DETERMINATION OF HEAVY METALS IN SEA CUCUMBER VARIETIES IN SABAH

REVATHI THILAKAR

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TAMAN UNRARA,
47100 PUCHONG, SELANGOR

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REVATHI THILAKAR

(BN 07110115)

CERTIFICATION

NAME	**: REVATHI THILAKAR**
MATRICE NO	**: BN07110115**
TITLE	**: DETERMINATION OF HEAVY METAL IN SEA CUCUMBER VARIETIES IN SABAH**
DEGREE	**: BACHELORS DEGREE IN FOOD SCIENCE WITH HONOURS IN (FOOD TECHNOLOGY AND BIOPROCESS)**
DATE OF VIVA	**: 24 MAY 2011**

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Signature

1. **SUPERVISOR**
   DR. IQBAL MOHD. HASHMI

2. **EXAMINER 1**
   ASSOC. PROF. DR. CHYE FOOK YEE

3. **EXAMINER 2**
   DR.MOHD ROSNI SULAIMAN

4. **DEAN**
   ASSOC. PROF. DR. SHARIFUDIN MD SHAARANI

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ABSTRACT

In Malaysia, the sea cucumbers have great value for their medicinal reimbursement and also as culinary delicacies. They are widely exploited due to their medicinal properties as some traditional medicines are processed from sea cucumber. This is due to the fact that sea cucumbers are useful in treatment of stomach ulcers, wound healing and as a pain killers. Sea cucumber fisheries are known to be important in Sabah, as it contributes to the livelihood for the supplement of income and significantly to foreign exchange. The main objective of this study was to determine the presence of heavy metals such as As, Pb, Cd, Zn, Cr, Cu and Mn concentration in the body wall of sea cucumber found around Sabah, to compare the concentration of each heavy metals between the different varieties of sea cucumber and to determine the safety level of metal accumulation in the sea cucumbers based on the Food Act and Regulations of Malaysia and other countries. The samples used in this study included *Holothuria edulis*, *Holothoria leueospilota, Actinopyga lecanora, Phyllophogius spiculata, Stichopus vastus, Bohadachio vitiensis, Thelenota anax and Thelenota ananas.* Heavy metal determination was done by using inductively coupled plasma mass spectrometer (ICP-MS). The outcome indicates that all the metal of study is present in the body wall of the selected sea cucumbers. Therefore the most abundant metal among all sea cucumbers were zinc, except manganese in *Phyllophogius spiculata.* The metal comparison between the sea cucumber species indicates that the highest concentration of Arsenic is found in *Holothoria leueospilota* with 2.43±0.01mg/g, Zinc in *Stichopus vastus* with 28.37±0.01mg/g, Manganese in *Phyllophogius spiculata* with 32.67±0.01mg/g and the species *Thelenota ananas* show the highest concentration of Lead with 0.24±0.01mg/g, Cadmium with 2.43±0.01mg/g, Copper with 1.35±0.01mg/g, and Chromium with 3.33±0.01mg/g. This shows that *Thelenota ananas* has the higher level of metal absorbance compared to other samples. To conclude, the metal concentration in all the samples are at the safe level when compared to the Food Act and Regulations of Malaysia and other countries. Therefore, the sea cucumbers found in Sabah are safe for human consumption.

ABSTRAK

ANALISIS STATUS LOGAM BERAT DALAM PELBAGAI JENIS TIMUN LAUT DI SABAH

Di Malaysia, timun laut dikatakan mempunyai nilai yang tinggi dari segi pengganti perubatan dan juga sebagai hidangan makanan. Timun laut dieksploitasi secara luas disebabkan oleh nilai perubatannya yang tinggi, dengan ini pelbagai jenis ubat telah dihasilkan daripada timun laut. Menurut kajian, telah terbukti bahawa timun laut amat berguna dalam merawat penyakit seperti ulser perut, menyembuhkan luka serta menenangkan rasa sakit. Perikanan timun laut menjadi penting di Sabah disebabkan sumbangannya sebagai sumber pendapatan rakyat Sabah dan juga bagi pertukaran mata wang asing. Tujuan utama kajian ini adalah bagi menentukan kewujudan logam berat As, Pb, Cd, Zn, Cr, Cu dan Mn dalam tubuh pelbagai jenis timun laut yang ditemui di Sabah, membandingkan nilai kepekatan setiap jenis logam diantara sampel yang terpilih. Serta membandingkan tahap keselamatan kandungan logam berat dalam timun laut berdasarkan piawaian yang ditetapkan oleh Malaysia serta Negara-negara lain. Kajian dilakukan terhadap species Holothuria edulis, Holothoria leueospilota, Actinopyga lecanora, Phyllophogius spiculata, Stichopus vastus, Bohadachio vitiensis, Thelenota anax dan Thelenota ananas. Kajian penentuaan tahap logam dilakukan dengan menggunakan inductively coupled plasma mass spectrometer (ICP-MS). Hasil kajian menunjukan bahawa semua logam tersebut wujud pada tubuh sampel. Didapati kepekatan logam zink adalah nilai tertinggi dalam semua spesis timun laut kecuali mangan dalam spesis Phyllophogius spiculata. Daripada perbandingan kepekatan logam diantara setiap spesis, didapati bahawa spesis Thelenota ananas mempunyai kepekatan yang tinggi bagi logam timah dengan 0.24±0.01mg/g, *kuprum dengan* 1.35±0.01mg/g, *kadmium dengan* 2.43±0.01mg/g *dan kromium dengan* 3.33±0.01mg/g. *Manakala, arsenik dalam Holothoria leueospilota dengan* 2.43±0.01mg/g, zink dalam *Stichopus vastus dengan* 28.37±0.01mg/g dan mangan dalam *Phyllophogius spiculata dengan nilai* 32.67±0.01mg/g. *Ini membuktikan bahawa spesis Thelenota ananas mempunyai tahap serapan logam yang tinggi berbanding spesis lain. Bagi menyimpulkan, boleh dikatakan bahawa semua timun laut di Sabah berada pada tahap selamat dimakan setelah dirujuk kepada piawaian yang ditetapkan oleh Malaysia serta negara-negara lain.*

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LIST OF ABBREVIATIONS AND SYMBOLS

AOAC	American official Analytical chemist
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
g	Gram
mg	Miligram
ppm	Part Per Million
p	Significant level
° C	Degree Celsius
FAO	Food and Agricultural Organization
WHO	World Health Organization
<	Less than
As	Arsenic
Pb	Lead
Cd	Cadmium
Zn	Zinc
Cu	Copper
Cr	Chromium
Mn	Manganese

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Malaysian sea cucumber fisheries are exploited off the costal waters around the coral reef regions in East Malaysia especially Sabah. This resource are minimally exploited in Peninsular Malaysia because more than 90% of the coral reef islands in both the east and west coasts have been gazette as fisheries prohibited areas, whereby it is prohibited in its surrounding areas (Forbes *et al.*, 1999). According to research in the 1980's at Sabah, the landings of sea cucumber recorded an annual catch of about 400 to 500 tonnes (inclusive of sea urchins), while landings in the 1990's had reaches to an annual catch of around 100 tonnes (Choo,2004).

Commercial species of sea cucumbers in many countries worldwide are overfished due to the fact that sea cucumbers can be collected from shallow reef flats throughout low tide. In Malaysia, the deficiencies in the fishing regulations such as the minimum legal permitted size for harvesting, closed season and catch quota are also some reasons that contribute to the overfishing. Study shows that, there are overfishing of a variety of sea cucumber species in both Peninsular Malaysia and Sabah. In fishery, the landings of sea cucumber have only contributed to a small part of the total marine landings in Malaysia (Choo, 2004).

In Malaysia, the sea cucumbers have great value for their medicinal reimbursement and also as culinary delicacies. The Chinese have been using the sea cucumbers way before as a wide range health tonic, valuable for treating tendonitis and arthritis, and as an aphrodisiac among with other medical claims. Other than that, they also consider it as culinary delicacies. The Malays have traditionally utilized certain species of *Stichopus* which are locally known as "gamat". They are used due to their medicinal properties as some traditional medicines are processed from sea cucumber. This is due to the fact that sea cucumbers are useful in treatment of stomach ulcers, wound healing and as a pain killers (Choo, 2004). Sea cucumber fisheries are known to be important in Sabah, because it contributes livelihood for the supplement of income and significantly to foreign exchange. Sea cucumbers landed in Sabah are mostly collected by hand from shallow reef flats or by snorkeling or diving. Fishing is carried out during the day or night as long as the weather permits and the tides are low to enable the fishers to walk on the reefs. The sea cucumbers such as teat fish, blackfish, elephant's trunk fish and bigger-sized sand fish are rarely found in the shallow reef flats due to overfishing (FAO, 2010).

Sea cucumbers which are often harvested by trawl gear are collected by trawler of 10-24.9 gross tonnage and 25-39.9 gross tonnage fishing in waters within 30 nautical miles of the coast (Anon, 2000). To collect sea cucumbers, it will take approximately 20 days in a month (Anon, 1994). The sea cucumbers fished at Sabah are mainly used as culinary delicacies, and then it is exported to Peninsular Malaysia, Sarawak and other foreign countries such as Singapore, Thailand, Hong Kong, Taiwan and China (Choo & Williams, 2004). Since the year of 1990's, Semporna has become the main attraction area in sea cucumber fishery, with catches of 29% to 62% of the total landings from Sabah. Sea cucumber landings in Malaysia and Sabah are showed in table 1.1 (Anon, 2000).

There are around 20 species of sea cucumber which are widely used commercially in the fishery industry. The price of the sea cucumbers depends on the thickness of the body wall and also the sizes of the sea cucumbers. The species of sea cucumber which has thick body wall such as teat fish are higher in price than the ones with thin body wall. For the species which looks like "worm", the large category consist of 25 to 30 pieces to a kilogram, the medium category with 70 to 80 pieces per kilogram, the small category with 130 to 140 pieces per kg and the extra small more than 180 pieces per kilogram (Anon, 2000).

Table 1.1: Landings of sea cucumber in Malaysia and Sabah (metric tones)

Year	Landings (metric tones)	
	Malaysia	Sabah
1980		300
1981	168	300
1982	430	400
1983	435	400
1984	367	300
1985	1169	900
1986	687	500
1987	800	600
1988	616	400
1989	800	200
1990	800	400
1991	780	37
1992	800	90
1993		64
1994		142
1995		155
1996		105
1997		90
1998		123
1999		178
2000		159

Source: Anon, 2000.

Table 1.2: Landings of sea cucumber in various districts in Sabah (metric tones)

Year	Landings (metric tonnes)						
	Semporna	Sandakan	Kudat	K. Marudu	K. Belud	K. Kinabalu	Total
1991	0	35	1	0	0	1	37
1992	0	74	4	10	0	2	90
1993	0	57	0	1	0	6	63
1994	30	24	41	0	0	47	142
1995	55	21	46	0	0	33	155
1996	34.38	23.75	24.04	0	3.10	19.49	104.76
1997	25.69	19.29	19.02	0	0	26.01	90.01
1998	52.36	24.04	14.68	0	0	31.48	122.56
1999	79.65	24.47	21.88	0	0	51.80	177.80
2000	98.65	22.67	15.88	0	0	21.71	158.91

Source: Annual Fisheries Statistics data of Sabah.

Since sea cucumbers are categorized as sea food, which is also apart of the aquatic ecosystem. Therefore, these sea cucumbers have higher possibilities to be contaminated by many pollutants. The major forms of pollutants are caused by industrial wastewaters, agricultural chemicals such as insecticides, agriculture and domestic sewage, herbicides and fungicides and heavy metals. Most of these contaminants enter the freshwater and marine ecosystem and since they are lipid soluble, it is likely to accumulate in the lipid of lives and then is passed down the entire food chain. Heavy metal contamination in food may cause hazard to the health of humans.

1.2 Justification of Study.

Research has been done in many countries on determining the nutritional composition of many types of sea cucumbers. However, there have been no further researches done regarding the heavy metal contents of sea cucumber around Malaysia especially in Sabah compared to other states. With this, eight species of sea cucumbers are selected randomly to determine the heavy metal accumulation in it.

The level of heavy metals in those sea cucumber are studied in order to determine the rate of contamination occur to sea cucumbers around Sabah. Sea cucumber are selected for this study, due to its high commercial value furthermore it has been widely consumed and used as traditional medicine among the locals. Therefore, the importance given by Sabah as the largest producer of sea cucumber compared to Peninsular Malaysia became the factor it has been chosen.

Heavy metals are often used as a group name for metals and semimetals (metalloids) which have been associated with contamination of healthcare products and foods. Furthermore, there are no appropriate meaning of heavy metals, but in most cases density is taken to be the defining factor. Heavy metals are basically defined as those having more than 5 g/cm^3 of specific density (Jiarup, 2003). Trace elements such as copper, iron, and zinc and toxic heavy metals such as arsenic, cadmium, lead and mercury may promote medical difficulties when they are present at excessive levels in the body.

1.3 Objectives

Since sea cucumbers in Sabah are rich with high commercial value. The sea cucumbers have to be evaluated further, in order to maintain its availability for the usage in many sectors such as the medical industry, food processing industry and many more. Sea cucumbers normally provide contribution towards the traditional medical industry in Sabah and also in Peninsular Malaysia. The general objective of this study is to determine the heavy metal content of varieties of sea cucumber around Sabah. The specific objectives of this study are listed below:

1. To determine the presence of heavy metals; Arsenic (As), Cadmium (Cd), Lead (Pb), Copper (Co), Chromium (Cr), Manganese (Mn), and Zinc (Zn) in selected eight species of sea cucumber and to compare the concentration of each heavy metals between the sea cucumber species.

2. To determine the safety level of sea cucumber found in Sabah based on the maximum level of heavy metals allowed by the Food Act and Regulations of Malaysia and other countries.

CHAPTER 2

LITERATURE REVIEW

2.1 Holothuroidea (Sea cucumber)

Echinoderms such as sea urchins, starfish, and sea cucumber are known to be the most interesting marine animals among other invertebrates and also commonly found in deep seas around the world (Abdel Razak *et al.*, 2005). Invertebrate represents any animals which present without a backbone. Earlier studies show that, invertebrates make up of 95 percent of all animal species on earth, therefore the diversity of invertebrates are massive. Basically, Echinoderms are divided into five main classes which are the *Asteroidea* (sea stars), *Ophiuroidea* (Brittle stars), *Crinoidea* (sea features), *Echinoidea* (sea urchins) and *Holothuroidea* (sea cucumbers) (Kamarudin *et al.,* 2009).

Sea cucumbers are known as soft-bodied marine dwelling echinoderm from the class Holothuroidea. These marine animals are special due to the evolved skeleton which looks like spicules or ossicles and ancient looked respiratory system known as the respiratory tree possessed by some species (Kamarudin *et al.,* 2009). According pervious research, there are six valid orders of class Holothuroidea around the world with total of 1430 species which are the *Apodida*, *Aspidochirotida*, *Elasipodida*, *Molpadiida*, *Dendrochirotida* and *Dactylochirotida* (Lambert, 1997). Till recent, around 1400 types of sea cucumber have been found around world (James, 2001). Therefore, studies have proved that there are approximately 1250 species of Holothuroidea which are then diverged into 200 genuses (Smiley *et al.*, 1991).

Holothuroidea are not shown into arms, thus their mouth and anus are located at the opposite poles of its body. Furthermore, there are also ambulacral and interambulacral areas arranged orderly at the polar axis. Normally, Holothuroidea are recognized with other echinoderms by having polar axis greatly lengthened, this eventually causes the body having an elongated cucumber shape. Whereby, the cucumber shape of these sea cucumbers results it to lie with the side of the body, besides the oral pole adjacent to the substratum. The reduction in the skeleton of microscopic ossicles and by the modification of the buccal podial into a circle of tentacle around the mouth is to further method of recognizing. The most outstanding features of this animal are their muscular body wall which almost devoid of large skeletal plates, the branching tentacle surrounding the mouth (Bhamrah & Kavita, 2001).

2.2 Morphology of Sea Cucumber.

Sea cucumbers are known as the most dominant marine animals among other groups of motile-invertebrate corals. Commonly, sea cucumbers are found to be black, brown or olive green in color. Thus, there are some species with orange, rose and violet in color. Some species present with patterns on the body. The sea cucumbers can be differentiated with their range of size, whereby the smallest species can be less than 3 centimeters in length, therefore, the Philippines species *Stichopus* may sometime reach the length of 1 meter and a diameter of 23 centimeters. *Cucumaria, Holothuria, Thyone, and Leptosynapta* is the most common North American and European Species which range from 10 to 30 centimeters in length (Bhamrah & Kavita, 2001).

The sizes of the sea cucumbers differ according to their morphological features. Research shows that, the species *Holothuria atra* are found to be achieving 60 cm in length with 2 kg of weight, the species *Actinopyga mauritiana* with 30 cm length and 2.8 kg, species *Thelenota ananas* with the length which could achieve 100 cm with weight 6 kg and the species *Holothuria scabra* with length at the range between 25 to 35 cm with 0.250 to 0.350 kg of weight (Martoyo *et al.,* 2010).

The body of sea cucumbers is normally cylinder shape with a little stretched posterior end, which then forms into a globule when it contracts. The tube feet of sea cucumbers are the one with numerous external, fluid filled muscular tubes of echinoderms, such as the sea urchin or starfish, which serves as organs of locomotion, food handling, and respiration (Levin & Gudimova, 2000). The tube feet are usually large and retractile which will be located in the ventral radials. The sea cucumbers breathing are with the help of the skin, internal respiratory trees or the tube feet. The body of sea cucumber frequently contains a toxin which are known as "holothurin", thus causes distasteful sensing (Colin, 1988).

The body walls of a sea cucumber are normally firm and rigid. This is due to its mass constitutes up to 20 % the sea cucumbers total mass (Levin & Gudimova, 2000). Those sea cucumbers are consists of a thin pigmented outer layer of connective tissue with mutable mechanical properties which can be altered by the nervous system (Motokawa, 1984a, 1984b). Hence, these are able to change the properties of the dermis which is composed of collagen fibrils, proteoglycans, a microfibillar network, nerve fibers, neurosecretory cells and other protein and glycoprotein in locomotion and protection (Motokawa, 1984; Wilkie, 1984).

Sea cucumbers are known to be a great deposit or suspension feeders. They use their twenty oral tentacles with an average of 4.8 mm in length to collect food (Conand *et al.*, 1998). The tentacles of sea cucumbers are curved or straight rods which a times present with single holes (Levin & Gudimova, 2000). The sea cucumbers vascular system is based on the water vascular system. The respiratory system is formed from the respiratory trees which is opened into the cloacae and extend into the coelomic cavity (Conand *et al.*, 1998).

Sea cucumbers are a type of ectodermic animal this is because it buries itself at the sea bed. The movement of the sea cucumbers is normally very slow, whereby it moves by changing the length of its body (Motokawa, 1982; Motokawa, 1984). The burrowing is done by the sea cucumbers by alternate contraction of longitudinal and circular muscle layers of the body wall in the way done by the earthworms (Bhamrah & Kavita, 2001). Sea cucumbers move in two ways of movements, which are move horizontally on the surface of substrate and move vertically or similar to scraping (Lokani *et al.*, 1996). This creature uses its muscles that are round and radius by swelling and shrinkage in the body wall.

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