

Portion of the digested sample solution (50cm^3) for skin, muscles, gills, liver, intestine, kidneys, brain and bone were spiked with theMESS to produced matrix spiked sample.The amount of MESS solutionrequired to produce Matrix Spike Samplewas calculated by using equation 3.1.

$$\text{Volume of MESS} = \frac{\text{Concentration of Spike desired} \times \text{Volume of sample to which the MESS is added}}{\text{Concentration of the MESS solution}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

3.2 Methods

3.2.1 Determination of sample population

Sample population was determinedbased on the calculation, following the use of student t-test. The t-test equation as given inequation 3.2 below was adapted to calculate the number of sample batches required for the analysis of *Trachurus trachurus*, *Scomber scobrus* and *Trachurus murphyi* species.

$$d \geq \frac{ts}{\sqrt{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Where d is the distance of sample mean ($\bar{x}$) from the population mean (μ), (i.e. variability allowed/taken for the investigation), t is the distribution (at 95% level of confidence for 3 replicate measurements is 3.182), S is the sample standard deviation and n in the number of samples drawn for investigation.

The sample mean ($\bar{x}$) taken for calculating the number of samples batches was based on the FAO (2003) stated mean limits for Pb, Hg and Cd in fresh edible part of fish, which are 0.4mgkg^{-1}, 0.5mgkg^{-1} and 0.1mgkg^{-1} respectively. The mean ($\bar{x}$) of these values, was calculated using equation 3.3 below and the population mean was taken to be zero (0) following the normal distribution of the mean and the variance, N(0,1). Then sample mean, $\bar{x}$was calculated as:

$$\bar{x} = \frac{\sum_{i=0}^{n} x_i}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where $x_i$is thesets of observation(i.e. mean limits of 0.1mgKg^{-1}, 0.5mgKg^{-1} and 0.4mgKg^{-1} for Cd, Hg and Pb respectively) andn is the number of measurements (i.e. 3).

$$\bar{x} = \frac{1.0}{3} \quad = 0.33$$

Also standard deviation, *S* was calculated using equation 3.4 below:

$$S = \sqrt{\frac{1}{n-1}\sum_{i=0}^{n} x_i^2 - \frac{(\sum_{i=0}^{n} x_i)^2}{n}} \quad \text{.....3.4}$$

$$S = \sqrt{\frac{1}{3-1}0.42 - \frac{1}{3}}$$

$$S = 0.35$$

d was estimated to be 0.32 for this experiment (i.e. the distance of sample mean from the population mean) and the number of samples drawn were also calculated using equation 3.6 below.

$$d \geq = \frac{ts}{\sqrt{n}} \quad \text{.....3.5}$$

$$n \geq \frac{(ts)^2}{d^2} \quad \text{.....3.6}$$

$$n \geq \frac{(3.182 \times 0.35)^2}{0.32^2} \geq 12.11 \approx 12$$

Hence, from the above calculation,it was found that an approximately twelve (12)number of sample batches were considered for the population selection with respects to each of the studied species; *Trachurus trachurus, Scomber scombrus* and *Trachurus murphyi* from two fishing zones; Russian (RS) and European zone (EU). Whereas, the incremental number of fish drawn from each batches was based on the FAO method (FAO, 2003). Since the cartons net weight was found to be less than 50kg, thereby three (3) incremental fish were drawn and composite from each batch of the cartons.

3.2.2 Sample collection

Samples of fish species were purchased at frozen fish depotin Sabon-Gari, Zaria, Nigeria. The samples were labeled and transported immediately to the laboratory in clean polythene bags. In the laboratory, the samples were stored in refrigerator and remained frozen prior to experiment. Sampling wasmade from the products processed under named companies with the following information such as, Name and address of the producer; Plant code number; Country of origin; Fishing zone; Production date; Expiry date; Product name; Scientific/Latin name; Size; Batch number; Net weight; Packing line/time and Storage temperature were considered.

3.2.3 Fish pre-treatment and dissection

Dissecting surgical blades, plastic containers and trays were washed with distilled water. Glass wares were soaked in 10% HNO_3 for 24hours and rinsed with distilled water. Frozen fish samples were thawed, rinsed with distilled water and then allowed to attain room temperature in desiccators before dissection. The skin and muscle were removed following *Tru-cut* method as described by Baker *et al*. (2004): few scales were removed from the dorsal region of the scute skin on the first side of the fish just below the dorsal fin using a sterilized notched needle. The outer barrel was inserted to a depth of about 1cm into the fish muscles tissue beneath the scale at an oblique angle (to minimize penetration depth). The 2cm long notched needle (inner barrel) was then extended into the flesh. The containment cover which is the sharp outer barrel slides over the extended needle to cut the tissue and capture it within the notch. The needle was withdrawn, the barrel opened and tissue slug removed with stainless steel tweezers (which were washed between samples) and placed in a labeled plastic Petri dish. While at the other side, few scales were removed and the skin was cut-off firmly making sure no part of the muscle was attached and placed in its labeled petri-dish.

The gills, brain, liver, intestine, kidney and bone tissues were dissected based on the modified method developed by Norwegian Institute for Water Research(Rosseland *et al.*2001). Dissections were done on plane plastic tray in to separate sample organs/tissues: The operculum gill cover was lifted up and cut outto expose the entire gill arch, filament and the rake. The abdominal wall was cut through the tail from the head, using tweezers and laid the fish on its right side with head to left. The abdominal wall tissue was laid with the scales down and use as to hold other organs. The bile bladder was removed from the liver and then whole liver was also freed onto the abdominal wall and cut out. The whole intestine was cut out and placed in to its drying container. The roof of the head was cut horizontally from the nostrils through the end of the skull in order to expose the brain, fetch out and cut accordingly. Dorsal bones were de-fleshed and cut out entirely from the head to the tail and sampled to its drying container. The tissues/organs were dried to constant weight at 80^0C on plastic petri-dish and cooled in desiccators and powdered using clean porcelain mortar and pestle.

3.2.4 Digestion

The digestion of the sample organs were based on the Microwave-assisted wet digestion method as described by Taghipour and Aziz (2011): one gram (1g) dry weight samples organs were placed in polytetrafluoroethylene (PTFE) tube and 8ml of a mixture of concentrated nitric acid(65%) and hydrogen peroxide(35%) in a ratio of 3:1 was added and placed in a microwave oven for 2minutes (Table 3.1), and then cooled to temperature of 25^0C. The cleared solutions were then filtered using Whatman filter paper (90mm)in a labelled plastic bottles and diluted with de-ionized water to $50cm^3$ for the skin, muscle, gills, liver, intestine, kidney, brain and liver sample respectively. The samples analyses were performed with AA240 Fast Sequential Atomic

Absorption Spectrophotometer (AAS) in Multi-User Science Research Laboratory (MSRL), Ahmadu Bello University Zaria.

3.2.5 Samples analysis by atomic absorption spectroscopy

AAS techniques for metal determinations involved the use of Hollow Cathode lamp (HCL) for each of the respective element. Portion of the digested solutions were pipette into a clean flask and the absorbance for each of the metals were determined, considering the optimum working standards. The mass concentrations of the metal contents in solutions were determined from the calibration curves (appendix). Metal with concentration above the optimum working standards of the Atomic Absorption Spectrophotometer, its solution was re-diluted (the effects of all dilution factor were also considered in the calculations).

Experimental analyses were carried out in triplicate to produce data between experimental variables that could be computed using statistics. The *mean* $\pm$*standard deviation* concentrations of each sorted triplicate were obtained by taking the average metal concentration between individuals using Excel Microsoft packages.

3.2.6 Data analysis and presentation

The mean concentration values for each of the metal in the samples were expressed as the arithmetic mean with the standard deviation. Figures were presented with the mean value indicated

Table 3.1 Microwave operation

Operation	Output
Machine model number	MW028A-MG720
Power (output)	700W
Turntable Diameter	255m

Function/power operation	High (100%)
Digestion time	2minutes

in a bar chart with standard deviation about the mean and tables to illustrate concentration profile over each group. The statistical significance of the mean values between different batches were determined by multiple comparison analysis of variation (Post-Hoc, Duncan) with the level of significance at $P<0.05$. The correlation between the metals were revaluated using Pearson correlation analysis. All statistical analyses were performed using computer IBM SPSS 20.0

version. The concentrations of all analyzed metals were presented in the result tables in the units of $mgkg^{-1}$ following the elimination from dilution (factor) effect. Dilution factor express the factor regarding the conversion of instrument readout from concentration in solution to concentration on mass basis. The results of ASS analysis obtained in this study were in the concentrations of ppm (mgL^{-1})which are expressed as concentrations in $mgkg^{-1}$ conside5ring the effects of dilution factor. The expression was aided by the equations below.

$$\text{Amount of metal in volume of digested sample } (mgdm^{-3}) = \frac{\text{Volume of digest solution } (cm^3) \times \text{Concentration of metal in the sample (ppm or } mgdm^{-3})}{1000\ (cm^3)} \quad \ldots..3.7$$

$$\text{Amount of metal in fish tissues } (mgkg^{-1}) = \frac{\text{Amount of metal in volume of digested sample} \times 1000g}{1g} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..3.8$$

3.3 Quality Assurance Experiment

Quality of analytical procedures and equipment used were evaluated by matrix spiking experiment. The experiment involved determination of percentage recovery (%R), limit of detection (LOD), limit of quantification (LOQ) and relative standard deviation for repeatability (RSD_r) as described by Ghanthimathi *et al.* (2012). Quality assurance experimental analysis for *Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi* were carried out on tissues of batches one.

3.3.1 Spiking (recovery) experiment

In the absence of reference standard materials, the nitrate salts of the metals were used to prepare multi-element standard solution (MESS) for spiking recoveries(IUPAC, 1997). Two portions of the samples were prepared for analysis (test and matrix spike samples). Matrix spike

is generated by adding a known amount of MESS(i.e. 5 cm^3 calculated from equation 3.1) to one portion of thetest samples and then testing the spiked sample together with the other portion to determining if we have recovered the amount of MESS added. The results of matrix spike should be higher by that known MESS concentration added as expected to validate the accuracy of analytical procedure. Detailed of MESS preparation, volume of MESS required for matrix spike and matrix spiking conditions are given in reagent prepation above (3.1.3).

The amount of spiked metals recovered were used to calculate the percentage recoveries (%R) following equation 3.9.

$$\%R = \frac{C1 - C2}{C3} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

Where C1 is the spiked sample result, C2 is the un-spiked sample result and C3 is the concentration of the spiked desired.

3.3.2 Limit of detection of the metals

The limit of detection is expressed as the smallest concentration that can be detected with reasonable certainty for a given analytical procedure (IUPAC, 1997). The limit of detection is expressed as the mean concentration corresponding to the sample blank plus three times standard deviation as determined by the measurement(s) of a standard (IUPAC, 1997). The value of LOD is given by the equation 3.10 below.

$$\bar{x}\text{blank} + 3S_a\text{blank} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation } 3.10$$

WhereS_a is the Standard deviation of the sample blank samples and $\overline{X}$ is the Mean concentration of the sample blank.

3.3.3 Limit of quantification of the metals

Limit of quantification is expressed as the smallest concentration that can be quantified with reasonable certainty and it is expressed as themean concentration corresponding to the

samples blank plus ten times the standard deviation as determined by the measurement(s) of a standard (IUPAC, 1997). The value of LOD is given by the equation 3.11 below.

$$\bar{x}\text{blank} + 10S_a\text{blank} \quad \ldots\ldots 3.11$$

Where $\bar{x}$blank is the mean of blank aqueous solution and $S_a$blank is the blank standard deviation

3.3.4 Relative standard deviation of the metals

The relative standard deviation (RSD_r) is defined as the standard deviation of a set of measurements divided by the mean of all the measurements(IUPAC, 1997). Notice that this is a unit less quantity and is often reported as the percent relative standard deviation ($\%RSD_r$).

$$\%\text{RSD}_\text{r} = \frac{\text{S}_\text{a}}{\overline{\text{X}}} \text{ x } 100 \quad \ldots\ldots 3.12$$

3.4 Risk Assessment Index

Risk assessment in this study was evaluated by using the following indices; Heath Quotient (HQ), Daily Intake Metal (DIM) and Health Risk Index (HRI) according to methods by Sajjad *et al*. (2009) and Okunola *et al*. (2011). The index assessment was designed based on three categories of individuals. Category A was designated for adults (aged 19years and above), category B designated for children (aged range from 7- 18years) and the last category C designated for (aged range 6years and below).

3.4.1 Hazard quotient (HQ)

The Hazard Quotient (HQ) is an estimates hazard of the studied metals on populations in the later future. It is a determined ratio of human hazard with respects to the metal reference dose (R_fD). Then if HQ results shows a value of less than one (1) then the metal will pose no risk on

the population due to fish consumption. However, if HQ value is greater than one (1) then the population would experience risk of hazardous metals. The equation 3.13 below was employed.

$$HQ = \frac{[W_{fish}] \times [M_{fish}]}{R_fD \times B_o} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.13$$

Where W_{fish} is the dry weight of edible fish consumed (gd^{-1}), M_{fish} is the concentration of metal in fish (mg kg^{-1}), R_fD is the metal reference dose ($mgkg^{-1}d^{-1}$) (given in Table 2.1) and B_o is the body mass (kg).Note: In this study, the daily intake of fish for nutritional requirement was100g intake rate for adults with average body weight of 70kg (aged 19years and above), 80g intake rate for children with average body weight of 48kg (aged range from 7- 18years) and 60g intake rate for individuals with body weight of 19kg (aged range 6years and below) using the methodby Portier *et al*. (2007).

3.4.2 Daily intakes of metals

The daily intake of metals (DIM) was calculated to detrmine the daily loading of metals into the human systemwith the consumption of fish muscles of this study. The DIM was determined according to equation 3.14.

$$DIM = \frac{C_{metal} \times [D_{fish}] \times [C_{factor}]}{B_o} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.14$$

Where C_{metal} is the concentration of heavy metals in the fish ($mgkg^{-1}$), D_{fish} is the daily nutritional intake of fish ($mgday^{-1}$), C_{factor} is the factor for conversion of fresh fish to dry constant weight. The$C_{factor}$for *Trachurus trachurus*, *Scomber scombrus* and *Trachurusmurphyi* species in this study, was given as 0.2990, 0.3659 and 0.2821 respectively as computed by the equations below (USEPA, 2008).

$$C_{factor} = IR_{ww} - IR_{dw} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.15$$

$$IR_{ww} = IR_{dw}\left[\frac{100-W}{100}\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.16$$

Where IR_{dw} is the dry weight intake rate; IR_{ww} is the wet weight intake rate and W is the percent water content of the raw musclesof *Trachurus trachurus, Scomber scombrus* and *Trachurusmurphyi* species which were and the values are given as TT = 71.79%,SS = 63.50% and TM = 70.10%respectively).

3.4.3 Health risk index

The health risk index (HRI) forindividuals of populations through consumption of contaminated fish were assessed based on DIM relative to R_fD for each metal. The following formula is used for the calculation of HRI.

$$HRI = \frac{DIM}{R_fD} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.17$$

CHAPTER FOUR

4.0 RESULTS

4.1 Quality Assurance Results

The resultsin Tables 4.1-4.3 presented the values of validation parameters for analytical procedures including; LOD, LOQ, RSDr and the percentage recoveries of the studied heavy metalsfor *Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi*species. The percentage recoveries for Cd, Pb, Hg, Fe and Ni in the tissues/organs of the studied fish species *Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi* varied between the ranges of 90.70 – 111.00%, 93.60 – 118.70% and 99.10 – 108.70% respectively.

For *Trachurus trachurus* species, the LODresults with regards to Cd, Pb, Hg, Fe and Ni concentrations in the tissues/organsvaried between the ranges of 0.031 - 0.071mgkg^{-1} for the kidneys and skin tissues, 0.004 – 0.006mgkg^{-1} for the brain and muscles tissues, 0.0006 – 0.001mgkg^{-1} for the gills and bones tissues, 0.0001mgkg^{-1} for the liver tissues and 0.002mgkg^{-1} for intestine tissuesrespectively.While LOQ results for Cd, Pb, Hg, Fe and Ni concentrations in the tissuesvaried between the ranges of0.051 – 0.230mgkg^{-1} for the kidneys and skin tissues, 0.012 – 0.024mgkg^{-1} for the brain and muscles tissues, 0.0024 – 0.005mgkg^{-1} for the gills and bone tissues, 0.00045mgkg^{-1} for the liver, and 0.007mgkg^{-1} for the intestine tissues, respectively. Also, the RSDr results for Cd, Pb, Hg, Fe and Nivaried between the ranges of 4.51 – 4.96% for skin and kidneys tissues, 2.07 – 4.96%for the muscles and brain tissues, 3.53 - 38.76% for gills and bone tissues, 1.55% for the liver tissues and 0.50% for the intestine tissues respectively.

For *Scomber scombrus* species, the LOD results for Cd, Pb, Hg, Fe and Ni concentrations in the tissuesvaried between the ranges of 0.009 - 0.058mgkg^{-1} for the kidneys and skin tissues, 0.001 – 0.003mgkg^{-1}forthe muscles and brain tissues, 0.001mgkg^{-1} forboth gills and bone tissues

Table 4.1 Quality assurance of metals in*Trachurus trachurus* species

Heavy metals	Tissues	Concentration of the sample (mgL^{-1})	Concentration of the spiked sample (mgL^{-1})	%R±SD	LOD ($mgkg^{-1}$) N=6	LOQ ($mgkg^{-1}$) N=6	$\%RSD_r$
Cd	Skin	0.020	1.130	111.0±0.05	0.071	0.230	4.51
Pb	Muscles	0.146	1.112	96.6±0.20	0.006	0.024	2.07
Hg	Gills	1.808	2.800	99.2±0.35	6.32E-4	2.47E-3	3.53
Fe	Liver	5.403	6.373	97.0±0.15	1.14E-4	4.50E-4	1.55
Ni	Intestine	0.151	1.150	99.9±0.05	0.002	0.007	0.50
Cd	Kidneys	0.062	1.111	104.9±0.31	0.013	0.051	29.55
Pb	Brain	0.185	1.092	90.7±0.45	0.004	0.012	4.96
Hg	Bone	1.760	2.792	103.2±0.40	0.001	0.005	38.76

Table 4.2 Quality assurance of metals in*Scomber scombrus* species

Heavy metals	Tissues	Concentration of the sample (mgL^{-1})	Concentration of the spiked sample (mgL^{-1})	%R±SD	LOD ($mgkg^{-1}$) N=6	LOQ ($mgkg^{-1}$) N=6	$\%RSD_r$
Cd	Skin	0.041	1.079	103.8±0.25	0.058	0.109	24.08
Pb	Muscles	0.294	1.302	100.8±0.10	0.001	0.009	9.92
Hg	Gills	1.693	2.880	118.7±0.25	0.001	0.004	23.56
Fe	Liver	7.348	8.409	106.1±0.55	8.19E-5	2.67E-4	51.84
Ni	Intestine	0.075	1.067	99.2±0.15	0.012	0.042	1.51
Cd	Kidneys	0.115	1.051	93.6±0.30	0.009	0.029	3.21
Pb	Brain	0.388	1.422	103.4±0.35	0.003	0.009	33.84
Hg	bone	1.760	2.890	113.0±0.15	0.001	0.003	13.27

Table 4.3 Quality assurance of metals in*Trachurus murphyi* species

Heavy metals	Tissues	Concentrations of the sample (mgL^{-1})	Concentrations of the spiked sample (mgL^{-1})	%R±SD	LOD ($mgkg^{-1}$) N=6	LOQ ($mgkg^{-1}$) N=6	$\%RSD_r$
Cd	Skin	0.040	1.127	108.7±0.32	0.061	0.262	0.29
Pb	Muscles	0.021	1.025	100.4±0.20	0.004	0.012	1.92
Hg	Gills	2.509	3.501	99.2±0.45	4.86E-4	0.002	4.54
Fe	Liver	12.828	13.900	107.2±0.15	6.78E-5	3.21E-4	13.99
Ni	Intestine	0.055	1.051	99.6±0.10	0.012	0.050	1.00
Cd	Kidneys	0.291	1.309	101.8±0.31	0.024	0.070	0.45
Pb	Brain	0.078	1.069	99.1±0.25	0.005	0.011	2.52
Hg	Bone	1.494	2.488	99.4±0.35	9.23E-4	0.003	3.52

0.000082mgkg^{-1} for liver tissues and 0.012mgkg^{-1} for intestine tissues respectively. While LOQ results for Cd, Pb, Hg, Fe and Ni concentrations in the tissues were between the ranges of 0.109 – 0.029mgkg^{-1} for skin and kidneys tissues, 0.009mgkg^{-1} for both muscles and brain tissues, 0.003 – 0.004mgkg^{-1} for gills and bone tissues, 0.00027mgkg^{-1} for liver tissues and 0.042mgkg^{-1} for intestine tissues respectively. Also RSDr results for Cd, Pb, Hg, Fe and Nivaried between the ranges of 3.21 – 24.08% for kidneys and skin tissues, 9.92 – 33.84% for muscles and brain tissues, 13.27 - 23.56% for bone and gills tissues, 51.84% for liver tissues and 1.51% for intestine tissues respectively.

For*Trachurus murphyi*species, the LOD results for Cd, Pb, Hg, Fe and Ni concentrations in the tissues were between the ranges of 0.024 - 0.0761mgkg^{-1} for the skin and kidneys tissues, 0.004 – 0.005mgkg^{-1} for the muscles and brain tissues, 0.00049 – 0.00092mgkg^{-1} for gills and bone tissues, 0.000069mgkg^{-1} for the liver tissues and 0.002mgkg^{-1} for the intestine tissues respectively. While LOQ results for Cd, Pb, Hg, Fe and Ni concentrations in the tissuesvaried between the ranges of 0.070 – 0.262mgkg^{-1} forthe kidneys and skin tissues, 0.011 – 0.012mgkg^{-1} for the brain and muscles tissues, 0.002 – 0.003mgkg^{-1} for the gills and bone tissues, 0.00032mgkg^{-1} for the liver tissues, and 0.050mgkg^{-1} for the intestine tissues respectively. Also RSDr results for Cd, Pb, Hg, Fe and Ni varied between the ranges of 0.29 – 0.45% for the skin and kidneys tissues, 1.02 – 2.52% forthe muscles and brain tissues, 3.52 – 4.54% for boneand gills tissues, 13.99% for the liver tissues and 1.000 % for the intestine tissues respectively.

4.2 Cadmium Concentrations in Tissues of *Trachurustrachurus* Species

The results presented in Table 4.4 showedthe concentrations of Cd in the tissues/organs of *Trachurus trachurus* species across the batches of RS and EU fishing origins. The ranges of Cd

Table 4.4 Cd concentration in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	1.000^{a}	0.950^{b}	0.950^{c}	1.050^{d}	1.100^{e}	0.500^{f}	0.925±0.025	0.100
Skin(EU)	0.300^{a}	0.450^{b}	0.350^{c}	0.350^{d}	0.450^{e}	0.500^{f}	0.400±0.015	
Muscles(RS)	1.050^{a}	1.050^{b}	1.050^{c}	1.050^{d}	1.000^{e}	0.500^{f}	0.950±0.045	
Muscles(EU)	0.250^{a}	0.400^{b}	0.350^{c}	0.350^{d}	0.350^{e}	0.500^{f}	0.367±0.020	
Gills(RS)	3.650^{a}	4.200^{b}	1.050^{c}	1.250^{a}	1.050^{b}	0.250^{d}	1.908±0.025	
Gills(EU)	0.350^{a}	0.500^{b}	0.450^{c}	0.350^{d}	0.400^{e}	0.450^{f}	0.417±0.040	
Liver(RS)	6.050^{a}	0.900^{b}	7.750^{c}	9.150^{d}	3.950^{cd}	3.050^{cd}	5.142±0.020	
Liver(EU)	2.950^{a}	3.450^{b}	6.750^{ac}	2.250^{b}	1.550^{c}	22.150^{d}	6.517±0.015	
Intestine(RS)	2.350^{a}	2.600^{b}	2.650^{b}	2.450^{c}	2.450^{d}	1.750^{e}	2.375±0.025	
Intestine(EU)	1.200^{a}	2.900^{b}	1.650^{c}	1.000^{d}	0.650^{e}	2.000^{b}	1.567±0.005	
Kidneys(RS)	3.100^{a}	1.650^{b}	1.550^{c}	3.150^{a}	1.350^{d}	0.650^{e}	1.908±0.005	
Kidneys(EU)	2.050^{a}	0.900^{b}	3.600^{c}	1.200^{d}	0.500^{e}	1.700^{c}	1.658±0.010	
Brain(RS)	1.200^{a}	1.300^{b}	1.550^{c}	1.300^{d}	0.600^{e}	1.100^{f}	1.175±0.025	
Brain(EU)	0.600^{a}	0.350^{b}	0.300^{c}	0.250^{d}	0.300^{e}	0.400^{f}	0.367±0.030	
Bones(RS)	1.300^{a}	2.000^{b}	2.050^{c}	1.500^{d}	1.200^{e}	1.700^{f}	1.6250.005	
Bone(EU)	0.850^{a}	0.700^{b}	0.500^{c}	0.350^{d}	0.500^{e}	3.500^{e}	1.067±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

concentration for the batches of RS fish species were between 0.500 – 1.100 $mgkg^{-1}$, 0.500 – 1.050 $mgkg^{-1}$, 0.250 – 4.200 $mgkg^{-1}$, 0.90 – 9.150 $mgkg^{-1}$, 1.750 – 2.650 $mgkg^{-1}$, 0.650 – 3.150 $mgkg^{-1}$, 0.600 – 1.550 $mgkg^{-1}$ and 1.200 – 2.050 $mgkg^{-1}$ for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, the ranges of Cd concentrations for the batches of EU fish species were between 0.300 – 0.500 $mgkg^{-1}$, 0.250 – 0.500 $mgkg^{-1}$, 0.350 – 0.500 $mgkg^{-1}$, 1.550 – 22.150 $mgkg^{-1}$, 0.650 – 2.900 $mgkg^{-1}$, 0.500 – 3.600 $mgkg^{-1}$, 0.300 – 0.600 $mgkg^{-1}$ and 0.350 – 0.850 $mgkg^{-1}$ for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Cd statistical analysis for *Trachurus trachurus* species showed that, significant differences ($P<0.05$) were recorded in some batches of RS fish species and these are shown in the batches of skin, muscle, brain and bone tissues. While for EU fish species, significant differences ($p<0.05$) were shown in the batches of skin muscles, gills and bone tissues.

The results of Cd correlation analysis in tissues of RS fish for *Trachurus trachurus* species are presented in Table 4.5. Significant ($p<0.05$) positive correlations were recorded between the skin and intestine, muscles and kidney tissues; muscles and gills, intestine and kidneys tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation was recorded between the gills and liver tissues only. While, the correlation results (shown in Table 4.6) for EU tissues, showed significant ($p<0.05$) positive correlation between skin and muscles, liver and bone tissues; muscles and liver and bone tissues; liver and bone tissues; and kidneys and bone tissues. Also, significant ($p<0.05$) negative correlation was not shown in any of the EU tissues.

4.3 Cadmium Concentrations in Tissues of *Scomber scombrus* Species

The results of Cd concentrations across the batches of RS and EU tissues/organs of

Table 4.5 Correlation of Cd in tissues of *Trachurus trachurus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.872**	1						
Gills	0.360	0.523*	1					
Liver	0.217	0.182	-0.621**	1				
Intestine	0.702**	0.730**	0.426	0.214	1			
Kidney	0.594**	0.628**	0.438	0.205	0.382	1		
Brain	-0.131	0.193	0.204	0.301	0.321	0.266	1	
Bone	-0.277	-0.106	-0.151	0.272	0.100	-0.274	0.607**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.6 Correlation of Cd in tissues of *Trachurus trachurus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.642**	1						
Gills	0.174	0.148	1					
Liver	0.489*	0.636**	-0.083	1				
Intestine	0.345	0.409	0.448	0.345	1			
Kidney	-0.419	-0.120	-0.172	0.223	0.077	1		
Brain	-0.277	-0.290	0.051	0.099	0.054	0.139	1	
Bone	0.524*	0.610**	-0.052	0.962**	0.311	0.027	0.232	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Scomber scombrus species are presented in Table 4.7. The ranges of concentrations recorded for RS fish species were between 2.050 – 2.200mgkg^{-1}, 1.950 – 2.450mgkg^{-1}, 2.150 – 2.650mgkg^{-1}, 6.300 – 35.800mgkg^{-1}, 3.250 – 19.100mgkg^{-1}, 2.700 – 5.800mgkg^{-1}, 2.100 – 5.650mgkg^{-1} and 2.450 – 22.350mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, concentrations ranges for EU tissues were between 0.600 – 2.600mgkg^{-1}, 0.400 – 9.000mgkg^{-1}, 0.500 – 2.650mgkg^{-1}, 4.950 – 30.250mgkg^{-1}, 0.950 – 9.900mgkg^{-1}, 1.450 – 16.850mgkg^{-1}, 0.650 – 5.000mgkg^{-1} for the skin, muscles, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Cd statistical analysis for *Scomber scombrus* species showed significant differences ($P<0.05$) in some batches of RS fish species. These are shown in the batches of the skin, muscles, gills and brain tissues.In contrast, significant differences ($p<0.05$) were not shown in any of the batches of EU fish species.

The results of Cd correlation analysis for*Scomber scombrus*in tissues of RS fish species are presented in Table 4.8.Significant($p<0.05$) positive correlation were recorded between gills and brain tissues; liver and bone tissues; and kidney and bone tissues. However, significant ($p<0.05$) negative correlationwere observed between muscles and liver and intestines tissues; and kidneys and bone tissues. The results of correlation analysis (as shown in Table 4.9) for EU, tissues showed significant ($p<0.05$) positive correlation between skin and muscles, gills, liver, muscles, gills, liver, intestine and kidneys tissues; muscles and gills, liver, intestine and kidneys tissues; gills and liver, intestine and kidneys tissues; liver and intestine and kidneys tissues; and intestine and kidneys tissues. However, significant ($p<0.05$) negative correlation was shown only between liver and bone tissues.

Table 4.7 Cd concentration intissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	2.050^{a}	2.100^{b}	2.150^{c}	2.150^{d}	2.150^{e}	2.200^{f}	2.133±0.025	0.100
Skin(EU)	2.600^{a}	0.600^{b}	0.800^{c}	1.100^{a}	0.850^{d}	1.000^{e}	1.158±0.035	
Muscles(RS)	1.950^{a}	2.450^{b}	2.200^{c}	1.950^{d}	2.150^{e}	2.450^{f}	2.192±0.005	
Muscles(EU)	9.000^{a}	0.400^{b}	1.050^{c}	0.750^{d}	1.900^{a}	1.150^{e}	2.375±0.015	
Gills(RS)	2.350^{a}	2.150^{b}	2.350^{c}	2.500^{cd}	2.550^{cd}	2.650^{d}	2.4250.015	
Gills(EU)	2.650^{a}	0.500^{b}	0.750^{c}	0.900^{d}	0.750^{a}	1.200^{e}	1.125±0.020	
Liver(RS)	24.900^{a}	6.300^{ab}	35.800^{c}	33.900^{ad}	8.500^{bd}	9.150^{c}	19.758±0.030	
Liver(EU)	30.250^{a}	4.950^{ab}	7.350^{c}	9.000^{cd}	5.900^{bd}	6.500^{ac}	10.658±0.015	
Intestine(RS)	19.100^{a}	3.750^{b}	6.900^{c}	3.250^{ac}	7.550^{d}	5.600^{ca}	7.692±0.015	
Intestine(EU)	9.900^{a}	6.000^{b}	8.750^{c}	1.750^{b}	0.950^{c}	5.600^{ac}	5.492±0.035	
Kidneys(RS)	5.750^{a}	4.350^{b}	2.700^{ac}	4.100^{bc}	2.900^{abd}	5.800^{d}	4.267±0.040	
Kidneys(EU)	16.850^{a}	1.950^{b}	5.900^{c}	1.450^{d}	1.800^{c}	4.500^{d}	5.408±0.030	
Brain(RS)	2.350^{a}	2.200^{b}	2.100^{c}	2.950^{d}	2.500^{ce}	5.650^{e}	2.958±0.015	
Brain(EU)	0.800^{a}	0.750^{b}	1.100^{c}	5.000^{d}	0.650^{d}	1.300^{e}	1.600±0.010	
Bone(RS)	3.000^{a}	2.950^{b}	22.350^{abc}	3.900^{d}	2.450^{e}	2.600^{b}	6.208±0.010	
Bone(EU)	1.550^{a}	1.250^{b}	1.850^{c}	1.600^{d}	1.050^{e}	2.350^{f}	1.608±0.020	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.8 Correlation of Cd in tissues of *Scomber scombrus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.165	1						
Gills	0.305	-0.072	1					
Liver	-0.055	-0.668**	-0.050	1				
Intestine	-0.354	-0.484*	-0.097	0.153	1			
Kidney	-0.115	0.092	0.109	-0.243	0.399	1		
Brain	0.337	0.437	0.677**	-0.341	-0.229	0.571*	1	
Bone	0.075	-0.016	-0.204	0.627**	-0.079	-0.573*	-0.329	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.9 Correlation of Cd in tissues of *Scomber scombrus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.948**	1						
Gills	0.984**	0.942**	1					
Liver	0.986**	0.950**	0.965**	1				
Intestine	0.495*	0.485*	0.547*	0.573*	1			
Kidney	0.922**	0.923**	0.939**	0.947**	0.769**	1		
Brain	-0.058	-0.307	-0.137	-0.110	-0.459	-0.326	1	
Bone	0.051	-0.098	0.164	-0.014	0.360	0.152	0.135	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

4.4 Cadmium Concentrations in Tissues of *Trachurus murphyi* Species

The results presented in Table 4.10 revealed the concentrations of Cd in tissues/organs of *Trachurus murphyi* across the batches of RS and EU fishing origins. The range of concentrations recorded in batches of RS fish species were between 1.050 – 2.000mgkg^{-1}, 1.050 – 1.750mgkg^{-1}, 1.100 – 2.400mgkg^{-1}, 1.250 – 56.300mgkg^{-1}, 4.250 – 10.150mgkg^{-1}, 6.800 – 17.800mgkg^{-1}, 1.050 – 2.050mgkg^{-1} and 1.600 – 2.900mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. Whilefor EU fish species, the concentration ranged between 1.600 – 2.750mgkg^{-1}, 1.850 – 2.900mgkg^{-1}, 1.800 – 6.550mgkg^{-1}, 2.200 – 67.550mgkg^{-1}, 3.900 – 27.200mgkg^{-1}, 5.450 – 12.900mgkg^{-1}, 1.650 – 9.350mgkg^{-1} and 1.800 – 3.000mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

Statistical results of Cd concentrations for *Trachurus murphyi* showedthere are significant differences ($p<0.05$) among the batches of both RS and EU fish species. For RS tissues, the significant differences ($P<0.05$) were recorded in the batches of skin and muscles tissues.While for EU tissues, significant differences ($p<0.05$) were recorded in the batches of bone tissues only.

The results of Cd correlation analysis for RS fish with respect to*Trachurus murphyi* species are presented in Table 4.11.Significant ($p<0.05$) positive correlationwere recorded between gills and braintissues; liver and bone tissues; and kidneys and brain tissues. However, significant ($p<0.05$) negative correlation werebetween muscles and liver, intestine and bonetissues; and kidneys and bone tissues were recorded. While for EU tissues, the results (in Table 4.12) showed significant ($p<0.05$) positive correlations were between skin and muscles tissues; gills and intestine and bone tissues; and kidneys and brain tissues. However, significant

($p<0.05$) negative correlation were between skin and kidneys and brain tissues; gills and liver tissues; liver and intestine tissues; and brain and bone tissues.

Table 4.10 Cd concentration in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO (2003)
Skin(RS)	2.000^{a}	1.050^{b}	1.350^{c}	1.450^{d}	1.950^{e}	1.750^{f}	1.592±0.065	0.100
Skin(EU)	1.600^{a}	2.300^{b}	2.550^{c}	2.500^{c}	2.150^{d}	2.750^{d}	2.308±0.020	
Muscles(RS)	1.750^{a}	1.050^{b}	1.200^{c}	1.400^{d}	1.600^{e}	1.400^{f}	1.400±0.025	
Muscles(EU)	1.850^{a}	1.750^{b}	2.100^{c}	2.350^{d}	2.100^{e}	2.900^{e}	2.175±0.015	
Gills(RS)	1.500^{a}	1.100^{b}	1.300^{c}	1.350^{d}	2.300^{e}	2.400^{f}	1.658±0.010	
Gills(EU)	3.335^{a}	1.800^{b}	1.900^{a}	6.550^{c}	1.950^{d}	2.100^{e}	2.939±0.005	
Liver(RS)	1.250^{a}	38.100^{b}	56.300^{c}	43.100^{d}	40.000^{c}	41.150^{c}	36.650±0.035	
Liver(EU)	39.700^{a}	67.550^{b}	26.050^{bc}	2.200^{a}	55.350^{d}	36.350^{cb}	37.867±0.030	
Intestine(RS)	8.550^{a}	4.250^{ab}	8.700^{c}	10.150^{ad}	7.100^{c}	6.200^{bd}	7.492±0.015	
Intestine(EU)	3.900^{a}	7.800^{b}	4.350^{bc}	27.200^{d}	7.200^{bd}	6.100^{c}	9.425±0.015	
Kidneys(RS)	14.550^{a}	17.800^{b}	17.050^{c}	6.800^{a}	10.950^{c}	7.800^{b}	12.492±0.015	
Kidneys(EU)	12.900^{a}	10.700^{b}	5.450^{ac}	8.750^{dc}	7.700^{b}	10.200^{ad}	9.283±0.005	
Brain(RS)	1.500^{a}	1.050^{b}	1.800^{c}	1.750^{d}	1.850^{e}	2.050^{f}	1.667±0.020	
Brain(EU)	9.350^{a}	1.650^{b}	1.650^{c}	4.650^{ac}	1.900^{d}	1.850^{e}	3.508±0.035	
Bone(RS)	1.600^{a}	1.250^{b}	2.900^{cd}	2.400^{d}	2.350^{e}	2.800^{d}	2.217±0.035	
Bone(EU)	1.800^{a}	2.750^{b}	2.150^{bc}	2.800^{d}	3.000^{e}	2.600^{c}	2.517±0.010	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.11 Correlation of Cd in tissues of *Trachurus murphyi* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.165	1						
Gills	0.305	-0.072	1					
Liver	-0.055	-0.668**	-0.050	1				
Intestine	-0.354	-0.484*	-0.097	0.153	1			
Kidney	-0.115	0.092	0.109	-0.243	0.399	1		
Brain	0.337	0.437	0.677**	-0.341	-0.229	0.571*	1	
Bone	0.075	-0.016	-0.204	0.627**	-0.079	-0.573*	-0.329	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 4.12 Correlation of Cd in tissues of *Trachurus murphyi* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.691**	1						
Gills	-0.014	0.134	1					
Liver	-0.316	-0.439	-0.779**	1				
Intestine	0.278	0.202	0.899**	-0.660**	1			
Kidney	-0.560*	-0.146	0.113	0.284	-0.104	1		
Brain	-0.769**	-0.284	0.470*	-0.252	0.070	0.652**	1	
Bone	0.459	0.240	0.064	0.155	0.446	-0.283	-0.634**	1

**Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

4.5 Lead Concentrations in Tissues of *Trachurus trachurus* Species

The results presented in Table 4.13 revealed the concentrations of Pbin tissues/organs of *Trachurus trachurus* species across the batches of RS and EU fishing origins. The range of Pb concentrations in batches of RS fish species were between 8.300 – 19.950mgkg^{-1}, 7.300 – 18.600mgkg^{-1}, 7.100 – 18.100mgkg^{-1}, 8.250 – 19.050mgkg^{-1}, 9.050 – 18.850mgkg^{-1}, 7.600 – 18.550mgkg^{-1}, 9.250 – 19.150mgkg^{-1} and 12.300 – 18.250mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While for EU fish species, the concentrations ranged between 16.550 – 19.650mgkg^{-1}, 14.950 – 18.350mgkg^{-1}, 16.450 – 18.700mgkg^{-1}, 16.050 – 19.950mgkg^{-1}, 15.700 – 19.500mgkg^{-1}, 14.800 – 19.500mgkg^{-1}, 16.150 – 20.950mgkg^{-1} and 15.250 – 21.350mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Pb statistical analysisfor *Trachurus trachurus* speciesshowed significant differences ($p<0.05$)were not shown in any of the batches of RS and EU fish species. Hence, non-significant differences ($p<0.05$) were found dominantly across the batches ofboth RS and EU fish species.

The results of Pb correlation analysisin tissues of RS fish species (as shown in Table 4.14) for *Trachurus trachurus* showed significant ($p<0.05$) positive correlationwere recordedacross theentire tissues. Hence significant ($p<0.05$) negative correlations were not shown in any of the tissues. The correlation results of Pb in tissues of EU fish species (Table 4.15) showed significant ($p<0.05$) positive correlationwere between skin and gills tissues; muscles and gills, liver, intestine, kidneys, brain and bone tissues; gills and liver, and intestine; liver and intestine and kidneys; intestine and kidneys, brain and bones; kidneys and brain and bones tissues; and

brain and bone tissues. But for EU tissues, significant ($p<0.05$) was shown between skin and bone tissues only.

Table 4.13 Pb concentration in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	8.300^{a}	10.500^{b}	17.150^{c}	15.000^{c}	19.950^{d}	19.250^{d}	15.025±0.025	0.400
Skin(EU)	18.550^{a}	18.700^{b}	16.550^{abc}	18.750^{cd}	16.100^{a}	19.650^{d}	18.050±0.015	
Muscles(RS)	7.300^{a}	10.350^{b}	14.600^{c}	18.600^{d}	18.550^{d}	16.600^{d}	14.333±0.010	
Muscles(EU)	15.100^{a}	14.950^{ab}	16.000^{bc}	16.000^{bd}	16.400^{c}	18.350^{d}	16.133±0.020	
Gills(RS)	7.100^{a}	12.800^{b}	14.700^{c}	17.500^{d}	18.100^{de}	16.950^{de}	14.525±0.030	
Gills(EU)	17.400^{a}	17.050^{b}	17.600^{ac}	17.150^{bc}	16.450^{ad}	18.700^{d}	17.392±0.010	
Liver(RS)	8.250^{a}	12.650^{b}	18.200^{c}	17.600^{c}	19.050^{d}	18.700^{d}	15.742±0.040	
Liver(EU)	18.100^{a}	16.050^{ab}	18.000^{c}	17.200^{ac}	17.050^{bd}	19.950^{d}	17.725±0.045	
Intestine(RS)	9.050^{a}	13.750^{b}	15.650^{c}	18.600^{d}	17.450^{de}	18.850^{e}	15.558±0.030	
Intestine(EU)	16.100^{a}	15.700^{a}	17.250^{b}	17.750^{c}	18.250^{d}	19.500^{e}	17.425±0.020	
Kidneys(RS)	7.600^{a}	13.350^{b}	17.200^{c}	18.400^{d}	18.550^{de}	18.200^{de}	15.55±0.005	
Kidneys(EU)	15.800^{a}	16.700^{b}	14.800^{ab}	14.100^{ab}	17.650^{c}	19.500^{d}	16.424±0.015	
Brain(RS)	9.250^{a}	13.300^{b}	17.350^{c}	17.150^{cd}	17.850^{de}	19.150^{e}	15.675±0.010	
Brain(EU)	16.700^{a}	17.150^{ab}	17.050^{c}	16.150^{ab}	20.950^{d}	20.350^{d}	18.058±0.025	
Bones(RS)	10.100^{a}	12.300^{b}	16.600^{c}	17.000^{d}	18.250^{de}	16.800^{de}	15.175±0.030	
Bones(EU)	16.100^{a}	15.250^{ab}	17.650^{ac}	15.650^{bc}	21.350^{d}	19.600^{d}	17.600±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.14 Correlation of Pb in tissues of *Trachurus trachurus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.879**	1						
Gills	0.876**	0.968**	1					
Liver	0.953**	0.938**	0.953**	1				
Intestine	0.861**	0.952**	0.973**	0.942**	1			
Kidney	0.899**	0.953**	0.984**	0.985**	0.971**	1		
Brain	0.936**	0.910**	0.941**	0.984**	0.961**	0.975**	1	
Bone	0.948**	0.972**	0.952**	0.984**	0.927**	0.968**	0.948**	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.15 Correlation of Pb in tissues of *Trachurus trachurus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.213	1						
Gills	0.604**	0.657**	1					
Liver	0.347	0.788**	0.865**	1				
Intestine	0.044	0.950**	0.448	0.667**	1			
Kidney	0.229	0.659**	0.420	0.469*	0.524*	1		
Brain	-0.213	0.693**	0.117	0.361	0.695**	0.854**	1	
Bone	-0.456	0.670**	0.046	0.392	0.737**	0.643**	0.933**	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

4.6 Lead Concentrations in Tissues of *Scomber scombrus* Species

The results presented in Table 4.16 revealedthe concentrations of Pb in the tissues of *Scomber scombrus* species across the batches of both RS and EU origins. The Pb concentrations showed for the tissues of RS fish species were between 13.350 – 20.300mgkg^{-1}, 9.950 – 20.250mgkg^{-1}, 15.000 – 20.000mgkg^{-1}, 14.050 – 21.850mgkg^{-1}, 15.800 – 21.200mgkg^{-1}, 13.400 – 21.600mgkg^{-1}, 13.700 – 19.400mgkg^{-1} and 14.150 – 21.200mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, concentrations of Pb shown in the tissues of EU fish species ranged between 3.500 – 21.800mgkg^{-}, 2.000 – 21.750mgkg^{-1}, 4.350 – 22.350mgkg^{-1}, 5.050 – 21.700mgkg^{-1}, 5.250 – 22.300mgkg^{-1}, 5.150 – 26.650mgkg^{-1}, 1.300 – 7.650mgkg^{-1} and 1.050 – 6.250mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Pb statistical analysis for*Scomber scombrus* speciesshowedthere aresignificant differences ($p<0.05$)in the batches of RS fish species. These includes the batches of gills, intestine, kidneys, bone and brain tissues. For EU tissues, significant differences ($p<0.05$) for Pb accumulations was found in the batches of brain tissues only.

The results of Pbcorrelation analysis for *Scomber scombrus* in tissues ofRS fish species are presented in Table 4.17. Significant ($p<0.01$) positive correlation were between skin and muscles, gills, liver, intestine, kidneys and brain tissues; muscles and gills, liver, intestine, kidneys, brain and bone tissues; gills and liver, intestine, kidneys, brain and bone tissue; liver and intestine, kidneys and bone tissues; intestine and kidneys, brain and bone tissues; kidneys and brain and bone tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation were not shown for RS tissues. While the correlation results in Table 4.18 for EU

tissues showed significant ($p<0.05$) positive correlation were between skin and muscles, gills, intestine and

Table 4.16 Pb concentration in tissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	16.600^{a}	19.150^{b}	13.350^{a}	15.000^{bc}	18.500^{bc}	20.300^{d}	17.150±0.35	0.400
Skin(EU)	21.800^{a}	3.500^{ab}	5.250^{c}	8.050^{d}	4.000^{ab}	4.400^{b}	7.833±0.005	
Muscles(RS)	14.700^{a}	16.950^{b}	9.950^{a}	13.500^{bc}	15.450^{bc}	20.250^{d}	15.133±0.010	
Muscles(EU)	21.750^{a}	2.000^{b}	5.450^{c}	5.200^{c}	4.600^{ab}	4.450^{b}	7.242±0.010	
Gills(RS)	15.400^{a}	16.150^{b}	12.000^{a}	15.000^{b}	16.700^{c}	20.400^{d}	15.942±0.005	
Gills(EU)	22.350^{a}	5.350^{b}	7.700^{c}	3.950^{ab}	4.350^{bc}	5.150^{ac}	8.142±0.015	
Liver(RS)	14.050^{a}	17.500^{ab}	15.300^{c}	13.400^{abd}	17.600^{de}	21.850^{e}	16.617±0.010	
Liver(EU)	21.700^{a}	5.050^{ab}	6.750^{c}	6.450^{d}	5.400^{bc}	6.100^{ac}	8.575±0.005	
Intestine(RS)	17.800^{a}	16.000^{b}	15.800^{ab}	13.900^{ac}	20.500^{cd}	21.200^{d}	17.533±0.020	
Intestine(EU)	22.300^{a}	5.450^{b}	5.900^{c}	5.400^{bd}	5.250^{a}	6.200^{d}	8.417±0.020	
Kidneys(RS)	19.850^{a}	13.400^{ab}	14.850^{a}	15.450^{a}	20.200^{b}	21.600^{b}	17.558±0.025	
Kidneys(EU)	26.650^{a}	5.400^{b}	5.200^{bc}	5.150^{ab}	5.350^{c}	4.600^{a}	8.725±0.015	
Brain(RS)	19.400^{a}	13.700^{ab}	14.650^{bc}	15.800^{cd}	18.900^{a}	19.200^{e}	16.942±0.035	
Brain(EU)	1.300^{a}	2.800^{b}	7.650^{c}	4.650^{cd}	2.900^{bcd}	7.200^{d}	4.417±0.025	
Bone(RS)	15.400^{a}	12.500^{ab}	14.150^{a}	17.850^{c}	19.150^{d}	21.200^{e}	16.708±0.030	
Bone(EU)	1.050^{a}	3.000^{b}	6.100^{c}	3.500^{cd}	3.800^{cde}	6.250^{e}	3.950±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.17 Correlation of Pb in tissues of *Scomber scombrus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.929**	1						
Gills	0.905**	0.941**	1					
Liver	0.803**	0.639**	0.802**	1				
Intestine	0.705**	0.565*	0.728**	0.769**	1			
Kidney	0.503*	0.475*	0.627**	0.524*	0.882**	1		
Brain	0.437	0.510*	0.633**	0.366	0.798**	0.913**	1	
Bone	0.393	0.483*	0.687**	0.512*	0.636**	0.663**	0.732**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.18 Correlation of Pb in tissues of *Scomber Scombrus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.983**	1						
Gills	0.946**	0.973**	1					
Liver	0.984**	0.995**	0.985**	1				
Intestine	0.970**	0.986**	0.987**	0.996**	1			
Kidney	0.972**	0.983**	0.982**	0.993**	0.997**	1		
Brain	-0.537*	-0.505*	-0.492*	-0.526*	-0.556*	-0.612**	1	
Bone	-0.711**	-0.647**	-0.628**	-0.673**	-0.686**	-0.735**	0.948**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

kidneystissues; muscles and gills, intestine and kidneys tissues; gills and liver, intestine and kidneys tissues; liver and intestine and kidneys tissues; intestine and kidneys tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation were between skin and brain and bone tissues; muscles and brain and bone tissues;liver and brain and bone tissues; intestine and brain and bone tissues; and kidneys and brain and bonetissues.

4.7 Lead Concentrations in Tissues of *Trachurus murphyi* Species

The results presented in Table 4.19revealed the concentrations of Pbin the tissues of *Trachurus murphyi* species across the batches of RS and EU fish species. The range of concentrations in batches of RS tissues were between 9.200 – 15.600mgkg^{-1}, 0.300 – 13.400mgkg^{-1}, 1.050 – 14.700mgkg^{-1}, 2.900 – 17.850mgkg^{-1}, 2.200 – 18.150mgkg^{-1}, 4.200 – 17.300mgkg^{-1}, 3.900 – 15.350mgkg^{-1} and 3.450 – 15.250mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While for EU fish species,the concentration rangedbetween 14.900 – 26.400mgkg^{-1}, 15.800 – 19.600mgkg^{-1}, 16.800 – 18.850mgkg^{-1}, 16.550 – 22.600mgkg^{-1}, 16.500 – 20.450mgkg^{-1}, 16.350 – 20.750mgkg^{-1}, 13.000 – 20.250mgkg^{-1} and 13.350 – 20.30 mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Pb statistical analysis for *Trachurus murphyi* species showed significance differences ($p<0.05$) was not established in any of the batches of both RS and EU fish species. Hence, non-significant difference ($p<0.05$) was found dominantly across the entire batches of both fishing origins.

The results of Pb correlation analysis in tissues of RS fish species (in Table 4.20) for *Trachurus murphyi* showedsignificant ($p<0.05$) positive correlation were recorded across the

entire tissues. Hence significance ($p<0.05$) negative correlation were not shown. While the results

Table 4.19 Pb concentration in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	9.400^{a}	7.250^{ab}	9.200^{ab}	10.600^{c}	12.450^{d}	15.600^{e}	10.750±0.015	0.400
Skin(EU)	14.900^{a}	19.950^{b}	19.700^{a}	18.500^{bc}	21.900^{bc}	26.400^{d}	20.225±0.035	
Muscles(RS)	0.300^{a}	1.200^{b}	9.250^{c}	8.900^{c}	12.200^{d}	13.400^{e}	7.542±0.005	
Muscles(EU)	16.150^{a}	15.800^{b}	19.400^{a}	18.250^{bc}	19.500^{bc}	19.600^{d}	18.117±0.020	
Gills(RS)	1.050^{a}	4.900^{b}	8.050^{c}	10.050^{d}	12.250^{e}	14.700^{f}	8.500±0.025	
Gills(EU)	16.800^{a}	17.600^{b}	17.250^{a}	17.050^{b}	18.850^{c}	17.300^{d}	17.475±0.010	
Liver(RS)	2.900^{a}	6.500^{b}	8.700^{c}	11.900^{d}	11.300^{d}	17.850^{e}	9.858±0.005	
Liver(EU)	18.050^{a}	17.600^{ab}	19.900^{c}	16.550^{abd}	22.600^{de}	16.500^{e}	18.533±0.045	
Intestine(RS)	2.200^{a}	6.900^{b}	7.950^{c}	12.750^{d}	13.150^{e}	18.150^{f}	10.183±0.040	
Intestine(EU)	16.500^{a}	19.350^{b}	20.450^{ab}	18.100^{ac}	19.000^{cd}	18.200^{d}	18.600±0.040	
Kidneys(RS)	4.200^{a}	6.950^{b}	9.650^{c}	12.650^{d}	14.200^{e}	17.300^{f}	10.825±0.020	
Kidneys(EU)	19.050^{a}	20.750^{ab}	19.100^{a}	17.300^{a}	19.450^{b}	16.350^{b}	18.667±0.030	
Brain(RS)	3.900^{a}	5.900^{b}	8.450^{c}	10.400^{d}	12.100^{e}	15.350^{f}	9.350±0.010	
Brain(EU)	19.500^{a}	17.750^{ab}	18.400^{bc}	17.000^{ac}	20.250^{a}	13.000^{d}	17.650±0.015	
Bone(RS)	3.450^{a}	6.800^{b}	7.550^{c}	11.200^{d}	14.100^{e}	15.250^{f}	9.725±0.030	
Bone(EU)	17.600^{a}	17.150^{ab}	17.650^{ab}	17.150^{c}	20.300^{e}	13.350^{f}	17.200±0.005	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.20 Correlation of Pb in tissues of *Trachurus murphyi* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.809**	1						
Gills	0.818**	0.956**	1					
Liver	0.852**	0.885**	0.962**	1				
Intestine	0.837**	0.887**	0.978**	0.988**	1			
Kidney	0.864**	0.943**	0.994**	0.975**	0.988**	1		
Brain	0.891**	0.943**	0.988**	0.978**	0.983**	0.996**	1	
Bone	0.837**	0.907**	0.979**	0.936**	0.974**	0.983**	0.974**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

presented in Table 4.21 for EU tissues showed significant ($p<0.05$) positive correlation between skin and muscles tissues; gills and liver and brain tissues; liver and kidneys, brain and bone tissues; intestine and brain tissues; kidneys and brain and bone tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlations wererecorded between skin and liver, brain and bone tissues; and muscles and kidney tissues.

4.8 Mercury Concentrations in Tissues of *Trachurus trachurus* Species

The results presented in Table 4.22 showed the concentrations of Hgin tissues of *Trachurus trachurus* species across the batches of RS and EU fish species. The range of Hg concentrations in the batches of RS fish species were between 53.300 – 97.500mgkg^{-1}, 51.150 – 98.900mgkg^{-1}, 52.100 – 95.600mgkg^{-1}, 48.150 – 93.700mgkg^{-1}, 51.700 – 96.400mgkg^{-1}, 1.550 – 100.200mgkg^{-1}, 60.550 – 99.100mgkg^{-1} and 55.500 – 95.300mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While the concentrations range in batches of EU species were between 56.050 – 85.450mgkg^{-1}, 54.250 – 79.700mgkg^{-1}, 60.050– 83.000 mgkg^{-1}, 51.500 – 82.950 mgkg^{-1}, 63.000 – 83.150 mgkg^{-1}, 56.550 – 83.050 mgkg^{-1}, 56.20 – 73.50 mgkg^{-1} and 59.800 – 83.850 mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The ANOVA results of Hg for *Trachurus trachurus* species showed significant differences ($p<0.05$) was not recorded in any of the batches of both RS and EU fish species. Hence, non-significant difference ($p<0.05$) remain dominantly across the entire batches of both fishing origins.

The results of Hg correlation analysis in tissues of RS fish species (Table 4.23) for *Trachurus trachurus* showed significant ($p<0.01$) positive correlationwere found across theentire tissues. Hence, significant ($p<0.05$) negative correlation were not recorded. While the

Table 4.21 Correlation of Pb in tissues of *Trachurus murphyi* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.656**	1						
Gills	0.404	0.366	1					
Liver	-0.005	0.354	0.776**	1				
Intestine	0.356	0.400	0.393	0.398	1			
Kidney	-0.446	-0.551*	0.340	0.488*	0.328	1		
Brain	-0.674**	-0.177	0.333	0.733**	0.003	0.628**	1	
Bone	-0.491*	-0.079	0.560*	0.803**	0.185	0.661**	0.941**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.22 Hg concentration in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	84.900^{a}	97.500^{b}	94.350^{c}	51.450^{a}	53.300^{bc}	54.950^{bc}	72.742±0.010	0.500
Skin(EU)	56.050^{a}	60.450^{b}	63.100^{c}	79.500^{a}	85.450^{bc}	63.450^{bc}	68.000±0.015	
Muscles(RS)	90.600^{a}	94.450^{b}	98.900^{c}	51.150^{a}	60.900^{bc}	56.650^{bc}	75.442±0.010	
Muscles(EU)	54.250^{a}	57.850^{b}	69.400^{c}	76.100^{a}	79.700^{b}	65.750^{bc}	67.175±0.020	
Gills(RS)	90.400^{a}	93.600^{b}	95.600^{c}	52.100^{a}	56.650^{b}	58.250^{c}	74.433±0.025	
Gills(EU)	60.050^{a}	66.550^{b}	67.950^{c}	82.900^{a}	83.000^{b}	65.750^{c}	71.033±0.005	
Liver(RS)	89.550^{a}	90.100^{b}	93.700^{c}	48.150^{a}	55.800^{b}	58.900^{c}	72.700±0.010	
Liver(EU)	51.500^{a}	68.250^{b}	71.750^{c}	79.950^{a}	82.950^{b}	59.600^{c}	69.000±0.025	
Intestine(RS)	90.600^{a}	89.650^{b}	96.400^{c}	51.700^{ab}	56.150^{ab}	59.550^{c}	74.008±0.010	
Intestine(EU)	63.400^{a}	63.400^{b}	71.250^{c}	83.150^{ab}	80.800^{ab}	63.000^{c}	70.833±0.030	
Kidneys(RS)	95.550^{a}	100.200^{b}	88.050^{c}	1.550^{ac}	55.500^{ab}	71.400^{bc}	68.708±0.015	
Kidneys(EU)	56.550^{a}	67.900^{b}	75.550^{c}	82.600^{ac}	69.900^{ab}	83.050^{bc}	72.592±0.010	
Brain(RS)	92.650^{a}	99.100^{b}	95.500^{ac}	60.700^{bc}	60.750^{b}	60.550^{a}	78.208±0.005	
Brain(EU)	56.200^{a}	68.450^{b}	73.500^{ac}	84.700^{bc}	64.100^{b}	72.700^{a}	69.942±0.015	
Bone(RS)	88.000^{a}	94.400^{b}	95.300^{c}	55.500^{a}	56.450^{bc}	56.200^{bc}	74.308±0.035	
Bone(EU)	59.800^{a}	66.550^{b}	72.800^{c}	83.850^{a}	69.000^{bc}	68.950^{bc}	70.158±0.020	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 4.23 Correlation of Hg in tissues of *Trachurus trachurus*from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.983**	1						
Gills	0.990**	0.994**	1					
Liver	0.977**	0.991**	0.996**	1				
Intestine	0.976**	0.991**	0.996**	0.997**	1			
Kidney	0.788**	0.819**	0.821**	0.858**	0.823**	1		
Brain	0.994**	0.982**	0.991**	0.978**	0.978**	0.770**	1	
Bone	0.995**	0.990**	0.995**	0.983**	0.986**	0.770**	0.997**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

correlation results (in Table 4.24) for EU tissues showed significant ($p<0.05$) positive correlationwere recordedbetween skin and muscles, gills, liver, intestine and bone tissues; muscles and gills, liver, intestine, kidneys, brain and bone tissues; gills and liver, intestine, kidneys, brain and bone tissues; liver and intestine, kidneys, brain and bonetissues; intestine and brain and bone tissues; kidneys and brain and bone tissues; and brainand bone tissues. Hence, significant ($p<0.05$) negative correlation were not shown.

4.9 Mercury Concentrations in Tissues of *Scomber scombrus* Species

The results presented in Table 4.25showed the concentrations of Hg in tissues of *Scomber scombrus* species across the batches of RS and EU fish origins. The concentrations of Hg inthe batches of RS fish species ranged between 57.800 – 92.150mgkg^{-1}, 58.400 – 91.500mgkg^{-1}, 57.250 – 96.650mgkg^{-1}, 56.20 – 86.950mgkg^{-1}, 55.250 – 92.050mgkg^{-1}, 54.70 – 90.150mgkg^{-1}, 57.100 – 92.800mgkg^{-1} 50.300 – 93.200mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While for EU batches, the concentrationsrangewere between 53.150 – 85.050 mgkg-1, 50.300 – 99.350 mgkg-1, 62.400 – 99.500 mgkg-1, 51.600 – 96.100 mgkg-1, 52.450 – 94.100 mgkg-1, 51.450 – 82.500 mgkg-1, 52.150 – 85.100 mgkg-1 and 56.200 – 83.200 mgkg-1 for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

Results of Hg statistical analysis for *Scomber scombrus* showed there is significant differences ($p<0.05$) in the batches ofbrain tissues of RS fish species. While for EU tissues, significant differences ($p<0.05$) was not shown with any of the batches. Hence, non-significance ($p<0.05$) was found dominantly across the entire batches of EU fish species.

The results of Hg correlation analysis for *Scomber scombrus*in the tissues of RS fish

Table 4.24 Correlation of Hg in tissues of *Trachurus trachurus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.922**	1						
Gills	0.979**	0.905**	1					
Liver	0.878**	0.860**	0.927**	1				
Intestine	0.920**	0.878**	0.951**	0.879**	1			
Kidney	0.398	0.624**	0.454	0.442	0.368	1		
Brain	0.380	0.537*	0.513*	0.530*	0.489*	0.890**	1	
Bone	0.604**	0.708**	0.723**	0.705**	0.741**	0.795**	0.945**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.25 Hg concentration in tissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	77.800^{a}	92.150^{b}	58.550^{bc}	57.800^{a}	67.450^{cd}	80.800^{b}	72.425±0.030	0.500
Skin(EU)	53.150^{a}	59.750^{ab}	53.250^{ab}	77.050^{d}	71.000^{d}	85.050^{e}	66.542±0.040	
Muscles(RS)	85.450^{a}	91.500^{b}	58.400^{ac}	62.750^{abd}	70.100^{bce}	85.950^{de}	75.692±0.054	
Muscles(EU)	50.300^{a}	99.350^{b}	57.550^{bc}	74.250^{cd}	74.800^{de}	84.150^{be}	73.400±0.015	
Gills(RS)	84.650^{a}	96.650^{b}	57.250^{ac}	64.150^{ab}	65.950^{cd}	86.900^{bd}	75.925±0.005	
Gills(EU)	93.150^{a}	99.500^{b}	62.400^{ac}	65.950^{bd}	71.150^{ac}	90.000^{d}	80.358±0.005	
Liver(RS)	86.950^{a}	56.200^{ab}	57.950^{ac}	69.600^{ab}	70.050^{b}	84.650^{c}	70.900±0.010	
Liver(EU)	96.100^{a}	51.600^{ab}	65.350^{bc}	73.250^{d}	73.100^{ce}	84.350^{ae}	73.958±0.020	
Intestine(RS)	92.050^{a}	55.250^{ab}	56.200^{bc}	66.100^{cd}	70.200^{d}	88.250^{a}	71.342±0.010	
Intestine(EU)	52.450^{a}	94.100^{b}	68.950^{bc}	71.650^{cd}	79.700^{d}	88.400^{b}	75.875±0.025	
Kidneys(RS)	90.150^{a}	54.700^{ab}	57.200^{bc}	69.600^{cd}	75.950^{d}	85.150^{a}	72.125±0.015	
Kidneys(EU)	54.700^{a}	51.450^{a}	64.950^{b}	65.850^{bc}	80.150^{cd}	82.500^{d}	66.600±0.030	
Brain(RS)	92.800^{a}	62.250^{b}	57.100^{ac}	71.350^{cd}	79.700^{ad}	92.800^{e}	76.000±0.010	
Brain(EU)	54.650^{a}	52.150^{a}	69.450^{b}	74.300^{c}	76.250^{d}	85.100^{e}	68.650±0.020	
Bone(RS)	88.000^{a}	52.800^{b}	50.300^{ac}	71.050^{cd}	81.750^{ad}	93.200^{e}	72.850±0.030	
Bone(EU)	56.200^{a}	63.000^{b}	63.950^{c}	77.750^{d}	79.250^{e}	83.200^{f}	70.558±0.020	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

species are presented in Table 4.26. Significant ($p<0.05$) positive correlation were shown between skin and muscles and gills tissues; muscles and gills tissues; liver and intestine, kidneys, brain and bone tissues; intestine and kidneys, brain and bone tissues, kidneys and brain and bone tissues; and brain and bone tissues.Hence significant ($p<0.05$) negative correlation were not found among RS tissues.The correlation results presented in Table 4.27 for EU tissues showed significant ($p<0.05$) positive correlation betweenskin and muscles, intestine, kidneys, brain and bone tissues; muscles and intestinetissues; kidneys and brain and bone tissues; and brain and bone tissues. However,significant($p<0.05$) negative correlationswere shown between muscles and liver tissues; gills and brain tissues and liver and intestine tissues.

4.10 Mercury Concentrations in Tissues of *Trachurus murphyi* Species

The results presented in Table 4.28 showed Hg concentrations in tissues of *Trachurus murphyi* species across the batches of RS and EU fish species. The range of Hg concentrations across the batches of RS fish species were between 73.700 – 160.300mgkg^{-1}, 66.500 – 117.850mgkg^{-1}, 88.550 – 125.450mgkg^{-1}, 102.350 – 126.500mgkg^{-1}, 96.600 – 124.000mgkg^{-}, 91.500 –126.800 mgkg^{-1}, 63.400 – 137.100 mgkg^{-1} and 74.700 – 137.100 mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While the range in the batches of EU tissues were between 57.350 – 94.300 mgkg^{-1}, 66.050 – 92.850 mgkg-^{1}, 65.050 – 88.000 mgkg-1, 66.950 – 96.550 mgkg^{-1}, 80.250 – 99.800 mgkg-1, 62.950 – 85.450 mgkg^{-1}, 55.150 – 92.400 mgkg^{-1} and 53.000 – 92.750 mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

Results of Hg statistical analysis for *Trachurus murphyi*in tissues of RS fish species showed significant differences ($p<0.05$) was found in the batches of bone tissues only. While for

Table 4.26 Correlation of Hg in tissues of *Scomber scombrus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.909**	1						
Gills	0.974**	0.900**	1					
Liver	0.164	0.206	0.284	1				
Intestine	0.214	0.239	0.318	0.993**	1			
Kidney	0.069	0.113	0.150	0.958**	0.957**	1		
Brain	0.265	0.307	0.353	0.974**	0.976**	0.972**	1	
Bone	0.123	0.199	0.208	0.943**	0.931**	0.974**	0.978**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.27 Correlation of Hg in tissues of *Scomber scombrus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.471*	1						
Gills	-0.070	0.404	1					
Liver	0.156	-0.621**	0.106	1				
Intestine	0.487*	0.949**	0.272	-0.643**	1			
Kidney	0.747**	0.087	-0.372	0.248	0.282	1		
Brain	0.804**	0.066	-0.496*	0.215	0.242	0.945**	1	
Bone	0.941**	0.408	-0.317	0.032	0.498*	0.877**	0.906**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.28 Hg concentration in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	FAO/WHO(2003)
Skin(RS)	160.30^{a}	73.700^{ab}	101.55^{bc}	109.60^{cd}	113.50^{de}	120.65^{ae}	113.22±0.005	0.500
Skin(EU)	94.300^{a}	93.300^{b}	57.350^{ac}	82.200^{b}	85.800^{cbd}	86.400^{ad}	83.225±0.010	
Muscles(RS)	117.85^{a}	66.500^{ab}	103.15^{c}	99.550^{bd}	115.50^{e}	115.15^{ad}	102.95±0.020	
Muscles(EU)	92.850^{a}	88.450^{b}	66.050^{ac}	79.350^{cd}	81.700^{bd}	78.700^{ab}	81.183±0.005	
Gills(RS)	125.45^{a}	88.550^{ab}	102.95^{bc}	103.25^{cd}	110.40^{d}	112.65^{a}	107.21±0.005	
Gills(EU)	84.200^{a}	88.000^{b}	65.050^{ac}	71.450^{bd}	80.600^{ac}	83.000^{bd}	78.717±0.030	
Liver(RS)	126.50^{a}	114.85^{b}	102.35^{ac}	107.10^{b}	114.35^{c}	126.10^{a}	115.21±0.040	
Liver(EU)	89.400^{a}	96.550^{b}	66.950^{ac}	78.450^{cd}	88.700^{e}	79.150^{ad}	83.200±0.035	
Intestine(RS)	101.65^{a}	110.90^{ab}	96.60^{abc}	110.95^{cd}	115.55^{de}	124.00^{e}	109.94±0.045	
Intestine(EU)	86.650^{a}	99.800^{b}	81.100^{bc}	87.100^{ad}	80.250^{ad}	84.000^{bc}	86.483±0.025	
Kidneys(RS)	91.500^{a}	111.15^{b}	102.10^{b}	113.50^{c}	112.05^{c}	126.80^{d}	109.51±0.035	
Kidneys(EU)	85.450^{a}	62.950^{ab}	68.950^{bc}	84.050^{d}	84.400^{e}	83.250^{ac}	78.175±0.015	
Brain(RS)	63.400^{a}	99.550^{b}	101.65^{c}	105.50^{d}	118.35^{e}	137.10^{f}	104.26±0.010	
Brain(EU)	86.450^{a}	55.150^{ab}	80.650^{bc}	85.150^{ac}	92.400^{d}	87.550^{d}	81.225±0.015	
Bone(RS)	74.700^{a}	95.150^{b}	99.800^{c}	116.90^{d}	116.15^{d}	137.10^{e}	106.63±0.020	
Bone(EU)	87.650^{a}	53.000^{ab}	75.500^{bc}	92.750^{ad}	88.250^{e}	81.450^{cd}	79.767±0.030	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

EU tissues, significant differences ($p<0.05$) were not found in any of the batches. Hence, non-significant differences ($p<0.05$) remain dominantly among the batches of EU fish species.

The results ofHg correlation analysis for *Trachurus murphyi*in tissues of RS fish species are presented in Table 4.29. Significant ($p<0.01$) positive correlations were recorded between skin and muscles, gills and liver tissues; muscles and gills tissues; gills and liver tissues; intestine and kidneys, brain and bone tissues; kidneys and brain and bone tissues; and brain and bone tissues. Hence significant ($p<0.05$) negative correlation was not recorded among RS tissues.The results of Hg correlation analysisin Table 4.30 for EU tissues showedsignificant ($p<0.05$) positive correlation between skin and muscles, gills, liver and intestine tissues; muscles and gills, liver and intestine tissues; gills and intestine tissues; liver and kidneys, brain and bone tissues; kidneys and brain and bone tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlationwere shown between intestine, brain and bonetissues.

4.11 Iron Concentrations in Tissues of *Trachurus trachurus* Species

The results presented in Table 4.31 revealedthe concentrations of Fe in tissues/organsof *Trachurus trachurus* species across the batches of RS and EU fish origins. The Fe concentrations in batches of RS fish species ranged between 7.900 – 68.250 $mgkg^{-1}$, 2.950 – 79.050 $mgkg^{-1}$, 227.150 – 298.400 $mgkg^{-1}$, 163.300 – 273.700 $mgkg^{-1}$, 42.450 – 258.800 $mgkg^{-1}$, 36.800 – 250.200 $mgkg^{-1}$, 83.000 – 175.900 $mgkg^{-1}$and 49.700 – 156.850$mgkg^{-1}$ for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While the concentrations of Fe in batchesof EU tissues ranged between 40.950 – 443.000$mgkg^{-1}$, 25.200 – 183.550$mgkg^{-1}$, 175.000 – 305.600$mgkg^{-1}$, 356.500 – 1102.050$mgkg^{-1}$, 54.200 – 1026.150$mgkg^{-}$

[1], 64.700 – 258.000mgkg^{-1}, 69.700 – 200.400mgkg^{-1} and 71.750 – 869.700mgkg^{-1} for skin, muscles, gills, liver,

Table 4.29 Correlation of Hg in tissues of *Trachurus murphyi* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.823**	1						
Gills	0.981**	0.902**	1					
Liver	0.584*	0.348	0.593**	1				
Intestine	-0.123	0.082	-0.036	0.456	1			
Kidney	-0.424	-0.086	-0.330	0.078	0.882**	1		
Brain	-0.433	0.052	-0.292	-0.087	0.760**	0.936**	1	
Bone	-0.256	0.191	-0.138	-0.027	0.794**	0.939**	0.959**	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.30 Correlation of Hg in tissues of *Trachurus murphyi* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.944**	1						
Gills	0.909**	0.851**	1					
Liver	0 .572*	0.557*	0.387	1				
Intestine	0 .519*	0.555*	0.555*	-0.363	1			
Kidney	0.299	0.209	0.037	0.859**	-0.581*	1		
Brain	-0.156	-0.223	-0.321	0.666**	-0.902**	0.876**	1	
Bone	-0.055	-0.095	-0.359	0.671**	-0.729**	0.914**	0.923**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.31 Fe concentration in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	CCFAC (2011)
Skin(RS)	36.050^{a}	20.250^{b}	12.500^{b}	7.900^{a}	68.250^{c}	43.550^{c}	31.417±0.005	0.800
Skin(EU)	57.700^{a}	66.100^{b}	53.900^{c}	54.250^{d}	443.00^{ab}	40.950^{ab}	119.32±0.10	
Muscles(RS)	26.900^{a}	2.950^{a}	3.050^{ac}	79.050^{d}	52.000^{d}	47.950^{c}	35.317±0.020	
Muscles(EU)	45.250^{a}	50.800^{b}	101.50^{ac}	183.55^{bd}	25.200^{ac}	26.200^{bd}	72.08±0-.035	
Gills(RS)	265.65^{a}	148.50^{b}	174.65^{bc}	127.15^{ac}	132.00^{ab}	298.40^{a}	191.06±0.025	
Gills(EU)	225.55^{a}	175.00^{b}	203.10^{c}	181.05^{c}	138.05^{a}	305.60^{d}	204.73±0.015	
Liver(RS)	270.15^{a}	183.80^{b}	163.30^{ac}	273.70^{d}	228.05^{ac}	261.85^{d}	230.14±0.035	
Liver(EU)	1000.5^{a}	1081.85^{b}	1102.05^{c}	910.70^{ac}	356.50^{ab}	565.95^{bc}	836.26±0.005	
Intestine(RS)	128.00^{a}	54.250^{b}	42.450^{ac}	258.85^{d}	63.400^{ce}	160.50^{de}	117.91±0.010	
Intestine(EU)	580.10^{a}	1028.15^{b}	799.85^{c}	205.00^{ac}	54.200^{ac}	96.750^{b}	460.68±0.045	
Kidneys(RS)	250.20^{a}	53.650^{b}	36.800^{c}	127.15^{d}	139.20^{e}	90.100^{ac}	116.18±0.035	
Kidneys(EU)	898.35^{a}	258.00^{b}	1156.3^{bc}	706.90^{d}	158.80^{ab}	64.750^{bc}	540.52±0.035	
Brain(RS)	142.75^{a}	66.950^{ab}	86.250^{c}	83.000^{ab}	143.20^{d}	175.90^{e}	116.34±0.005	
Brain(EU)	200.40^{a}	90.200^{b}	69.700^{ab}	92.000^{c}	110.20^{d}	74.250^{ab}	106.13±0.005	
Bone(RS)	58.800^{a}	63.750^{ab}	49.700^{ac}	79.850^{d}	156.80^{e}	134.95^{e}	90.642±0.015	
Bone(EU)	190.30^{a}	869.70^{b}	124.60^{c}	779.00^{bd}	133.25^{ad}	71.750^{a}	361.43±0.010	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

intestine, kidneys, brain and bone tissues respectively.

The results of Fe statistical analysis for *Trachurus trachurus*showed, significance differences ($p<0.05$) was not shown in any of the batches of both RS and EU fish species.

The results of Fe correlation analysis for *Trachurus trachurus* (as presented Table 4.32) showed the concentration in the tissues of RS fish species recorded significant ($p<0.05$) positive correlation between skin and brain and bone tissues; muscles and liver, intestine and bone tissues; gills and braintissues; liver and intestine, kidneys and bone tissues; and brain and bone tissues. Hence significant ($p<0.05$) negative correlation were not shown. While for EU tissues, the correlation results presented in Table 4.33 showed significant ($p<0.05$) positive correlation between skin and liver, intestine and bones tissues; muscles and kidneys tissues; liver and intestine and kidneystissues; and intestine and bone tissues. Hence significant ($p<0.05$) negative correlations were not shown in the EU tissues.

4.12 Iron Concentrations in Tissues of *Scomber scombrus* Species

The resultsin Table 4.34 revealed the concentrations of Fe in tissues of *Scomber scombrus*species across the batches of RS and EU fish origins. The range of Fe concentrations in batches of RS species were between 3.240 – 26.120 $mgkg^{-1}$, 6.040 – 18.80 $mgkg^{-1}$, 151.50 – 303.50 $mgkg^{-1}$, 94.080 – 806.800$mgkg^{-1}$, 41.560 – 822.320$mgkg^{-1}$, 40.720 – 104.000 $mgkg^{-1}$, 37.960 – 66.200 $mgkg^{-1}$ and 21.080 – 77.480 $mgkg^{-1}$ for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While the concentrations in batches of EU tissues ranged 8.040 – 23.800$mgkg^{-1}$, 3.440 – 59.160$mgkg^{-1}$, 184.600 – 282.920$mgkg^{-1}$, 49.600 – 308.440$mgkg^{-1}$, 20.720 – 65.840$mgkg^{-1}$, 68.120 – 541.520$mgkg^{-1}$, 29.960 – 57.280$mgkg^{-1}$ and

43.200 – 62.200mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

Table 4.32 Correlation of Fe in tissues of *Trachurus trachurus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.198	1						
Gills	0.184	-0.169	1					
Liver	0.234	0.789**	0.378	1				
Intestine	-0.279	0.818**	0.073	0.817**	1			
Kidney	0.356	0.346	0.414	0.727**	0.329	1		
Brain	0.761**	0.321	0.679**	0.560*	0.114	0.463	1	
Bone	0.827**	0.547*	-0.046	0.335	0.067	0.054	0.673**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.33 Correlation of Fe in tissues of *Trachurus trachurus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.258	1						
Gills	-0.369	-0.236	1					
Liver	0.842**	0.442	-0.021	1				
Intestine	0.863**	0.016	-0.163	0.849**	1			
Kidney	0.381	0.546*	-0.102	0.703**	0.421	1		
Brain	0.241	-0.241	-0.051	0.101	0.035	0.229	1	
Bone	0.756**	-0.220	-0.288	0.424	0.734**	-0.252	-0.044	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.34 Fe concentration in tissues of *Scomber scombrus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	CCFAC (2011)
Skin(RS)	14.160^{a}	23.760^{ab}	19.480^{c}	13.440^{b}	26.120^{d}	3.240^{a}	16.700±0.030	0.800
Skin(EU)	9.760^{a}	14.600^{b}	23.800^{c}	17.760^{cd}	21.400^{d}	8.040^{a}	15.893±0.020	
Muscles(RS)	6.040^{a}	16.280^{b}	18.800^{c}	7.320^{bd}	8.640^{bc}	11.64^{cd}	11.453±0.020	
Muscles(EU)	45.640^{a}	6.400^{b}	12.040^{bc}	4.560^{bc}	59.160^{ad}	3.440^{ad}	21.873±0.025	
Gills(RS)	280.00^{a}	151.5^{ab}	300.68^{c}	198.0^{ac}	303.5^{cd}	175.3^{bd}	234.81±0.025	
Gills(EU)	223.44^{a}	282.92^{b}	201.20^{bc}	251.84^{ad}	184.60^{ad}	207.48^{cb}	225.25±0.010	
Liver(RS)	293.92^{a}	237.76^{b}	806.8^{bc}	726.3^{ad}	96.80^{bd}	94.08^{ac}	375.93±0.025	
Liver(EU)	308.44^{a}	70.000^{b}	106.20^{c}	112.76^{d}	149.12^{e}	49.60^{a}	132.69±0.040	
Intestine(RS)	822.32^{a}	88.720^{b}	111.32^{c}	176.60^{d}	41.56^{ad}	162.8^{ad}	233.88±0.045	
Intestine(EU)	65.840^{a}	29.720^{b}	53.720^{bc}	62.640^{cd}	20.720^{ad}	27.560^{ab}	43.367±0.045	
Kidneys(RS)	48.960^{a}	95.040^{ab}	40.720^{ac}	104.00^{bd}	48.560^{bd}	87.400^{c}	70.780±0.010	
Kidneys(EU)	205.20^{a}	170.24^{b}	541.52^{c}	68.120^{ab}	91.840^{ab}	105.00^{c}	196.99±0.035	
Brain(RS)	57.280^{a}	54.760^{b}	66.200^{c}	72.880^{ad}	37.960^{ac}	56.400^{cd}	57.580±0.025	
Brain(EU)	47.920^{a}	57.280^{b}	43.520^{c}	29.960^{a}	55.920^{d}	31.560^{b}	44.360±0.040	
Bone(RS)	47.880^{a}	43.040^{b}	77.480^{ac}	70.880^{d}	21.080^{ad}	56.480^{cd}	52.807±0.015	
Bone(EU)	43.200^{a}	62.200^{b}	57.160^{c}	61.600^{d}	49.920^{b}	49.920^{b}	54.000±0.015	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

The results of Fe statistical analysis for *Scomber scombrus* showed, significance differences ($p<0.05$) was not shown in any of the batches of both RS and EU fish species.Hence, non-significant difference ($p<0.05$) remain dominantly across the batches ofRS and EU tissues.

The results of Fe correlation analysis presentedin Table 4.35 for *Scomber scombrus*showed the concentration in tissuesRS fish species recorded significant ($p<0.05$) positive correlation between skin and liver tissues; liver and brain and bones tissues; intestine and bone tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation were shown between skin and bone tissues; muscles and intestine tissues; gills and kidneys tissues; and liver and kidneys tissues.The correlation results presented in Table 4.36 for Fe concentration in tissues of EU fish species showed significant ($p<0.05$) positive correlation between muscles and liver and brain tissues; gills and bone tissues; and liver and intestine and kidneys tissues. However, the significant ($p<0.05$) negative correlation were shown between muscles and gills and bone tissues; and liver and bone tissues.

4.13 Iron Concentrations in Tissues of *Trachurus murphyi* Species

The results presented in Table 4.37 revealed the concentrations of Fe in tissues of *Trachurus murphyi* across the batches of RS and EU fish origins. The concentrations of Fe in batches of RS tissues ranged between 49.050 – 1103.600mgkg^{-1}, 38.050 – 60.250mgkg^{-1}, 374.150 – 595.60mgkg^{-1}, 636.700 – 775.40mgkg^{-1}, 112.850 – 242.250mgkg^{-1}, 380.40–1205.45mgkg^{-1}, 41.750 – 114.700mgkg^{-1} and 36.400 – 95.400mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.While the range of concentrations in batches of EU tissues were between 35.950 – 257.500mgkg^{-1}, 0.00 – 33.900mgkg^{-1}, 137.350 –

516.700mgkg^{-1},300.050 – 635.550mgkg^{-1}, 22.900 – 368.600mgkg^{-1}, 58.450 – 514.500mgkg^{-1}, 31.850 –

Table 4.35 Correlation of Fe in tissues of *Scomber scombrus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.261	1						
Gills	0.361	-0.124	1					
Liver	0.006	0.277	0.197	1				
Intestine	-0.313	-0.536*	0.240	-0.060	1			
Kidney	-0.342	-0.081	-0.929**	-0.031	-0.283	1		
Brain	-0.459	0.142	-0.262	0.823**	0.124	0.367	1	
Bone	-0.483*	0.337	-0.123	0.831**	0.010	0.175	0.941**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.36 Correlation of Fe in tissues of *Scomber scombrus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.147	1						
Gills	-0.246	-0.534*	1					
Liver	-0.167	0.703**	-0.179	1				
Intestine	-0.004	-0.103	0.179	0.557*	1			
Kidney	0.459	-0.130	-0.230	0.030	0.315	1		
Brain	0.244	0.571*	0.063	0.254	-0.384	0.117	1	
Bone	0.429	-0.682**	0.626**	-0.678**	-0.24	0.091	-0.089	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.37 Fe concentration in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	CCFAC (2011)
Skin(RS)	86.950^{a}	49.050^{ab}	75.250^{c}	58.350^{ab}	1103.6^{d}	117.15^{f}	248.39±0.020	0.800
Skin(EU)	36.200^{a}	37.650^{b}	47.550^{ac}	35.950^{b}	257.50^{cd}	25.800^{ad}	73.442±0.045	
Muscles(RS)	60.250^{a}	43.600^{b}	51.250^{bc}	42.950^{c}	54.400^{d}	38.050^{a}	48.417±0.040	
Muscles(EU)	22.250^{a}	10.900^{b}	0.000^{c}	17.000^{cd}	33.900^{d}	22.200^{d}	17.708±0.045	
Gills(RS)	374.15^{a}	595.60^{b}	379.35^{bc}	394.65^{cd}	521.15^{e}	408.10^{d}	445.50±0.030	
Gills(EU)	443.85^{a}	318.95^{b}	221.20^{bc}	137.35^{a}	516.70^{ad}	342.30^{d}	330.06±0.020	
Liver(RS)	641.40^{a}	655.60^{b}	775.40^{bc}	730.00^{d}	705.60^{d}	636.75^{ac}	690.79±0.025	
Liver(EU)	635.55^{a}	641.90^{b}	315.70^{bc}	300.05^{ad}	363.20^{ad}	334.65^{bc}	431.84±0.005	
Intestine(RS)	112.85^{a}	164.00^{b}	163.10^{bc}	242.25^{bd}	166.40^{d}	133.30^{bd}	163.65±0.010	
Intestine(EU)	70.650^{a}	84.650^{ab}	22.90^{ac}	368.60^{d}	79.150^{bd}	75.100^{cd}	116.84±0.020	
Kidneys(RS)	1205.45^{a}	1192.7^{b}	1108.1^{c}	380.40^{ac}	631.50^{cb}	390.20^{ab}	818.05±0.025	
Kidneys(EU)	325.15^{a}	320.85^{b}	58.45^{ab}	520.80^{a}	165.85^{bc}	514.50^{c}	317.60±0.005	
Brain(RS)	114.70^{a}	41.750^{ab}	79.800^{bc}	102.10^{d}	105.45^{e}	83.100^{ac}	87.817±0.010	
Brain(EU)	349.75^{a}	31.850^{ab}	324.10^{c}	43.650^{cd}	70.950^{cd}	33.650^{ab}	142.33±0.010	
Bone(RS)	58.000^{a}	36.400^{ab}	76.250^{b}	95.100^{bc}	68.050^{bd}	78.950^{cd}	68.792±0.015	
Bone(EU)	26.950^{a}	23.500^{ab}	28.900^{bc}	68.050^{d}	34.250^{c}	40.400^{c}	37.008±0.020	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

349.750mgkg^{-1} and 23.500 – 68.050mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Fe statistical analysis for *Trachurus murphyi* showed, significance differences ($p<0.05$) was not shown in any of the batches of both RS and EU fish species. Hence, non-significant difference ($p<0.05$) remain dominantly across the batches of RS and EU tissues.

The results of Fe correlation analysis for *Trachurus murphyi* in the tissues of RS fish species are presented in Table 4.38. Significant ($p<0.05$) positive correlationwere recordedbetween skin and brain tissues; muscles and kidneys and brain tissues; liverand intestine and bonetissues; intestine and bone tissues; and brain and bone tissues. However, the significant ($p<0.05$) negative correlation were between skin and intestine tissues; gills and brain and bone tissues; intestine and kidneys tissues; and kidneys and bone tissues. The correlation results presented in Table 4.39 for EU tissues showed significant ($p<0.05$) positive correlation between skin and muscles and gills tissues; muscles and gills tissues; intestine and kidneysand bonetissues; and kidneys and bonetissues. However, significant ($p<0.05$) negative correlation were recorded between gills and intestine and bone tissues; liver and bone tissues; intestine and brain tissues; and kidneys and brain tissues.

4.14 Nickel Concentrations in Tissues of *Trachurus trachurus* Species

The results presented in Table 4.40 showed the concentrations of Ni in tissues of *Trachurus trachurus* species across the batches of RS and EU fishing origins. The concentration in batches of RS tissues ranged between 6.60 – 10.80mgkg^{-1}, 6.750 – 12.250mgkg^{-1}, 7.30 – 11.050mgkg^{-1}, 7.700 – 12.600mgkg^{-1}, 7.650 – 13.450mgkg^{-1}, 8.350 – 13.450mgkg^{-1}, 7.200 – 13.050mgkg^{-1}

Table 4.38 Correlation of Fe in tissues of *Trachurus murphyi* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.085	1						
Gills	-0.283	-0.170	1					
Liver	-0.297	0.112	-0.242	1				
Intestine	-0.543*	-0.385	0.059	0.588*	1			
Kidney	-0.408	0.567*	0.182	-0.042	-0.478*	1		
Brain	0.461	0.545*	-0.626**	0.094	-0.011	-0.321	1	
Bone	0.253	-0.249	-0.699**	0.493*	0.516*	-0.746**	0.548*	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.39 Correlation of Fe in tissues of *Trachurus murphyi* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.638**	1						
Gills	0.635**	0.714**	1					
Liver	-0.207	0.010	0.399	1				
Intestine	-0.158	0.099	-0.580*	-0.319	1			
Kidney	-0.463	0.272	-0.280	-0.021	0.626**	1		
Brain	-0.186	-0.355	0.089	0.215	-0.425	-0.547*	1	
Bone	-0.029	0.198	-0.527*	-0.633**	0.911**	0.590**	-0.444	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.40 Ni concentration in tissues of *Trachurus trachurus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	WHO(2008)
Skin(RS)	7.450^{a}	7.100^{b}	6.600^{a}	9.450^{c}	9.150^{c}	10.800^{d}	8.425±0.015	0.200
Skin(EU)	15.200^{a}	13.850^{a}	17.950^{b}	19.400^{c}	22.600^{d}	21.300^{d}	18.383±0.010	
Muscles(RS)	6.750^{a}	6.800^{ab}	7.900^{bc}	9.800^{cd}	11.000^{de}	12.250^{e}	9.083±0.010	
Muscles(EU)	13.100^{a}	14.950^{b}	17.250^{c}	19.500^{d}	21.900^{e}	20.600^{e}	17.883±0.020	
Gills(RS)	8.000^{a}	7.750^{ab}	7.300^{ac}	8.900^{cd}	9.300^{de}	11.050^{e}	8.717±0.005	
Gills(EU)	14.000^{a}	13.350^{b}	18.250^{ac}	18.250^{c}	21.800^{d}	1.300^{a}	14.492±0.025	
Liver(RS)	7.700^{a}	8.650^{b}	9.050^{c}	8.450^{bd}	10.900^{de}	12.600^{e}	9.558±0.005	
Liver(EU)	13.250^{a}	15.850^{ab}	20.200^{bc}	20.000^{c}	23.100^{d}	3.700^{a}	16.017±0.005	
Intestine(RS)	7.550^{a}	11.050^{b}	7.650^{ab}	8.100^{bc}	11.800^{cd}	13.100^{d}	9.875±0.010	
Intestine(EU)	13.600^{a}	13.850^{a}	18.250^{b}	21.650^{c}	22.300^{d}	5.700^{e}	15.892±0.005	
Kidneys(RS)	8.350^{a}	8.700^{ab}	9.250^{bc}	9.650^{cd}	12.450^{de}	13.450^{e}	10.308±0.005	
Kidneys(EU)	14.350^{a}	15.950^{ab}	18.600^{bc}	20.900^{c}	22.350^{c}	4.950^{a}	16.183±0.010	
Brain(RS)	7.200^{a}	8.600^{b}	9.500^{c}	8.800^{bd}	10.750^{de}	13.050^{e}	9.650±0.020	
Brain(EU)	14.750^{a}	15.500^{ab}	18.550^{bc}	20.450^{cd}	22.200^{d}	4.000^{a}	15.908±0.010	
Bone(RS)	6.600^{a}	7.500^{b}	9.100^{c}	8.850^{c}	10.400^{d}	12.850^{e}	9.217±0.015	
Bone(EU)	12.550^{a}	16.050^{ab}	19.000^{bc}	21.450^{cd}	21.650^{d}	5.450^{a}	16.025±0.010	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

and 6.600 – 12.850mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, the concentrations in the batches of EU fish species ranged between 13.850 – 22.600mgkg^{-1}, 13.100 – 21.900mgkg^{-1}, 1.300 – 21.800mgkg^{-1}, 3.700 – 23.100mgkg^{-1}, 5.700 – 22.300mgkg^{-1}, 4.950 – 22.350mgkg^{-1}, 4.00 – 22.200mgkg^{-1} and 5.450 – 21.650mgkg^{-1} for the skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of statistical analysisfor *Trachurus trachurus* species showed, significant differences ($p<0.05$) were not recorded in any of the batches of both RS and EU fish species. Hence non-significant difference ($p<0.05$) were recorded across the entire batches of both RS and EU tissues.

The results of Ni correlation analysis for *Trachurus trachurus*in tissues of RS fish species are presented Table 4.41. Significant ($p<0.05$) positivecorrelation were recorded across the entire tissues. Hence significant ($p<0.05$) negative correlation was not shown in any of the tissues.While the correlation results for EU fish speciespresented in Table 4.42 showed significant ($p<0.05$) positive correlationbetween skin and muscles, gills, intestine and brain tissues; musclesand gills and brain tissues; gills and liver, intestine, kidneys, brain and bone tissues; liver and intestine, kidneys, brain and bone tissues; intestine and kidneys, brain and bone tissues; intestine and kidneys, brain and bone tissues; kidneys and brain and bone tissues; and brain and bone tissues. Hence significant ($p<0.05$) negative correlations were not recorded in any of the tissues.

4.15 Nickel Concentrations in Tissues of *Scomber scombrus* Species

The results of Niconcentrations across the batches of RS and EU tissues/organs of *Scomber scombrus* species are presented in Table 4.43. The ranges of concentrations recorded for RS fish

Table 4.41 Correlation of Ni in tissues of *Trachurus trachurus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.915**	1						
Gills	0.960**	0.903**	1					
Liver	0.734**	0.874**	0.840**	1				
Intestine	0.610**	0.648**	0.726**	0.841**	1			
Kidney	0.823**	0.947**	0.885**	0.961**	0.788**	1		
Brain	0.717**	0.872**	0.808**	0.969**	0.783**	0.923**	1	
Bone	0.782**	0.931**	0.843**	0.953**	0.706**	0.942**	0.976**	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.42 Correlation of Ni in tissues of *Trachurus trachurus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.951**	1						
Gills	0.010	0.024	1					
Liver	0.048	0.104	0.978**	1				
Intestine	0.176	0.215	0.966**	0.962**	1			
Kidney	-0.003	0.049	0.946**	0.937**	0.936**	1		
Brain	0.004	0.044	0.993**	0.980**	0.978**	0.960**	1	
Bone	0.075	0.163	0.957**	0.974**	0.981**	0.942**	0.978**	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.43 Ni concentration in tissues of *Scomber Scombrus* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	WHO(2008)
Skin(RS)	1.850^{a}	0.300^{b}	4.400^{c}	7.900^{d}	14.250^{e}	8.250^{e}	6.158±0.010	0.200
Skin(EU)	9.850^{a}	11.150^{b}	10.400^{c}	9.050^{ac}	7.450^{bc}	6.250^{ab}	9.025±0.010	
Muscles(RS)	1.350^{a}	2.300^{b}	4.400^{c}	8.000^{d}	11.200^{e}	9.000^{e}	6.042±0.005	
Muscles(EU)	11.200^{a}	11.800^{b}	9.250^{c}	8.100^{ab}	6.600^{ab}	6.700^{a}	8.942±0.05	
Gills(RS)	3.600^{a}	2.150^{a}	5.550^{b}	9.350^{c}	9.700^{d}	9.550^{d}	6.650±0.010	
Gills(EU)	10.650^{a}	12.050^{b}	11.150^{c}	7.900^{bc}	6.300^{ab}	6.350^{a}	9.067±0.015	
Liver(RS)	3.700^{a}	2.500^{a}	5.350^{b}	7.950^{c}	10.450^{d}	9.950^{d}	6.650±0.020	
Liver(EU)	11.550^{a}	11.150^{b}	9.500^{c}	6.100^{c}	5.750^{b}	4.600^{a}	8.108±0.015	
Intestine(RS)	3.750^{a}	3.050^{a}	5.400^{b}	8.700^{c}	10.100^{d}	9.800^{d}	6.800±0.020	
Intestine(EU)	10.400^{a}	11.550^{b}	9.950^{c}	7.750^{ab}	7.900^{b}	3.800^{a}	8.558±0.010	
Kidneys(RS)	1.150^{a}	3.450^{b}	6.750^{c}	11.400^{d}	9.100^{de}	10.250^{de}	7.017±0.005	
Kidneys(EU)	9.900^{a}	10.550^{ab}	10.750^{b}	6.350^{a}	7.350^{a}	7.000^{a}	8.650±0.005	
Brain(RS)	0.450^{a}	3.000^{b}	7.300^{c}	9.100^{d}	8.600^{d}	10.650^{e}	6.517±0.10	
Brain(EU)	10.000^{a}	10.150^{b}	8.450^{c}	6.650^{ab}	8.350^{c}	5.800^{a}	8.233±0.015	
Bone(RS)	2.300^{a}	2.550^{b}	7.150^{c}	9.850^{d}	8.250^{d}	10.500^{e}	6.767±0.010	
Bone(EU)	10.700^{a}	11.150^{b}	8.300^{c}	6.550^{ac}	7.400^{ac}	4.900^{ab}	8.167±0.010	

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

species were between0.300 – 14.250mgkg^{-1}, 1.350 – 11.200mgkg^{-1}, 2.150 – 9.700mgkg^{-1}, 2.500 – 10.450mgkg^{-1}, 3.050 – 10.100mgkg^{-1}, 1.150 – 11.400mgkg^{-1}, 0.450 – 10.650mgkg^{-1} and 2.300 – 10.500mgkg^{-1} forthe skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, concentration range for EU tissues were between 6.250 – 11.150mgkg^{-1}, 6.600 – 11.800mgkg^{-1}, 6.300 – 12.050mgkg^{-1}, 4.600 – 11.550mgkg^{-1}, 3.800 – 11.550mgkg^{-1}, 6.350 – 10.750mgkg^{-1}, 6.650 – 10.150mgkg^{-1} and 4.900 – 11.150mgkg^{-1} forthe skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of Ni statistical analysis for *Scomber scombrus* species showed that, significant differences ($p<0.05$) were recorded in the batches of RS fish species and these includes the batches of skin, muscles, gills, liver and bones tissues. While for EU tissues, significant differences ($p<0.05$) was not shown with any of the batches.

The result of Ni correlations analysis for *Scomber scombrus* in the tissues of RS fish species are presented in Table 4.44. Significant ($p<0.05$) positive correlation were recordedacross the entire tissues. Hence significant ($p<0.05$) negative correlation were not recorded in any of the tissues.Similarly the correlations results for EU tissues are presented in Table 4.45 which showedsignificant ($p<0.05$) positive correlation were shown across the entire tissues. Hence significant ($p<0.05$) negative correlation were not recorded in any of the tissues.

4.16 Nickel Concentrations in Tissues of *Trachurus murphyi* Species

The results of Niconcentrations across the batches of RS and EU tissues/organs of *Trachurus murphyi* species are presented in Table 4.46. The ranges of concentrations recorded for RS species were between 0.250 – 2.300mgkg^{-1}, 0.300 – 1.150mgkg^{-1}, 0.500 – 2.100mgkg^{-1}, 0.300 – 2.30 mgkg^{-1}, 0.150 – 4.600mgkg^{-1}, 0.250 – 6.750mgkg^{-1}, 0.550 – 2.650mgkg^{-1},

Table 4.44 Correlation of Ni in tissues of *Scomber scombrus* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.961**	1						
Gills	0.907**	0.949**	1					
Liver	0.942**	0.970**	0.972**	1				
Intestine	0.922**	0.963**	0.983**	0.979**	1			
Kidney	0.751**	0.871**	0.909**	0.840**	0.910**	1		
Brain	0.755**	0.879**	0.895**	0.867**	0.929**	0.973**	1	
Bone	0.756**	0.865**	0.938**	0.884**	0.943**	0.974**	0.978**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.45 Correlation of Ni in tissues of *Scomber scombrus* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.882**	1						
Gills	0.947**	0.932**	1					
Liver	0.883**	0.958**	0.942**	1				
Intestine	0.935**	0.847**	0.869**	0.900**	1			
Kidney	0.582*	0.596**	0.674**	0.654**	0.594**	1		
Brain	0.760**	0.824**	0.769**	0.897**	0.914**	0.590**	1	
Bone	0.842**	0.920**	0.850**	0.950**	0.933**	0.606**	0.972**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.46 Ni concentration in tissues of *Trachurus murphyi* ($mgkg^{-1}$) wet weight

Tissue	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean±SD	WHO(2008)
Skin(RS)	0.950^{a}	0.250^{b}	1.050^{c}	0.300^{d}	1.250^{e}	2.300^{f}	1.017±0.005	0.200
Skin(EU)	3.400^{a}	4.450^{b}	4.200^{ac}	3.500^{c}	1.750^{b}	1.200^{d}	3.083±0.010	
Muscles(RS)	0.950^{a}	0.300^{b}	0.150^{c}	1.200^{d}	1.150^{e}	0.900^{f}	0.775±0.005	
Muscles(EU)	3.850^{a}	4.100^{b}	3.550^{ac}	3.500^{bc}	0.350^{d}	2.600^{b}	2.992±0.015	
Gills(RS)	0.900^{a}	0.500^{b}	1.020^{c}	2.100^{d}	0.850^{e}	0.700^{f}	1.012±0.020	
Gills(EU)	4.300^{a}	3.100^{b}	5.550^{bc}	3.150^{ac}	0.150^{c}	1.400^{d}	2.942±0.005	
Liver(RS)	1.350^{a}	0.400^{b}	1.200^{c}	2.300^{d}	0.300^{e}	1.450^{f}	1.167±0.010	
Liver(EU)	3.550^{a}	4.000^{b}	4.050^{c}	2.400^{a}	1.150^{bc}	0.500^{d}	2.608±0.025	
Intestine(RS)	2.750^{a}	0.150^{b}	2.750^{a}	4.600^{c}	1.150^{d}	2.100^{e}	2.250±0.010	
Intestine(EU)	2.450^{a}	3.850^{ab}	3.600^{bc}	0.500^{d}	2.100^{e}	2.500^{b}	2.500±0.010	
Kidneys(RS)	5.300^{a}	6.450^{b}	6.750^{c}	1.350^{a}	2.550^{cb}	0.250^{c}	3.775±0.010	
Kidneys(EU)	4.200^{a}	4.850^{b}	6.400^{abc}	2.200^{c}	4.250^{c}	3.350^{b}	4.208±0.005	
Brain(RS)	0.550^{a}	0.750^{b}	0.850^{c}	1.750^{d}	0.650^{e}	2.650^{d}	1.200±0.010	
Brain(EU)	5.000^{a}	4.150^{b}	4.200^{bc}	1.600^{a}	2.650^{b}	3.750^{c}	3.558±0.005	
Bone(RS)	0.100^{a}	0.400^{b}	0.300^{c}	0.000^{d}	0.150^{e}	1.150^{f}	0.350±0.020	
Bone(EU)	4.300^{a}	4.650^{b}	4.400^{c}	0.650^{ac}	1.300^{b}	2.300^{c}	2.933±0.015	

Values in each row marked by the same superscript letter are not significantly different at P<

and 0.000 – 1.150mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively. While, the concentration range in batches of EU fish species were between 1.200 – 4.450mgkg^{-1}, 0.350 – 4.100mgkg^{-1}, 0.150 – 5.550mgkg^{-1}, 0.500 – 4.050mgkg^{-1}, 0.500 – 3.850mgkg^{-1}, 2.200 – 6.400mgkg^{-1}, 1.600 – 5.000mgkg^{-1} and 0.650 – 4.650mgkg^{-1} for skin, muscles, gills, liver, intestine, kidneys, brain and bone tissues respectively.

The results of statistical analysis for *Trachurus murphyi* species showed significant differences ($p<0.05$) were recorded across the entire batches of both RS and EU fish origins.

The result of Ni correlation analysis for *Trachurus murphyi* in tissues of RS tissues are presented in Table 4.47. Significant ($p<0.05$) positive correlation were between skin and brain and bone tissues; gills and liver and intestine tissues; liver and intestine tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation were between skin and liver and kidneys tissues; muscles and kidneys tissues; and kidneys and brain tissues. While thecorrelationanalysis for EU tissues in Table4.48 showed significant ($p<0.05$) positive correlation between skin and muscles, gills, liver and bone tissues; muscles and gills, liver and bone tissues; gills and liver, kidneys and bone tissues; liver and intestine kidneys, brain and bone tissues; intestine and kidneys, brain and bone tissues; kidneys and brain and bone tissues; and brain and bone tissues. However, significant ($p<0.05$) negative correlation were not recorded in the EU tissues.

4.17 Profiles of Heavy MetalinTissues of Fish Species

The statistics of heavy metals; Cd, Pb, Hg, Fe and Ni concentrations in the tissues/organs

(skin, muscle, gills, liver, intestine, kidneys, brain and bones) of *Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi* across Russia (RS) and Europe (EU) fishing zonesare summarized in Figure 4.1 to 4.3.

Table 4.47 Correlation of Ni in tissues of *Trachurus murphyi* from RS fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.215	1						
Gills	-0.374	0.435	1					
Liver	-0.030	0.369	0.785**	1				
Intestine	-0.275	0.372	0.906**	0.848**	1			
Kidney	-0.483*	-0.784**	-0.343	-0.419	-0.182	1		
Brain	0.534*	0.302	0.219	0.545*	0.121	-0.781**	1	
Bone	0.699**	-0.197	-0.452	-0.005	-0.467	-0.338	0.714**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4.48 Correlation of Ni in tissues of *Trachurus murphyi* from EU fishing zone

Parameter	Skin	Muscles	Gills	Liver	Intestine	Kidney	Brain	Bone
Skin	1							
Muscles	0.745**	1						
Gills	0.791**	0.796**	1					
Liver	0.949**	0.722**	0.839**	1				
Intestine	0.348	0.245	0.298	0.469*	1			
Kidney	0.386	0.078	0.478*	0.541*	0.782**	1		
Brain	0.242	0.421	0.462	0.473*	0.741**	0.602**	1	
Bone	0.583*	0.599**	0.629**	0.740**	0.842**	0.697**	0.897**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

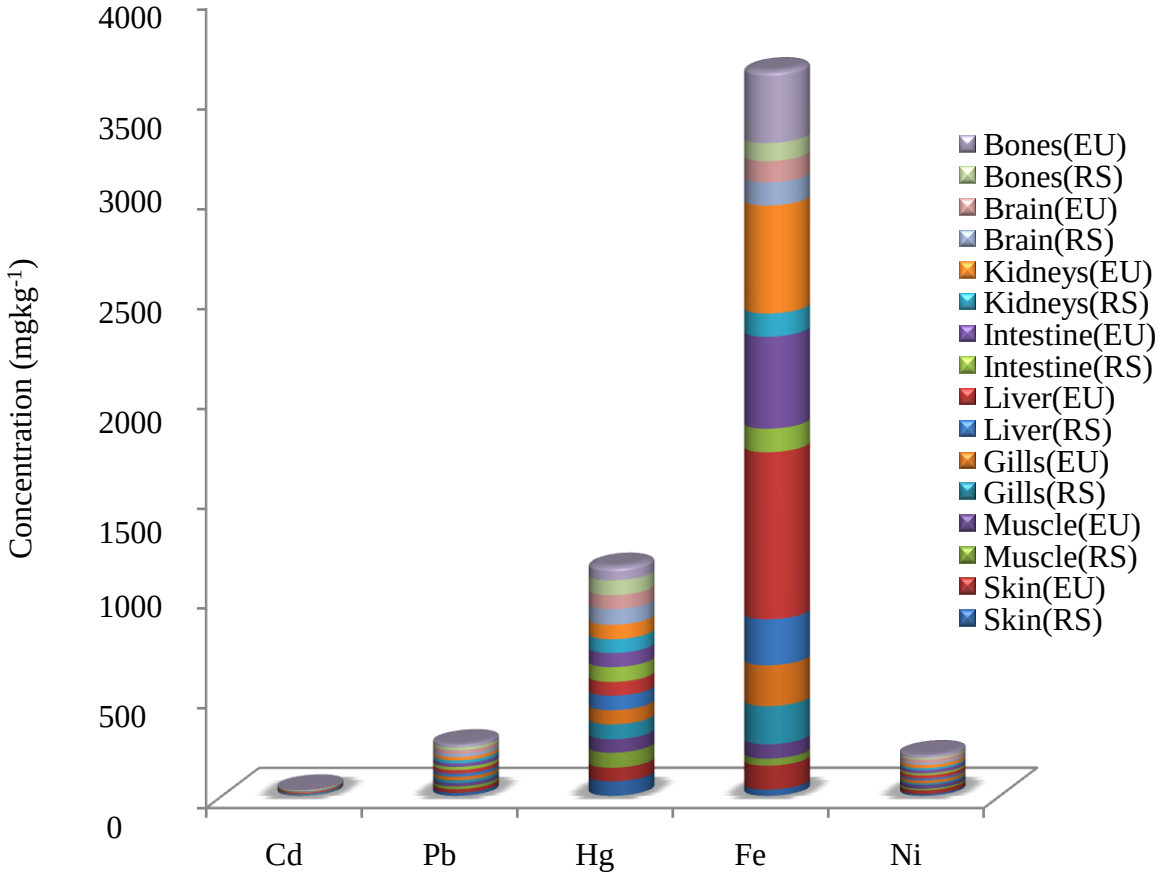


Figure 4.1 Mean concentrations of metals in*Trachurus trachurus* species

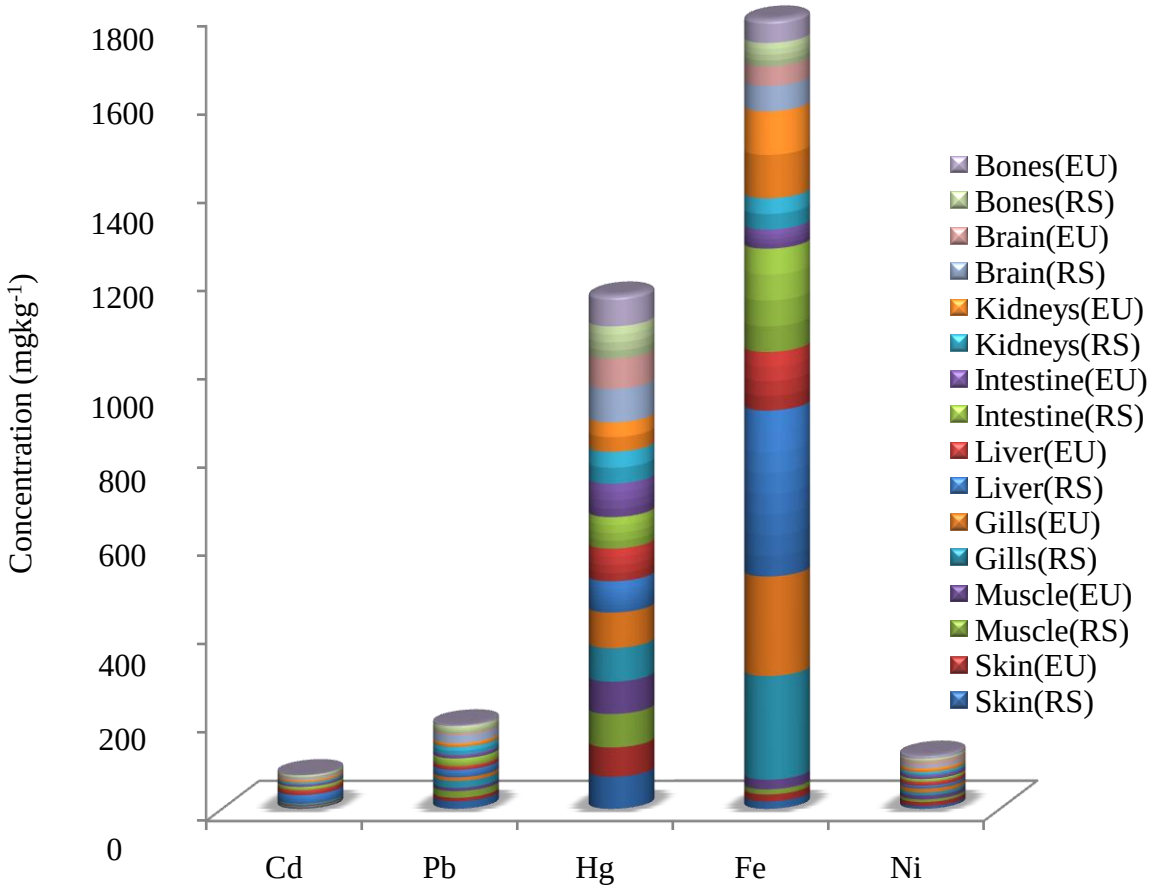


Figure 4.2 Mean concentrations of metals in *Scomber scombrus* species

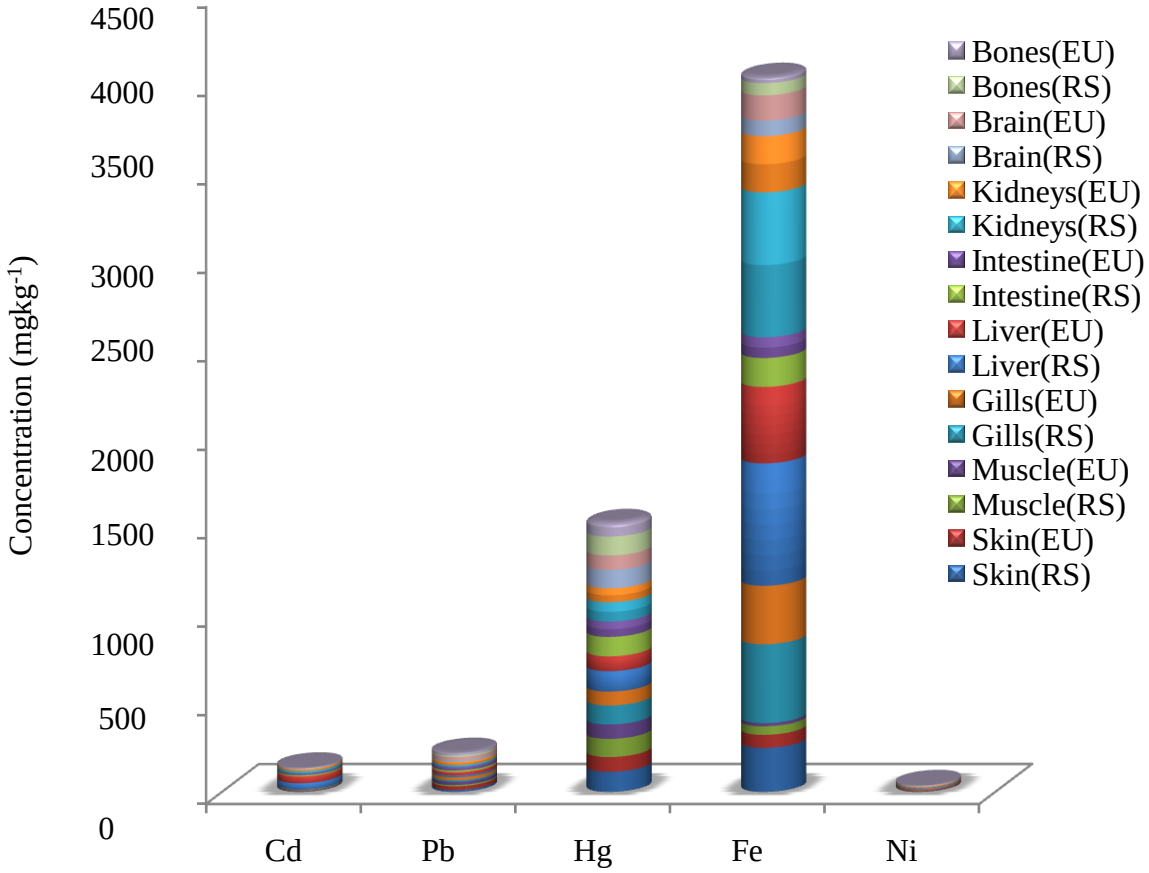


Figure 4.3 Mean concentrations of metals in*Trachurus murphyi* species

4.18 Comparative Mean Concentration of Cd in Fish Species

The graphical representation in Figure 4.4 showed the accumulation of Cd in various tissues of *Trachurus murphyi*,*Scomber scombrus* and *Trachurus trachurus*. The graph showed*Trachurus murphyi* presents higher level of accumulations, followed by *Scomber scombrus* and*Trachurus trachurus* species with the lowest levels. However, the variation of tissues accumulation between fishing origins presents there is no significant differencesbetween RS and EU tissues with respects to each of the species. Although, thetissues accumulation for each of the species showed highest concentrations of Cdwas shownin the liver tissues, followed by the kidney tissues and then the intestine tissues. While the skin, muscles, brain and bone tissues showed comparable concentrations.

4.19 Comparative Mean Concentration of Pb in Fish Species

Graphical representation of Pb accumulations in various tissues of fish species are presented in Figure 4.5. The graph showed a comparable level of Pb accumulation between the fish species; *Trachurus murphyi*, *Scomber scombrus* and *Trachurus trachurus*.However, the tissues accumulations with respects to fishing origins revealed*Trachurus murphyi* species showed EU fish species accumulate highly than the RS species. While in the case of *Scomber scombrus* species, higher accumulations were shown with the tissues of RS fish origins. And the bars for*Trachurus trachurus* species showed higher accumulation are presented with the tissues of EU fish origins.

4.20 Comparative Mean Concentration of Hg in Fish Species

The graphical representation of Hg concentration (as presented in Figure 4.6)

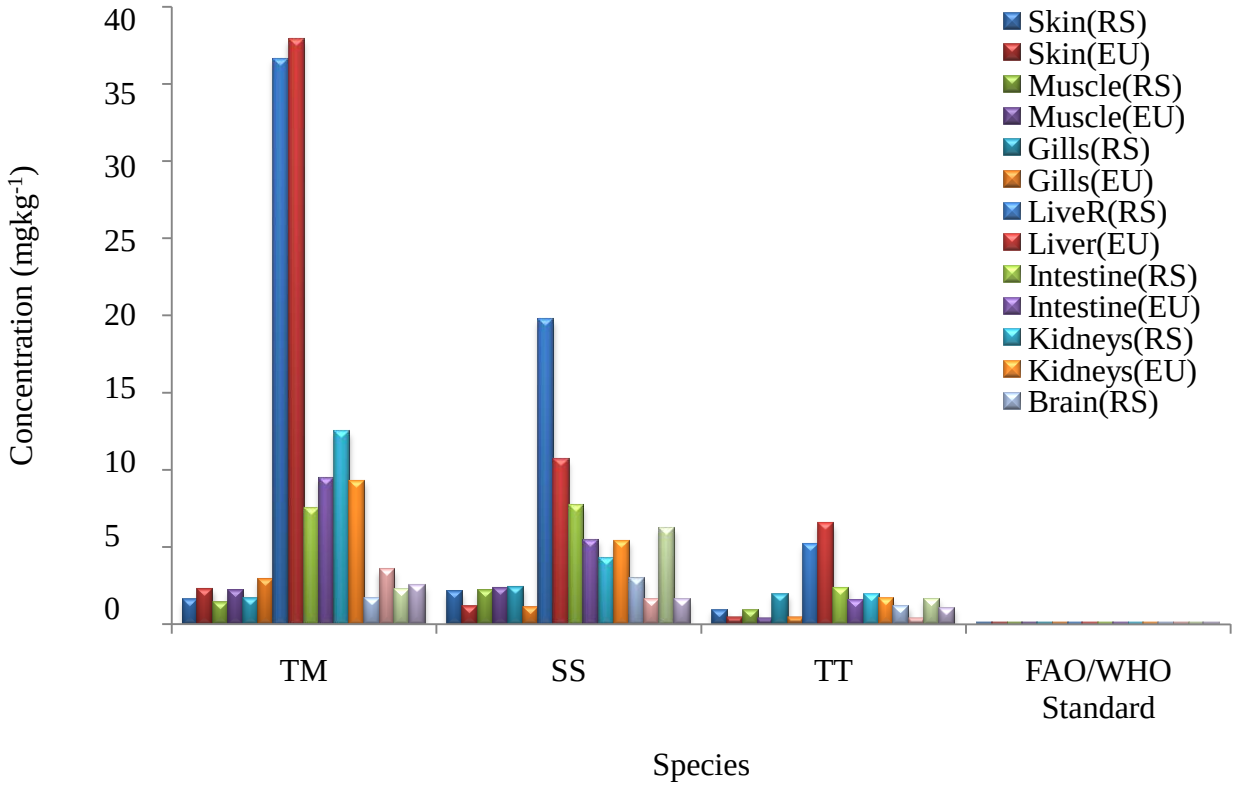


Figure 4.4 Comparative mean concentration for Cd in the tissues of fish species

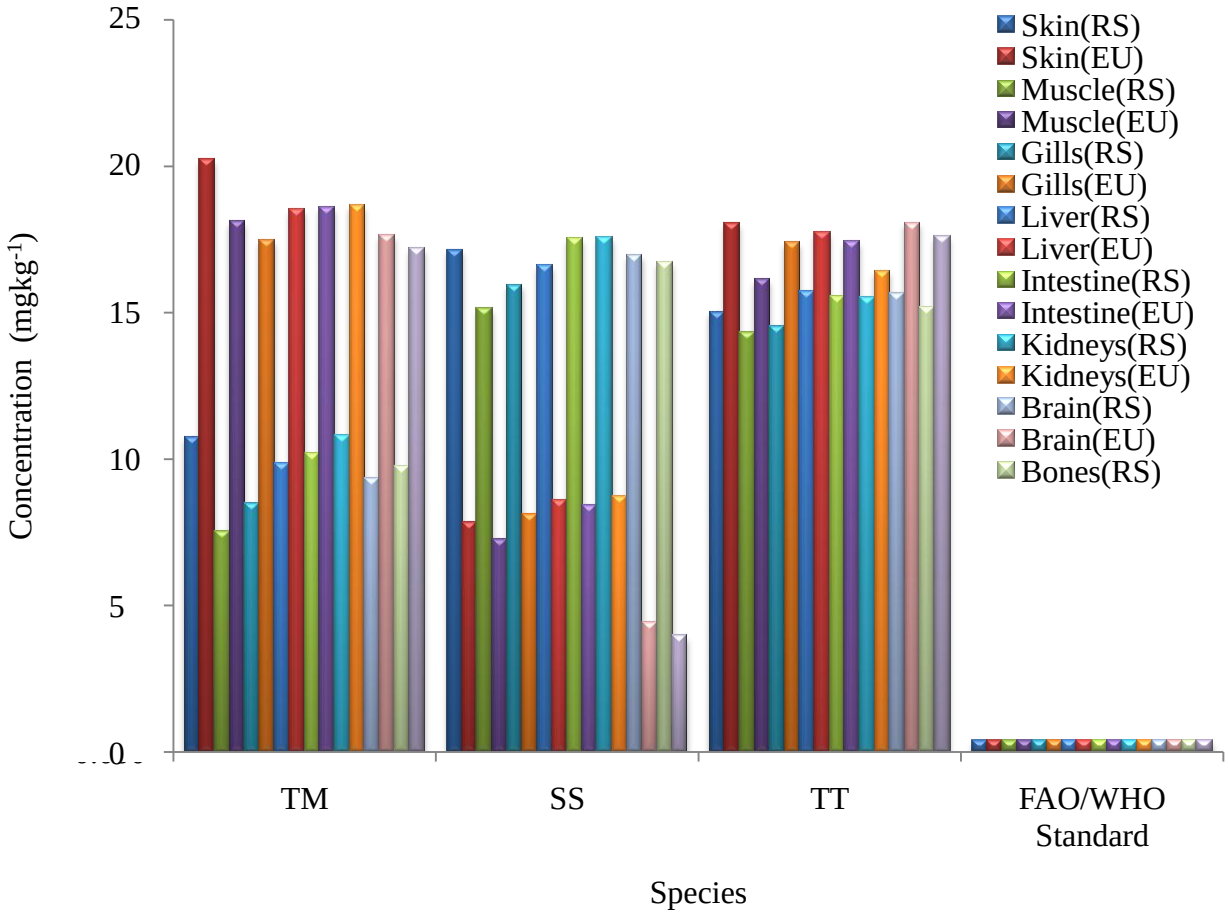


Figure 4.5 Comparative mean concentration for Pb in tissues of fish species

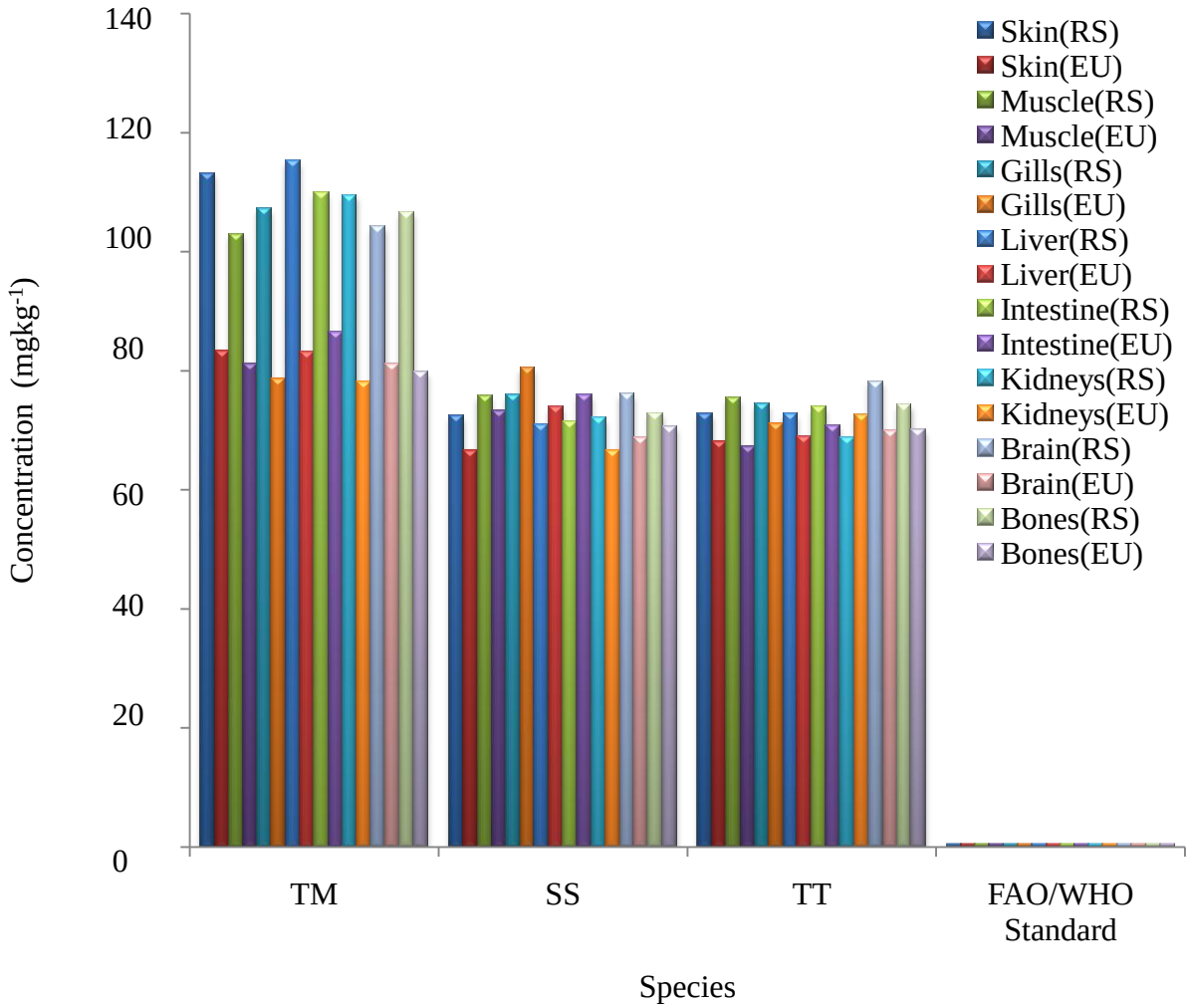


Figure 4.6 Comparative mean concentration for Hg in tissues of fish species

showed*Trachurus murphyi* species recorded high level of accumulations than the corresponding *Trachurus trachurus* and*Scomber scombrus* species. The bars for *Trachurus trachurus* and *Scomber scombrus*were found to have comparable levels of accumulations. However, the tissues accumulations with regards to fishing origins showed RS tissues for*Trachurus murphyi* species accumulate highly than the corresponding EU tissues. While in the case of *Scomber scombrus*species and *Trachurus trachurus* fish species, both RS and EU fish origins shows there is no significant differences in the accumulations level among tissues.

4.21 Comparative Mean Concentration of Fe in Fish Species

The graphical representation in Figure 4.7 for Fe accumulation in various tissues of fish species revealed *Trachurus murphyi* and *Trachurus trachurus* accumulates highly, followed by *Scomber scombrus* which was found with the least levels.However, the tissues accumulations with respects to fishing origins revealed *Trachurus murphyi* species showed RS fish species accumulate highly than the EU species. While in the case of *Scomber scombrus* species, comparablelevel of accumulations were shown between the tissues of RS and EU fish origins. And the bars for *Trachurus trachurus* species showed higher accumulation are presented with the tissues of EU fish origins.

4.22 Comparative Mean Concentration of Ni Fish Species

The graphical representation in Figure 4.8 showed the accumulation of Niin various tissues of fish species; *Trachurus murphyi*, *Scomber scombrus* and *Trachurus trachurus.*The higher level of Ni accumulations was found by the tissues of *Trachurus trachurus* followed by

Scomber scombrus, and *Trachurus murphyi* with the least levels. However, the accumulation with regards

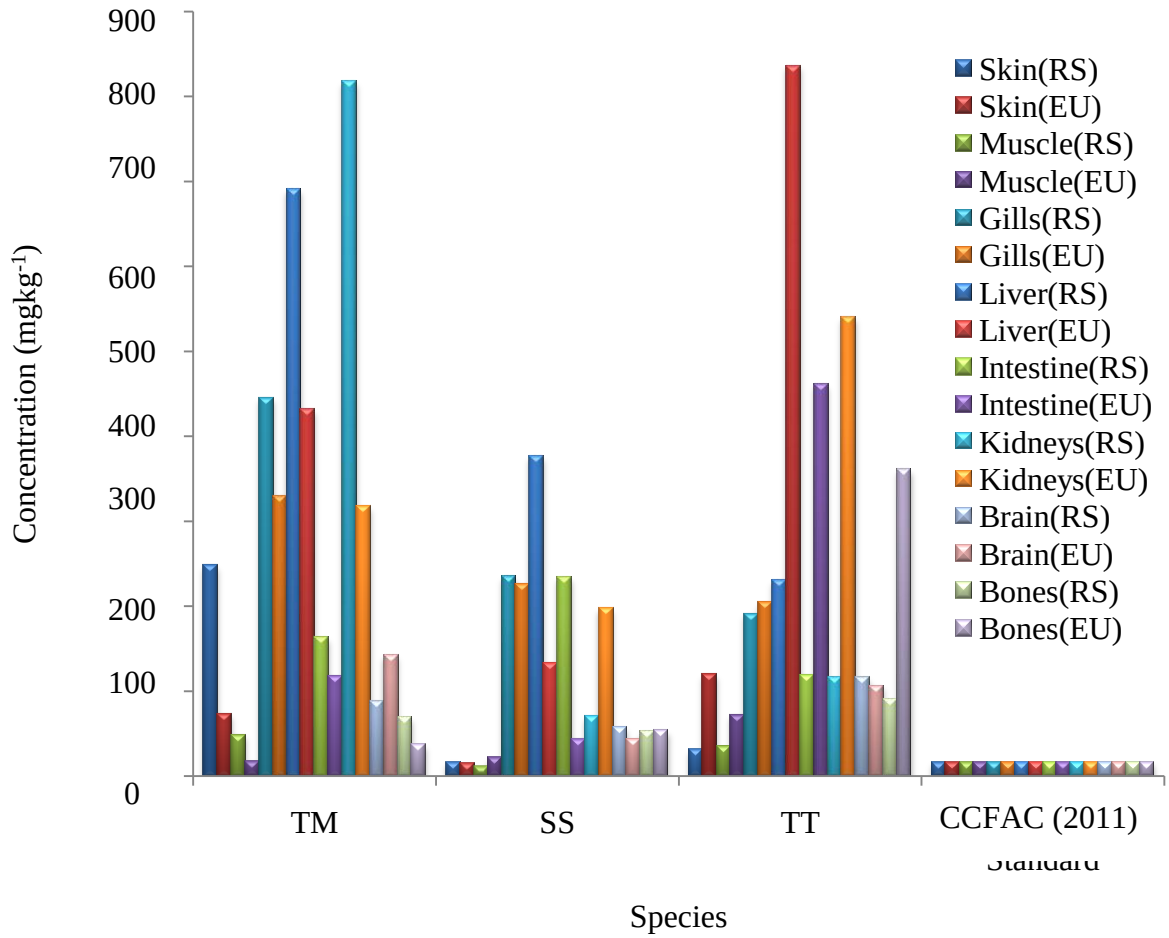


Figure 4.7 Comparative mean concentrations for Fe in tissues of fish species

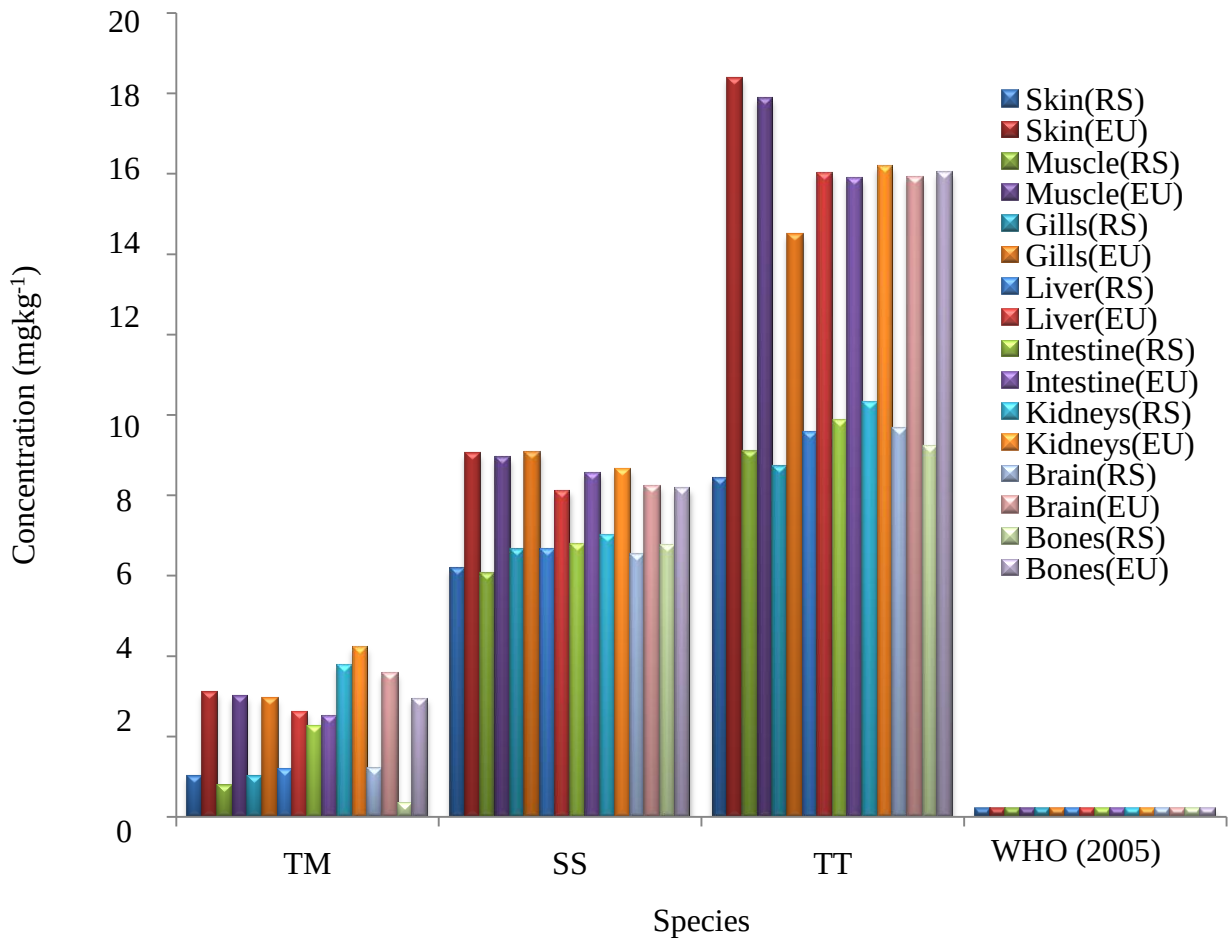


Figure 4.8 Comparative mean concentration for Ni in tissues of fishspecies

to fishing origins for each of the species showed RS tissues accumulates highly than the corresponding tissues of EU fish species.

4.23 Risk Assessment Index

The results of risk index for heavy metals; Cd, Pb, Hg, Fe and Ni concentrations ($mgkg^{-1}$) in muscles tissues of *Trachurus trachurus*, *Scomber scombrus* and*Trachurus murphyi*across the batches of RS and EU fish species; including health quotient (HQ), daily intake of metal (DIM) and health risk index (HRI) of different age groups are summarized in Table 4.4 - 4.6. The index assessments on individuals was categorized in to three groups. Category A was designated for adults (aged 19years and above), category B designated for children (aged range from 7-18years) and the last category C designated for (aged range 6years and below).

4.23.1 Risk assessment Index for *Trachurus trachurus* species

The results in Table 4.49 showed the Cd mean concentration of 0.66 $mgkg^{-1}$ in muscles tissues of *Trachurus trachurus* species. The results revealed HQ ratio of 0.941, 1.172 and 2.081; DIM with concentration of $2.656x10^{-4}$ $mgkg^{-1}day^{-1}$, $3.305x10^{-4}$ $mgkg^{-1}day^{-1}$ and $5.870x10^{-4}$ $mgkg^{-1}day^{-1}$; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C, respectively.

The Pb mean concentration of 15.23 $mgkg^{-1}$ in muscles tissues of *Trachurus trachurus* species give rise to HQ ratio of greater than one (1); DIM with concentration of $6.139x10^{-3}$ $mgkg^{-1}day^{-1}$; $7.638x10^{-3}$ $mgkg^{-1}day^{-1}$ and $1.360x10^{-2}$ $mgkg^{-1}day^{-1}$; and HRI ratio of greater than one (1),corresponding to individual's in category A, B and C, respectively.

The Hg mean concentration of 71.31 $mgkg^{-1}$ in muscles tissues of *Trachurus trachurus*

Table 4.49HQ, DIM and HRI index for individual's response of heavy metals in muscles tissues of *Trachurus trachurus* species

Heavy metals	Range ($mgkg^{-1}$)	Mean±SD ($mgkg^{-1}$)	Individuals	HQ	DIM ($mgkg^{-1}day^{-1}$)	HRI
Cd	0.25-1.05	0.66±0.33	Category A	0.941E+0	2.656E-4	0.266E+0
			Category B	1.172E+0	3.305E-4	0.331E+0
			Category C	2.081E+0	5.870E-4	0.587E+0
Pb	7.30-18.60	15.23±0.02	Category A	6.217E+0	6.139E-3	1.754E+0
			Category B	7.737E+0	7.638E-3	2.283E+0
			Category C	1.374E+1	1.360E-2	3.877E+0
Hg	51.15-98.9	71.31±0.02	Category A	1.018E+3	2.871E-2	2.874E+2
			Category B	1.267E+3	3.576E-2	3.576E+2
			Category C	2.252E+3	6.350E-2	6.353E+2
Fe	2.95-101.5	53.70±0.20	Category A	0.109E+0	2.164E-2	0.309E-1
			Category B	0.136E+0	2.693E-2	0.385E-1
			Category C	0.242E+0	4.783E-2	0.683E-2
Ni	6.75-21.90	13.48±0.02	Category A	0.963E+0	5.433E-3	0.272E+0
			Category B	1.198E+0	6.762E-3	0.338E+0
			Category C	2.129E+0	1.200E-2	0.601E+0

A = adults age 19years and above, B = children age 7- 18years, C = children age 6years and below.

Table 4.50 HQ, DIM and HRI index for individual's response of heavy metals in muscles tissues of *Scomber scombrus* species

Heavy metals	Range ($mgkg^{-1}$)	Mean±SD	Individuals	HQ	DIM ($mgkg^{-1}day^{-1}$)	HRI
Cd	0.40-9.00	2.28±0.010	Category A	3.263E+0	1.162E-3	1.056E+0
			Category B	5.076E+0	1.482E-3	1.482E+0
			Category C	7.213E+0	2.633E-3	2.632E+0
Pb	2.00-21.75	11.19±0.010	Category A	4.567E+0	5.833E-3	1.667E+0
			Category B	5.683E+0	7.559E-3	2.074E+0
			Category C	1.009E+1	1.289E-2	3.684E+0
Hg	50.3-99.35	74.55±0.035	Category A	1.064E+3	3.887E-2	3.887E+2
			Category B	1.325E+3	4.837E-2	4.837E+2
			Category C	2.354E+3	8.592E-2	8.592E+2
Fe	6.04-59.16	16.66±0.023	Category A	0.340E-1	8.689E-3	0.124E-1
			Category B	0.423E-1	1.081E-2	0.154E-1
			Category C	0.751E-2	1.921E-2	0.274E-2
Ni	1.35-11.80	7.49±0.053	Category A	0.535E+0	3.906E-3	0.195E+0
			Category B	0.666E+0	4.861E-3	0.243E+0
			Category C	1.183E+0	8.636E-3	0.432E+0

A = adults age 19years and above, B = children age 7- 18years, C = children age 6years and below.

Table 4.51HQ, DIM and HRI index for individual's response of heavy metals in muscles tissues of*Trachurus murphyi* species

Heavy metals	Range ($mgkg^{-1}$)	Mean±SD	Individuals	HQ	DIM ($mgkg^{-1}day^{-1}$)	HRI
Cd	1.05 - 2.90	1.79±0.020	Category A	2.554E+0	7.595E-4	0.759E+0
			Category B	3.179E+0	9.504E-4	0.950E+0
			Category C	9.442E+0	1.688E-2	1.688E+1
Pb	0.30-19.6	12.83±0.020	Category A	5.237E+0	5.480E-3	1.566E+0
			Category B	6.517E+0	6.820E-3	1.989E+0
			Category C	1.158E+2	1.211E-1	3.461E+1
Hg	66.5-117.9	92.07±0.015	Category A	1.315E+3	3.932E-2	3.933E+2
			Category B	1.636E+3	4.893E-2	4.894E+2
			Category C	2.907E+4	8.693E-1	8.693E+3
Fe	10.9-60.3	33.06±0.043	Category A	0.675E-1	1.412E-2	0.202E-1
			Category B	0.840E-1	1.757E-2	0.251E-1
			Category C	1.492E+0	3.122E-1	0.446E-0
Ni	0.30 - 4.10	1.884±0.010	Category A	0.135E+0	8.047E-4	0.402E-1
			Category B	0.168E+0	9.993E-4	0.499E-1
			Category C	2.995E+0	1.779E-2	0.889E+0

A = adults age 19years and above, B = children age 7- 18years, C = children age 6years and below.

species give rise to HQ ratio of greater than one (1);DIM with concentration of $2.871x10^{-2}mgkg^{-1}day^{-1}$, $3.576x10^{-2}mgkg^{-1}day^{-1}$ and $6.350x10^{-2}mgkg^{-1}day^{-1}$; and HRI ratio of greater than one (1), corresponding to individual's in category A, B and C respectively.

The Fe mean concentration of $53.70mgkg^{-1}$ in muscles tissues of *Trachurus trachurus* species give rise to HQ ratio of less than one (1);DIM with concentration of $2.164x10^{-2}mgkg^{-1}day^{-1}$, $2.693x10^{-2}mgkg^{-1}day^{-1}$ and $4.783x10^{-2}mgkg^{-1}day^{-1}$; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

The Ni mean concentration of $13.48mgkg^{-1}$ in muscles tissues of *Trachurus trachurus* species give rise to HQ ratio of 0.963, 1.198, and 2.129; DIM with concentration of $5.433x10^{-4}mgkg^{-1}day^{-1}$, $6.762x10^{-4}mgkg^{-1}day^{-1}$ and $1.200x10^{-4}mgkg^{-1}day^{-1}$; and HRI ratios of less than one (1)corresponding to individual's in category A, B and C respectively.

4.23.2 Risk assessment index for *Scomber scombrus* species

The results in Table 4.50 showed the Cd mean concentration of $2.28mgkg^{-1}$ in muscles tissues of *Scomber scombrus*species. The results give rise to HQ ratio of greater than one (1); DIM with concentration of $1.162x10^{-2}mgkg^{-1}day^{-1}$, $1.482x10^{-3}mgkg^{-1}day^{-1}$ and $2.633x10^{-3}mgkg^{-1}day^{-1}$; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

The Pb mean concentration of $11.19mgkg^{-1}$ in muscles tissues of *Scomber scombrus*species give rise to HQ ratio of greater than one (1); DIM with concentration of $5.833x10^{-3}mgkg^{-1}day^{-1}$, $7.559x10^{-3}mgkg^{-1}day^{-1}$ and $1.289x10^{-2}mgkg^{-1}day^{-1}$; and HRI ratio of greater than one (1), corresponding to individual's in category A, B and C respectively.

The Hg mean concentration of 74.55mgkg^{-1} in muscles tissues of *Scomber scombrus*species give rise to HQ ratio of greater than one (1);DIM with concentration of 3.887x10^{-2}mgkg^{-1}day^{-1}, 4.837x10^{-2}mgkg^{-1}day^{-1} and 8.592x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of greater than one (1), corresponding to individual's in category A, B and Crespectively.

The Fe mean concentration of 16.66mgkg^{-1} in muscles tissues of *Scomber scombrus*species give rise to HQ ratio of less than one (1);DIM with concentration of 8.689x10^{-3}mgkg^{-1}day^{-1}, 1.081x10^{-2}mgkg^{-1}day^{-1} and 1.921x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

The Ni mean concentration of 7.49mgkg^{-1} in muscles tissues of *Scomber scombrus*species give rise to HQ ratio of 0.535, 0.666 and 1.183; DIM with concentration of 3.906x10^{-3}mgkg^{-1}day^{-1}, 4.861x10^{-3}mgkg^{-1}day^{-1} and 8.636x10^{-3}mgkg^{-1}day^{-1}; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

4.23.3 Risk assessment index for *Trachurus murphyi* species

The results in Table 4.51 showed the Cd mean concentration of 1.79mgkg^{-1} in muscles tissues of *Trachurus murphyi* species. The results give rise to HQ ratio of greater than one (1); DIM with concentration of 7,595x10^{-4}mgkg^{-1}day^{-1}, 9.504x10^{-4}mgkg^{-1}day^{-1} and 1.688x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

The Pb mean concentration of 12.83mgkg^{-1} in muscles tissues of *Trachurus murphyi*species give rise to HQ ratio of greater than one (1); DIM with concentration of 5.480x10^{-3}mgkg^{-1}day^{-1}, 6.820x10^{-3}mgkg^{-1}day^{-1} and 1.211x10^{-1}mgkg^{-1}day^{-1}; and HRI ratio of greater than one (1), corresponding to individual's in category A, B and C respectively.

The Hg mean concentration of 92.07mgkg^{-1} in muscles tissues of *Trachurus murphyi*species give rise to HQ ratio of 1, 1 and 2; DIM with concentration of 3.932x10^{-2}mgkg^{-1}day^{-1}, 4.893x10^{-2}mgkg^{-1}day^{-1} and 8.693x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of greater than one (1), corresponding to individual's in category A, B and C respectively.

The Fe mean concentration of 33.06mgkg^{-1} in muscles tissues of *Trachurus murphyi*species give rise to HQ ratio of 0.067, 0.084 and 1.492;DIM with concentrationsof 2.164x10^{-2}mgkg^{-1}day^{-1}, 2.693x10^{-2}mgkg^{-1}day^{-1} and 4.783x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of less than one (1), corresponding toindividual's in category A, B and C respectively.

The Ni mean concentration of 1.884mgkg^{-1} in muscles tissues of *Trachurus murphyi*species give rise to HQ ratio of 0.135, 0.168 and 2.995;DIM with concentration of 8.047x10^{-4}mgkg^{-1}day^{-1}, 9.993x10^{-4}mgkg^{-1}day^{-1} and 1.779x10^{-2}mgkg^{-1}day^{-1}; and HRI ratio of less than one (1), corresponding to individual's in category A, B and C respectively.

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Quality Assurance

Thepercentage recoveries presentedin Table 4.1 – 4.3showed acceptable recoveries wereobtained with each of the species and that the digestion method used and the AAS analysis were reliable. Thepercentage recoverieswith respects to each species falls within the range of 90 – 120%,thereforethe results showed compliance with the requirements of Commission Decision (2002),European Commission Regulation (2007) and Standard Operating Procedure (SOP) by (USEPA, 2011). Also, the results of LOD, LOQ and RSD_robtained with respects to each speciesshowed compliance with the practical guide for within-lab repeatability of International Standard Organization (ISO) 17025 (2005). Therefore,the consistent results on the lower values ofLOD and LOQ obtained, indicates that the results obtained are reliable.

5.2 Cadmium Concentrations in Tissues of *Trachurus trachurus*Species

The results presented for *Trachurus trachurus*species showed the concentrations of Cd inthe tissues of RS fish species were varied between the range of 0.925 to 5.142mgkg^{-1}for the skin and liver tissues respectively. While in EU fish species,the concentrations werevaried betweenthe range of 0.367to 6.517mgkg^{-1} for the muscles and liver, tissues respectively. These concentrations revealedthat fish from RS and EU fishing origins bio-concentrate Cd highly in their liver tissues. Fromthe literature of fish studies, it was found in coinciding sequence that highCd bio-concentrationsin various fish tissues are usually accumulatedin the liver tissues. For

instance,the study byAbolfaz and Maryam (2012) on*Platycephalus indicus* speciesshowed liver tissues represents the major organ of Cdbio-accumulation with concentration of 4.67mgkg^{-1}.Similarly the study by Yilmaz (2009) on *Mugil cephalus* species revealedhighest concentrations of Cd (3.32mgkg^{-1}) in the liver tissues.TheCd accumulation with different array of species have been found with concentration higher than revealed in this study.In their studies, Storelli *et al.* (2003) revealed that*Sphyrma zygaena*species showed high Cd concentration of 9.810mgkg^{-1}and similarly*A. thalassinus*species was found withCd concentration of 13.35mgkg^{-1}in liver tissues (Simon *et al.,*2013). This indicates that Cdaccumulations in fish tissues are largely depended on the nature of the fishing origins, the types of the fish speciesas well as differences in tissues accumulation. The higher level of Cd shown in the liver relative to other tissues may be attributed to the high coordination of metallo-thionein protein with the Cd (Uzairu *et al.*, 2009). In addition, the liver is the principal organ responsible for detoxification, transportation and storage of toxic substances metals such as Cd (Fazli *et al.*, 2009). Liver is one of the active site of pathological effects induced by contamination (Storelli *et al.*, 2012). However, the concentrations of Cdshown in the intestine and kidneys tissues irrespective of the batch were higher than the concentrations obtained in the skin, gills, bones and brain tissues. This is because the tissues that are involved in the process of absorption, storage and excretion of feed ingestion by the fish are reported to accumulate high metals concentration (Storelli *et al.*, 2012). Therefore, Cd storage in tissues of *Trachurus trachurus* species of this study are frequently shown with the tissues/organs that are involved in the process of digestion.

In contrast, the lowest levels of Cdaccumulation in the entire tissues for RS and EU fish species are revealed in the skin and muscles tissues with concentration of 0.925mgkg^{-1}and 0.367mgkg^{-1}respectively. These are related to the fact thatthey are inactive tissues andsuch

inactive tissues can only store low concentrations of metals because of the absence of metallo-thionein proteins (Storelli *et al.,* 2012). This report was found in agreement withthe studies reported on *A. thalassinus* and *J. belangeri* species which shows low Cd accumulations in the muscles ($0.027 mgkg^{-1}$) and skin ($0.035 mgkg^{-1}$) tissues (Simon *et al.*, 2013).This is similar to study byYazkan (2002), Kalay *et al*. (1999) and Copet *et al.* (2013) that showed low accumulation of Cd in fish tissues are found in the muscles and skin tissues. Furthermore, the concentrations of Cd shown with the entire tissues across the batches of both fishing zoneswereabovethesafety limit specified by FAO (2003).

From the results of statistical analysisfor*Trachurus trachurus* species, significant differences ($p<0.05$) were shown in the batches of both RS and EU tissues. The significant differences shown in the batches of RS(skin, muscle, brain and bones) and EU (skin, muscles, gills and bone) tissuesimplies that, contaminations with Cd across these batches are related to impacts of different sources. While, the non-significant differences ($p<0.05$) shown among the batches of RS (gills, liver, intestine and kidneys) and EU (liver, intestine, kidneys and brain) tissues gives an indication that, contamination with Cd in these batches were due to homogeneity from similar sources. Also the non-significant difference shown among batches with respects to both origins, involves the fish digestive tissues and this gives an idea that Cd contamination are majorly from the fish feeds. The fish digestive organs are the major sites responsible for Cd storage or detoxification during digestion process.

The results of correlation analysis for Cd contamination in *Trachurus trachurus species*shows that,both significant ($p<0.05$) positive and negative correlations were shown in the batches of RS and EU fish species. Positive correlation shown among tissues indicate that, contaminations with Cd were from similar sources or the accumulation routes into these tissues

are via similar mechanism since uptake was governed by similar physico-chemical conditions.While, negative correlations among other tissues implies contamination with Cd were from different sources.

5.3 Lead Concentrations in Tissues of *Trachurus trachurus*Species

The results presented for *Trachurus trachurus* species showsthat, the concentrations of Pb in the tissues of RS fish species varied between the range of 14.333to 15.742mgkg^{-1} for the muscles and liver tissues respectively. While, the concentrations recorded in the tissues of EU fish species ranged between 16.133to 18.058mgkg^{-1} for the muscles and brain tissues respectively.Accumulations of Pb with respect to tissues of RS fish origins showed higher concentrations in the liver tissues (15.742mgkg^{-1}). Several studies on fish species shows that high Pb accumulation are usually found in liver tissues. For instance, the study by Usero *et al.* (2004) shows that high Pb concentration (5.050mgkg^{-1}) was recorded in the liver tissues of common sold frozen *Solea vulgaris* species from the Southern Atlantic coast of Spain.Although an unusual report in this study that run contrary to the literature shows Pb accumulation in thetissues of EU fishspecies was highly shown in the brain tissues (18.058mgkg^{-1}).With this regard therefore,EU fishing origins showedhigh contamination with Pb, because, *Trachurus trachurus* among other species have beenreported as a good indicator of Pb in the aquatic environment (Joana *et al.*, 2011).

In contrast to the high level of Pbshown in the liver and brain tissues, the lowest Pb accumulations with respects to both RS and EU fish specieswere shown in the muscles tissues. This is in agreement with several reportsthat fishmuscles tissues, is not an active organ andcannot store Pb to a greater extend that can reflects the Pb concentration in the surrounding water habitat (Hulya*et al.* 2004; Eneji *et al.*, 2011; Reena *et al.*, 2011). This is due to the fact

that, ingestion of Pb and other metals take place via eating by mouth and absorption through gills or skin. The entry through the ingestion path, flows to the intestine, then absorbed, processed and distributes by the liver and exit through kidneys. These parts are referred to as target active organs where bioaccumulation of Pb can takes place in the presence of mucus and metallo-thionein protein. Therefore, the higher accumulative of Pb in the liver tissues, the lesser its concentration would be in the inactive muscles tissues. This was also in line with the studies reported by Uzairu *et al.* (2009)and Noik *et al*. (2011).The concentrations of Pbshown with the entire tissues were found to showed remarkable concentrations far above the safety limits of 0.4mgkg^{-1} recommended by FAO (2003).

The results of statistical analysis for Pb in tissues of *Trachurus trachurus* species with respects to both RS and EU fishing zones recorded that, the non-significance differences ($p<0.05$) were predominantly shown across most of the batches and hence, these implied contaminations are due to impact of similar sources across the entire batches. The pronounced positive correlationamong the entire tissues with regards to both RS and EU species were due to contamination from similar sources.Therefore, the mechanisms for Pb uptake across these tissues were aided by similar physico-chemical factors. Also the results of correlations analysis were in lined with the results of statistical test of significance and hence, these indicate that lead accumulations were homogeneously contaminated by similar sources across the entire batches (with respects to both fishing zones).

5.4 MercuryConcentrations in Tissues of *Trachurus trachurus*Species

The resultsof Hg presentedfor *Trachurus trachurus* species shows the concentrations inthe tissues of RS fish origin were varied between the range of 68.708to 78.208mgkg^{-1}for the

kidneys and brain tissues respectively. While the concentrations recorded in EU fish species ranged between the values of 67.175to 72.592mgkg^{-1} for the muscles and kidney tissues respectively. High concentrations of Hg accumulation was found in the brain tissues (78.208mgkg^{-1}) and least were shown in the kidney tissues (68.708mgkg^{-1}) of RS fish origin. Mieiro *et al.* (2009) pointed out that brain is a vital organ in fish which can give rise to high accumulation of Hg. In contrast, the level of Hgshown in the brain tissues (of RS fish species) of this study, were higher than the level of 0.9949mgkg^{-1} and 0.21mgkg^{-1} Marioara *et al*. (2012) and(Storelli *et al.*, 2012). While for the EU tissues, kidneys exhibit higher accumulations (72.592mgkg^{-1}) and least were shown in the muscles (67.175mgkg^{-1}) tissues. Kidney tissues in fish are organs for excretion, where absorption, selective re-absorption, storage and eliminations were essentially (Durali *et al.*,2010). The study reported by Mieiro *et al.* (2009) shows that *Liza aurata* species recorded high Hg concentrationin the kidney (4.02mgkg^{-1}) tissues. Hg gets into the fish through ingestion or absorption via skin or gills from the surrounding water body. Fish ingest food (contaminated with Hg), flown through gastrointestinal tract, absorbed and distributed by the liver and finally accumulates or detoxified by the kidney (Jabeen *et al.*, 2012; Zubair *et al.*, 2013). However, high Hg levels shown in the kidney tissues correspond to low detoxification process in the kidney system.

In contrast, the lower concentrations of Hg shown in the kidneys tissues of RS species implies high detoxification process in the kidney system. While the lower concentrations of Hg shown in the muscles tissues of EU fish species were as a result of its role as inactive tissues. This is because the more Hg became accumulated in the digestive tissues the low its mobility would be to the inactive tissues (such as muscles, bones tissues etc.). Also, concentration of Hg shown with respect to entire tissues of both RS and EU fish species were above the levels

reported by related studies on other fish species (Lillian and Lorraine, 2012; Jayashree *et al.*,2013) and as well as the safety limits of0.5mgkg^{-1}recommended by FAO (2003).

The statistical results for Hg in the tissues of*Trachurus trachurus*with respect to both RS and EU fish origin showed the entire batches recorded non-significance differences ($p<0.05$) and hence, impliesthat contaminations with Hg across these batches were highly homogeneous which can be due to impacts from similar sources. While the results of correlation analysis with regards to both RS and EU tissues also shows significant ($p<0.05$) positive correlation were recorded across the entire tissues and hence contamination with Hg are due to impacts from similar source. Therefore, the correlation results was found in lined with the results of statistical analysis, in which both analyses reported that Hg contaminations were as a result of similar sources. This justifies that the route of tissues uptake and accumulations of Hg are homogeneously contaminated across the entire batches or the uptake mechanisms were governed by similar physico-chemical factors.

5.5 Iron Concentrations in Tissues of *Trachurus trachurus*Species

The concentrations of Fe presented for*Trachurus trachurus* species show that, the accumulation in the tissues of RS fish speciesvaried between 31.417 to 230.140mgkg^{-1} for the skin and liver tissues respectively. While the concentrations recorded for the tissues of EU fish origins, were ranged between the values of 72.080to 836.260mgkg^{-1} for the muscles and liver tissues respectively. Accumulation of Fein the tissues with respects to both RS and EU fish species were higher in the liver tissues.This is attributed to high coordination of Fe with metallo-thionien protein present in the liver tissues (Uzairu *et al.*, 2009). This is similar to report byHulya *et al*. (2004) on *Silurus triostegus* and *Liza abu*species that highest Fe concentrations with the

values of 34.670mgkg^{-1} and 9.240mgkg^{-1} respectively were found in the liver tissues and described liver tissues as a major organ for Feaccumulation. Also,Hjeltnes *et al*. (1992) reported a high concentration of 246.00mgkg^{-1} was found in the liver tissues of*Salmo salar* species. The results presented for both RS and EU species showed high level of Feis found in the liver tissues, therefore such can be related to the large volume of blood present in it which was not accountedforinthisstudy.

With regards totheother tissues, the Feconcentrations shown in the intestine and gills tissues were higher than aswith the skin, brain and bones tissues. The concentration of Fe recorded for gills tissues predicts an estimates of the concentrations of Fe present in the surrounding water body where the fish lives (Hulya *et al.*, 2004). Whereas, the skin, brain and bone, show low concentration of Fe even though the skin tissues is in direct contact with the metals present in the surrounding water body.Generally, the level of Fe shown with the entire tissues of bothRSandEUfishoriginswerehigherthanthesafetylimits recommended by CCFAC (2011).

The results of statistical analysis (ANOVA) recorded for Fe in*Trachurus trachurus* species showedthat non-significant differences ($p< 0.05$) were found with the entire batches of both RS and EU tissues. This implied thesource that lead to Fe contamination are homogeneous across the entire batches for both fishing origins. However, the results of correlation analysis obtained with regards to both RS and EU tissuesshowed significant positive correlations and hence this resultswere in lined with the results of ANOVA,in which Fe contamination was due to similar sources. These also justifies that the route of tissues uptake and accumulations of Fe are homogeneously contaminated across the entire batches and the uptake mechanisms were governed by similar physico-chemical factors.Reports on Fe inter-

actionsinbiologicaltissueshasshowedthatexcessamount ofFecausesrapidincreaseinpulserateandcoagulationof bloodinbloodvessels,hypertension,anddrowsiness(FAO, 2012).Ironisanessentialsourceformostformsoflife.In humans,Feisanessentialcomponentofproteinsinvolved inoxygentransportedfromthelungstothetissues (FAO 2012).

5.6 Nickel Concentrations in Tissues of *Trachurus trachurus*Species

The results of Ni concentrations presented for*Trachurus trachurus* species shows that, the concentrations in tissues of RS fish speciesvaried between the range of 8.425to 10.308mgkg^{-1} for the skin and kidneys tissues respectively. While for EU fish species the Ni concentrations shown in the tissues ranged between 14.492to 18.383mgkg^{-1} for the gills and skin tissues respectively. Ni accumulations were highly shown in the kidneys (10.308mgkg^{-1}) and skin (18.383mgkg^{-1}) tissues of RS and EU fish species respectively. While, low Ni accumulations were shown in the muscles (7.542mgkg^{-1}) and bone (17.200mgkg^{-1}) tissues of RS and EU fish species respectively. The higher levels of Ni shown in the kidneys tissues of RS fish species is similar to Hg, this is related to high coordination of Ni with the metallo-thionien protein presents in the kidneys. Kidney is an organ next to liver that plays a vital role in digestion process. Detoxifications of trace metals by the liver tissues mostly get accumulated in the kidneys (Lillian and Lorraine, 2012). This reportis similar with the study by Sahar *et al*. (2014) that Niwere highly accumulated in the kidney tissues. In contrast, the highest accumulations shown in the skin tissues with respects to EU species are related to the fact that, skin is directly in contact with the metals present in the surrounding water. Thus, concentrations of Ni shown in the skin tissues can also reflect its concentration in the body of waters where the fish lives (Uzairu *et al.*, 2009).

However, the levels of nickel recorded for the liver, intestine and kidneys tissues are higher than those in the brain, bones and gills. This is because metabolic tissues such as liver, intestine and kidneys that are involved in the process of digestion accumulate trace metals more than any other tissues (Denise *et al.*, 2012; Israa *et al.*, 2013; Sahar *et al.*, 2014).

In contrast, the low levels of Ni shown in the muscles and bones tissues of *Trachurus trachurus* species in this study agreed with other findings that muscles and bone tissues bioaccumulate low level of trace metals due to the fact that they are inactive tissues (Durali *et al.*, 2010; Storelli *et al.*, 2012; Jabeen *et al.*,2012). Although, the Ni concentrations in this study for both RS and EU fish species were above the safety limits of 0.2mgkg^{-1}recommendedby WHO (2005).

From the results of Analysisof Variance (ANOVA)for Ni in*Trachurus trachurus* species, the batches with respect to RS and EU tissues showed non-significant differences ($p< 0.05$). Since the entire tissues show similarities of Ni uptakes across the batches, hence contaminations were as a result of similar sources. However, the results of correlation analysis presented pronounced positive correlation across the tissues for both RS and EU fish species. This indicates that the route for Ni contamination was strongly from similar sources.

5.7 CadmiumConcentrations in Tissues of *Scomber scombrus*Species

The results presented forCd concentration in tissues of *Scomber scombrus* showedRS fish speciesvaried between the range of 2.133 to 19.758mgkg^{-1} for the skin and liver tissues respectively. While in EU fish species, the concentrationswere varied between the range of 1.125 to 10.658mgkg^{-1} for the gills and liver tissues respectively. The highest Cd accumulations in both RS and EU species were found in batches of livers tissues, with the concentration value of

19.758mgkg^{-1} and 10.658mgkg^{-1} respectively. High levelofCdshowninthelivertissueswassimilar to 19.81mgkg^{-1}found in liver tissues of*Sphyrma zygaena* species(Storelli *et al.*, 2003) and 13.35mgkg^{-1}in the liver tissues of *A. thalassinus* species (Simon *et al.*,2013). From the literature of fish studies, several reported that high level of Cd are usually accumulated in the liver tissues (Yilmaz, 2009; Uzairu *et al.*, 2009; Abolfazl *et al.*, 2012; Simon *et al.*,2013).

However, the concentrations of Cd shown in the intestine and kidneys tissues, irrespective to any batch, were higher than the results obtained for skin, gills, brain, and bone. This is becauseoftheirresponsiblerolesintheprocessofabsorption, storage, and excretion of ingested feed by the fish. This also entails that Cd is stored in a state of binding form in the digestive tissues. Fish studies also show that, absorption of Cd from the gills or gastro-intestinal tract was mainly accumulated in the liver and kidneys (Fazli *et al.*, 2002; Kalay and Canli, 2000). Furthermore, the concentrations of Cd shown with the entire tissues across the batches of both fishing zoneswereabovethesafetylimit specified by FAO (2003).

From the results of statistical analysis(ANOVA) presented for *Scomber scombrus* species, significant differences ($p<0.05$) were recorded among the batches of RS(in the skin and muscles tissues) andEU(in bones tissues only) fish species. However, the ANOVA results showed non-significant differences ($p<0.05$) dominates most of the batches for RS and EU fish species and hence these implies that contamination with Cdare due to impacts of related sources. Similarly the results of correlation analysis on the other sides showed, both RS and EU fish species presented significant ($p<0.05$) positive correlationacross most of the tissues. Hence, these gives indication that the uptake routes of Cd among tissues were due to similar sources of contamination. Although the significant ($p<0.05$) negative correlationshown among few tissues indicate that, there are other non-related factor which contributesto Cd contaminations. Although

the significant ($p<0.05$) negative correlations shown amongfew tissues indicates that there are other non-related factors which contributes to Cd contaminations.

5.8 LeadConcentrations in Tissues of *Scomber scombrus*Species

The results presented forPb concentrations in tissues of*Scomber scombrus* species showed RS fish originsvaried between the ranges of 15.133to 17.558mgkg^{-1} for the muscles and kidney tissues respectively. While, for EU fish species the concentration were varied between ranges of 3.950to 8.725mgkg^{-1} for the bone and kidney tissues respectively. Pb accumulations in the tissues of both RS and EU fishing zones were highly shown in the kidney tissues with a concentrationof 17.558mgkg^{-1} and 8.725mgkg^{-1} respectively. The high levels of Pb shown in the kidneys may be attributed to the high coordination of Pb with the metallo-thionein protein present. Kidney is an organ next to liver that plays a vital role in the process of digestion. Detoxifications of metals by the liver tissues mostly get accumulated in the kidneys (Yousafzai *et al.*, 2009). The higher concentrations of Pb shown in the kidneys than the liver tissues of RS fish species indicate that, liver undergoes more detoxification process (Sahar *et al*., 2014).

While the muscles and bones tissues on the other hand were found to accumulate lowest level of Pb concentrations. This is due to the fact that muscles and boneare inactive tissues and cannot accumulates Pb that can reflects the concentration in the surrounding fish habitat (Fredrick *et al.*, 2014; Eneji *et al.*,2011; Hulya *et al.*, 2004). *Scomber scombrus* species generally showed some affinity for Pb accumulation in all the analyzed tissuesof both RS and EU fish species. The concentrations of Pb in all the tissues were found to exceed the safety limits of 0.4mgkg^{-1} recommended by FAO (2003). The concentrations were found far above the levels

reported by others studies in Mediterranean Sea on fish tissues (Yazkan *et al.*, 2002; Soegianto*et al.*, 2009; Ogundiran *et al.*,2014).

The statistical results for *Scomber scombrus* species with respect to both RS and EU tissues showed non-significance differences($p<0.05$) were recorded across the batches and hence, these implied that the source of lead contaminations across the batches were highly homogeneous. While the results of correlation analysis on other hand shows that, significant ($p<0.05$) positive correlation were shown across the tissues of RS and EU fish species and these corresponds with the results of statistical analysis in which both analysis reported Pb were homogeneously contaminated across the entire batches.

5.9 MercuryConcentrations in Tissues of *Scomber scombrus*Species

The results of Hg concentrations in tissues of*Scomber scombrus* species recorded RS fish originsvaried between the range of 70.900 to 76.000mgkg^{-1} for the liver and brain tissues respectively. While for EU fish origins, the concentrations were varied between the range of 66.542 to 80.358mgkg^{-1} for the skin and gills tissues respectively. The concentrations in the tissues of RS fish origins revealed high accumulation of Hg in the brain tissues (76.000mgkg^{-1}) and least was shown in the liver tissues (70.900mgkg^{-1}). For EU tissues, gills exhibit higher accumulations (80.358mgkg^{-1}) and least was shown in the skin tissues (66.542mgkg^{-1}). Brain is a vital organ in fish that acts a major storage of mercury (Mieiro*et al.*, 2009). Fish brain serve as major critical target of mercury in the environmentally exposed fish (Mieiro*et al.*, 2011). Mercury gets into the fish through ingestion or absorption via skin or gills from the surrounding water. The high concentrations of Hg shown with the gills of EU species were due to direct exposure between the gills tissues and the surround water body. However, the accumulation

levels of Hg with the entire tissues of both RS and EU species were more pronounced and hence were above the concentrations reported by related studies on other fish species(Hjeltnes and Kiire, 1992; Lillian and Lorraine 2012). They were also found to exceed the recommended safety limit by FAO (2003).

The results presented for both RS and EU tissues showed non-significant differences ($p<0.05$) across the entire batches with the exception of the batches of bone tissues. This implies that Hg contamination were highly homogeneous across the entire batches due to impacts of similar sources. While on the other hand, the results of correlation analysis shows pronounced significant ($p<0.05$) positive correlation across the batches of RS tissues.Therefore, this implies that contamination with Hg was due to impactsof similar sources. Also the profound significant ($p<0.05$) positive correlation shown among EU tissues indicate that, Hg contaminations were from similar sources.

5.10 IronConcentrations in Tissues of *Scomber scombrus*Species

The results of Fe concentrationsfor *Scomber scombrus* species shows thatthe concentrations recorded in the tissues RS speciesvaried between the range of 11.443 to 375.93mgkg^{-1} for the muscles and liver tissues respectively. While the concentrations recorded in the tissues of EU species ranged between the values of 15.893to 225.250mgkg^{-1} for the skin and gills tissues respectively. With regards to both RS and EU fish species, higher concentrations of Fe were found in the liver (375.930mgkg^{-1}) and gills (225.250 mgkg^{-1}) tissues respectively. While, the lower Fe accumulations were shown in the muscles (11.443mgkg^{-1}) and skin (15.893mgkg^{-1}) tissues of RS and EU fish species respectively. High levels of Fe shown in the liver tissues were similar to246mgkg^{-1}, 200.860mgkg^{-1} of Fe in fish liver reported by Hjeltnes

and Kiire (1992) and Erhan *et al*. (2004),respectively. Also, Fathi *et al*. (2012) showed*A. thalassinus* and *P. anea* recorded 1007.100mgkg^{-1} and 1975.000mgkg^{-1}in the liver tissues, respectively.Similarly to *Trachurus trachurus*species, *Scomber Scombrus* species also stored high concentration of Fe in the liver tissues. The higher level of the Fe in the liver relative to other tissues may be attributed to the high coordination of Fe with metallo-thionien protein (Uzairu *et al.,* 2009). Kumar and Mukherjee (2011) and Marioara *et al*. (2012) found in their studies that liver tissues accumulates Fe more than any other tissues. With respects to EU tissues, gills were found to accumulate high levels of Fe.This is similar to the 538.600mgkg and 299.500mgkg^{-1} in the gills tissues reported by Fathi *et al*. (2012). However, considerable levels of Fe were accumulated in the kidneys and intestine than the skin, brain and bone. The lowest level of Fe shown in the brain and bone tissues, were due to the fact that they are not active tissues. Generally, the level of Fe in all the tissues of both RS and EU zones were higher than the safety limitsof 0.8mgkg^{-1}recommended by CCFAC (2011).

From the results of statistical analysis(ANOVA) for *Scomber scombrus* species, non-significant differences ($p<0.05$) are shown with respects to entire batches of both RS and EU fish species. These were resulted due to homogeneity in the source of Fe contaminations. While the results of correlation analysis with regards to both RS and EU tissues shows significant ($p<0.05$) positive correlation were recorded across the entire batches and this coincide with the results of statistical analysis, which indicates that Fe contaminations were as a result of similar sources.

5.11 NickelConcentrations in Tissues of *Scomber scombrus*Species

Nickel results for *Scomber scombrus* species shows that, the concentrations recorded across the tissues of RS fish speciesvaried between the range of 6.042to 7.017mgkg^{-1} for the

muscles and kidney tissues respective. Whereas for EU tissues, the accumulation ranged between the values of 8.108 to 9.067mgkg^{-1} for the liver and gills tissues respectively. RS fish species showed high Ni accumulation in kidney tissues with a concentration of 7.017mgkg^{-1}. Kidney is regarded as the major gateway for heavy metal detoxification in the body of fish. While, the lowest levels of Ni in muscle tissues of RS fish species observed in this study coincides with the findings of trace metals bioaccumulation in several fish species by (Han *et al*., 1998; Erhan *et al.*, 2012; Israa *et al*., 2013). In contrast, gills of EU tissues shows significant high levels of nickel accumulations. Generally, the gills perform the function of respiration and are directly in contact with the metals present in the surrounding water and thus, concentrations of nickel in gills reflect itsimmediate concentration in the water body where the fish lives (Uzairu *et al.*, 2009). However, liver tissues with respect to EU fish species shows low level of Ni accumulation as compared to others. Also, the level of Ni found in the brain and bonetissues was less pronounced compared to other tissues and this could be as a result of low Ni mobility. However, the concentrations of Ni in the tissues with regards to both fishing zones were above the concentrations reported on marine fish (Khaled, 2004; Kojadinovic *et al.*,2007; Damodhar and Vikram, 2012) and were also found above the safety limits of 0.2mgkg^{-1}recommended by WHO (2005).

The results of statistical analysis (ANOVA) for *Scomber scombrus* species showed non-significant differences ($p<0.05$) in all the batches of both RS and EU fish species. Therefore, the similarities of Ni uptakes across the entire batches, implies that contaminations with Ni were due to impacts of related source. From the results of correlation analysis, the significant ($p<0.05$) positive correlation shown with respects to both RS and EU fish species implies, the route for Ni contamination are strongly from similar sources of contaminations.

5.12 Cadmium Concentrations in Tissues of *Trachurus murphyi* Species

The results presented for *Trachurus murphyi* species showedthe concentrations of Cd in the batches of RS fish speciesvaried between the range of 1.400 to 36.650mgkg^{-1} for the muscles and liver tissues respectively. While for EUfish species, the concentration ranged between the values of 2.175 to 37.867mgkg^{-1} for the muscles and liver tissues respectively. Accumulations of Cd intissues of both RS and EU fish species arefound highly in the liver tissues. However, lower accumulations are found in the muscles tissues. This pattern of Cd accumulation was found similar to the reported studies by Canli *et al.* (1997), Lopa and Adhikari (2006), Asegbeloyin *et al*. (2010), Shahat *et al*. (2011) and Muniyan and Ambedkar (2012). The higher concentration of Cd in the liver relative to other tissues can be attributed to the high coordination of Cd with metallo-thionein protein (Uzairu *et al.,* 2009; Boominathan *et al.*,2014). In addition, the liver is the principal organ responsible for detoxification, transportation, and storage of toxic substances.It is also an active site of pathological effects induced by contamination (Rauf *et al.*, 2009).The low concentrations of Cd found in the muscles tissues were due to the fact that it is an inactive tissue. This was in lined with some studies that foundfish muscles isan inactive tissues and hence it accumulate less Cd (Wim *et al.*, 2007; Fathi *et al.*, 2012; Amtul *et al.*, 2013). The level of Cd found in muscles tissues are mostly determine by its concentrationsin the liver tissues (Amtul *et al.*, 2013). The more accumulative of Cd in the liver tissues, the lower chance of its mobility to the inactive tissues such as muscles and bones tissues (Shariati *et al.*,2012; Amtul *et al.*, 2013; Nadeem *et al.*, 2014).

The concentration of Cd found in the intestine and kidneys tissues were higher than the level found in skin, gills, brain and bone tissues. This is because intestine and kidneys tissues are involved in the process of metabolism (i.e. in the absorption, storage and excretion of ingested

feed during the process of digestion by the fish)Shariati *et al.*,2012. Ahmad and Bibi (2010) found that, Cd accumulations in the storage tissues of fish were due to presence of biding protein. Similarly the study by Wim *et al.* (2007) showed that, absorption of Cd from the mouth or gills via gastrointestinal tract was mainly accumulates in the liver and kidneys tissues. Generally the concentration of Cd found in tissues of *Trachurus murphyi* across the batches ofRS and EU fish species are more pronounced and also found above the levels reported in tissues ofsome wild captured fish species (Gregory *et al.*, 2005; Bervoets *et al.*,2013). The high levels of Cdfoundacross the tissues of both RS and EU fish species were more pronounced and found above the safety limit of 0.1mgkg^{-1}specified by FAO (2003).

The ANOVA results showed significant differences ($p<0.05$) among the batches of RS tissues (skin, muscle, gills and brain) gives an indication that, contaminations with Cd are due to the impacts of contaminants from non-related activities. While, the non-significant differences ($p<0.05$) among other batches (liver, intestine, kidneys and bones) implied, contamination with Cd are due to impact of related sources of activities leading to homogeneous contaminations cross the batches. While for EU fish species, the non-significant differences ($p<0.05$) shown in the batches impliedcontaminations with Cd are strongly from the impact of related sources of activities.Hence the entire batches are said to be homogeneously contaminated. Also theresults of correlation analysis showedsignificant ($p<0.05$) positive correlations among most tissues implies, the uptake routes of Cd were from similar sources of contaminations.Whilesignificant ($p<0.05$) negative correlations shown among the other tissues indicate that, the uptakes of Cd involved impacts of contaminant from non-related sources.The ANOVA and correlation analysisresults showedCd contaminations inRS and EU fishing origins are strongly from the impacts of non-related sources.

5.13 Lead Concentrations in Tissues of *Trachurus murphyi* Species

Results presented for *Trachurus murphyi* species showed Pb accumulations in the batches of RS fish species were ranged between the values of 7.542 to 10.825mgkg^{-1} for the muscles and kidney tissues respectively. While for EU fish species, the accumulations in the batches varied between the range of 17.200 to 20.225mgkg^{-1} for the bone and skin tissues respectively. Leadaccumulations recorded for RS and EU fish species found higher was in the kidneys (10.825mgkg^{-1}) and skin (20.225mgkg^{-1}) tissues respectively. While, low accumulations was found in the muscles (7.542mgkg^{-1}) and bones (17.200mgkg^{-1}) tissues respectively. Kidney is an organ next to liver that plays a vital role in the process of digestion anddetoxification of Pb by the liver tissues in turns accumulation in the kidneys (Yousafzai *et al.*,2009). The higher concentrations of Pbfound in the kidneys relative to liver tissues for RS fish species indicates that, liver under goes more detoxification process and this is found similar to study reported by Yilmaz *et al.* (1997).

In contrast, the low accumulation of Pbas shown in the muscles tissues was found similar tostudy that showed muscles tissues is an inactive tissues and cannot accumulates Pb as it does with the active tissues (Erhan *et al.*, 2004; Simon *et al.*, 2013; Jalal *et al.*, 2013). The intestine, liver and kidneys are regarded as organs that are involve in the digestion process and this parts are referred to as target active organs where bioaccumulation of Pb can takes place (Simon *et al.*, 2013).Also, the skin and gills tissues were shown to accumulate high level of Pb because they are directlyin contact with the Pb in the surrounding water body (Vesna *et al.*, 2010; Ezekiel *et al.*,2012; Saima *et al.*, 2013). Generally, the concentrations of Pb found in the this study was

higher than the results of*Trachurus murphyi* found in study by Ikem *et al.* (2003) and Ogundiran *et al.* (2014) and also higher than the safety limits of 0.4mgkg^{-1} recommended by FAO (2003).

The results of ANOVAfor Pb in tissues of *Trachurus murphyi* species showed significant differences ($p<0.05$) in the batches of RS tissues (gills, intestine, kidneys, bone and brain) and this implied impacts of different sources of activities that govern the contaminations of Pb. For EU tissues non-significant differences ($p<0.05$) are dominantly shown across the batches and these implied homogeneity in the sources that leads to Pb contaminations across the batches. Also, the results of correlation analysis showed significant ($p<0.05$) positive correlationin the tissues of RS species indicates Pb contamination are due to impact of similar sources. While for EU tissues, the significant ($p<0.05$) positive and negative correlations among tissuesshowed contamination with Pb are related to contributions from different sources of activities.

5.14 Merury Concentrations in Tissues of *Trachurus murphyi* Species

The results of Hg presented for *Trachurus murphyi* species showedthe concentrations in batches of RS fish speciesvaried between the range of 102.950 to 115.210mgkg^{-1} for the muscles and liver tissues respectively. While for EU fish species,the concentrations ranged between the values of 78.717 to 86.483mgkg^{-1} for the gills and intestine tissues respectively.Higher accumulations of Hg for RS fish species are found in the liver tissues with concentration of 115.210mgkg^{-1} and lowest accumulations in the muscles tissues with concentration of 102.950mgkg^{-1}). While for the EU tissues, higher accumulation are found in intestine tissues with concentration of 86.483mgkg^{-1} and lowest accumulations in gills tissues with the concentration of 78.717mgkg^{-1}. The major route of Hg contamination in fish are due to ingestion of contaminated feed(Marcela *et al.*, 2008). While other channels of contaminations involves

absorption via skin or gills from the surrounding water body (Marcela *et al.*, 2008). The high levels of Hg in the liver tissues of RS fish species were attributed to the high coordination of Hg with the metallo-thionein protein present in the liver.The liver is the principal organ responsible for the detoxification, transportation, and storage of toxic substances and it the active site of pathological effects induced by contamination (Marcela *et al.*, 2008).). However, lower levels of Hg in the muscles tissues of RS fish species was as a result of its role as an inactive tissues.

While for EU tissues, intestines tissues was found with thehigh concentrations of Hgand lower concentrations was found in the gills tissues. The higher concentrations shown with intestine tissues are related to its role in the digestion process. Naoko *et al.* (2012) found that, intestine tissues is one of the most active sites in fish that accumulations high Hg because of its role in the digestion process. Digestive tissues are referred to as target or active organs where higher Hg bio-accumulation are found due to presence of binding mucus and metallo-thionein protein (Naoko *et al.*, 2012). Although,the high level of Hg found in theother tissues, are largely related to contamination from the feed of the fish (Daniele *et al.*, 2009; Ahmed and Bibi, 2010). However, the concentrations of Hgfound in all the tissues of *Trachurus murphyi*for both RS and EU fish species were above the levels reported in the studies by Tulay *et al*. (2014) Haluk *et al.* (2012) and Ekeanyanwu *et al.* (2012) and as well were above the safety limits of 0.5mgkg^{-1} recommended by FAO (2003).

From the results of statistical analysis (ANOVA) for *Trachurus murphyi* species showed significant difference ($p<0.05$) inthe batches of brain tissues of RS fish species. Whilethe non-significant differences ($p<0.05$) shown in the batches of RS and EU tissues indicates contaminations with Hgare due to impacts of similar sources of activities. However, the results of correlation analysis for both RS and EU with the results of ANOVA and this imlied Hg

contaminations are due to impacts of similar contamination sources. Hence, the route of Hg uptake across the batches are homogeneously.

5.15 Iron Concentrations in Tissues of *Trachurus murphyi* Species

The results presented for *Trachurus murphyi* species showed, the Fe concentrations in the batches of RS fish species ranged between the values of 48.417 to 818.05mgkg^{-1} for the muscles and kidney tissues respectively. While for EU fish species, the concentrations ranged between 17.708 to 431.84mgkg^{-1} for the muscles and liver tissues respectively. Higher accumulations of Fe in tissues of RS and EU fish species are found in the kidneys and liver tissues with concentrations of 818.05mgkg^{-1} and 431.84mgkg^{-1} respectively. The lower Fe accumulations in tissues of both RS and EU species are found in the muscles tissues with concentrations of 48.417mgkg^{-1} and 17.708mgkg^{-1} respectively. Liver as the principal organ for storage, also stores iron to a considerable higher levels in fish tissues. The higher level of Fe in the liver tissues are attributed to the high coordination of Fe with the metallo-thionein protein (Ozgur *et al.*, 2014). Also, high concentrations of Fe are shownin the kidneys tissues and this is due to excess detoxification by the liver tissues. The concentration of Fe found in the gills would fairly entail the level of Fe present in the surrounding water body where the fish lives. Because gills tissues are directly in contact with the iron present in the water body. Generally, the level of Fe found in all the tissues of both RS and EU fish species are higher than the safety limits of 0.8mgkg^{-1}, recommended by CCFAC (2011).

The ANOVAresults for Fe in tissues of *Trachurus murphyi* showedsignificant differences ($p<0.05$) was found in the batches of skin tissues forRS fish species and this however, are due to differences in the sources of contamination.While other batches showed non-significant differences ($p<0.05$) and this implied the sources of contaminations are due to similar sources of

activities. Whereas for EU tissues, the entire batches presented non-significant differences ($p<0.05$) in the accumulation of Fe and this was as a results of homogeneous contaminations due to impacts of related sources of activities. Also the results of correlation analysis shows significant ($p<0.05$) positive correlationin tissues of both RS and EU fish species. Thisimplied that uptakes of Fe was from similar sources of contamination.

5.16 Nickel Concentrations in Tissues of *Trachurus murphyi* Species

The results presented for *Trachurus murphyi* showed concentrations of Ni inthe batches of RS fish speciesvaried between the range of 0.350to 3.775mgkg^{-1} for the bone and kidney tissues respective. While for EU fish species, the concentration ranged between 2.500to 4.208mgkg^{-1} for the intestine and kidneys tissues respectively.Nickel accumulation in tissues of both RS and EU fish species shows higherconcentration in the kidneys tissues with values of 3.775mgkg^{-1} and 4.208mgkg^{-1} respectively. While lower Ni accumulations are found in the boneand intestine tissues with values of0.350mgkg^{-1}and 2.500mgkg^{-1}for RSand EU fish species, respectively. Kidney tissuesis considered as one of the major organ of Ni accumulation due to high coordination with metallo-thionein protein (Sahar *et al.*, 2014). The high concentrations of Ni accumulationfound in the kidneys relative to other tissues was coincide with the reportsby Sahar *et al*. (2014).Similarly the study by Shaikh (2013) on*Cirrhina mrigala* species showed Ni accumulations was highly found in the kidney tissues.Also high concentration of Ni were accumulatedin the skin, gills, liver and brain tissues of both RS and EUfish species. The presence of concentration of Ni in the gills tissues because it perform the function of respiration and are directly in contact with the water body. Thus, concentrations of Ni found in gills tissues reflects the levels that are present in the surrounding water body (Uzairu *et al.*, 2009; Sahar *et al*., 2014).

In contrast, EU tissues showed lower Ni accumulations are found in the intestine tissues. This should be as a result of high detoxification process that took place within the intestine (Erhabor and Obasohan, 2011; Shaikh *et al.*, 2013). Generally the concentrations of Ni shown in tissues of both RS and EU fish species were above the safety limit of 0.2mgkg^{-1}stated by WHO (2005).

The results of statistical analysis (ANOVA) for Ni in batches of *Trachurus murphyi* species showed significant differences ($p<0.05$) in batches of RS tissues (skin, muscles, gills, liver and bone) and this implied there is impacts of different sources of activities. On the other hand, the EU tissues recorded non-significant differences ($p<0.05$) with all the batches and these also implied homogeneity in the sources that leads to Ni contamination. Also the results of correlation analysis showed significant ($p<0.05$) positive correlationin most of the tissues which implieduptake of Niare from similar sources of contaminations.

5.17 Profile of MetalsConcentration in Tissues of Fish Species

The profile of heavy metals in the analyzed tissues samples of *Trachurus trachurus Scomber scombrus* and *Trachurus murphyi* species were summarized in Figure 4.1 to 4.3. The trends in accumulation pattern are in these orders:

i. Fe > Hg > Pb > Ni > Cd (for *Trachurus trachurus* species)

ii. Fe > Hg > Pb > Ni > Cd (for *Scomber scombrus* species)

iii. Fe > Hg > Pb > Cd > Ni (for *Trachurus murphyi* species)

The accumulation patterns of heavy metals trends in tissues of*Trachurus trachurus* and *Scomber scombrus* species were found to showed similarity in order of distribution. While, for *Trachurus murphyi*species,the distribution portrays similarly as with the following metals; Fe,

Hg and Pb. But the only variation was noticed in the case for Ni in tissues of *Trachurus murphyi*species which show least accumulations. The results of Fe concentrations recorded for the entire tissues samples were extremely higher, as compared to the concentrations of other analyzed metals. The higher levels of Fe can be related to contributions from both natural and anthropogenic activities which has high impacts on the water body where these fish are grown. Natural activities such as Meteor rays deposition, is an example of frequent occurrence in the Mediterranean and Russian ocean that can contributes to level of Fe concentrations. Iron is an essential trace element required by all forms of life. The effects of high doses of Fe in animal, includes symptoms of initial depression, coma, convulsion, respiratory failure and cardiac arrest (Annune and Iyaniwura, 1993). Post-mortem examination reveals adverse effects on the gastrointestinal track with excess Fe intake, may result in siderosis (deposition of iron in tissues); in liver, pancreas, adrenals, thyroid, pituitary and heart (Taweel *et al.*,2012). These are characterized by initial depression, coma, convulsion, respiratory failure and cardiac arrest (Annune and Iyaniwura, 1993).

In contrast to Fe, Hg was second in metal accumulation in the tissues with respects to each of the studied fish species. Mercury as a non-essential element is not expected to have its uptake/elimination actively regulated and subsequently its tissue accumulations can vary in a wide range, reflecting fish exposure from its immediate environmental and feeding behavior (Capelli *et al.*,2008). Hence, Hg body burdens in bio-indicator species provide sensitive indications of aquatic pollution as well as the potential impact in human health (Kotze *et al.*,1999). However, the metal distribution within the body depends on both the fish species and the metal's properties (Gaspic *et al.*, 2002).

The profile results with respects to entire fish species showed accumulation of Pb. were highly shown. Lead is one of the major non-essentials elementsthat can be harmful to humans when ingested or inhaled, particularly to children under the age of six (6). Lead poisoning can cause a number of adverse human health effects, but is particularly detrimental to the neurological development of both adults and children.

In contrast, Cd distribution shows least accumulation across the tissues of *Trachurus trachurus* and *Scomber scombrus* species. While, in *Trachurus murphyi* species it appeared second to the least. The acute effects of Cd in humans through food exposure consist mainly ofeffects on the lung, such as pulmonary irritation (CalEPA). Chronic (long-term) inhalation or oral exposure to Cd leads to a build-up of Cd in the kidneys that can cause kidney disease (Eva *et al.*, 2014). Cadmium has been shown to be a developmental toxicant in animals, resulting in fetal malformations and other effects, but no conclusive evidence exists in humans. An association between Cd exposure and an increased risk of lung cancer has been reported from human studies, but these studies are inconclusive due to confounding factors (CalEPA). Animal studies have demonstrated an increase in lung cancer from long-term inhalation exposure to Cd. EPA has classified Cd as a Group B1, probable human carcinogen.

Excess Ni exposures at high concentrations were associated with lung and nasal cancer (Tom *et al.*, 2002). Nickel compounds are considered to be mutagenic as human cells treated with Ni compounds in vitro have shown an increase in chromosome damage or chromosome number, as well as DNA strand breaks, mutations and cell transformation. Thus, these compounds have mutagenic potential (Ekine *et al.*, 2012). Two chronic studies in rats have been reported that provide some insight into the carcinogenic potential of orally administered Ni, only one of which presented increases in the incidence of leukemia and of benign tumors of the

prostate and testis. The positive rat study however, was deemed of questionable relevance to humans due to the anatomical differences between human and rodent prostate (Ekine *et al.*, 2012).

High levels of heavy metals in the tissues of fish irrespective of the fishing zones. In general, the levels of the heavy metal varied significantly in different tissues of the fish and the concentration levels of all the investigated metals were considerably higher than the safety limits recommended by FAO (2003), CCFAC (2011) and WHO (2008) irrespective of the batch and fishing area.

5.18 Comparative Mean Concentrations of Cd in Tissues of FishSpecies

The graphical presentationin Fig 4.4for Cd accumulation in various tissues of the three (3) fish species shows that, *Trachurus murphyi* was found to presents higher level of Cd accumulations, followed by *Scomber scombrus* and the lowest accumulations was found in the tissues of*Trachurus trachurus*species.The higher levels of Cd shown with the entire tissues of *Trachurus murphyi* can be explained by their nature as being *benthopelagic* organisms that remain entirely bottom feeders. Bottom feeders are the most likely organisms that feeds on the depositscontaining the metals presents in the water body.However, thevariations of Cd accumulation in tissues of the species showed little accumulation of metals among*Trachurus trachurus, Scomber scombrus* and *Trachurus murpyi*with respects to RS and EU fish species.Although higher Cd accumulations recorded for each of the speciesare found in the liver tissues, followed by kidneys and then intestine tissues. Whereasthe skin, muscles, brain and bone tissues was found to showed comparable level of accumulations. Also the level of Cd accumulations across the entire tissues with regards to each species were higher than the safety limits stated by FAO (2003).

5.19 Comparative Mean Concentrations of Pb in Tissues of FishSpecies

As presented in Figure 4.5, Pb concentration in the tissues of the three fish species;showeda comparable accumulations across the entire tissues. However, the accumulations with respects to*Trachurus murphyi* species recorded for EU tissues were of high Pb concentrations compared tothe RS tissues. The high levels of Pbin EU fish origins indicates that Pb is more concentrated in the bottom sediment of the water body.While in the case of *Scomber scumbrus*species, higher accumulations were found in the tissues of RS fish origins and this implies waters of RS fishing zones were rich in Pb. This is support with the fact that the species *Scomber Scombrus* are mesopelagic organisms that remain in the meso-layer of the water body. Also for *Tracurus trachurus* species,EU tissues shows slightly higher levels of Pb accumulations than the corresponding tissues of RS fish species.The differences of Pb accumulation among fishing zones could be as a results of the impacts of natural and anthropogenic activities. However, the levels of Pbin the tissues with respects to each species were higher than the corresponding safety limits as specified by FAO (2003).

5.20 Comparative Mean Concentrations of Hg in Tissues of FishSpecies

The graphical presentation in Figure 4.6 for Hg accumulations in the tissues of fish species shows that, *Trachurus murphyi*recorded high level of accumulations than the*Scomber scombrus* and *Trachurus trachurus* species.Although RS tissues for *Tracurus murphyi* speciesaccumulates highly than the corresponding EU tissues. While the tissues of both RS and EU with respects to *Scomber scombrus* and *Trachurus trachurus* species show comparable levels of accumulations andthis implies that the sources of Hg contamination are distributed uniformly

throughout the water body. However, the accumulations ofHg recorded across the entire tissues with regards to each species were higher than the corresponding safety limits by FAO (2003).

5.21 Comparative Mean Concentrations of Fe in Tissues of FishSpecies

The graphical representation for Fe accumulation in Figure 4.7 for tissues of the three species showed comparable accumulations between*Trachurus murphyi* and *Trachurus trachurus* species and the least levels are found with*Scomber scombrus* species. All the three species shows that, the highest concentrations of Fe accumulations were shown in the liver tissues. Kidney tissues were the second highest in metals accumulation, followed by the intestine, while skin, muscles, brain and bone tissues showed comparable concentrations.

5.22 Comparative Mean Concentrations of Ni in Tissues of FishSpecies

The graphical presentation (as shown Fig 4.8above) for Ni accumulation in various tissues of the three (3) fish species showed that, *Trachurus trachurus* was found to presents the higher level of Ni accumulations, followed by *Scomber scombrus* and the lowest accumulations was found with*Trachurus murpyi* species. The fish *Trachurus trachurus*are *epipelagic* species that localized at the upper layer of the water body and feeds directly with the immediate sources as it dumps in the water. However, the variations of Ni accumulation among tissuesfor each of the speciesshowed EU tissues recorded significant higher accumulations than the corresponding RS tissues. Also the level of Niaccumulations shown across the entire tissues with regards to each species were higher than the safety limits by WHO (2005).

5.23 Risk Assessment Index

5.23.1 Risk assessment index for*Trachurus trachurus* species

The results of Cd HQ analysis for *Trachurus trachurus* showeda ratio value of less than one (1) was found for category A and values of greater than one (1) for category B and C, respectively. This implies that, the adult population(category A) would not be expose to high dose of Cd with the consumption of fish muscles. While the populations of fish consumers in category A and Bwould have risk of Cd exposure in the latter time. The DIM results for individual's daily loading of Cd were recorded with the values $2.656x10^{-4}$,$3.305x10^{-4}$ and $5.870x10^{-1}mgkg^{-1}day^{-1}$ for category A, B and C respectively. These values correspond to HRI results in which each of the categories recorded a ratio value of less than one (1) and this indicates that individuals with respects to each of the categories would not expose to high dose of Cd with the consumption of *Trachurus trachurus* fish muscles as specified for daily intake.

The results of Pb HQ analysis for *Trachurus trachurus*shows that a ratio values of greater than one (1) were found with each of the individual's category A, B and C respectively. This implies that, the entire population with respects to each individual's category would expose to high dose of Pbat unacceptable concentrations in the latter timewith the consumption of fish muscles. The DIM results shows that individual's daily loading of Pb were given as $6.139x10^{-3}$,$7.638x10^{-3}$ and $1.360x10^{-2}mgkg^{-1}day^{-1}$for category A, B and C respectively. This correspond to HRI with ratio of greater than one (1) shown for each of the individual's category. This indicates that, the entire categories would expose to hazard of Pb based on consumption of *Trachurus trachurus* fish muscles as specified for daily intake.

The results of Hg HQ analysis for *Trachurus trachurus*shows that, ratio values of greater than one (1) were found with each of the individual's categories. Hence the entire population

would expose to high dose of Hg at unacceptable concentrations in the latter time with the consumption of fish muscles. The results of DIM recorded for individual's daily loading of Hg are $2.871x10^{-3}$,$3.567x10^{-3}$ and $6.350x10^{-1}mgkg^{-1}day^{-1}$for category A, B and C respectively. This corresponds to HRI with ratio values of greater than one (1) with each of the individual categories. This implies that the individuals from any of the category would expose to hazard of Hg if consume *Trachurus trachurus* fish muscles of equal or above the recommended amountspecified for daily intake.

The results of Fe HQ analysis for *Trachurus trachurus*showed a ratio value of less than one (1) are found with each of the category A, B and C respectively. This implies that, the entire population of fish consumers would not expose to high level of Fe with the consumption of fish muscles in the latter time. The results of DIM shown for individual's daily loading of Fe are given as $2.164x10^{-2}$,$2.693x10^{-2}$ and $4.783x10^{-3}mgkg^{-1}day^{-1}$for category A, B and C respectively. These also correspond to HRI values of less than one (1) with each of the categories. However, the individuals from any of the category would not expose to high level of Fe if consume fish muscles based on the recommended daily intake as specified in this study.

The results of Ni HQ analysis for*Trachurus trachurus* showed a ratio values of greater than one (1) was found for category A and for category B and C showed a value of greater than one (1). This implies that, only adult population (category A) would not exposed to high dose of Ni.However, the populations of both children in category B and C would expose to high level of Ni in the latter time if continue to consume fish muscles as specified in this study. The results of DIM shown for each of the individual's daily loading of Ni were given as $5.433x10^{-3}$,$6.762x10^{-3}$ and $1.200x10^{-1}mgkg^{-1}day^{-1}$for category A, B and C respectively. These correspond to HRI values of less than one (1) found for each of the categories. This also indicates that individuals

belonging to any of the category would not expose to high dose of Ni with the consumption of fish muscles based on the recommended daily intake as specified in this study.

5.23.2 Risk assessment index for*Scomber scombrus* species

The results of Cd HQ analysis for *Scomber scombrus* shows that a ratio values of greater than one (1) was found for category A, B and C, respectively. This implies that, the population of fish consumers would exposed to high dose of Cd in the latter time, if they continue to consumefish muscles recommended for daily intake.The DIM results for individual's daily loading of Cd were recorded with the values given as $1.162x10^{-1}$, $1.482x10^{-1}$, and $2.633x10^{-1}$ $mgkg^{-1}day^{-1}$for category A, B and C respectively. These correspond to HRI ratio of greater than one (1) as shown for each of the individual's categories. The results also justify that individuals from any ofthecategorieswouldbeexposedtohighdoseofCd withtheconsumptionoffishmusclesofequalorabove the recommended daily intake values as specified in this study.

Similar to Cd, the results of Pb HQ analysis for *Scomber scombrus*shows that a ratio values of greater than one (1) was found for category A, B and C, respectively. This implies that, the population of fish consumers would exposed to high dose of Pb in the latter time, if they continue to consumefish muscles recommended for daily intake.TheresultsofDIMshowsindividual'sdaily loadingofPbare givenas$5.833x10^{-3}$,$7.559x10^{-1}$,and$1.289x10^{-2}mgkg^{-1}day^{-1}$ for category A, B and C respectively. These corresponds to HRI with the ratio values of greater than one (1) each of the three categories. This results indicate that each category of the individuals wouldexpose to the hazard of Pb metals with the consumption of *Scomber scombrus* fish musclesbasedontheamountrecommendedfordailyintake asspecifiedinthisstudy.

Also the results of Hg HQ analysis shows that a ratio values of greater than one (1) was found for category A, B and C respectively. This implies that, the population of fish consumers would exposed to high dose of Hg in the latter time, if they continue to consume*Scomber scombrus*fish muscles recommended for daily intake. The results of DIM recorded for individual's dailyloadingofHg were given as $3.887x10^{-2}$, $4.837x10^{-2}$,and$8.592x10^{-2}mgkg^{-1}day^{-1}$for category A, B and C respectively.ThesecorrespondtoHRIwith the values of greater than one (1), as shown with each of the individual categories. Thisindicatesthattheentirecategories of individualsconsuming*Scomber scombrus* fish muscles of equal or above the recommendedamount,would expose to thehazardofHg.

The results of Fe HQ analysis for *Scomber scombrus*shows that a ratio values of less than one (1) was found for category A, B and C respectively. This implies that, the population of fish consumers would not exposed to high dose of Fe in the latter time, if they continue to consume*Scomber scombrus*fish muscles recommended for daily intake. The results of DIM recorded for individuals daily loading of Fe were given as $8.689x10^{-3}$, $1.081x10^{-2}$,and$1.921x10^{-2}mgkg^{-1}day^{-1}$for category A, B and C respectively.ThesealsocorrespondtoHRI with values of less than one (1) as shown with each of the categories.However,theentireindividual'spopulationwould not be exposed to high level of Fe with the consumption of fish muscles based on the recommended daily intake as describedinthisstudy;otherwise,individualswouldbenefit fromFe assupplement.

The results of Ni HQ analysis for *Scomber scombrus*shows that a ratio values of less than one (1) was found for category A and B respectively. While category C was found with a ratio of greater than one (1).This implies that, the population of fish consumers from A and B category would exposed to high dose of Ni in the latter time, if they continue to consume*Scomber*

*scombrus*fish muscles recommended for daily intake.The results of DIM recorded for individual's daily loading of Ni were given as $3.906x10^{-3}$, $4.861x10^{-3}$, and $8.636x10^{-3}$ $mgkg^{-1}day^{-1}$for category A, B and C respectively. These correspond to HRI in which each of the categories shows a value of less than one (1). Hence, this indicates that individuals belonging to any categorieswouldnotbeexposedtohighdoseofNiwith the consumption of fish muscles based on the recommended daily intake as describedinthisstudy.

5.23.3 Risk assessment index for*Trachurus murphyi* species

The results of Cd HQ analysis for *Trachurus murphyi*shows that a ratioof greater than one (1) was found with each of the individual category A, B and C, respectively. This implies that, the entire population of fish consumers would be expose to high dose of Cd in the latter time. The results of DIM showed for individual's daily loading of Cd are given as $7.595x10^{-4}$, $9.504x10^{-4}$ and $1.688x10^{-2}$. These correspond to HRI with ratio values of less than one (1) shown for the category A and B respectively. While a ratio values of greater than one (1) is shown with category C. This indicate individuals in category C would expose to hazard of Cd with the consumption of fish muscles as specified for daily intake in this study.

The results of Pb HQ analysis for *Trachurus murphyi*shows that a ratio values of greater than one (1) are found with each of the category A, B and C respectively. This implies that, the population of fish consumers any of the individual's category would expose high dose of Pb in the latter time. The results of DIM shown for individual's daily loading of Pb are given as $5.480x10^{-3}$, $6.820x10^{-3}$ and $1.211x10^{-1}$. These correspond to HRI with ratio values of greater than one (1), shown for each of the individual's category. This indicates that the categories of the entire individuals would expose to high dose of Pb metals based on the consumption of *Trachurus murphyi* fish muscles as recommended for daily intake in this study.

The result of Hg HQ analysis for *Trachurus murphyi*shows that a ratio values of greater than one (1) are found with each of the individual's category A, B and C respectively. The results of DIM recorded for individuals daily loading of Hg are given as $3.932x10^{-2}$, $4.893x10^{-2}$ and $8.693x10^{-1}$. These correspond to HRI with ratio values of greater than one (1), shown with each of the individual's categories. Hence, this indicates that the entire categories of individuals would expose to high dose of Hg metals with the consumptions of *Trachurus murphyi* fish muscles as recommended for daily intake.

The results of Fe HQ analysis for *Trachurus murphyi*shows that a ratio value of less than one (1) was found for category A and B, respectively. While a ratio values of greater than one (1) was found for category C. This implies that, the population of fish consumers in category C would expose to high dose of Fe in the latter time if they continue toconsume fish muscles. The results of DIM shown for individual's daily loading of Fe were given as $1.412x10^{-2}$, $1.757x10^{-2}$ and $3.122x10^{-4}$. These correspond to HRI with ratio values of less than one (1) for all the categories. Hence,the population of individuals from any categories would not exposed to high dose of Fe, otherwise they may benefit with Fe as nutrient from the consumption of fish muscles as recommended for daily intake.

The results of Ni HQ analysis for *Trachurus murphyi*shows that a ratio value of less than one (1) was recorded for the category A and B, respectively. However, a ratio value of greater than one (1) was found with category C. This impliesthat, only the population of category C would experience high exposure of Niin the latter time with the consumption of fish muscles. The results of DIM recorded for individual's daily loading of Ni were given as $8.047x10^{-4}$, $9.993x10^{-4}$ and $1.779x10^{-2}$. These correspond to HRI with the ratio values of less than one (1) shown with all the categories. This indicate that the population of fish consumers in all the

categories would not expose to high dose of Ni with consumption of fish muscles recommended for daily intake.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Three major consumable imported frozen fish species (*Trachurus trachurus*, *Scomber scombrus* and *Trachurus murphyi*) sold in Zaria metropolis were extensively assessed for heavy metals; cadmium, lead, mercury, iron and nickel (Cd, Pb, Hg, Fe and Ni) contaminations in this study. The health status of human with respect to contaminations by heavy metals was evaluated on the basis of health quotient (HQ), daily intake of metals (DIM) and health risk index (HRI) analysis to justify the population and individual's index of risk partaken in the latter time on

consumption of these fish species. Based on the analysis obtained, the risk for human exposure to heavy metal contamination through fish consumption was significant.

6.2 Conclusion

The levels of the studied heavy metals in all the analyzed tissues (irrespective to fish species) were above their corresponding permissible limits recommended by FAO (2003), CCFAC (2011) and WHO (2005). The health risk indices of population of fish consumers regarding the consumption of fish muscles tissues showed the chance for lead and mercury exposure were highly significantthan for cadmium and then less risk were found with iron and nickel. However,the levels of metals in fish tissues regarding their import batches recorded significant variation and consumption of fish livers, intestine and kidneys were associated with high risk from ingestion of toxic metals at unacceptable concentrations. Generally, the risk of fish consumers for the studied heavy metals cannot be excluded in relation with the consumption of imported *Trachurus trachurus, Scomber scombrus* and *Trachurus murphyi* from RS and EU fishing origins; it reinforced the importance to define the regulatory thresholds taking into consideration, the fish consumption rate, in order to efficiently protectpossible exposurefrom hazardous heavy metals.

6.3 Recommendations

i. In Nigeria, importation and distribution of contaminated fish species across the country are still continues, despite the efficiency of food regulatory agencies.The food agencies should establish national food control goals, put into effect the strategic and operational activities necessary to achieve the goals (which are based on contaminated food).

ii. They should also revise and update the national food control strategy as needed; advise on policy matters including determination of priorities and use of resources; draft regulations, standards and codes of practice and promote their implementation towards formulation of risk assessment and management.

iii. The agency should consider the role of private analytical, inspection and certification services particularly for food export trade.

iv. The federal department of fisheries has to ensue that fish and fishery products imported into Nigeria should conform to international quality standards of wholesomeness as stipulated in the Sea Fisheries (Regulation of 1995) and enhancement of aquaculture practice across states and local government areas.

REFERENCES

Abdullah, E., Mehanna, E. E. and Meki, A. (2008). Evaluation of lead and cadmium levels in fresh water fish farms at Qassim region, K.S.A. *Journal of Agricultural and Veterinary Sciences, Qassim University*,**1(2):** 59 - 77.

Abolfazl, A. S. and Maryam, M. (2012). Comparison of mercury and cadmium toxicity in fish species from Marine water. *Research Journal of Fisheries and Hydrobiology*,**7(1):** 14 - 18.

Abowei, J. F. N. and Ekubo A.T. (2011). A review of conventional and unconventional feeds in fish nutrition. *British Journal of Pharmacology and Toxicology*, **2(4):** 179 – 191.

Ada, F. B., Ekpenyong, E. and Bayin, P. B. (2012). Heavy metal concentration in some fishes (Chryscihthys nigrodigitatus, Clarias gariepinus and Oreochromis nitoticus) in the great Kiva River, Cross-River state, Nigeria. *Global Advanced Research Journal of Environmental Science and Toxicology*,**1(7):** 184 – 189.

Adefemi, S.O., Asaolu, S. S. and Olaofe, O. (2008). Major elements in fish (Illisha Africana), sediments and water from selected Damsin Ekiti State. *Reserve Journal of Environmental Science*,**2(1):** 63 - 67.

Aderinola, O. J, Clarke, E. O., Olarinmoye, O. M., Kusemiju, V. and Anatekhai, M. A. (2009). Heavy Metals in Surface Water, Sediments, Fish and Perwinkles of Lagos Lagoon. *American-Eurasian Journal of Agriculture and Environmental Sciences*,**5(5):** 609 - 617.

Afiza, A. S., Faizal, W. I. Nurliyana, M.H. Laili, S. and Amirah, M. N. (2013). Human health risk assessment of metal contamination through consumption of fish.*Journal of Environment Pollution and Human Health*, **1(1):** 1 - 5. http://dx.doi:10.12691/jephh-1-1-1

Aftab, A. S., Iram, A. S., Mudassir, S., Pir, A., Muhammad. Y., Ali, M. and Muhammad, Z. (2013). White muscle as a bio-indicator of cadmium (Cd) pollution across Kalpani River Mardan, Khyber Pakhtunkhwa.*Pakistan International Journal of Biosciences*,**3(10):** 105 - 116. http://dx.doi.org/10.12692/ijb/3.10.105-116

Agency for Toxic Substances and Disease Registry (ATSDR)(1999). Toxicological profile for lead. Atlanta: US Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/tp13.pdf

Agency for Toxic Substances and Disease Registry (ATSDR) Mission Statement, (2001).Toxicologic Profile for heavy metals. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Agency for Toxic Substances and Disease Registry (ATSDR), (2008). ATSDR Medical Fact Sheet; Division of Toxicology and Environmental Medicine ToxFAQs; Accessed November 19, 2014. http://www.atsdr.cdc.gov/tfacts5.pdf.

Ahmed, M. K., Islam, M. M., Bang, S., Kyoung-Woong, Kim and Jannat, M. (2010). Heavy metals in frozen and canned marine fish of Korea. *Journal of scientific research*,**2(3):** 549 - 557.

Ahmed, M. S. and Bibi, S. (2010). Uptake and bioaccumulation of water borne lead (Pb) in the fingerlings of a freshwater Cyprinid, Catla Catla L. *The J. of Animal and Plant Sciences*, **20(3):** 201 - 207.

Ahsan, K., Muhammad, S., Musarrat, S., Aftab, Alam Sthanadar, Douglas P., Chivers, Ali andMuhammad, Yousafzai (2014).A comparative study of bioaccumulation of heavy metals in twofresh water species, Aorichthys seenghala and Ompokbimaculatous at River Kabul, Khyber Pakhtunkhwa, Pakistan. *Journal of Biodiversity and Environmental Sciences*,**4(3):** 40-54.

Akan, J. C., Abdulrahman F. I., Sodipo, O. A. and Akandu, P. I. (2009). Bioaccumulation of some heavy metals of six fresh water fishes caught from Lake Chad in Doron Buhari,

Maiduguri, Borno state, Nigeria. *Journal of Applied Sciences in Environmental Sanitation*,**4(2):** 103 - 114.

Akintunde, N. A. (2012). Fish species diversification in aquaculture for the success of the agriculture transformation agenda: The Role of Tilapia Production, Fisheries Society of Nigeria, Annual Public Lecture.

Allen, G. T, Gill, S. M. and Martynov, V. G. (1995). Heavy metals burdens in nine species of freshwater and anadro- mous fish from the Pechora River, Northern Russia. *Science Total Environment*, **161:** 653 - 659.

Allison, E. H., Perry, A. L., Badjeck, M. C., Adger, W. N., Brown, K., Conway, D., Halls, A. S., Pilling, G. M., Andrew, N. L. and Dulvy, N. K.. (2009). Vulnerability of national economies to the impacts of climate change on fisheries. *Fish and Fisheries*, **1(10):** 73 - 196.

Amtul, B. T., Sidra, B., Abdullah, Y. and Saleha, M. (2013). Heavy metals concentrations in water, sediment and fish in river Sutlej at Sulemanki Headworks. *Pakistan Journal of Zoology*, **45(6):** 1663 - 1668.

Annune, P. A. and Iyaniwura, T. T. (1993). Accumulation of two trace metals in tissues of freshwater fishes, Clarias gariepinus (Burch) and Oreochromis niloticus (Trewavas). *Journal of Aquatic food product and technology*, **1(2):** 5 - 18.

Asegbeloyin, J. N., Onyimonyi, A. E., Ujam, O. T., Ukwueze, N. N. and Ukoha, P. O. (2010). Assessment of toxic trace metals in selected fish species and parts of domestic animals. *Pakistan Journal of Nutrituion*,**9(3):** 213 – 215.

Ashoka, S., Peake, B. M., Bremner, G., Hageman, K. J. and Reid, M. R. (2009). Comparism of digestion methods for ICP-MS determination of trace elements in fish tissues. *Analytica Chimica Acta*,**653:** 191-199.

Aynur, C. (2009). Mercury in the Izmir Bay: An assessment of contamination, *Journal of marine system*, **61(7):** 67 - 78.

Babalola, A. F., Olusola, A. O., Akande, G. R., Ezekiel, M. O., Adeleke, T. A. and Ozor, P. A. (2014). Mercury, cadmium content and organoleptic quality of some canned seafood sold in Nigerian market. *Journal of Agriculture and Veterinary Science*, **7(4):** 14 – 17.

Babatunde, A. M., Waidi, O. A. and Adeolu, A. A. (2012). Bioaccumulation of heavy metals in fish (Hydrocynus forskahlii, Hyperopisus, Bebe occidentalis and Clarias gariepinus) organs in downstream ogun coastal water, Nigeria. *Transnational Journal of Science and Technology*, **2(5):** 119 – 133.

Baker, R. F., Blanchfield, P. J., Paterson, M. J., Flett, R. J., and Wesson, L. (2004). Evaluation of non-lethal methods for the analysis of mercury in fish tissue. Trans. *American Fish Society,***1(33):** 568 - 576.

Bat, L., Gokkurt, O., Sezgin, M., Ustun, F. and Sahin, F. (2009). Evaluation of the Black Sea Land basedsources of pollution the coastal region of Turkey. *The Open Marine Biology Journal*, **3(1):** 112-124.

Beetseh, C. I. and Abrahams, J. O. (2003). Dangers of bioaccumulation of some heavy metals consumed in sardine and mackerel (ice fish) in Benue state Nigeria. *Civil and Environmental Research*, **3(5):** 60 – 65.

Bervoets, L., Knapen, D., De-Jonge, M., Van-Campenhout, K. and Blust, R. (2013) Differential hepatic metal and metallothionein levels in three feral fish species along a metal pollution gradient. *Plos One*, **8(3):**60805 - 60811.http://dxdoi:10.1371/journal.pone.0060805

Boque, R.,Maroto, A., Riu, J. and Rius, X.F. (2002). Validation of analytical methods. *Grasasy Aceites*, **1(53):** 128-143.

Boominathan, M., Ramalingam, M. and Karuppiah, V. (2014). Metallothionein expression in marine catfish Arius Arius liver on exposure to cadmium using immunohistochemistry and western blot. *International Journal of Pharmaceutical Sciences*, **6(2):** 818 – 821.

Borgmann, U., Couillard, Y., Doyle, P., and Dixon, D. G., (2005). Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness.*Environmental Toxicology and Chemistry*, **24(3):** 641–652.

Brown, F. M., Balls, J. andPaul, W. (1997). Trace metals in fish and shellfish from Scottish waters. Scottish Fisheries Research Report Number 60. The Scottish office agriculture, environment and fisheries department, pp. 1 – 37.

Bushra, K., Hizbullah, K., Said, M., andTariq, K. (2012). Heavy metals concentration trends in three fish species from shah Alam River (Khyber Pakhtunkhwa province, Pakistan). *Natural Environmental Science*, **3(1):** 1 - 8.

California Environmental Protection Agency (CEPA) (1997). *Technical Support Document for the Determination of Non-cancer Chronic Reference Exposure Levels. Draft for Public Comment.* Office of Environmental Health Hazard Assessment, Berkeley, CA.

Campel, P. H. and Nikel, H. (2006).Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and C*larias garicpinus*, from the Olifants River, Mpumalanga, South Africa. *Water SA*, **25:** 99 – 110.

Canli, M., Stagg, R. M. and Rodger, G. (1997). The induction of metallothionein in tissues of Norway Lobster, Nephrops Norvegicus following exposure to cadmium, copper and zinc:

the relationship between metallothionein and the metals. *Environmental Pollution*, **1(96):** 343 - 350.

Canli M., and Atli, G., (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental Pollution***1(21):** 129 – 136.

Capelli, R., Das, K., De-Pellegrini, R., Drava, G., Lepoint, G., Miglio, C., Minganti, V. and Poggi, R. (2008).Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. *Total Environmenyt*, **390(2):** 569-578.

CCFAC (Codex Committee on Contaminants in Foods) (2011). Food Standards Programed: Joint FAO/WHO. Fifth Session, The Hague, The Netherlands; 21 – 25th March.ftp://ftp.fao.org/codex/meetings/CCCF/cccf5/cf05_INF.pdf

Chiara, C., Giovani, A., Maria, F., Caterina, L., Roberto, F., Salvatore, S. and Margherita, C. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. *Food and Chemical Toxicology*, **1(53):** 33 – 37. http://dx.doi.org/10.1016/j.fct.2012.11.038

Clarkson, T. W. (1997). The toxicology of mercury. *Critical Reviews in Clinical Laboratory Sciences*,**34(3):** 369 - 403.

Cordova, J., Bahamonde, R. andCatasti, V. (2008). Jack mackerel (*Trachurus murphyi*, Nichols, 1920) acoustic survey in the central coast of Chile, Paper presented to the SPRFMO Chilean Jack Mackerel Workshop, Santiago, 30th June -4th July 2008.

Collette, B. B. (1986). Scombridae. In Fishes of the North-eastern Atlantic and the Mediterranean Volume III (Whitehead P. J. P., Bauchot M. L., Hureau J. C., Neilsen, J., and Tortonese, E., eds.) UNESCO, Paris, pp 981-997.

Copet, C., Giovani, A., Maria, F., Caterina, L., Roberto, F., Salvatore, S., and Margherita, F. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea:Consumption advisories. *Food Chemical Toxicology***53:** 33-37. http://dx.doi.org/10.1016/j.fct.2012.11.038

Commission Decision 2002/657/EC. (2002). Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. European Union. faolex.fao.org/cgi- http://dx.bin/faolex.exe?rec_id=040116,accessed 21 November, 2014.

Dalhatu, M. and Ala, A. L. (2010). Analysis of fish demand in Sokoto metropolis, Sokoto, Nigeria, *Nigerian Journal of Basic and Applied Science*,**18(2):** 154 - 159.

Damodhar, U. and Vikram, M. (2012). Assessment of heavy metals concentrations in water and four fish species from the Uppanar River at Cuddalore (Tamil Nadu, India). *Continental Journal of Environmental Sciences*, **6(3):** 32 – 41.

Daniel, N. W. and Wendy M. D. (1997). Alterations in neurobehavioral responses in fishes exposed to lead and lead-chelating agents. *American Zoology*, **1(37):** 354 - 362.

Daniele, K., Elisabete, F., Albuquerque, P., Ana, Carolina, M., Iozzi, D., Gustavo, L. F., Rafael, P.,Christina, W., Castelo, B. and Olaf, M. (2009). Mercury distribution in different tissues and trophic levels of fish from a tropical reservoir, Brazil. *Neotrophical Ichthyology*, **7(4):** 751 - 758. http://dx.doi.org/10.1590/s1679-62252009000400025

Denise, G., Bruno, L. B., Maria, F. H. C. and Fernando, B. J. (2012). Evaluation by ICP-MS of essential, nonessential and toxic elements in Brazilian fish and seafood samples. *Food NutritionScience*,**3(1):** 1252-1260.http://dx.doi.org/10.4236/fns.2012.39165

Diagomanolin, V., Farhang, M., Ghazi-Khansari, N and Jafarzadeh, M. (2004). Heavy metals (Ni, Cr, Cu) in the Karoon waterway river, Iran. *Toxicology Letter*, **151(1):** p63 – 71.

D'Ilio, S., Petrucci, F., D'Amato, M., Di Gregorio, M., Senofonte, O. and Violante, N. (2008). Method validation for determination of arsenic, cadmium, chromium and lead in milk by means of dynamic reaction cell inductively coupled plasma mass spectrometry. *Analytica Chimica Acta* **624:** 59-67.

Domingo, L. J., Falcoa, M., Llobet, J. and Ana, B. (2006). Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. *Journal of Agricultural Food Chemistry*,**1(54):** 6106 - 6112.

Durali, M., Zafer, D., Mustafa, T. and Mustafa, S. (2010). Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey. *Food and Chemical Toxicology***1(48):** 865 **–** 870.

Eisler, R. (1985). Cadmium hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. fish and wildlife service biological report 85 (1.2), contaminant hazard reviews report No.2, pp46.

Eisler, R. (2000). Hand book of chemical risk assessment: Health hazard to human, plant and animals. Vol. 1, Boka raton, lewis publisher.

Ekeanyanwu, C., Raphael, O., Augustina, C. andEtienajirhevwe, O. k. (2012).Trace metals distribution in fish tissues, bottom sediments and water from Okumeshi River in Delta State, Nigeria. *Environmental Research Journal*, **5(1):** 6 - 10. http://dx.doi:10.3923/erj.2011.6.10

Ekine, D. I., Albert, C. O. and Peregba, T. A. (2012). Expenditure pattern for beef consumption in selected households in southern Nigeria. *Developing Country Studies*, **2(7):** 1 - 7.

Ekpenyong, E., Abraham, J. T., Ndome, C. B., Okon, O. E., and Ibor, C. I. (2013). Capillariasis in Chrysicthys Nigrodigitatus (Catfish), Cynoglossus Senegalensis (Sole) and Pseudotolthus Elongatus (Bobo Croaker) from Cross River Estuary and Adjacent Coastal Waters. *Afrrev Stech*, **2(1):**2225 – 2227.

El-Ghasham, A., Mehanna, E. E. and Meki, A. (2008). Evaluation of lead and cadmium levels in fresh water fish farms at Qassim region, K.S.A. *Journal of Agricultural and Veterinary Sciences, Qassim University*, **1(2):** 59 - 77.

Elizarov, A. A., Grechina, A. S., Kotenev, B. N. and Kuzetsov, A. N. (1993). Peruvian jack mackerel, *Trachurus symmetricus murphyi*, in the open waters of the South Pacific. *Journal of Ichthyology* **1(33):** 86–104.

Eneji, S. I., Rufus, S. and Annune P. A. (2011). Bioaccumulation of heavy metals in fish (Tilapia Zilli andClarias Gariepinus) organs from river Benue, north central Nigeria. *Pakistan Journal of Analytical Environmental Chemistry*, **12(1):** 25 - 31.

Erhan, U., Hulya, K. and Seyit, A. O. (2004). Heavy metals in mullet, Liza Abu, and catfish, silurus triostegus, from the Ataturk dam lake (Euphrates), Turkey. *Environment International*, **1(30):** 183 – 188. http://dx.doi:10.1016/s0160-4120(03)00169-7

Erhabor, C. U. and Obasohan, E. E. (2011). The distribution of heavy metals in water and fish tissues of Chrysichthys Nigrodigitatus and Parachanna Obscura from Ibiekuma stream, Ekpoma, Edo State, Nigeria. *African Scientist*, **12(3):** 133 - 141.

Essuman, K. M. (1992). The role of inland fish in food consumption in West Africa, A review preceeding of the symposium on post-havest fish technology FAO corporate document repository. Technical Report investigation vol. 2. pp 470 – 477.

Etornyo, A., Fredrick, A., Georgina, A. and Albert, K. Q. (2014). Bioaccumulation of heavy metals in water, sediments and tissues of some selected fishes from the red Volta, Nangodi in the upper east region of Ghana. *British Jornal of Applied Science and Technology*, **4(4):** 594 – 603.

European Commission Regulation 333/2007. (2007).Laying down the sampling methods and the methods of analysis for the official control of the lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32007R0333, accessed 22 November, 2014.

Ezekiel, O. A., Benedict, O. O. and Fidelis, B. A. (2012). Heavy metal profile of water, sediment and freshwater cat fish, Chrysichthys Nigrodigitatus (Siluriformes: Bagridae), of Cross River, Nigeria. *Reserve of Biology and Tropical*, **60(3):** 1289 - 1301.

FAO (2003). Heavy Metals Regulations: Part 1. Legal Notice No. 66. www.**fao**lex.**fao**.org/docs/pdf/eri42405.pdf

FAO (2012). The state of world review of fisheries and aquaculture. Part 1. www.fao.org/docrep/016/i2727e/i2727e01.pdf

Farkas, A., Salanki, J. and Specziar, A. (2002). Relation between growth and the heavy metal concentration in organs of bream, L. populating Lake Balaton. *Archieve of EnvironmentalContamination and Toxicology*, **43(11):** 236 - 243.

Fathi, A. B., Mohammad, S., and Mazlan, A. G. (2012). Evaluation of trace metal levels in tissues of two commercial fish species in Kapar and Mersing coastal waters, Peninsular Malaysia. *Journal of Environmental and Public Health*,**1(20):** 1 - 10. http://dx.doi.org/10.1155/2012/352309

Fazli, H., Zhang, C. I., Hay, D. E. and Lee, C. W. (2009). Stock assessment and management implications of anchovy Kilka in Iranian waters of the Caspian Sea. *Fisheries*, **1(100)**: 103 – 108.

Fish base (2014). Fish species, common names, pictures, references and collaborators. Ver. 04/2014. http://www.fishbase.org/search.php

Fredrick, A., Etornyo, A., Georgina, A. and Albert, K. Q. (2014). Bioaccumulation of heavy metals in water, sediments and tissues of some selected fish from the Red Volta, Nangodi in the upper east region of Ghana. *British Journal of Applied Science and Technology*, **4(4):** 594 - 603.

Gaspic, Z. K., Zvonaric, T., Vrgoc, N., Odzak, N. and Baric, A. (2002). Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea. *Water Reserve*, **(36):** 5023–5028.

Ghanthimathi, S., Aminah, A., Salmijah, S., Ujang, T. and Nurul-Izzah, S. (2012). Comparison of microwave assisted acid digestion methods for ICP-MS: Determination of total arsenic in fish tissue. *Sains Malaysiana*, **4(12):** 1557 – 1564.

Gholam, R., Jahed, K., Inteaz, A., Ebrahim, N. and Ramin, N. (2005). Mercury contamination in fish and public health aspects: A Review. *Pakistan Journal of Nutrition*, **4(5):** 276 - 281.

Gil, F., Olmedo, P., Pla, A., Hernández, A.F., Barbier, F. and Ayouni, L. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environment International*, **(59):** 63 – 72.

Glanze, W. D. (1996). Mosby Medical Encyclopedia, Revised Edition, C.V. Mosby, St. Louis, MO,hh.http://www.amazon.com/Mosby-Medical-Encyclopedia-The-Signet/dp/0451184092

Gregory, G. P., James, W. R. and Patrice, C. (2005). Effects of- industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxicol Environmental Safety*, **61(3):**287-312.

Guilherme, S., Pereira, M. E., Santos, M. A. and Pacheco, M. (2010). Mercury distribution in key tissues of caged fish (Liza Aurata) along an environmental mercury contamination Gradient. *Biological Responses to Contaminants*, **1(1):** 165 – 173.

Hajep P., Jinap S., Ismail A., Fatima A. B., Hajeb P., Jinap S., Ismail A., Fatimah A. B., Jamilah B., and Abdul Rahim B. (2009). Assessment of mercury level in commonly consumed marine fishes in malaysia.*Food Control*, **20:** 79 - 84. http://dx.doi:10.1016/J.Foodcont.2008.02.012

Haluk, O., Gulsin, A. and Emine, A. (2012). Determination of some metal levels in muscle tissue of nine fish species from Beysehir Lake, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*,**1(12):** 761 - 770. http://dx.doi:10.4194/1303-2712-v12_4_04

Han, B. C., Jeng, W. L., Chen, R. Y., Fang, G. T., Hung, T. C. and Tseng, R. J. (1998). Estimation of Target Hazard Quotients and Potential Health Risks for metals by consumption of Seafood in Taiwan. *Archieve of Environmental Contamination Toxicology***35(1):** 711 – 720.

Hansen, J. A., Paul, G., Welsh, J. L., Dave, C., and Anne, D. D. (2002). Relative sensitivity of bull trout (*Salvelinus confluentus*) and rainbow trout (*Oncorhynchus mykiss*) to acute exposures of cadmium and zinc. *Environmental Toxicology and Chemicals*, **21(1):** 67–75.

Hayat, S. and Javed, M. (2008). Regression studies of planktonic productivity and fish yield with reference to physico-chemical parameters of the ponds stocked with sub-lethal metal stressed fish. *International Journal of Agricultural Biology*, **10(2):** 561 - 565.

Hjeltnes, B. and Kiire, J. (1992). Concentrations of iron, copper, zinc and selenium in liver of Atlantic salmon *Salmo salar* infected with *Vibrio salmonicida*.*Dissaster aquatic*,**1(2):** 147 - 149.

Hulya, K., Seyit, A. O. and Erhan, U. (2004). Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Ataturk Dam Lake (Euphrates), Turkey. *Environment International*, **1(30):** 183 - 188. http://dx.doi:10.1016/S0160-4120(03)00169-7

Ikem, A., Egiebor, N. O. and Nyavor, K. (2003). Trace elements in water, fish and sediment from Tuskegee Lake, Southeastern USA. *Water, Air and Soil Pollution*, **149(4):** 51 - 74.

International Council for the Exploration of the Sea(ICES) (2001). Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy. ICES C. M. 2001/ACFM: 6. www.archimer.ifremer.fr/doc/00112/22335/20007.pdf

International Union of Pure and Applied Chemistry (IUPAC), (1997). Compendium of Chemical Terminology: Limit of detection, limit of quantification and relative standard deviation. 2nd Edition. http://old.iupac.org/goldbook/L03540.pdf

ISO-International Standards Organization. (2005). ISO/IEC 17025. *General requirement for the competence of testing and calibration laboratories.*

Israa, A. J., Luma, H. Z. and Rana, M. A. (2013). Bacterial and heavy metals analyses in fish at Shawaka area of Tigris River. *Chemistry and Materials Research*, **3(7):** 94 - 100.

Iwegbue, G. E., Nwajei, F. O., Arimoro, O. E. and Chukwujindu, M. A. (2009). Characteristic levels of heavy metals in canned sardines consumed in Nigeria. *Environmentalist*, **29:** 431 – 435. http://dx.DOI10.1007/s10669-009-9233-5:

Jabeen, G., Javed, M. and Azmat, H. (2012). Assessment of heavy metals in the fish collected fromthe river Ravi, Pakistan. *Pakistan Veterinary Journal*, **32(1):** 107 - 111.

Jalal K.C.A., Akbar J. B. and Habab M., Mohd A.Y. And B.Y. Kamaruzzaman B. Y. (2013). Bioaccumulation Of Selected Metals In Fresh Water Haruan Fish (Channa Striatus) Collected From Pahang River Basin, Malaysia. *Orientational Journal of Chemistry*, 29, **(4):** 1553 - 1558. http://dx.doi.org/10.13005/ojc/290436

Jane, M. Hightower, M. and Moore, D. (2003). Mercury Levels in High-End Consumers of Fish. *Environmental Health Perspectives*, **111(4):** 604 – 608.

Jayakumar, P. and Paul, V. I. (2006). Patterns of cadmium accumulation of the catfish Clarias Batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. *Veterinarshki Archivment*, **1(76):** 167 - 177.

Jayashree, S. M. and Sarita, M. (2013). Mercury accumulation in different tissues of fish from Ulhas River Estuary and Thane Creek and the pattern of fish consumption among fish eaters. *Indian journal of Geo-marine science*, **42(6):** 812 - 816.

Jefferies, D. J. and Freestone, P. (1984). Chemical analysis of some coarse fish from a Suffolk River carried out as part of the preparation for the first release of captive-bred otters. *JournalOtter Trust*, **1(8):** 17 – 22.

Jensen, C. S., Menne, T., Lisby, S., Kristiansen, J. and Veien, N. K. Experimental (2003). Systemic contact dermatitis from nickel: a dose-response study. *Contact Dermatitis*, **49(3):** 124 – 134.

Jinadasa, K. and Kolita, B. K. (2010). Preparation of a standard operation procedure for validation of laboratory methods for trace metal analysis in seafood for national aquatic resources research and development agency (Nara), Sri Lanka. *United Nation Universal Fisheries Training Program*, **(1):** 1 - 46.

Joana, R., Patricia, P., Miguel, C., Maria, T. C. and Carlos, V. (2011). Decrease of Zn, Cd and Pb concentrations in marine fish species over a decade as response to reduction of anthropogenic inputs: The example of Tagus estuary. *Marine Pollution Bulletin*,**1(62):** 2854 - 2858. http://dx.doi:10.1016/j.marpolbul.2011.09.020

Johensen, p., Asmund, G., Martin, M. H. and Palle, B. N. (1989). Marine organism as indicator of heavy metals in 16years monitoring, Papers from the Eighth International Ocean Disposal Symposium, 9-13 Oct 1989, Dubrovnik, Yugoslavia (Part II).

Johnson, G. M. (2015) Mercury and lead contamination in three fish species and Sediments from Lake Rukwa and Catchment areas in Tanzania. *Journal of Health and Pollution*, **5(8):** 7 - 18.

Kalay, M., AY, O. and Canli, M. (1999). Heavy metal concentrations in fish tissues from the northeast Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology*, **63(5):** 673 - 681.

Kalay, M. and Canli, M. (2000). Elimination of essential (Cu and Zn) and non-essential (Cd and Pb) metals from tissues of a fresh water fish, Tilapia zillii, *Tropical Journal of Zoology*, **24(1):** 429 - 436.

Kannan, K. S., Jr, R. G., Lee, R. F., Windom, H. L., Heitmuller, P. T., Macauley, J. M. and Summers, J. K. (1998). Distribution of total mercury and methyl mercury in water, sediment, and fish from south Florida estuaries. *Archieveof Environmental Contaminationand Toxicology*,**1(34):** 109 – 118.

Katherene, R.(2001). Pelagic-benthic coupling: Are we asking the right questions.*Senckenbergiana maritime***31(2):** 215–224.

Kenneth, J. G. (1997). Regulation of body temperature in the white shark, Carcharodon carcharias,*Journal of Comparative Physiology*,**167(6):** 423-429.

Khaled, A. (2004). Heavy metal concentrations in certain tissues of five commercially important fishes from El-Mex Bay, Alexandria, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*.**8(4):** 51 - 64.

Khare, S. and Singh, S. (2002). Histopathological lessons induced by copper sulphate and lead nitrate in the gill of freshwater fish nundus.*Journal of Ecotoxicology Environment and Marine Pollution*, **6(3):** 5760 - 5760.

Kojadinovic, J., Patier, M. and Le-Corre, M. (2007). Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean.*Journal of Environmental Pollution*, **146(4):** 548 - 566.

Kotze, P., Preez, H. H., van J. and Vuren H. J. (1999). Bioaccumulation of copper and zinc in Oreochromis mossambicus and Clarias gariepinus from the Olifants River, Mpumalanga, South Africa. *Water SA*, **1(25):** 99–110.

Kumar, B. & Mukherjee, D. P. (2011). Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. *Advances in Life Science and TechnologyJournal*, **1(2):** 13 - 25.

Kurien, J., (2006). International fish trade and food security: issues and perspectives,variations in cast net catches in a tropical brackish water pond. *Journal of Science Technology Environment***5(2):** 6-14.

Laila, D. L. Wahidu, Z. and Tajul, A. Y. (2013). Effect of chilled-frozen storage on the physic-chemical, microbial and sensory quality of farmed bighead carp (Hypophthalmichthys Nobilis). *Journal of fisheries and aquatic science*, **2(3):** 1 – 7. http://dx.doi:10.3923/jfas.2013

Lasheen, R. M., Fagr, A., Aly, A. A. and Hassan, M. H. (2012). Fish as bio indicators in aquatic environmental pollution assessment: A case study in Abu-Rawash area, Egypt. *World Applied Sciences Journal*, **19(2):** 265 - 275.

Lee, Y. H. and Stuebing, R. B. (1990) Heavy metal contamination in the RiverToad, Juxtasper (Inger), near a copper mine in East Malaysia. *Bulletin of Environmental Contamination andToxicology*, **45:**272 – 281.

Li, X. D., Zhang, G., Won, C.S.C. and Zhang,W.L. (2005). Heavy metal and Pb isotopic compositions of aquaticorganisms in the Pearl River Estuary, *South ChinaEnvironmental Pollution,***138(25):** 494-504.

Lillian, H. and Lorraine, E. (2012). Assessment of mercury in fish tissue from select lakes of North-eastern Oregon, U.S. Environmental Protection Agency Region 10, EPA-910-R-12-001.

Lieven, B., Dries K.,Maarten, D.,Karen, V. andRonny, B. (2013). Differential Hepatic Metal and Metallothionein Levels in Three Feral Fish Species along a Metal Pollution Gradient. *Plos one*, **8(3):** 1 - 11. http://dx.DOI:10.1371/journal.pone.0060805

Lofts, S., Tipping, E. and Hamilton-Taylor, J. (2008). The chemical speciation of Fe (III) in freshwaters. *Aquatic Geochemistry*,**14:** 337 − 358.

Lokhande, R. S., Singare, P. U. and Pimple, D. S. (2011). Quantification Study of Toxic Heavy Metal Pollutants in Sediment Samples Collected From Kasardi River Flowing Along the Taloja Industrial Area of Mumbai, India.*New York Science Journal*, **4(9):** 6 - 14.

Lopa, G. and Adhikari, S. (2006). Accumulation of heavy metals in freshwater fish-an assessment of toxic interactions with calcium. *American Journal of Technology*,**1(2):** 139 - 148. http://dx.doi:10.3923/ajft.2006.139.148

Mahaffey, K. R. and Rice, G. E. (1998). Environmental Protection Agency Office of Air Quality Planning and Standards. Mercury Study Report to Congress. Govt Reports Announcements and Index (GRA and 1), Issue 09, Available: http:// www.epa.gov/oar/mercury.html.Accessed 2 November 2014.

Malm, J., William, R., and Rull, F. (1995). Effect of mercury on mortality and growth. *Marine Pollution Bulletin*, **2(12):** 182 – 189.

Mansour, S. A. and Sidky, M. M. (2002). Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chemistry*, **78(5):** 15 - 22.

Marcela, H., Ladislav, D., Danka, N., Gorzyslaw, P. and Zdenka, S. (2008). Comparison of mercury distribution between liver and muscle – A bio-monitoring of fish from lightly and heavily contaminated localities. *Sensors*, **1(8):** 4095 - 4109. http://dx.doi:10.3390/s8074095

Marioara, N. P., Negrea, I., Gergen, M., Harmanescu, I., Gogoasa,I. andLunca, M. (2012). Mercury bioaccumulation in tissues of fresh water fish *carassius auratus gibelio* (Silver Crucian Carp) after chronic mercury intoxication. *University de-Ştiinţe Agric and Medicinal Veterinary Iasi Lucrari Ştiinţifice*,**1(52):** 676 - 679.

Maureen, H., (2010). New estimate puts Gulf Oil Leak at 205 Million Gallons - The Rundown News Blog PBS News Hour - PBS. Retrieved from:http://www.pbs.org/newshour/rundown/2010/08/new-estimate-puts-oil-leak-at-49-million-barrels.html.

Mieiro, C. L., Pacheco, M. Eduarda, P. and Armando, C. D. (2009). Mercury distribution in key tissues of fish (Liza Aurata) Inhabiting a contaminated estuary-implications for human and ecosystem health risk assessment. *Journa of Environmental Monitering*, **1(11):** 1004 – 1012. http://dx.doi:10.1039/b821253h

Mieiro, C. L., Pereire, M. E., Duarte, A. C. andPacheco, M. (2011). Brain as a critical target of mercury in environmentally exposed fish (Dicentrarchus labrax)bioaccumulation and oxidative stress profiles.*Aquatic Toxicology*, **3(4):** 233 - 240. http://dx.doi:10.1016/j.aquatox.2011.03.006

Mieiro, C. L., Coelho, J. P., Pacheco, M. , Duarte, A. C. and Pereira, M.E. (2012). Trace elements in two marine fish species during estuarine residency: Non-essential versus essential.*Marine Pollution Bulletin*, **3(4):** 56 – 62.http://dx.doi.org/10.1016/j.marpolbul.2012.08.033

Mukesh, K. R., Puneet, K., Manoj, S. and Anand, S. (2008). Toxic effect of heavy metals in livestock health. *Veterinary World*, **1(1):** 28 - 30.

Muniyan, M. and Ambedkar, A. (2012). Analysis of heavy metals in water, sediments and selected freshwater fish collected from Gadilam River, Tamilnadu, India. *International Journal ofToxicology and Applied Pharmacology*, **2(2):** 25 – 30.

Nadeem, A. K., Nermin, E., Ibrahim, A., Ahmed, A., Abd-Allah, E. and Ahmed, E. (2014). Effect of heavy metal pollutants on fish population in two Egyptian lakes. *International Journalof Advanced Research*, **2(1):** 408 – 417.

Nadkarmi, R. A.(1984). Application of microwave ovun sample dissolution in analysis. *Analytical Chemical*, **56(12):** 2233 – 2237.

Nam, S.h., Oh, h.J., Min, h. S. and lee, J.h. 2(010). A study on the extraction and quantification of total arsenic and arsenic species in seafood by hPlC-ICP-MS. *Microchemical Journal*,**95:** 20-24.

Naoko, W., Misato, T., Minoru I. and Akira, Y. (2012). Distribution and chemical form of mercury in commercial fish tissues. *The Journal of Toxicological Sciences*, **37(2):** 853 - 861. http://dx.doi.org/10.2131/jts.37.853

National Academy of Sciences(2000). Toxicological Effects of Methylmercury. Washington, DC: National Academy of Sciences. Available: http://nap.edu/books/0309071402/htmlaccessed 2 February 2014.

Nellen, W. and Dulcic, J. (2008). Evolutionary steps in ichthyology and new challenges. *Actaadriatica*, **49(3):** 210-232.

Nobrega, J. A., Trevizan, L. C., Donati, G. L. and Nogueira, A. R. A. (2007). Microwave-assisted procedures for sample preparation: recent developments, in: Marco Aurelio Zezzi Arruda (Ed.), Trends in Sample Preparation, Nova Science Publishers, Sao Paulo, Brazil, pp. 29 – 52.

Noik, H., James, A. K., Noik, V., The, Y. Y., Lee, C. Y. and PearlineNg, H. C. (2011). Assessment of cadmium (Cd) and lead (Pb) levels in commercial marine fish organs between wet markets and supermarkets in Klang Valley, Malaysia. *International Food Research Journal*, **1(18):** 795 – 802.

Obasohan, E. E. (2008). Bioaccumulation of chromium, copper, maganese, nickel and lead in a freshwater cichlid, hemichromis fasciatus from Ogba River in Benin City, Nigeria. *African Journal of General Agriculture*, **4(3):** 1595 - 1605.

Obasohan, E. E. and Erhabor, C. U. (2011). The distribution of heavy metals in water and fish tissues of Chrysichthys nigrodigitatus and Parachanna obscura from Ibiekuma stream, Ekpoma, Edo State, Nigeria.*African Scientist*, **12(3):** 133 – 140.

Occupational Safety and Health Administration (OSHA) (1998). Occupational Safety and Health Standards, Toxic and Hazardous Substances. *Code of Federal Regulations.* 29 CFR 1910.1000.

Ogbuehi, G. I. and Eze, S. O. (2015). Assessment of heavy metals and polycyclic aromatic hydrocarbon concentration in some stock fish species sold in five major markets of aba south, Abia state, Nigeria. *European Journal of Pure and Applied Chemistry*, **2(1):**11 - 18.

Ogundiran, M. A., Adewoye, S. O., Ayandiran, T. A. and Dahunsi, S. O. (2014). Heavy metal, proximate and microbial profile of some selected commercial marine fish collected from two markets in south western Nigeria. *African Journal of Biotechnology*, **13(10):** 1147 - 1153. http://dx.doi:10.5897/ajb2013.13240

Okunola, O. J., Alhassan, Y., Yebpella, G. G., Uzairu, A., Tsafe, A. I. , Abechi, E. and Apene, S. E. (2011). Risk assessment of using coated mobile recharge cards in Nigeria. *Journal of Environmental Chemistry and Ecotoxicology*, **3(4):** 80 - 85.

Olaifa, F. E., Olaifa, A. K., Adelaja, A. A. and Owolabi, A. G. (2004). Heavy metal contamination of Clarias garpinus from a lake and Fish farm in Ibadan, Nigeria. *African Journal of Biomedical Research*,**1(7):** 145 - 148.

Oluwaniyi, O. O. and Dosumu, O. O. (2009). Preliminary Studies on the effect of processing methods on the quality of three commonly consumed marine fishes in Nigeria. *Nigerian Society for Experimental Biology*, **21(1):** 1 - 7.

Oluyemi, E. A. and Olabanji, I. O. (2011). Heavy metals determination in some species of frozen fish Soldat Ile-Ife main market.*Ife Journal of Science*, **13(2):** 86 - 93.

Omima, A. S. and Aboud, A. (2010). Impact of pollution with lead, mercury and cadmium on the immune response of Oreochromis niloticus. *New York Science Journal*, **3(9):** 16 – 26.

Ozgur, C. (2013). The determination of some heavy metals and minerals in the tissues and organs of the Capoeta Umbla fish species in relation to body size, sex, and age. *Ekoloji*,**22(87):** 64 - 72. http://dx.doi:10.5053/ekoloji.2013.879

Picot, J. C. and Gill, D. A. (1996). "The Exxon Valdez Oil Spill and Chronic Psychological Stress", In *Proceedings of the Exxon Valdez Oil Spill Symposium,* eds. American Fisheries Society.

Portier, K., Tolson, J. & Roberts, S. (2007). Body weight distributions for risk assessment. *Risk Analysis*, **27(1):** 11- 26.

Rahman, K., Zara, S., Tanisha, T. and Sayka, K. (2014). Impact of cadmium exposure on human health with a focus on Bangladesh Eva. *European Journal of Toxicological Science*, **3(1):** 20 – 23.

Rauf, A., Javed, M. and Ubaidullah, M. (2009). Heavy metal levels in three major carps (Catla Catla, Labeo Rohita and Cirrhina Mrigala) from the river Ravi, Pakistan. *Pakistan Veterinary Journal*, **29(1):** 24 - 26.

Reena, S., Neetu, G., Anurag, M. and Rajif, G. (2011). Heavy metals and living system: An over view.*Indian Journal of Pharmacology*, **43(3):** 246 - 253. http://dx.doi.org/10.4103%2F0253-7613.81505

Richard, G., Frank, S., Kenneth, W., Humera, A., Lorraine, B., Lena, L. and John, R. (1986). The use of microwave ovens for rapid organic synthesis. *Tetrahedron letters*,**27(3):** 279 - 282 doi:10.1016/S0040-4039(00)83996-9

Rosseland, B. O., Massabuau, J. C., Grimalt, J., Hofer, R., Lackner, R., Raddum, G., Rognerud, S. and Vives, I. (2001). *Fish ecotoxicology, The EMERGE fish sampling manual for live fish. The EMERGE Project* (European Mountain Lake Ecosystems: Regionalisation, Diagnostic and socio-economic valuation). http://www.mountain-lakes.org/emerge/methods/29.pdfl

Sabdono, A., (2009). Heavy metal level and their potential toxic effects on coral galaxea fasciculars, from Java Sea, *Indonesia, Reserve Journal of Environmental Science*, **3(1):** 96-102.

Saima, N.and Muhammad, J. (2013). Evaluation of acute toxicity of metals mixture and bioaccumulation in freshwater fish. *Bioscience Methods*, **4(3):** 11 - 18. http://dx.doi:10.5376/bm.2013.04.0003

Sahar, M. B., Alireza, P. and Reza, A. (2014). Analysis and determination of trace metals (Ni, Cd, Cr, and Pb) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran. *Journal of Toxicology*, **57(64):** 96 - 6. http://dx.doi.org/10.1155/2014/576496

Shahat, M. A., Amer, O.S.O., Abdallah, A.T., Abdelsater, N. and Moustafa, M.A. (2011). The distribution of certain heavy metals between intestinal parasites and their fish hosts in the river Nile at Assuit province, Egypt. *The Egyptian Journal of Hospital Medicine*, **1(43):** 241 – 257.

Sajjad, K., Robina, F., Shagufta, S., Mohammad, A. K. and Maria, S. (2009). Health risk assessment of heavy metals for population via consumption of vegetables. *World Applied Sciences Journal*, **6(12):** 1602 - 1606.

Serra, R. and Glubokov, A. (2008). Population structure of Chilean jack mackerel, *Trachurus murphyi*, in the South Pacific Ocean: Full proposal for an international joint research

programme. Paper presented to the SPRFMO Chilean Jack Mackerel Workshop, Santiago, 30th June -4th July, 2008.

Shaikh, M. J. (2013). Analysis of heavy metals in water and fish Cirrhina mrigala of River Godavari, at Nathsagar Dam in Maharashtra, *Indian Bioscan*,**8(3):** 1025 - 1027.

Shariati, F., Esmaili, A. S., Mashinchian, M. A. and Pourkazemi, M. (2012). Metal bioaccumulation in Persian sturgeon after sublethal exposure. *International Journal of Marine Science and Eng*ineering, **2(1):** 81 - 90.

Shesterin, I. S. (1996).Water Pollution and its impact on fish and aquatic invertebrates - Water pollution and its impact on fish and aquatic invertebrates: Interactions: *Food, agriculture and environment*, **1:1** – 10.

Shirvani, E. and Jamili, S. (2009). Assessing Cd, Pb Accumulation in the tissues of *Chalcalburnus chacoides*. *Research Journal of Environmental Science*, **3(5):** 522-529.

Shimshack J. P., Ward, M. B. and Beatty, T. K. M. (2007). Mercury advisories: Information, education, and fish consumption. *Journal of Environmental Economic Management*, **53:** 158 – 179.

Sidra, B., AmtulBari, T., Abdullah, Y. and Jaffar, H. (2014). Concentration of Metals in Frozen and Canned Fish in Pakistan Sidra Siddique. *Pakistan Journal of Zoology*, **46(6):** 1521-1527.

Simon, K. D., Bashir, F. H., Othman, M. S., Mazlan, A. G. and Rahim, S. M. (2013). Heavy metal concentration in fish from the coastal waters of Kapar and Mersing, Malaysia. *Turkish Journal of Fisheries and Aquatic Sciences*,**1(13):** 375 - 382. http://dx.doi:10.4194/1303-2712-v13_2_21

Soegianto, A., Irawan, B. andHamami, I. (2009). Bioaccumulation of heavy metals in aquatic animals collected from coastal waters of Gresik Indonesia. *Asian Journal of Water, Environment and Pollution*,**1(6):**95-100.

Solomon, K. R., Baker, D. B., Richards, R. P., Dixon, D. R., Klaine, S. J. and Lapoint, T. W. (1996). Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemicals*, **15(1):**31 – 74.

Souza, S., Pinto, V.C., Carlos T. and Junqueira, R.G. (2007). In-house method validation: Application in arsenic analysis. *Journal of Food Composition and Analysis*,**1(20):** 241-247.

Soylak, M., Tuzen, M., Narin, I. and Sari, H. (2004). Comparison of microwave, dry and wet digestion procedures for the determination of trace metal contents in spice samples produced in Turkey. *Journal of Food and Drug Analysis*,**1(12):** 254-258.

Standard Operating Procedure (SOP) A03-005. (2008). Method Validation in Chemical Analysis, Issue 3, Rev.0. Sungai Buloh: National Public Health Laboratory.

Staniskiene, B., Matusevicius, P., Budreckiene, R. and Skibniewska, K. A. (2006). Distribution of heavy metals in tissues of freshwater fish in Lithuania. *Polish Journalof Environmental Study*, **15(4):** 585-59.

Storelli, M. M., Ceci, E., Storlli, A. and Marcotrigiano, G. O. (2003). Polychlorinated biphenyl, heavy metal and methylmercury residues in hammerhead sharks: contaminant status and assesment. *Marine Pollution Bulletin*,**1(46):** 1035 - 1039.

Storelli, M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs).*Food Chemical Toxicology*,**46(8):** 2782 - 8. http://dx.doi:10.1016/j.fct.2008.05.011

Storelli, M. M., Normanno, G., Barone, G., Dambrosio, A., Errico, L. and Garofalo, R. (2012). Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: suitability for human consumption. *Journal of Food Protechtion*,**75(1):** 189 – 94. http://dx.doi:10.4315/0362-028X.JFP-11-212

Taghipour, V. and Aziz, S. N. (2010). Determination of trace elements in canned Kilka fish marketed in Islamic Republic of Iran. *World Applied Science Journal*,**9(6):** 704 - 707.

Tanee, T., Chaveerach, A., Narong, C., Pimjai, M., Punsombut, P. and Sudmoon, R. (2013). Bioaccumulation of heavy metals in fish from the Chi River, Maha Sarakham province, Thailand. *International Journal of Biosciences*, **3(8):** 159 - 167. http://dx.doi.org/10.12692/ijb/3.8.159-167.

Tawari, P. and Kkaye, S. A. (2007). Fish species diversity as indicator of pollution in Ikpoba River, Benin city, Nigeria. *Reverse ofFisheries and Biological Fisheries*, **17(5):** 21 - 30.

Taweel, A., Shuhaimi-Othman, L. M. and Ahmad A. K. (2012). Heavy metals concentration in tilapia fish (Oreochromis Niloticus) from four selected markets in Selangor, Peninsular Malaysia. *Journal of Biological Science*,**10(55):** 1562 - 1566.

Taverniers, I., De Loose, M. and Van-Bockstaele, E. (2004). Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Journal of Trends in Analytical Chemistry.* **1(23):** 535 - 552.

Taylor, P. R. (2002). Stock structure and population biology of the Peruvian jack mackerel, Trachurus symmetricus murphyi. New Zealand Fisheries Assessment Report, 2004/28 pp22.

The World Fish Center, (2009).Reducing poverty and hunger by improving fisheries and aquaculture. Publications Catalog: Penang, Malaysia.www.worldfishcenter.org

Tom, K. G., Steinar, R. B., Tor, H. and Aage, A. (2002). Exposure to different forms of nickel and risk of lung cancer. *American Journal of Epidemiology*,**156(12):** 1123 - 1132. http://dx.doi:10.1093/aje/kwf165

Trites, A. W., Boyd, L., Wanless, S., Camphuysen C. J., Christensen, V. and Pauly, D. (2006). Effects of fisheries on ecosystems: just another top predator? In: Top predators in marine ecosystems. Their role in monitoring and management. Cambridge University Press, Cambridge: Pp11 – 27.

Tulay, K., Huseyin, A. andMustafa, I. (2014). Assessment of trace element levels in muscle tissues of fish species collected from a river, stream, lake, and sea in Sakarya, Turkey. *The Scientific World Journal*, **1(14):** 1 - 7. http://dx.doi.org/10.1155/2014/496107

United Nations Environmental Program (2003).Environment for development. Annual report http://www.unep.org/annualreport/2003/

USEPA (1998). Microwave assisted acid digestion of sediments, sludge, soils, and oils, Method 3051a, Office of Solid Waste and Emergency Response, U.S. Government Printing Office, Washington, DC.

USEPA (2008). Child-specific exposure factors handbook, Chapter 10 - Intake of fish and shellfish. Washington, D. C: Office of research and development.

USEPA (2009). United States Environmental Protection Agency, Risk-Based concentration Table. Philadelphia: United States Environmental Protection Agency, Washington, D. C.

USEPA (2011). Exposure Factors Handbook: 2011 Edition. EPA/600/R-090/052F. Office of Research and Development, Washington, DC.

USEPA (2014). Provisional Peer-Reviewed Toxicity Values (PPRTV); on-line database. Accessed via http://hhpprtv.ornl.gov/ (January).Washington Department of Ecology (WDOE). Fish Consumption Rates Technical Support Document: A Review of Data and Information about Fish Consumption in Washington, Ver. 1.0 (Pub. No.11-09-050). Washington Department of Ecology, Toxics Cleanup Program. Olympia, Washington.

Usero, J., Izquierdo, C., Morillo, J. and Gracia, I. (2004). Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. *Environmental International*,**29(7):** 949 – 56.

Uzairu, A., Harrison, G. F. S., Balarabe, M. L. and Nnaji J. C. (2009). Concentration levels of trace metals in fish and sediment from Kubanni River, northern Nigeria. *Bulletin of Chemical Society Ethiopia*,**23(1):** 9 - 17.

Vesna, P., Mirjana, L., Ivan, Jaric, Dragana, D., Zoran, G., Gorcin, C. and Bozidar, R. (2010). Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet

(Acipenser Ruthenus Linnaeus, 1758). *Environmental Toxicology and Chemistry*, **1(29):** 515 – 521. http://dx.doi:10.1002/etc.82

Vilano, M. and Rubio, R. (2001). Determination of arsenic in seafood by focused microwave digestion and hydride generation-atomic fluorescence detection. *Journal of AOAC International*,**1(84):** 551-555.

Vinodhini, R. and Narayanan, M. (2008).Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). *International Journal of Environmental Science and Technology*, **5(2):** 179 - 182.

Wafaa, M., Bakr, K., Walaa, A., Hazzah, A. F. and Abaza, F. (2011). Detection of Salmonella and Vibrio species in some seafood in Alexandria. *Journal of American Science*, **7(9):** 663 - 668.

Wim, V., Isabelle S., Karen, B., Stefaan, D. and John, V. C.(2007). Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights from Belgium. *Aquaculture International*,**15(2):**121 – 136.

WHO (2005). Background document for development of WHO guidelines for nickel in drinking-water quality and fish. WHO/Sde/Wsh/05.08/55 English Only.

Wladiana, O. M., Eveline, A., Menezes, M. H., Gonzalez, L. M., Costa, L. C., Trevizan, R. and Nogueira, A. (2009). Partial microwave-assisted wet digestion of animal tissue using a baby-bottle sterilizer for analyte determination by inductively coupled plasma optical emission spectrometry. *Spectrochimica Acta Part B*,**1(64):** 615 – 618.

Yaowu, H. Y., Hong, S. H., Haowen, T. H., Olaf, N. O., Wu, L. W., Zhao, C., Yu, J., Wang, C., Trinkaus, E. and Richards, M. (2009). *Stable isotope dietary analysis of the Tianyuan early modern human.*Proceedings of the National Academy of Sciences, **106(27):** 10971 - 10974.

Yazkan, M. O., Zedemir, F., and Golukcu, M. (2002). Cu, Zn, Pb and Cd content in some fish species caught in the Gulf of Antalya. *Turkish Journal of Veterinary Animal Science*,**26(6):** 1309 – 13. http://dx.doi.org/10.1016/j.marpolbul.2012.08.033

Yousafzai, A. M., Khan,A.R. and Shakoori, A.R. (2009). Trace metal accumulation in the liver of an endangered south Asian fresh water fish dwelling in sub-lethal pollution. *Pakistan Journal of Zoology*, **41(1):** 35 - 41.

Yilmaz, A. B. (2003). Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of Mugil cephalus and Trachurus mediterraneus from Iskenderun Bay, Turkey. *Environmental Reserves*,**1(92):** 277 – 281.

Yilmaz, A. B. (2005). Comparison of heavy metal levels of grey mullet (Mugil cephalus) and sea bream (Sparus aurata) caught in Iskendrun Bay (Turkey). *Turkish Journal of Veterinary Animal Science*,**29(1):** 257-262.

Yilmaz, F. (2009). The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Koycegiz Lake-Mugla (Turkey). *Turkish Journal of Science Technology*, **4(1):** 1 – 15.

Zubair, A., El-Amin, M. S., Khalid, A. A. and Shahid, M. (2013). Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of Clarias Gariepinus. *Brazilian archieveof biological technology*, **56(2):** 45 – 56. http://dx.doi.org/10.1590/S1516-89132013000200015

APPENDICES

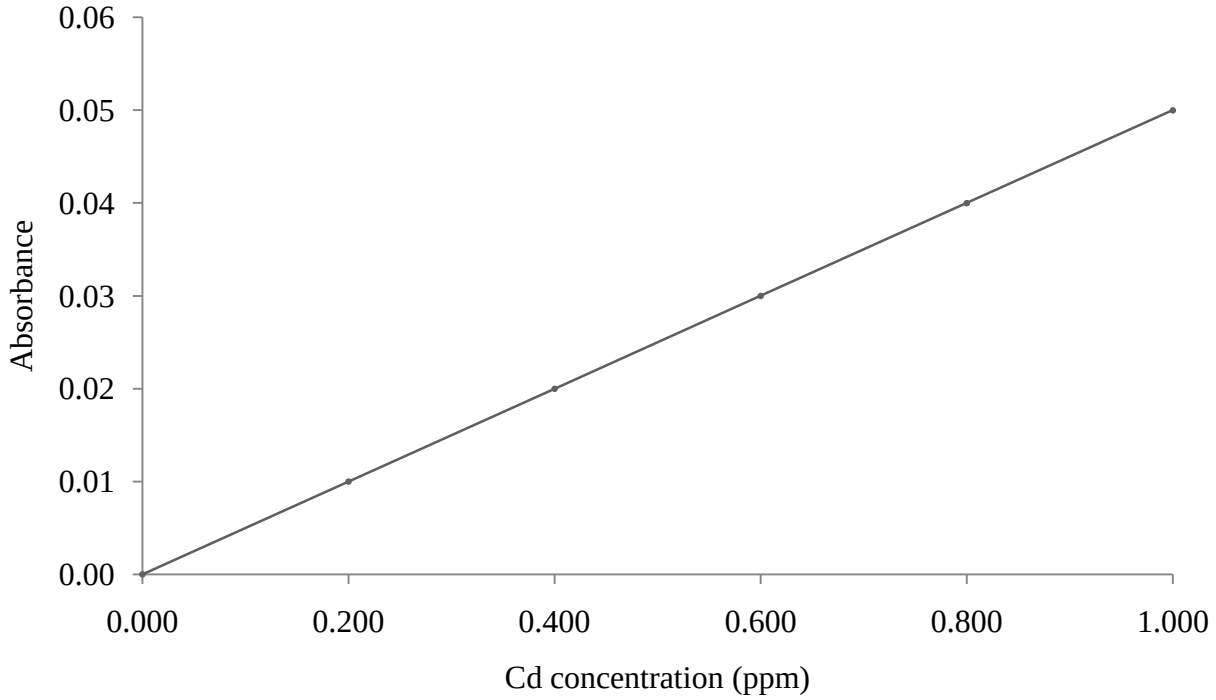


Figure 3.2 Cadmium calibration graph

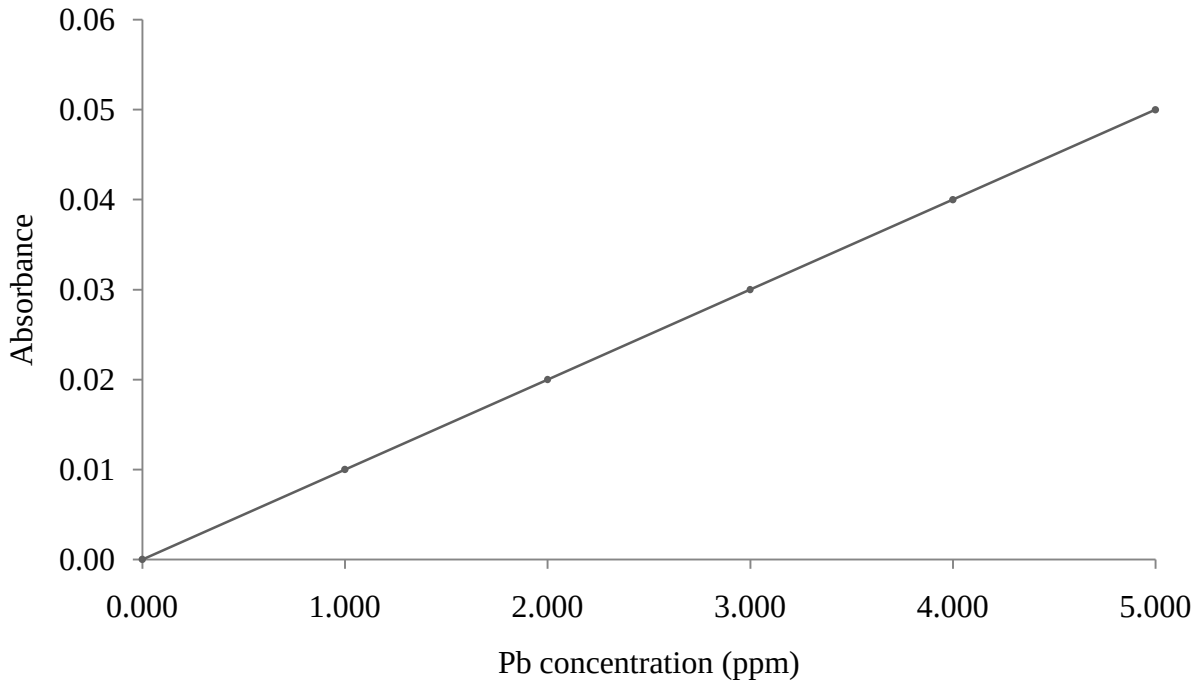


Figure: 3.3 Lead calibration graph

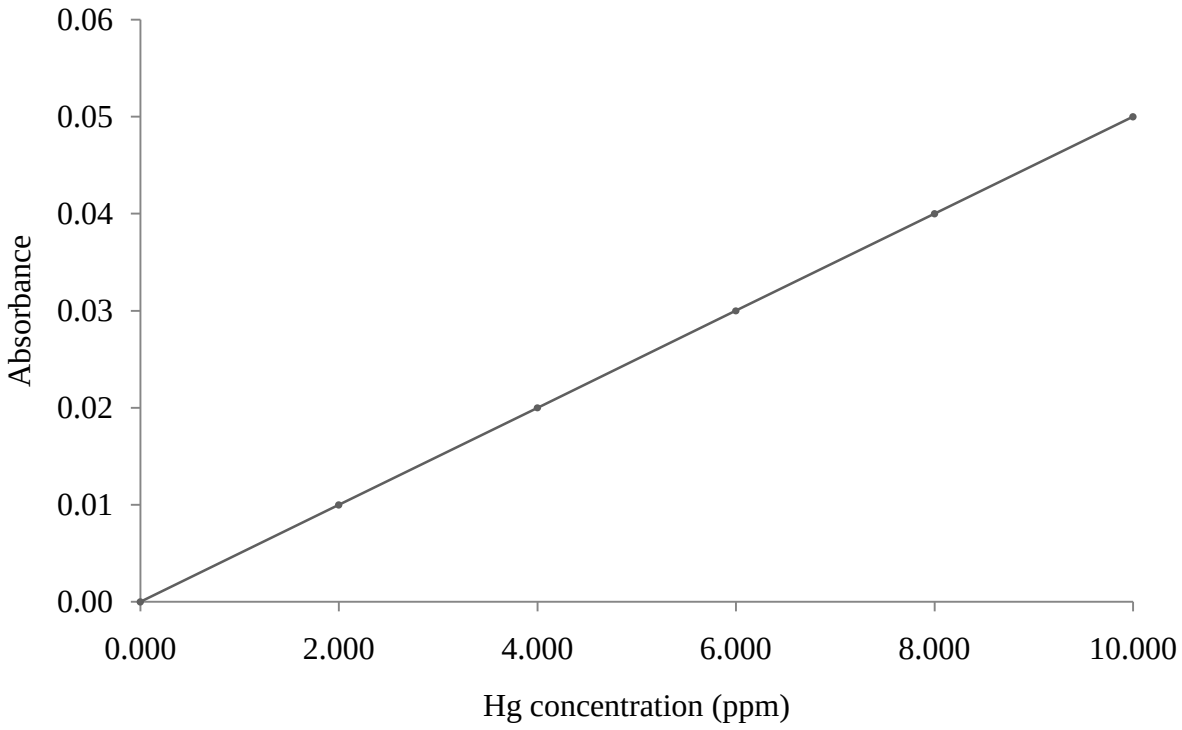


Figure 3.4 Mercury calibration graph

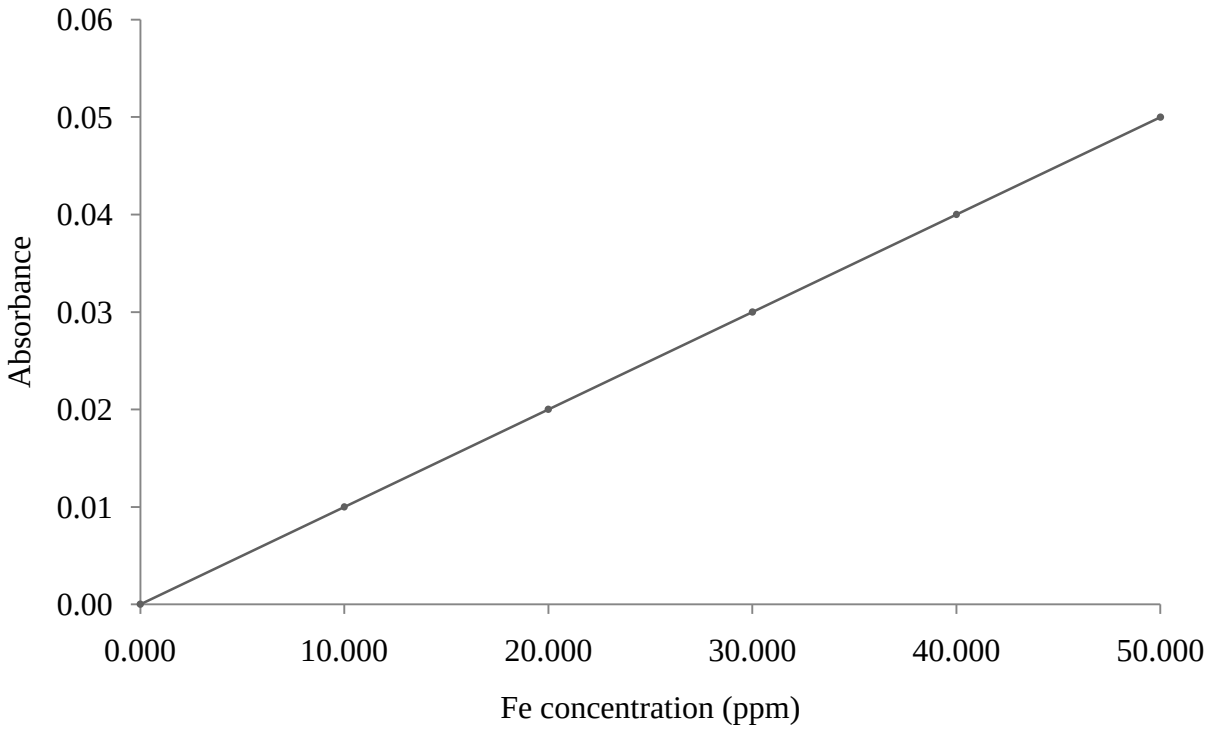


Figure 3.5 Iron calibration graph

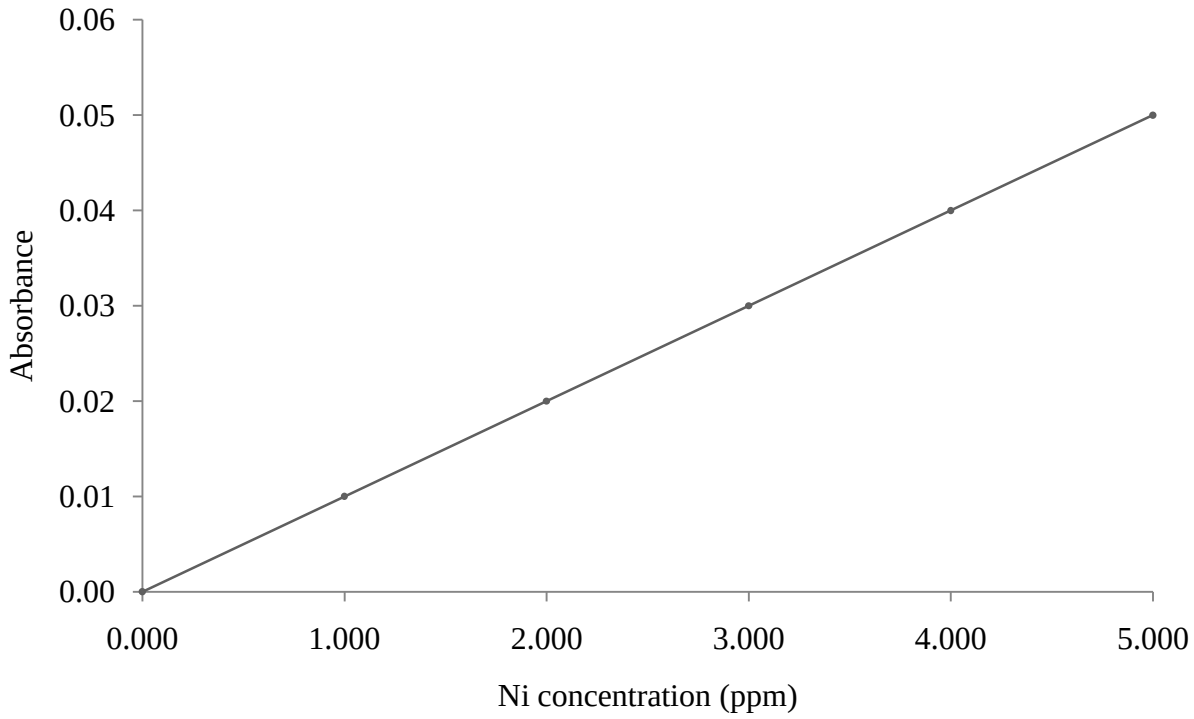


Figure 3.6 Nickel calibration graph

0