DETERMINATION OF FE, ZN, CU, CD AND PB IN SIX SPECIES OF SELECTED FISH MUSCLES

SITI FATIMAH MD ZAIN

THESIS SUBMITTED IN PARTIAL FULLFILLMENT FOR THE REQUIREMENT FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2007

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: DETERMINATION OF FE, ZN, CU, CD AND PB IN SIX SELECTED OF FISH MUSCLES

IJAZAH: SARJANA MUDA SAINS MAKANAN DENGAN KEPUJIAN (TEKNOLOGI MAKANAN DAN BIOPROSES)

SESI PENGAJIAN: 06/07

Saya SITI FATIMAH MD. ZAIN
(HURUF BESAR)

mengaku membenarkan tesis (LPS/ Sarjana/ Doktor Falsafah) ini di simpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. ** Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukakan oleh organisasi/badan di mana penyelidikan dijalankan)
[/]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 613 MAKLOM, SG DUA, 13800 BUTTERWORTH

DR. MOHD IQHAL HASHIMI
Nama Penyelia

Tarikh: 11/5/2007

Tarikh: 6/5/2007

CATATAN: * Potong yang tidak berkenaan.
* Jika tesis ini SULIT atau TERHAD, sila lampiran surat daripada pihak berkuasa/organsasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
* Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the thesis is my own work, except for certain quotations and references that have been duly acknowledged.

29 Mac 2007

SITI FATIMAH MD ZAIN
HN2004-2047

EXAMINERS CERTIFICATION

CERTIFIED BY

Signature

1. SUPERVISOR

   (DR. MUHAMMAD IQBAL HASHMI) --------------------------

2. EXAMINER 1

   (DR. CHYE FOOK YEE) --------------------------

3. EXAMINER 2

   (MR. SHARIFUDIN MD SHAARANI) --------------------------

4. DEAN

   (ASSOC. PROF. DR. MOHD. ISMAIL ABDULLAH) --------------------------

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my thesis supervisor, Dr. Mohd. Iqbal Hashimi, who accepted me and supported my scientific endeavors. Dr. Iqbal gave me the freedom to think creatively and critically throughout the study, this has no doubt cultivated a strong enthusiasm in analytical science in me. His expertise in the field of analysis has guided me well, corrected my mistakes and not to mention, provided valuable solutions to challenging problems.

Much of my research was facilitated by En. Awang, Pn. Zainab and En. Panjiman who are in-charged of SSMP and SKTM laboratory. They provided the instruments and reagents necessary for my work. Besides, I would like to thank my friends whom freely offer me their advice, both scientifically and personally.

Last but not the least, my warmest thanks must go to my parent whose patience, support, and understanding allowed me to write this thesis. I thank them for their support throughout my entire educational process. They have been my greatest source of moral support and unqualified love and have provided me with a lifetime of both. For all their guidance, both with the mechanics of the written text and with the implications that lay therein, I wish to express my sincerest appreciation

ABSTRAK

Analisis telah dijalankan untuk menentukan kepekatan logam Ferum, Zink, Kuprum, Kadmium dan Plumbum dalam beberapa sampel ikan yang terdapat di pasar ikan Kota Kinabalu. Enam spesies isi ikan yang dinamakan ialah Kembong (*Rastrelliger kanagurta),* Mata besar (*Selar crymenophthalmus)*, Selar kuning *(Selaroides leptolepis)*, Kerisi *(Namipterus spp)*, Merah *(lutianus argentimaculatus)* dan Siakap (*Lates calcarifer)* telah dianalisa. Semua sampel telah menjalani pengabuan basah sebelum dihadamkan dengan menggunakan campuran asid nitrik pekat (HNO_3 70%) dan hidrogen peroksida (H_2O_2 30%). Hasil penghadaman dituras menggunakan kertas turas Whatman bersaiz 0.45 µm. Seterusnya dianalisis menggunakan Spektrometri Serapan Atom jenis nyalaan (FAAS). Keputusan analisis dari kajian telah menunjukkan bahawa semua spesies ikan yang dikaji mempunyai kepekatan logam yang berbeza. Kepekatan keseluruhan logam dalam unit µg/g berasaskan berat bersih adalah paling tinggi didapati dalam spesies ikan Kembung (Fe 31.55, Zn 20.35, Cu 2.54) diikuti oleh Selar kuning (Fe 22.55, Zn 29.10, Cu 0.51) > Mata besar (Fe 11.79, Zn 15.45, Cu 0.51) > Merah (Fe 11.40, Zn 15.15) > Siakap (Fe 9.90, Zn 15.45, Cu 0.80) dan nilai yang paling rendah adalah di dalam Kerisi (Fe 6.85, Zn 12.60). Sementara itu, pengesanan dua jenis logam toksik, Cd dan Pb adalah di bawah had instrumental untuk kesemua enam spesies isi ikan. Perbandingan kesemua logam yang dianalisis adalah didapati pada kepekatan min bawah daripada had maksima yang ditetapkan oleh FAO 1983.

ABSTRACT

DETERMINATION OF FE, ZN, CU, CD AND PB IN SIX SELECTED FISH MUSCLES

This analysis was conducted to determine the metal concentrations of Fe, Zn, Cu, Cd and Pb in six selected fish sample obtained from Kota Kinabalu fish market. Six species of fish muscles, namely Indian mackerel (Rastrelliger kanagurta), Big-eye scad (Selar crymenophtalmus), Yellow striped (Selaroides leptolepis), Threadfin bream (Namipterus spp), Red snapper (Lutianus argentimaculatus) and Giant sea perch (Lates calcarifer) were examined. All samples had undergone wet ashing prior to digest with combination of concentrated nitric acid (HNO_3 70%) and hydrogen peroxide (H_2O_2 30%). The digestion mixtures were filtered using Whatman filter paper 0.45 µm. Then the supernatants were analyzed for the selected metals using Flame Atomic Absorption Spectrometry (FAAS). The analytical results of the study revealed that all the studied fish species contains different metal concentrations. The highest total metal concentrations in µg/g fresh weight basis were observed in Indian mackerel (Fe 31.55. Zn 20.35, Cu 2.54) followed by Yellow striped (Fe 22.55, Zn 29.10, Cu 0.51) > Big–eye scad (Fe 11.79, Zn 15.45, Cu 0.51) > Red snapper (Fe 11.40, Zn 15.15) > Giant sea perch (Fe 9.90, Zn 15.45, Cu 0.80) and the lowest values was in Threadfin bream (Fe 6.85, Zn 12.60). While the detection of two toxic metals, Cd and Pb values were under instrumental limit for all six species of fish muscles. Comparation of all the analyzed metals in fish species were found at mean concentrations lower than the maximum permissible limits proposed by FAO 1983.

TABLE OF CONTENTS

CHAPTER 3: MATERIALS AND METHODS

LISTS OF TABLES

LIST OF ABBREVIATIONS

μ	Micro
°C	Celcius
>	More than
%	Percent
/	Per
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
Fe	Ferum
Zn	Zinc
Cu	Cuprum
Cd	Cadmium
Pb	Plumbum
cm	centimetre
IDA	Iron Deficiency Anemia
kg	kilogram
mg	Miligram
mL	Milliliter
FAAS	Flame Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
RDA	Recommended Daily Allowance
FAO	Food and Agriculture Organization
EEZ	Economic Exclusive Zone
km	kilometre
et al	et alia (and other)
GDP	Gross Domestic Product
WHO	World Health Organization
PTWI	Provisional Tolerable Weekly Intake
LDL	Low Density Level
HDL	High Density Level
UL	Upper Level
MT	Metallothionein
DOF	Department Of Fisheries
C	Concentration
V	Volume
M	Mass
HCL	Hollow Cathode Lamp
ppm	part per million
SD	Standard Deviation

LIST OF APPENDIX

CHAPTER 1

INTRODUCTION

1.1 Fish demands

Fish is the best source of omega-3 fatty acids. Fish constitutes about 45% of the total animal proteins intake and contains valuable trace minerals (Dunne, 2002). Throughout human history, fish have been an important source of foods. More than 1 billion people rely on fish alone as an important source of animal proteins. It is generally believed that fish is particularly important to poorer population groups of many countries. However, increases in population have increased demands for fish to the point that the Earth's oceans are being depleted.

Fish demands are estimated to increase from 1.14 million metric tons in 2004 to 1.93 million in 2010 (Biusing, 2003). In developed countries, increasing consumption may be due to rises in per capita income and growing awareness of the nutritional values in fishery products. On the other hand, in the under developed countries there is also a strong efforts by national and international bodies to provide more protein to the population through increased fish supply.

1.2 Fisheries potential in Sabah

The state of Sabah located on the northern part of Borneo Island. The population of Sabah including the transient population is around 2.45 million of which 75% of them live and works in the coastal zone. The territorial waters of Sabah cover about 32% of the Malaysian Economic Exclusive Zone (EEZ). Considering all coasts with direct marine exposure, the total length of Sabah coastline becomes around 4315 km making it being the longest coastline in Malaysia (Chuan *et al.* 1996).

The fisheries sector is an essential part of Sabah's economy. The fisheries and aquaculture industries produce about 200,000 metric tons of fish annually. This translates to a contribution of 2.8% to the Sabah's annual Gross Domestic Product (GDP). The major contributor to this is the marine capture fisheries which accounts for about 80%. Combined with 50% of the fish landings in Labuan, Sabah ranked second (16.2%), with Perak as the top marine fish contributor in the country, 17.6%. In 2003, it contributed about 84% of the total production 180, 000 metric tons. Sabah is also a net exporter of fish and fish products (Biusing, 2003). It is estimated that Sabahans consumed fish at a high 50 kilograms per capita.

1. 3 Marine pollutions

As a result of mining, forestry, waste disposal and fuel combustion, our environment is becoming increasingly contaminated with heavy metals. The heavy metal pollution of the marine environment has long been recognized as a serious environmental concern. Consumption of seafood is a significant pathway to metal exposure in the human population living in coastal areas. Increased levels of minerals in the coastal zone enhance the metal load of inshore waters and of aquatic animals.

Generally, the ocean margin is a critical land sea interface at the boundary of which several sources may affect the chemical characteristics of the waters and bring pollutant materials. Continental sources such as river runoff and atmospheric transport, oceanic sources and diagenetic exchanges at the water-sediment interface have been identified as the main factors (Hashmi *et al.* 2002). Carbon compounds, nutrients and heavy metals that are delivered to the ocean margin are important in the living processes of marine organisms but they are potential source of marine contamination as well. Marine animals are sensitive to metals when the concentration of these substances reaches a certain level in the water.

The aquatic environment receives waste products from such activities and may be the final depository for these heavy metals. Oil spills and dumping can occur offshore and sewage. Pesticides, organic pollutants and sediments from erosion wash into coastal waters where most fish spend at least part of their lives. Siltation in the coastal areas may also pose a threat in the long run (Chuan *et al.* 1996). Most of the rivers are heavily affected by the silting process and these rivers empty into coastal estuaries where many species of fish spend part of their life cycle.

Contaminating substances may enter the food chain at many different stages. In the sea, pollutants are potentially accumulated in marine organisms and sediments, and subsequently transferred to a man through the food chain. Large fish at the top of the food chain are more likely to contain high levels of industrial contaminants, but shellfish also accumulate contaminants because they feed by passing large volumes of water through their bodies (Ruiter, 1995).

Many countries have established limits for the toxic trace elements in dietary products, including seafood. Malaysia permits in predatory fish higher levels of toxic trace elements arsenic, mercury, lead and cadmium than in other fish (Table 1.3). The FAO/WHO recommended PTWI of trace elements which includes from all sources and values for an adult of 70 kg are given in Table 1.3. Exact levels of tolerable metals that are safe for marine organisms are still confusing because the concentration of a metal required to produce toxicity may differ.

1.4 Objectives:

a) To determine the metal concentrations of Fe, Zn, Cu, Cd and Pb in common edible fish of Kota Kinabalu fish market.

b) To compare the concentration of analyzed metals among species observed.

c) To compare the concentration of analyzed metals with which indicated by international regulations (FAO 1983).

Table 1.3: Permissible toxic trace elements levels in seafood (mg/kg) set by Malaysia Food Act 1983 & Regulations in fish and fishery product (mg/kg) and Provisional Tolerable Weekly Intake (PTWI) for an adult weighing 70 kg (mg/person) set by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (WHO 1996).

	Arsenic (As)	*Lead (Pb)*	*Mercury (Hg)*	*Cadmium (Cd)*
Malaysian Food Act 1983 (Act 281) & Regulations				
Fish and fishery product				
(i) Predatory	1	2	1	1
(ii) Others	1	2	0.5	1
Joint FAO/WHO Expert Committee				
PTWI[a]	1.05[a]	1.75	0.35	0.49

[a]PTWI for inorganic arsenic.

Source: Food Act 1983 (Act 281) & Regulations

CHAPTER 2

LITERATURE REVIEWS

2.1 Metals

About 80 of the 103 elements listed in the Periodic Table of the Elements are metals. All the elements in the *s, d* and *f* blocks of the table are metals (Reilly, 2004). Metals are typically solids that are lustrous, malleable, ductile and electrically conducting. In their metallic state, they are insoluble in water and therefore cannot be used by biological systems. The metallic characteristics increase with the number of electron shells. There is also an intermediate group of elements termed as metalloids by IUPAC that are neither clearly metals nor non-metals.

2.2 Trace metal in foods and diets

The metals and metalloids present in the earth's crust, soils, water, atmosphere, biosphere and consequently end in foods. Metals arise through absorption processes of naturally-occurring soil components. Trace elements were acccordingly classified as essential, non-essential or toxic. Trace elements were at first considered as undesireable contaminants of food, but later it was realized that some of these elements, particularly iron and copper, were essential for normal health.

All essential element are found among lighter elements and present in all healthy tissue. An essential element is a metabolic or functional nutrient that is required in a minute amount for the maintenance of life. At the present time, nine metals such as iron, copper, zinc, chromium, cobalt, nickel, molybdenum, selenium and manganese are included in group of essential elements. The physiological role and deficiency of copper, iron and zinc are well recognized. Other elements including cadmium and lead have also been claimed essential, but this remains to be confirmed (Ruiter, 1995).

To be considered as essential, its concentration must be relatively constant between different organisms. The deficiency causes an impairment of a function from optimal to suboptimal. The impairment can lead to disease, metabolic anomalies or perturbations in development. However, the essential metals can be toxic at high concentration.

Non-essential elements were also termed as heavy metals as they are toxic even in traces. Scientific definition of heavy metal is one that has a specific gravity of more than 5 g/cm^3 (Ebdon *et al.* 2001). Arsenic, cadmium, lead, mercury and nickel are the most prevalent heavy metals with health concerns. Many metals including heavy metals end up in our food supply and can be traced back to industry via effluent, sewage, dumps and dust into the environment and eventually into the food chain.

2.2.1 Iron (Fe)

Iron is the second most abundant metal after aluminium in the earth's crust and found in all foods. The chemical symbol for iron is Fe. The release and utilization of energy from food depend on iron-containing enzymes. Iron is used in the synthesis of blood pigments and in many other essential activities of cells. Iron in the diet comes from both plant and animal sources. Much of the iron in animal products is heme iron. Heme iron accounts for about 10 to 15% of the dietary iron in industrialized countries (Dunne, 2002).

Animal offal especially liver is one of the foods with the highest concentrations of iron. Other animal product particularly red meats are also rich in iron. Shellfish are good sources as are fish that are eaten whole such as sardines and pilchards but not most other fish are good sources. Leafy green vegetables, legumes, nuts, whole and enriched grains are also good sources of non heme iron. In another way, iron cooking utensils also contribute non heme iron to the diet because the iron leaches from the cookware into food.

2.2.1.1 Iron deficiency

Iron deficiency can cause Iron Deficiency Anemia (IDA). Symptoms of IDA include fatigue, weakness, headache, decreased work capacity, an inability to maintain body temperature in a cold environment, changes in behavior, decreased resistance to infection, adverse pregnancy outcomes, impaired cognitive developments in young children and an increased risk of lead poisoning in young children.

One strange symptom related to iron deficiency is *pica.* This is a compulsion to eat non food items such as clay, ice, paste, laundry starch, paint chips and ashes. Pica can cause the consumption of substances containing toxic minerals and can introduce substances into the diet that inhibit mineral absorption. Mostly, athletes, adolescent are girls and pregnancy women are more susceptible to iron deficiency.

Table 2.2.1: RDAs for iron in some Southeast Asian countries

Age (years)	Southeast Asian countries				
	Indonesia	Malaysia	Philippines	Thailand	Vietnam
Children (1-9)	3-10	10	9-15	6-10	6-12
Boys (10-19)	14-23	10-18	16-18	10-12	11-12
Girls (10-19)	14-25	10-28	17-25	15	12-24
Men (20 ≥)	13	9	12	10	11
Women (20 ≥)	14-26	9-28	11-26	10-15	9-24
Pregnancy	+30	+0	+41	+30	+6
Lactation	+2	+0	+23	+0	+0

Source:Trace Elements in Human Nutrition and Health

2.2.1.2 Iron toxicity

Iron toxicity can be acute, resulting from ingestion of a single large dose at one time or chronic due to accumulation of iron in the body over time. Iron overload is not likely to result from a high dietary intake but it does occur in individuals with hereditary abnormalities in iron absorption. A disease which requiring frequent blood transfusions. Iron is toxic in large amounts causing damage to the intestinal lining, abnormalities in body pH, shock and liver failure. Iron toxicity from supplements is one of the most common forms of poisoning among children under age six and is the leading cause of liver transplants in children.

Iron stores that are above normal but below toxic levels have been associated with a greater risk of both heart disease and cancer. One study found that elevated serum ferritin; an indicator of high iron status is related to an increased risk of heart attacks (Grosvenor & Smolin, 2002). Because iron can act as a pro-oxidant, it can increase the formation of oxidized LDL cholesterol which then leads to atherosclerosis. Excess iron is suggested to increase cancer incidence by boosting the formation of free radicals, suppressing the activity of the immune system and promoting cancer cell multiplication.

Hemochromatosis is an inherited disordrer, characterized by abnormal absorption of iron from the gastrointestinal tract with progressive accumulation of the metal in various organs especially the heart, endocrine organs and liver. It is the most common inherited disease in Caucasian populations in North America, Australia and Europe, occurring in about 1 in 300 north-west European descent. The accumulation of excess iron that occurs in hemochromatosis causes oxidative changes that result in heart damage, liver cancer, diabetes, cirrhosis, cardiomyopathy and arthritis. Iron deposits also can darken the skin. Based on the appearance of gastrointestinal symptoms, a UL has been set at 45 mg per day of iron consumed from all sources (Larsen *et al.* 2002).

2.2.2 Zinc (Zn)

Zinc is an essential for humans and forms an integral part of many enzymes. However, it has a density of 7.14 making it one of the heavy metal (Ihnat, 2006). Zinc is found in both plant and animal foods. Though zinc is widely distributed in both plant and animal foodstuffs, the amounts in different kinds of foods show a wide variation from les than 1mg/kg in some fruits to more than 1000 mg/kg in certain shellfish.

Zinc from animal sources is better absorbed than that from plants because the zinc in plant foods is often bound by phytate. Zinc is abundant in red meat, liver, eggs, dairy products, vegetables and some seafood. Whole grains are a good source but not in refined grains, because zinc is lost in milling and not added back in enrichment.

2.2.2.1 Zinc deficiency

Zinc deficiency has been seen in individuals with a genetic defect in zinc absorption and metabolism called acrodermatitis enteropathica, in those fed TPN solutions lacking zinc and in individuals consuming low-protein but high-phytate diets. It may also occur in a number of disease states including kidney disease, sickle cell anemia, alcoholism, cancer and AIDS (Grosvenor & Smolin, 2002).

The symptoms of zinc deficiency include growth retardation, loss of appetite, taste change, delayed sexual maturation, dermatitis, hair loss, skeletal abnormalities, malabsorption and night blindness. In addition, because zinc is required for vitamin A transport, a deficiency can affect vitamin A status. The impact of zinc deficiency on immune function is rapid and extensive. It results in atrophy of the thymus and a decrease in the number and function of lymphocytes in the blood. The risk of zinc deficiency is greater in groups such as the elderly, children, female and vegetarians.

REFERENCES

Act. 2006. *Food Act 1983 (Act 281) & Regulations.* Kuala Lumpur: International Law Book Services.

Andrew, A. V. 2006. *Atomic Spectroscopy. Introduction to the Theory of Hyperfine Structure.* New York: Springer Books.

Braunbeck, T., Hinton, D. E. & Streit, B. 1998. *Fish Ecotoxicology.* Basel: Birkhauser.

Campbell, C. R. & Plank, C. O. 1998. *Handbook of Reference Methods for Plant Analysis.* Boca Raton: CRC Press.

Chuan, T. T., Mustapha, O. & Fung, S. 1996. *Development & Management of Fisheries Resources in Malaysia.* Sabah: Institute for Development Studies (Sabah).

Cid, B. P., Boia, C., Pombo, L. & Rebelo, E. 2001. Determination of Trace Metals in Fish Spesies of the Ria de Aveiro (Portugal) by Electrothermal Atomic Absorption Spectrometry. *Food Chemistry.* **75**: 93-102.

Cocchi, M., Faeti, V., Manfredini, M., Manzini, D., Marchetti, A. & Sighinolfi, S. 2005. Determination of Metal Concentration in Fat Supplements for Swine Nutrition by Atomic Absorption Spectroscopy: *Journal of AOAC International.* **88**(2): 393-402.

Connell, J. J. 1995. *Control of Fish Quality.* Cambridge: Fishing News Books.

Dean, J. R. 2003. *Methods for Environmental Trace Analysis.* England: John Wiley & Sons Ltd.

De Bievre, P. & Gunzler, H. 2005. *Traceability in Chemical Measurement.* New York: Springer Books.

Dunne, L. J. 2002. *Nutrition Almanac. Fifth Edition.* New York: McGraw- Hill.

Ebdon, L., Pitts, L., Cornelis, R., Crews, H. & Donard, O. F. X. 2001. *Trace Element Speciation for Environment, Food and Health.* Cambridge: The Royal Society of Chemistry.

Gaya UMS. 2006. *Panduan Penulisan Ilmiah Gaya UMS.* Kota Kinabalu: Universiti Malaysia Sabah.

Harris, D. A. & Bashford, C. L. 1987. *Spectrophotometry and Spectrofluorimetry a Practical Approch.* Oxford: IRL Press.

Hashmi, M. I., Mustafa, S. & Tariq, S. A. 2002. Heavy Metal Concentrations in water and Tiger Prawn from Grow-Outs Farms in Sabah, North Borneo*: Journal of Food Chemistry.* **79**: 151-156.

Hilborn, R. & Walters, C. J. 2001. *Quantitative Fisheries Stock Management. Choice, Dynamics & Uncertainty.* London: Kluwer Academic Publishers.

Hollas, J. M. 2002. *Basic Atomic & Molecular Spectroscopy.* New York: John Wiley & Sons Ltd.

Ihnat, M. 2006. Metal and Other Elements: *Journal of AOAC International.* **89**(1): 290-295.

Jorhem, L. & Engman, J. 2000. Determination of Lead, Cadmium, Zinc, Copper and Iron in Foods by Atomic Absorption Spectrometry after Microwave Digestion: *Journal AOAC International.* **83**(5): 1189-1203.

Juresa, D. & Blanusa, M. 2003. Mercury, Arsenic, Lead and Cadmium in Fish and Shellfish from the Adriatic Sea*: Journal of Food Additives and Contaminants.* **20**(3): 241-246.

Kaul, B. L. 2004. *Advances in Fish & Wildlife Ecology & Biology.* Delhi: Daya Publishing House.

Kurfust, U. (ed.). 2001. *Solid Sample Analysis. Direct & Slurry Sampling Using GF-AAS & ETV-ICP.* New York: Springer Books.

Langston, W. J. & Bebianno, M. J. 1998, *Metal Metabolism in Aquatic Environments.* London: Chapman and Hall.

Larsen, E. H., Andersen, N. L., Moller, A., Petersen, A., Mortensen, G. K. & Petersen, J. 2002. Monitoring the Contents and Intake of Trace Elements from Food in Denmark. *Food Additives and Contaminats.* **19**(1): 33-46.

Luten, J. B., Oehlenschalager, J. & OLafsdottir, G. 2003. *Quality of Fish from Catch to Consumer. Labelling, Monitoring & Traceability.* The Netherlands: Wageningen Academic Publishers.

Mansour, S. A & Sidky, M. M. 2002. Ecotoxicological Studies. 3. Heavy Metals Contaminating water and Fish from Fayoum Governorate, Egypt: *Journal of Fooc Chemistry.* **78**: 15-22.

Murano, P. S. 2003. *Understanding Food Science and Technology*: Wadsworth Thomson Learning.

Potter, N. N. & Hotchkiss, J. H. 1995. *Food Science. Fifth ed.* New York: Chapman and Hall

Rasco, B. A. & Bledsoe, G. E. 2005. *Bioterrorism and Food Safety*. New York: CRC Press.

Reilly, C. 2002. *Metal Contamination of Food. Its Significance for Food Quality and Human Health*. Oxford: Blackwell Science Limited.

Ruiter, A. 1995. *Fish and Fishery Products.* New York: CAB International.

Schlenk, D. & Benson, W. H. 2001. *Target Organ Toxicity in Marine & Freshwater Teleosts.* London: Taylor & Francis.

Sepe, A., Ciaralli, L., Ciprotti, M., Giordano, R., Funari, K. & Costantini, S. 2003. Determination of Cadmium, Chromium, Lead and Vanadium in Six Species from the Adriatic Sea*: Journal of Food Additives and Contaminants*. **20**(60): 543-552.

Storelli, M. M. & Marcotrigiano, G. O. 2001. Consumption of Bivalve Mollusks in Italy:Estimated Intake of Cadmium and Lead*: Journal of Food Additives and Contaminants*. **18**(4): 303-307.

Tuzen, M. 2003. Determination of Heavy Metals in Fish Samples of the Middle Black Sea (Turkey) by Graphite Furnace Atomic Absorption Spectrometry: *Journal of Food Chemistry*. **80**: 119-123.

Voegborlo, R. B., El-Methnani, A. M., & Abedin, M. Z. 1999. Mercury, Cadmium and Lead Content of Canned Tuna Fish. *Food Chemistry*. **67**: 341-345.

Watson, D. H. 2004. *Pesticide, Veterinary and other Residues in Food*. Cambridge: Woodhead Publishing Limited.