METAL CONTAMINATION IN COMMERCIALLY IMPORTANT FISH AND SHRIMP SPECIES COLLECTED FROM ACEH (INDONESIA), PENANG AND PERAK (MALAYSIA)

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METAL CONTAMINATION IN COMMERCIALLY IMPORTANT FISH AND SHRIMP SPECIES COLLECTED FROM ACEH (INDONESIA),PENANG AND PERAK (MALAYSIA)

by

SOFIA

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**KONTAMINASI LOGAM DALAM SPESIES IKAN DAN UDANG BERNILAI
KOMERSIAL YANG DIPEROLEHI DARIPADA ACEH (INDONESIA),
PULAU PINANG DAN PERAK (MALAYSIA)**

ABSTRAK

Kajian ini dijalankan untuk mengkaji kontaminasi empat logam berat dalam tisu ikan dan udang yang bernilai komersial yang diperolehi dari Aceh (Indonesia) serta Pulau Pinang dan Perak (Malaysia). Seterusnya kajian juga dijalankan untuk menaksir risiko pemakanan makanan laut terhadap kesihatan. Secara khusus, matlamat kajian ini adalah untuk menyediakan maklumat tentang kepekatan logam Cd, Pb, Cu dan Cr dalam otot, tisu hati dan ginjal bagi lima spesies ikan (*A. mate*, *R. kanagurta*, *E. affinis*, *L. surinamensis* and *E. coioides*) dan di dalam otot udang (*P. coromandelica*). Sampel ikan dan udang diperolehi dari 2 lokasi di Malaysia (Batu Maung di Pulau Pinang dan Kuala Sepetang di Perak) dan 3 lokasi di Aceh, Indonesia (Lampulo, Lambada dan Ulee Lheue). Sebagai tambahan, kajian ini juga cuba membandingkan nilai yang diukur dengan nilai piawai makanan dan kesihatan nasional dan serantau.

Kandungan Cd didapati berjulat di antara 0.01 ke 0.83 µg/g (berat kering) dengan kandungan tertinggi tercatat di dalam spesies ikan tuna (*E. affinis*). Kandungan Pb berjulat di antara 0.02 ke 0.73 µg/g (berat kering) dengan kandungan yang tertinggi tercatat dalam spesies yang sama. Kepekatan antara 0.03 ke 4.7 µg/g (berat kering) dicatat untuk logam Cu dengan kandungan tertinggi didapati bagi spesies ikan kerapu (*E. coioides*). Kandungan Cr berjulat di antara 0.03 ke 2.09 µg/g (berat kering) dengan kandungan tertinggi dikesan di dalam tisu ikan kembong (*R. kanagurta*).

Kajian ini mendapati bahawa kandungan Cd, Pb, Cu dan Cr tinggi di dalam hati berbanding dengan otot dan ginjal. Sampel yang diperolehi dari Aceh (Indonesia) mengandungi logam berat yang tinggi berbanding sampel yang dikumpulkan dari Pulau Pinang dan Perak (Malaysia). Bagi sampel udang (*P. coromandelica*) kandungan Cu

didapati paling tinggi dengan berjulat di antara 1.25 ke 2.84 µg/g (berat kering), diikuti dengan Cr yang berjulat di antara 0.14 ke 1.1 µg/g (berat kering). Kandungan Cd berjulat di antara 0.01 hingga 0.06 µg/g (berat kering) manakala Pb berjulat di antara 0.01 hingga 0.09 µg/g (berat kering).

Secara keseluruhannya, kajian ini menunjukkan bahawa kandungan Cd, Pb, Cu dan Cr di dalam tisu ikan dan udang didapati lebih rendah daripada paras maksima yang dibenarkan seperti yang ditetapkan oleh Kementerian Kesihatan Malaysia (1998), Kementerian Pertanian, Perikanan dan Makanan, UK (MAFF, 1995) dan Organisasi Makanan dan Pertanian Sedunia (FAO, 2002). Kajian ini juga mendapati bahawa kandungan logam berat berada pada paras yang tinggi didalam tisu hati dan ginjal bagi beberapa sampel. Bagaimanapun, pada kebiasaannya hati dan ginjal ikan tidak dimakan. Perbandingan hasil kajian ini dengan data kandungan logam berat dalam spesies ikan dan udang dalam otot, tisu hati dan ginjal bagi spesies ikan laut dan pantai dari berbagai wilayah di dunia dilakukan untuk mengamati arah aliran dan statusnya dalam kontek serantau dan global.

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The fish samples were found to contain Cd levels ranging between 0.01 to 0.83 µg/g (dry wt.) with the highest level recorded in the tuna (*E. affinis*). The highest Pb levels were recorded in the same species and concentrations ranged between 0.02 to 0.73 µg/g (dry wt.). Concentrations of between 0.03 to 4.7 µg/g (dry wt.) were recorded for Cu with the highest levels found in the grouper (*E. coioides*). Levels of between 0.03 to 2.09 µg/g (dry wt.) were detected for Cr with the highest accumulation recorded in the mackerel (*R. kanagurta*).

The results from this study showed that the concentrations of Cd, Pb, Cu and Cr were relatively high in the liver compared to the kidney and muscle. Samples collected from Aceh (Indonesia) recorded relatively higher metal concentrations than those collected from Penang and Perak (Malaysia). In the shrimp (*P. coromandelica*) samples,

the highest metal content was detected for Cu, which ranged from 1.25 to 2.84 µg/g (dry wt.) followed by Cr, the values for which, ranged between 0.14 to 1.1 µg/g (dry wt.). Cd levels ranged from 0.01 to 0.06 µg/g (dry wt.) while Pb levels ranged from 0.01 to 0.09 µg/g (dry wt.). Shrimp samples collected from Aceh again recorded higher metals levels compared to samples collected from Penang and Perak.

Overall, the findings from this study revealed that Cd, Pb, Cu and Cr concentrations in the tissues were lower than the maximum permissible limit as recommended by the Ministry of Health Malaysia (1998), Ministry of Agriculture, Fisheries and Food, UK (1995) and Food and Agricultural Organization (2002). This study found that levels of the metals in the liver and kidney tissues in some samples, were higher than the recommended concentrations. However, consumers do not routinely consume the viscera. A comparative study was also carried out between the available recent data on the metals in the fish and shrimp species, muscle, liver and kidney tissues of marine and coastal fish species from different regions of the world with those of the present study, to observe their trend and status in regional and global contexts.

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CHAPTER 1

INTRODUCTION

1.1   Background of the Study

Fish is an important source of food for humans and is a key component in many natural food webs. Fish is also one of the sources of biologically valuable protein, fats and fat-soluble vitamins (Belitz and Grosch, 1987). The high quality protein of fish is better for health than that in meat and poultry. Fish consists of 15-24% protein; 1-3% carbohydrate; 0.1-22% lipid; 0.8-2% inorganic substances and 66-84% water (Suzuki, 1981). Each of these is important for human health, growth and intelligence.

In human nutrition, fish plays an important role as it provides an important source of trace minerals and calcium. Fish also provides calories, nutrients such as fat, vitamins (B complex and D), elements such as, phosphorus, sodium as well as trace elements (Mn, Mg, I, Zn, etc.) (Abdullah and Idrus, 1977).

Fish has an important place in the diet of Malaysians and most of the ASEAN countries. It provides approximately 49% of the total animal protein consumed and 12 % of the total protein intake in the Malaysian diet. Chua (1986) reported that the fish consumption in Malaysia was approximately 31.8 to 43 kg/person/year. In Indonesia, the consumption was approximately 13.1 to 18.8 kg/person/year. In a human health perspective, an ASEAN per capita seafood consumption rate of 40 kg/person/year (i.e., 110g/person/day) was adopted for calculation of water quality criteria for protection of human health from contaminated seafood consumption. This is based on the agreement of the ASEAN Marine Water Quality Criteria-Working Group (Chongprasith *et al.*, 1999).

Fish may easily absorb pollutants from the ambient water and from their food and then deposit them in the tissue through the effects of bioconcentration, bioaccumulation and the food chain process especially Hg (Chen and Chen, 2001). In this regard, heavy metals have long been recognized as an important pollutant due to their toxicity and ability to accumulate in marine organisms. Some of the listed toxic metals are arsenic, beryllium, cadmium, chromium, cobalt, tin, zinc, copper, iron, lead, manganese, aluminium, mercury, nickel and selenium (Ornektekin *et al.*, 1997). Humans as consumers of seafood may be affected by consuming them. The effects include chronic and acute diseases (Al-Yousuf *et al.*, 2000). An example of these diseases was reported in Japan when the local population of Toyama consumed cadmium-contaminated fish taken from rivers near the smelting plants. The affected people suffered from a combination of severe kidney damage and painful bones and joints (Ernest and Patricia, 1997). Other metals that cause similar problems are lead and arsenic. Acute lead poisoning can cause *plumbism*, where the typical symptoms are intestinal cramps, renal failure, sterility, and irreversible brain damage (Forstner and Wittmann, 1983). In children, lead is suspected as contributing to hyperactivity, behavior disorders, and learning disabilities (Francis, 1994). Arsenic on the contrary, in chronic state, may cause loss of appetite, leading to weight loss and is followed by gastrointestinal disturbance and conjunctivitis (Reilly, 1991). Based on the incidents as listed in Table 1.1, the health and environmental issues related to metal contamination have attracted many environmentalists to carry out research on metal pollution in the aquatic environment, including in marine organisms.

Monitoring heavy metal content in organisms can better give meaningful information on the pollution status of a water body than merely monitoring the metals in water and sediments (Law and Singh, 1988). There are several disadvantages when analysing water samples for heavy metal. Metal concentrations in natural waters are normally very low which makes it difficult to quantify while avoiding extraneous contamination. Temporal variability is also a problem, rendering mean levels of pollutants difficult to determine.

Analysis of sediments offers several advantages, particularly in terms of providing time-averaged values for pollutant abundance. However, it is difficult to account for the effects of particle size and organic carbon content on metal levels. In addition, no simple methods exist to determine the bioavailability of metals in sediments. On the basis of these problems, the use of organisms is most widely employed as a method to monitor heavy metals in the marine environment (Phillips, 1990).

Much research has been conducted to assess the level of trace metals in fish tissues, such as in liver, kidney, stomach, skin, gill, bone, muscle and spleen (Pagenkopf and Neuman, 1974; Brown and Chow, 1977; Wharfe and Van Den Broek, 1977; Buggiani and Vannucchi, 1980; Szefer *et al.*, 1990; Singh *et al.*, 1991; Al-Ghais, 1995; Kargin, 1998; Romeo *et al.*, 1999; Al-Yousuf *et al.*, 2000; Karadede and Unlu, 2000; Chen and Chen, 2001; Wong *et al.*, 2001; Canli and Atli, 2003).

Muscle is analyzed because of the implications it carries for human consumption and health risk. Liver is often recommended, however, because it concentrates many contaminants in higher levels than muscle. Liver has an important role in contaminant storage, redistribution, detoxification or transformation, and it is an important site of pathological effects induced by contaminants. Fish livers and kidneys are used as the target tissues in monitoring both organic and inorganic contaminants in the Benthic Surveillance Project of the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program of coastal environmental assessment (Evans *et al.*, 1993).

Data and publications on heavy metal pollution in Malaysia and Indonesia are still limited. In Malaysia, results of a few studies on heavy metal pollution show that the

Table 1.1 Some incidents of heavy metal poisoning in humans after consumption of contaminated fish

Country	Year	Disease	Metal	Victims
Fugawa, Japan	1947	*Itai-itai* disease ❑ Beginning with simple symptoms such as 'lumbago' or 'joint paints' (Tucker, 1972). ❑ A yellow discoloration of the teeth, the sense of smell is lost and the mouth becomes dry. Subsequently, the number of red blood cells is diminished which results in impairment of bone marrow (Tucker, 1972). ❑ The most characteristic features of the disease are lumbar pains and leg *myalgia*, these conditions continue for several years until the patient becomes bed-ridden and the clinical conditions progress rapidly (Friberg *et al.*, 1974).	Cd	At the end 1965, 100 deaths had been recorded (Friberg *et al.*, 1974).
Minamata, Japan	1953	❑ Neurological disorder (Tucker, 1972). ❑ *Delirium* (a mental disturbances characterized by confusion, disordered speech, and hallucinations), speech disturbance and difficulties in walking (Tucker, 1972). ❑ Numbness and followed with tremor, slurred speech, tunnel vision, blindness, loss of hearing, and finally death (Armstrong, 1979). ❑ Intellectual disturbances, slow reflex (Armstrong, 1979). ❑ *Dysarthria*, a constriction of the visual fields ultimately leading to tunnel vision and impairment of hearing (Armstrong, 1979).	Hg	By 1978, the number of victims recognized by Japan was 1303 with an additional 200 deaths (Tollefson, 1989).

Table 1.1 continued

		❑ *Parenthesis*, a numbness and tingling sensation around the mouth, lips, fingers and toes (Armstrong, 1979). ❑ *Hyperkinesias* and h*yper salivation* (Armstrong, 1979)*.* ❑ *Ataxia*, a stumbling gait and difficulty in articulating words is the next progressive symptom along with *neurasthenia*, a generalized muscle weakness, fatigue, headache, irritability, and inability to concentrate often occur (Tollefson, 1989).		
Niigata, Japan	1965	❑ The symptoms of this poisoning are loss of feeling or coordination of limbs, numbness of mouth and face and loss of vision (Tollefson, 1989).	Hg	6669 victims were recognized for relief measures by 1978 and there were 55 deaths (Tollefson, 1989).
Sweden	1965	❑ Increase in the frequency of chromosome breakage (Bryan, 1976).	Hg	

problem is getting worse. Over the last 15 years, research on heavy metal content in fish tissues, molluscs, prawn and in the water column and sediment has been conducted in Malaysia (Babji *et al.*, 1979; Sivalingam and Ahzura, 1980; Ramachandran *et al.*, 1985; Law and Singh, 1988; Din, 1992; Din and Jamaliah, 1994; Ismail *et al.*, 1995; Ong and Din, 1995; Saed *et al.*, 1995; Shazili *et al.*, 1995). In Indonesia, data and publication on metal contamination are even more limited. Only a few studies on heavy metal content in water, sediment and organisms have been carried out (Hutagalung, 1994; Abdullah *et al.*, 1995; Sunoko, 1995; Kambey, *et al.*, 2001).

A survey on the distribution of heavy metals in consumable fish species in these countries is urgently needed. Therefore, this study was designed to investigate the concentrations of cadmium, lead, copper and chromium in the muscle, liver and kidney of five commercially important fish species.

1.2   Objective of the Study

The objectives of this study were :

1. To determine the concentrations of heavy metals (Cd, Pb, Cu and Cr) in the muscle, liver, and kidney tissues of five commercially important fish species and one species of shrimp.

2. To determine among the tissue (liver, kidney, and muscle), which is able to accumulate the highest level of the metals.

3. To compare the level of heavy metal contamination in fish and shrimp collected from three locations, namely Aceh in Indonesia, and Penang and Perak in Malaysia.

4. To ascertain whether concentrations of heavy metals in consumable fish exceed the national and international standards for food and human health.

1.3   Assumptions

Fish samples were either bought from fisherman at landing sites or purchased from the wet markets. The fisherman and fishmongers were interviewed as to where the fish came from. It is assumed that the information provided was correct.

CHAPTER 2

LITERATURE REVIEW

2.1   Heavy Metals

Heavy metals are defined as all metals of atomic weight greater than sodium with specific gravity of more than 5.0 (MARC, 1980). Hashim (1995a) pointed out that heavy metals are also a group of toxic metals with strong affinity for biological tissues, and which are slowly eliminated from the biological systems.

The term 'heavy metal' has been used extensively to describe metals that are environmental pollutants (Walker *et al.*, 2001). According to Francis (1994), even though some metals are essential when taken up by organisms, their excessive presence will reverse the effect so that benefit becomes toxicity.

Heavy metals can be critically important to the life processes of marine organisms. Aluminium, arsenic, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc are essential heavy metals for one or more organisms. Usually, they are present in living organisms in trace amount not exceeding 1 μg/g. Copper and zinc are necessary in trace amounts for the functioning of biological systems (Markert, 1994). The non-essential heavy metals include cadmium, gold, lead, mercury, silver, and metals (including radionuclides) of higher atomic weight (Rainbow and Furness, 1990). Lead and cadmium are known to interfere with the functioning of the biological systems (Villareal-Trevino *et al.*, 1986).

Due to the fact that even trace amounts of some heavy metals can generally exhibit high toxicity to marine biota and human, there is an increasing interest in studying these metals in the marine environments (Sadiq, 1992). Among these heavy metals are

cadmium, lead, copper, and chromium. These metals represent the greatest potential concern to the environment and human health (Hashim, 1995b).

2.1.1 Cadmium (Cd)

Cadmium is a relatively rare earth element that is almost uniformly distributed in the earth's crust with an average concentration of 0.15-0.2 mg/kg (Sadiq, 1992) and ubiquitously present in food, water and air (Passwater and Cranton, 1983).

Cadmium also has an extremely long residence time (over 20 years) in the human body and a significant proportion of the body burden is stored in liver and kidney, and bound to metallothionein (Urieta *et al.*, 2001). Cadmium circulates in the blood primarily bound to the red cells. It is evidently bound partly to hemoglobin and partly to metallothionein (Miettinen, 1975). Once in the blood, it binds to large proteins (e.g. albumin) for distribution to tissues, primarily the kidneys. Cadmium can be transferred from mother to fetus but its concentration in newborn blood is on the average 30-50% lower than that in the maternal blood because placenta plays a barrier role. Placenta has been shown to be able to accumulate cadmium where at delivery its concentration in the placenta is about 10 fold higher than in the maternal blood (on a wet weight basis) (Piscator, 1979).

Cadmium is a highly toxic metal causing long-term risks (renal tubular damage and hypertension) in human, even though its concentration in the body fluids may not be greater than approximately 10 μg/L (Boiteau and Pineau, 1988). Most cadmium remains in the plasma for the first few hours after administration.

Fish, mollusks and crab are the main transport of cadmium intake by man (Miettinen, 1975). In the general population, the main exposure to cadmium is via food, e.g. kidney, liver, grains and cereals, fish, oysters and clams (Friberg *et al.*, 1974).

Ingestion of small amounts of contaminated fish that contain cadmium over long periods of time may lead to some form of cadmium intoxication. The first sign of chronic cadmium intoxication is the appearance in urine of low molecular weight proteins, known as tubular proteinuria and later may occur *aminoaciduria, glucosuria* and *phosphaturia*. This in turn implies that the liver and other organs of most fish are not fit for human consumption (Moore and Ramamoorthy, 1984).

Nutritional status and the adverse effects of calcium may influence the absorption of cadmium. In particular a less adequate intake of zinc may increase the gastrointestinal absorption of cadmium (Miettinen, 1975).

Cadmium is very efficiently retained in the organism and normally only a very small quantity is excreted daily. The main route of excretion is via the urine. Excretion is low, less than 0.01% of the total body burden per day. Cadmium can also be excreted through other routes (feces, saliva, hair) but at a much lower rate than in the urine (Piscator, 1979).

Cadmium in the aquatic environment is generally taken up into the human body via the gastrointestinal tract as drinking water and food. Absorption rate from the gastrointestinal tract has been documented to be between 3 to 6% (Clarkson, 1979).

Complexation of Cd is known to modify bioaccumulation and biotoxicity in a marine environment (Sadiq, 1992). Cadmium is accumulated primarily in major organ tissues of fish such as liver and kidney rather than in muscle. In general, residues in fish muscle cannot be related to concentrations in water (Moore and Ramamoorthy, 1984). Cadmium is very efficiently retained in the organism and accumulates with age (Piscator, 1979).

Clarkson (1979) stated that it has been estimated that long-term exposures with daily intakes of 300-480 µg of cadmium may cause renal tubular dysfunction. Anemia and disturbed liver function may also result from excessive cadmium exposure. The toxicity of cadmium to human may be seen in Table 2.1.

Table 2.1 Acute oral toxicity of cadmium

Doses	Effects
3-90 mg	Reported non fatal incidents
15 mg	Experimentally induced vomiting
10-326 mg	Severe toxic symptoms but not fatal
350-500 mg	Estimated lethal doses
1580-8900 mg	Reported lethal doses

Source: Lauwerys (1979).

Cadmium is one of the most biotoxic elements and is regarded as a priority pollutant. Moore and Ramamoorthy (1984) found that cadmium is widely used in various industrial products and processes including electroplating, pigments, plastic stabilizers, batteries and packing industries. In the electroplating process, cadmium is deposited on objects to provide bright appearance and resistance to corrosion. Cadmium stearates are used as stabilizers in the production of polyvinyl chloride plastics (PVC) and cadmium phosphors are used as tubes in television sets, fluorescent lamps, x-ray screen, cathode-ray tubes, and phosphorescent tapes. Cadmium pigments are used in traffic paints and in the glass enamel red label on for example the 'Coca-Cola' bottles. In electrical and electronic applications, cadmium is widely used in many functions such as heavy-duty relays, switches, automobile distributors contacts, and solar and photocells. Because of its wide variety of uses, anthropogenic inputs into the marine environment are considered the principal source of cadmium contamination (Sadiq, 1992).

Cadmium contamination in the environment has caused severe human sufferings. The most significant example of cadmium intoxication is the Itai-itai disease, which was caused by exposure to Cd discharged into the local rivers. It was diagnosed in residents of Toyama Prefecture (Japan) from the 1940's to the 1960's. Patients suffering from the Itai-itai disease showed signs of *osteomalacia* in bones and are characterized by extreme fragility of the bones, with spontaneous fractures, bone deformity, and severe pain of bones and joints (Francis, 1994).

2.1.2 Lead (Pb)

Lead is one of the oldest metals known to man and has been used in piping, brass, red lead manufacture, building materials, solders, paint, metal products, ammunition, castings, storage batteries, chemicals, pigments and also in the production of synthetic polymers (Moore and Ramamoorthy, 1984). One of the alkyl compounds, tetraethyl lead (TEL), is widely used as an anti-knock agent in motor fuels. The amount used generally is about 0.1% of the fuel content. Combustion of TEL in motor fuel is a main source of lead in ambient air and TEL poisoning is usually associated with concentrations of 300 µg/L or more. The earliest symptom of TEL poisoning is insomnia, and the main organ affected is the central nervous system. The poisoning is usually acute, developing into toxic psychosis with hallucinations, delusions, excitement and bad dreams, and may result in death. Abdominal pain and peripheral neuropathy, which are common symptoms of inorganic lead poisoning, have seldom been observed in cases of TEL poisoning (Tsuchiya, 1979).

Anthropogenic activities are responsible for most of the lead pollution and anthropogenic inputs greatly exceed those from natural sources. Most of the lead in the environment is in inorganic form (Sadiq, 1992).

In human blood, lead increases the fragility of erythrocytes or its precursor cells in the bone marrow. The metal increases the permeability but inhibits active transport by blocking K-Na sensitive transport ATPase. Also, lead inhibits biosynthesis of heme, particularly in conversion of δ-amino levulinic acids to prophobilinogen and the formation of heme from iron and photoporhyrin (Cross *et al.*, 1970). Although lead is bound to the red cells, it passes into the brain more easily than cadmium. However it does not accumulate there. The concentration of lead is higher in cortical gray matter and basal ganglia than in the cortical white matter in cases of lead poisoning (Miettinen, 1975).

Toxicity of lead is not immediately seen, since it can remain in bone for years. Lead is present in all organs and tissues of mammals, although it is not essential to their nutrition (Cross *et al.*, 1970). This metal may cause both acute and chronic effects, which usually result from its accumulation in the body over a certain period of time. The major effects are related to four organs systems, i.e., hematopoietic, nervous, gastrointestinal and renal systems. Anemia is an early manifestation of chronic lead intoxication (Tsuchiya, 1979).

In fish, lead residues in muscle tissue are usually only slightly lower than those in organs, contradictory to cadmium. This reflects the relatively low rate of binding to SH-groups. In addition, the low solubility of lead salts restricts movement across cell membranes. Wong *et al.* (1975) demonstrated that tetramethyl lead could be accumulated rapidly from water by the rainbow trout. Highest residues were found in the intestinal fat, skin and gills. There was no relation between species, feeding habits and size of fish and concentration of tetraalkyl lead in tissues (Moore and Ramamoorthy, 1984).

About 10% of ingested lead is absorbed in the gastrointestinal tract. This fraction may be higher for infants and children. With 10% absorption, food would contribute 20-30 µg/day. Absorbed lead is transported by blood and initially distributed in various organs

and tissues. It is then gradually redistributed to form an exchangeable compartment (blood and soft tissues) and a storage compartment, essentially bone. In humans subjected with low-level exposure, about 90% of the total body burden is found in bone. Lead in blood is mainly bound to erythrocytes where its concentration is about 16 times higher than in plasma (Tsuchiya, 1979).

Absorbed lead is excreted in milk, sweat, hair, and nails. About 90% of ingested lead is eliminated unabsorbed through feces. The kidneys also excrete lead, but a small fraction may be lost in sweat or by gastrointestinal excretion. This includes lead returned to the gastrointestinal tract in bile and in secretions from the salivary glands and other intestinal glands (Goyer and Mushak, 1977). Tsuchiya (1979) found that absorbed lead is excreted primarily in urine (about 76%) and other excretion routes are gastrointestinal secretions (about 16%) and hair, nails and sweat (<8%). The gastrointestinal excretion of lead is low; of the order of one or a few percent (Miettinen, 1975).

Food is a major pathway of lead intake for the general adult population, contributing more than 80-85% of the total daily intake. The values reported by Tsuchiya (1979) may serve as an example of lead concentration in food, i.e., condiments contained about 1 mg/kg; fish and seafood 0.2-2.5 mg/kg; meat and eggs 0.2-0.4 mg/kg; and grains and vegetables contained up to 1.4 mg/kg. Estimated daily intake via food is about 200-300 µg for adults and total body lead content of a 70-kg man is approximately 100-400 mg and increases with age. As for the average daily intake in food, estimation from different countries and different investigators vary from about 110 µg to about 520 µg.

2.1.3 Copper (Cu)

Copper is an essential metal for all living organisms and is found in all body tissues. It is widely distributed in nature in free state and in sulfides, arsenides, chlorides, and carbonates. It is also an essential part of several enzymes, e.g. tyrosinase, which is

necessary for the formation of melanin pigments, superoxide dismutase, amine oxidases and uricase and copper also plays a catalytic role for many enzyme systems such as cytochrome oxidase (Sadiq, 1992). In the biological system, copper takes the place of an essential metal for the utilization of iron in the formation of hemoglobin. Besides, copper and iron are also involved in the natural selection of aerobic cells and the evolution of metalloproteins and metalloenzymes (Piscator, 1979).

Presently, there are many industrial, agricultural, and domestics uses for Cu. Because of its widespread use, Cu is one of the most common environmental pollutants. Approximately 17,000 metric tons of solid Cu wastes are deposited annually into the oceans. It has been shown that anthropogenic inputs are the major sources of Cu contamination (Sadiq, 1992).

Absorption of copper in human occurs primarily in the stomach and upper part of the small intestine (Moore and Ramamoorthy, 1984). Mercury, lead, vitamin C, sulfides, raw meat, silver and possibly calcium and molybdenum may prevent the absorption of copper while amino acids and fresh vegetables may promote its absorption (Passwater and Cranton, 1983).

Passwater and Cranton (1983) observed that copper is transported from the liver primarily with a high molecular weight protein, ceruloplasmin (a copper-containing protein), which is produced in the liver and also has ferridoxase activity (Hill, 1977). The liver controls copper storage and excretion. The amount of copper stored in the liver is approximately 30 percent of the total body copper content.

Excessive storage of Cu in the liver can cause Wilson's disease, an inborn error of metabolism, also called *hepatolenticular* degeneration. Wilson's disease heightens the urinary excretion of copper considerably. Main excretion route of copper is via the bile and

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only a few percent of the absorbed amount is found in urine. Copper is mainly stored in the liver and muscle and the biological half-life in human beings is about four weeks (Piscator, 1979).

Generally, low Mo, Zn, and SO_4^{2-} intake may increase copper toxicity (Forstner and Wittmann, 1983). In adults, the ingestion of about 1 g of copper sulfate (about 400 mg of copper) can induce vomiting. Accidental ingestion of large amounts of copper salts causes gastrointestinal disturbances, systemic effects, especially hemolysis, liver damage and renal damage (Piscator, 1979). Excessive copper and iron and/or zinc and manganese deficiencies are primary factors in one type of schizophrenia, called 'histapenia' (low blood histamine) (Passwater and Cranton, 1983).

A U.S. Geological Survey Bulletin suggests that 2 to 5 mg consumption per day of Cu in human will not change the body's copper balance. However, intake of 250 to 500 mg per day of the metal would be toxic. The Food and Nutritional Board of the National Academy of Sciences (U.S.) assumes that an occasional intake of up to 10 mg of Cu is safe for adults (Passwater and Cranton, 1983). In addition, Moore and Ramamoorthy (1984) also recommended dietary allowance of copper for adults to be 2-3 mg per day corresponding to about 15-45 µg/kg body weights and daily requirements have been estimated to be about 30 µg/kg body weight for adults, 40 µg/kg body weight for older children and 80 µg/kg body weight for infants.

The primary use of copper is in electrical equipment and as a component of many alloys where it may occur together with other metals, such as silver, cadmium, tin and zinc. Other important uses of the metal are in plumbing and heating industries and in marine environment, copper was used as an anti-fouling paints. In addition, copper salts may also serve as pesticides (Piscator, 1979).

2.1.4 Chromium (Cr)

Chromium is ubiquitous in nature and one of the trace elements that have functions in both animal and human nutrition. Chromium occurs in biological materials in trivalent and hexavalent form. Trivalent chromium (Cr^{3+}), in ionic form does not penetrate the cell membrane, while the hexavalent chromium (Cr^{6+}), because of its very high solubility does (Rondia, 1979). In solutions, Cr^{3+} easily forms hydroxy complexes and coprecipitate easily with many metal hydroxides (Kumpulainen, 1988). Hexavalent chromium forms chromates (CrO_4^{2-}) and dichromates ($Cr_2O_7^{2-}$), both of which are potential oxidizing agents and are, therefore, relatively toxic to living organisms (Kumpulainen, 1988).

Trivalent chromium plays an important role in insulin metabolism as the glucose tolerance factor (GTF). GTF may have a role as a cofactor for the initiation of peripheral insulin action. It increases the effectiveness of insulin. It also plays an important role in the activity of different enzymatic reactions such as thromboplastic activity, β-glucuronidase activity and bacterial urease activity (Norseth, 1979). In the Cr^{6+} state, chromium acts as a strong oxidizing agent and reacts readily with organic materials, leading to a reduction to the Cr^{3+} form (Rondia, 1979).

The uses of chromium are commonly in tanning industries. The others are in pigment production and application, also in the graphics industry. Ferrochromium and chromium metal are the most important classes of chromium used in the alloy industry (Norseth, 1979). Presence of chromium in iron casting improves the resistance to corrosion and oxidation and also increases the ability to withstand stress at elevated temperatures (Rondia, 1979).

Chromium compounds are widely used as mordant and dyes in textile industry, chrome electroplating, anodizing, and dipping and it is employed as oxidants and catalysts

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in the manufacture of products such as saccharin, in bleaching and purification of oils, fats and chemicals and as agents to increase the anti-wetting by water insolubility of various products such as glues, inks and gels (Brooks and Rumsey, 1974). Because of the wide industrial use of Cr metal and its compounds, anthropogenic activities have become the most significant contributor to environmental contamination (Sadiq, 1992). Vercoutere and Cornelis (1995) reported that chromium and its compounds occurring in the work environment are associated with such process as welding and grinding of stainless steel, chrome plating, tanning, wood preservation, painting and pigment production.

Buchanan (1979) documented that insoluble or slightly soluble compounds of chromium in human are likely to be retained in the lungs. In particular soluble chromates can cause liver damage or dysfunction when ingested. Norseth (1979) reported that hexavalent chromium may cause skin ulceration, irritative dermatitis, allergic skin reactions and allergic asthmatic reactions. It may also cause ulcerations in the mucous membranes and perforation of the nasal septum.

Chromium is not acutely toxic to human. This is due to high stability of natural chromium complexes in the abiotic matrices. However, Cr^{6+} is more toxic than Cr^{3+} because of its high rate of adsorption through intestinal tracts (Moore and Ramamoorthy, 1984). Reduction of the hexavalent form to the trivalent may be of importance for the toxicity of chromium compounds (Norseth, 1979).

Chromium is transported in the blood in at least two forms. One form is glucose tolerance factor (GTF) and the other form is trivalent chromium bound to β-globulin. Glucose tolerance factor is a complex of chromium with vitamin B3 (niacin) and three amino acids. The excretion of chromium occurs mainly through the kidneys (Passwater and Cranton, 1983) and urine (Rondia, 1979).

Food is a more significant source of chromium than water. Food items vary considerably in concentration of chromium. Among large sources are meat, vegetables and unrefined sugar, while fish, vegetable oil and fruits contain smaller amounts (Norseth, 1979). In the U.S., the mean daily chromium intake with food has been estimated to be 5 to 100 µg/day for an adult, of which 99% are excreted in the feces (Rondia, 1979).

2.2   Heavy metals pollution in the marine environment

Pollution of the marine environment by heavy metals has long been recognized as one of the important pollutants posing a threat to the marine organisms (Al-Yousuf *et al.*, 2000). Heavy metals are non-biodegradable and do not break down in the environment. Heavy metals can accumulate in fish via the food chain. For example, copper, cadmium, mercury and silver are toxic to phytoplankton and may influence the overall primary productivity or alter phytoplankton species composition. Higher organisms are exposed to heavy metal both in solution and in food. Many invertebrates, in particular, accumulate large amounts of heavy metals, which may then be taken up and accumulated by predatory fish (Blaber, 2000).

The United Nations Joint Group of Experts on the Scientific Aspects of Marine Pollution, GESAMP (1982) has defined marine pollution as:

"introduction by man, directly or indirectly, of substances or energy into the marine environment, resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fisheries, impairment of quality for use of seawater, and reduction of amenities".

High concentrations of heavy metals are normally associated with urban and industrial development. For example, the Cukurova region, which is at the southern part of Turkey, is known as an area that is rapidly expanding in population, agriculture and industry. A study by Kargin (1998) was conducted near the region, in the Seyhan River

that assess metal contents (Cu, Zn, Fe, Cd, and Pb) in the liver, gill and muscle tissues of the freshwater fish *Capoeta barroisi.* The results found that all the untreated industrial, chemical pollutants and domestic sewage were thrown through the river. And the metal concentration was found higher from downstream of this river (2.9 - 260 µg/g, dry wt.).

Law and Singh (1987; 1988) studied metal contents in tissues of several fish species including *Arius thalassinus, Plotosus anguillaris, Dasyatis zugei, Lagocephalus lunaris, Setipinna taty* and *Johnius carutta* in the Kelang Estuary, Malaysia. They found that the overall mean for mercury content in the fish tissue was 0.22 µg/g and for lead it was 0.27 µg/g. They concluded that mercury content was five times greater and lead content was two times more than in an adjacent unpolluted estuary. However, the values were still below the maximum permitted levels for human consumption of 0.5 µg/g for mercury and 1.5 µg/g for lead. Levels of zinc and copper were similar in fish from both estuaries.

2.2.1 Source of heavy metals pollution

Heavy metals have been recognized as one of the major factors posing serious threat to marine biota and its contamination in the marine environment have been reported to cause toxicity to aquatic organisms (Buggiani and Vanucchi, 1980). According to Al-Ghais (1995), heavy metals may enter marine environments from a variety of natural and anthropogenic sources. Kennish (2001) differentiated the natural and anthropogenic sources of pollution as follows:

1. Natural sources include weathering of rocks, leaching of soils, eruptions of volcanoes, and emissions of hydrothermal vents;
2. Anthropogenic sources include leaching of antifouling paints, dredged materials disposal, spills from coastal installations and vessel, mining of metal ores, smelting operations, refining, electroplating, and the manufacture of dyes, paints, and textiles.

In addition, Thomson *et al.* (1984) stated that leachates from landfills, shipping and boating activities, aerial deposition also contribute to the anthropogenic input, as well as industrial and domestic sewage, fossil fuel combustion, waste incineration, agricultural utilization of pesticides and herbicides (Chester and Murphy, 1990).

2.2.2 Transport of heavy metals in the environment

The atmosphere represents an active environmental compartment for heavy metals. The other environmental compartments are land surface, ocean, sediments and biosphere. It is known that heavy metals in the atmosphere originate from both natural and anthropogenic sources. Each year large quantities of potentially toxic metals are introduced into the world's ocean from both sources (Martin *et al.*, 1976).

The atmosphere can also be viewed as a transient environmental compartment whereby heavy metals pass through at a rapid rate from stationary and mobile sources, on their way to other environmental compartments (Hashim, 1995a). Besides, human activities also result in the release of large quantities of different contaminants, which are dispersed along various pathways through the biosphere.

Beijer and Arne (1979) has divided the transport of heavy metals in the marine environment into three parts:

1. Atmospheric transport

In atmospheric transport, metals are emitted into the atmosphere and are transported by wind over vast distances, depending on their state (gaseous, vapor or particulate). The atmosphere is a major route for the transport of heavy metals to the open oceans (Patterson *et al.*, 1976).

2. Aquatic transport

Generally, the greater part of metal load emitted into the environment is transported by water. Most of it eventually reaches lakes and the coastal areas via river transport. Part of the total metal load carried into a lake systems are transported by absorption or adsorption onto particles of different types. The metals may then be released again into the systems through microbial activity or due to changes in various physical and chemical factors including pH and redox potential.

3. Biological transport

Transport of metals by living organisms also plays a role in the total transport. In biological transport, plankton in coastal areas may have a quantitative influence on metal transport. A large numbers of metals may be absorbed by phytoplankton and zooplankton and are brought to the coastal areas via the river systems. The metals may then settle and become incorporated in sediments instead of being transported further into the oceans.

Heavy metals transported into the marine environment may be incorporated into the marine food chain and eventually reach human consumers (Hashim, 1995a).

2.2.3 Transformation of heavy metals in aquatic environment

Beijer and Arne (1979) clearly described that the physical and chemical form of metals in the aquatic environment is controlled by such environmental variables as pH, redox potential (dissolved oxygen, ionic strength), salinity, alkalinity and hardness, the presence of organic compounds and particulate matter, and biological activity.

Walker *et al.* (2001) reported that six principal factors, which contribute to the movement and distribution of heavy metals, are polarity and water solubility, partition

coefficients, vapor pressure, partition between different compartments of the environment and molecular stability and recalcitrant molecules.

There are considerable variations in heavy metals toxicity to the marine organisms because the uptake, storage, detoxification, and removal of the heavy metals vary greatly among different marine species. According to Rainbow (1993), intrinsic and extrinsic factors that may affect bioavailability and trace metal uptake by these organisms are:

(1) Intra- and interspecifically variable intrinsic factors, e.g. nutritional state, stage of molt cycle, throughput of water by osmotic flux, and surface impermeability, and

(2) Extrinsic physical-chemical factors, e.g. temperature, salinity, dissolved metal concentration, presence or absence of other metals, and presence or absence of chelating agents.

Zakrzewski (1991) described the fate of heavy metals in an individual organism as shown in Figure 2.1.
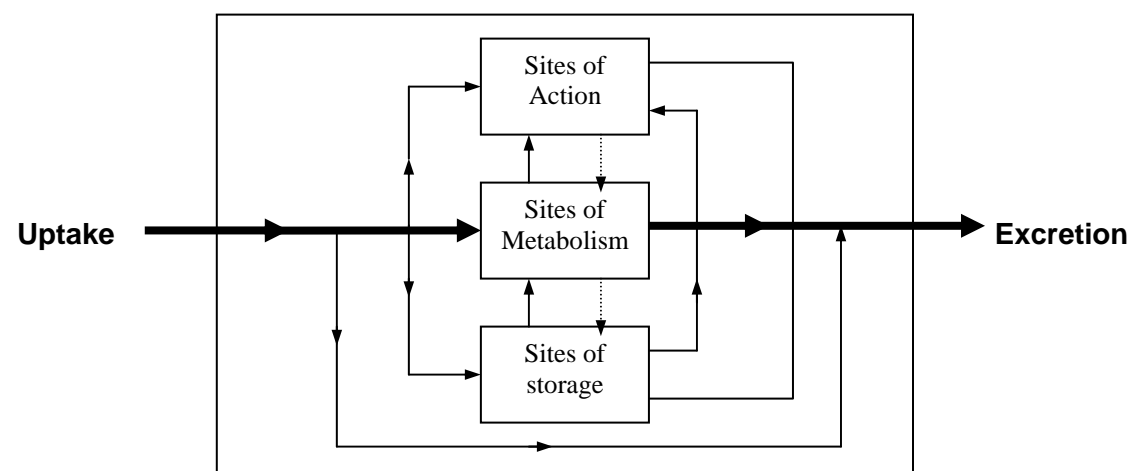

Figure 2.1 General model describing the fate of heavy metals in living organisms
(Walker *et al.*, 2001)

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In this figure, an integrated picture is given of the movements, interactions and biotransformations that occur after an organism has been exposed to heavy metals. Heavy metals absorbed from the gastrointestinal tract are carried by the portal vein to the liver, where storage, metabolism, and biosynthetic activities take place.

The model identifies five types of sites, namely sites of uptake, metabolism, action, storage and excretion. The arrows identify the movements of the chemicals between the sites. Once a metal enters an organism, four types of site, which it may reach, are identified, as follows:

1. <u>**Sites of action**</u>

The toxic form of a pollutant interacts with an endogenous macromolecule (e.g. protein or DNA) or structure (e.g. membrane) and this molecular interaction leads to the appearance of toxic manifestations in the whole organism.

In vertebrates, absorbed pollutants may travel in the blood stream and in the lymph. But if the absorption occurs from the gut, much of the absorbed pollutant will initially be taken to the liver by the hepatic portal system. Commonly, a high proportion of the circulating pollutant will then be taken into hepatocytes. Entry into hepatocytes may be by diffusion across the membrane or by co-transport with lipoprotein fragments, which are taken up by endocytosis (Walker *et al.*, 2001).

2. <u>**Sites of metabolism**</u>

The enzymes metabolize the pollutants. Usually metabolism causes detoxification. For oxidation, hydrolysis, hydration and reduction of lipophilic xenobiotics, many enzymes are responsible in the initial biotransformation. These enzymes are located in the endoplasmic reticulum. Lipophilic xenobiotics tend to move into the endoplasmic reticulum, but their more polar biotransformation products tend to partition out into the