THE PREVALENCE OF METALS (Cu, Fe, Mn, Zn, Cd and Pb) IN SOME VEGETABLES AND SEAWEEDS OF SABAH.

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DECLARATION

I hereby declare that the materials in this thesis are original except for quotations and references, which have been duly acknowledged.

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ABSTRAK

KEHADIRAN LOGAM-LOGAM (Cu, Fe, Mn, Zn, Cd & Pb) DALAM SAYUR-SAYURAN DAN RUMPAI LAUT DI SABAH

Sebanyak 154 sampel sayur-sayuran seperti tomato, kobis, sawi bunga, timun, kobis Cina telah dikumpul dari pelbagai ladang di Kundasang, Keningau, Penampang dan Papar yang merupakan kawasan utama penanamannya di Sabah dan juga dari pasar borong di Kota Kinabalu. Sampel tanah dan air saliran (71 sampel) juga diambil dari ladang-ladang tersebut. Sebanyak 18 sampel spesies rumpai laut kormesil (*Eucheuma cottonii*) dan 18 sampel air laut telah diperoleh dari Kudat dan Semporna. Sampel sayur dan rumpai laut telah dihadam menggunakan asid nitrik dan H_2O_2 sehingga larutan menjadi jernih. Sampel tanah telah diekstrak menggunakan air dan dihadam dengan asid nitrik. Larutan-larutan ini, sampel air saliran dan air laut telah ditapis untuk mendapatkan larutan yang jernih. Paras logam-logam (Cu, Fe, Mn, Zn, Cd dan Pb) dalam larutan sampel telah ditentukan menggunakan Spektrofotometer Serapan Atom Nyalaan. Hasil kandungan logam dalam semua jenis sampel seperti sayur-sayuran, rumpai laut, tanah, air saliran dan air laut menyerupai keputusan kajian-kajian lepas yang telah dilakukan sebelum ini. Kandungan Ferum dalam kobis (1.723-7.339 μg/g berat basah) didapati paling tinggi, diikuti dengan sawi bunga (1.232-6.275 μg/g) dan tomato (2.137-4.132 μg/g). Namun, timun pula mempunyai kandungan zink yang tertinggi (0.564-2.117μg/g) berbanding dengan paras logam lain yang dikaji. Ferum juga merupakan logam yang paling banyak ditemui dalam air saliran iaitu antara 0.305-1.591 μg/ml dan juga dalam tanah yang diekstrak dengan asid (34.993-23 245.65 μg/g). Ini diikuti dengan plumbum (6.155-42.421μg/g) manakala Mn merupakan logam yang mempunyai paras tertinggi dalam tanah ekstrak air (0.118-44.349 μg/g). Rumpai laut yang dikaji didapati kaya dengan ferum (9.677-23.676 μg/g) dan zink (0.148-2.524 μg/g) manakala sample air laut juga menunjukkan bahawa ferum mempunyai paras yang tertinggi (30.588-47.368 μg/ml) diikuti oleh mangan (0.314-0.733μg/ml). Julat logam-logam Cd, Pb dan As dalam sayuran dan rumpai laut adalah lebih rendah daripada paras maksimum yang dibenarkan oleh Akta Makanan Malaysia 1983 dan Peraturan Makanan 1985 serta WHO/FAO. Oleh itu, dapat disimpulkan bahawa sayur-sayuran dan rumpai laut di Sabah adalah selamat sebagai sumber makanan kepada manusia.

ABSTRACT

THE PREVALENCE OF METALS (Cu, Fe, Mn, Zn, Cd & Pb) IN SOME VEGETABLES AND SEAWEEDS OF SABAH

A total of 154 samples of vegetables such as tomato, cabbage, Chinese mustard, cucumber, Chinese cabbage were collected from various farms in Kundasang, Keningau, Penampang and Papar which were the main areas for their cultivation in Sabah as well as from the wholesale market in Kota Kinabalu. Soil and irrigation water samples (71 each) were also collected from the corresponding vegetables farms. A total of 18 samples of commercial seaweed species (*Eucheuma cottonii*) and 18 samples of seawater were obtained from Kudat and Semporna. The vegetable and seaweed samples were digested with nitric acid and H_2O_2.until a clear solution was formed. The soil samples were extracted with water as well as digested with nitric acid. These solutions, samples of irrigation water and seawater were filtered to obtain clear solutions. The levels of the metals (Cu, Fe, Mn, Zn, Cd and Pb) in the solutions were determined by Flame Atomic Absorption Spectrometry. The metal concentrations in all types of samples such as vegetables, seaweeds, soil, irrigation and seawater were in a similar range with previous studies that were reported. Ferum had the highest concentration range in cabbages (1.723-7.339 μg/g fresh weight) as well as in Chinese mustard (1.232-6.275 μg/g) and tomatoes (2.137-4.132 μg/g). Cucumbers were found to contain the highest level of zinc (0.564-2.117μg/g) compared with other metals studied. Ferum was also found to be the most concentrated metal in the irrigation water with a range of 0.305-1.591 μg/ml and in acid extractable soil (34.993-23 245.65 μg/g) as well. This is followed by plumbum with a range of 6.155-42.421μg/g whereas manganese (0.118-44.349 μg/g) was found to have the highest concentration range in water extractable soil. Seaweeds were found to be richest in ferum (9.677-23.676 μg/g) followed by zinc (0.148-2.524 μg/g) while the seawater samples showed that ferum had the highest concentration range (30.588-47.368 μg/ml) followed by manganese (0.314-0.733μg/ml). The range of concentrations of these metals (Cd, Pb and As) in vegetables and seaweeds were lower than those permitted by the Malaysian Food Act 1983 and Regulation 1985 as well as the maximum level set by WHO/FAO. Therefore, it can be concluded that vegetables and seaweeds in Sabah were safe for human consumption and contain some beneficial effects due to the essential metals present in the samples.

ABBREVIATIONS

%	:	Percent
µg/g	:	microgram per gram
µg/mL	:	microgram per millimetre
g	:	gram
g/L	:	gram per litre
kg	:	kilogram
mg/kg	:	milligram per kilogram
mg/L	:	milligram per litre
mL	:	millilitre
ppm	:	Part per million
AAS	:	Atomic Absorption Spectrophotometer
ANOVA	:	One Way Analysis of Variance
FAO	:	Food and Agriculture Organization
IUPAC	:	International Union of Pure and Applied Chemistry
U.S.A	:	United States of America
WHO	:	World Health Organization

CONTENTS

CHAPTER 3 MATERIALS AND METHODS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Humans require a number of minerals for maintaining good health. Vegetables are one of the good and natural sources for supply of minerals to humans. Vegetables may also contain some toxic metals in addition to essential metals. Therefore there has been a great interest for the determination of the concentrations of essential and toxic metals in vegetables and other natural products such as fruits, seaweeds etc. Metals are an important component of a healthy diet. The earlier work on the biochemical aspects and functions, toxicity, occurrence and determinations of metals in vegetables has been adequately covered by Monier-Williams (1949). A number of comprehensive and critical reviews have already been published during 1970's (Somers, 1974; Crosby, 1977). The metal concentrations in vegetables may be far less as compared with other minor component such as vitamins and major components such as carbohydrate, lipids and proteins. However, their nutritive value partly depends on the soil and climate in which they are grown (Luke, 1984). Concentrations of metals occurring in foodstuffs including vegetables can be found from the food Tables prepared by Ministry of Agriculture, Fisheries, and Food MAFF (1998).

The role of metals obtained through vegetables and fruits in human nutrition has attracted the interest of researchers. Numerous publications on the metals contents of vegetables and fruits have appeared in scholarly journals during the past few years. Thus, for example, the metals contents of selected fruits commonly consumed in the United States had been presented by Miller-Ihli 1996a and 1996b. The concentrations of metals found in the samples were in the range of concentrations set by FDA for daily consumption. Tahvonen (1993) has reported the contents of metals in fruits and vegetables available in the Finish market, his results showed that there were significant differences in the metal contents of the domestic and imported varieties and values were similar to those reported earlier from that region (Varo *et al.* 1980; Tahvonen & Kumpulainen, 1999). Similarly, Schumhmacher *et al.* (1993) have reported the concentrations of chromium, copper and zinc in vegetables from Spain. Tinggi *et al.* (1997) has mentioned the content of manganese and chromium in various types of food products available in Australia. Masud and Jaffar (1997) has mentioned the concentrations of the selective metals such as As, Cd, Cr, Fe, Hg and Ni in some local vegetables. Their results indicated that all the concentrations were in the normal range. In another study, Sattar *et al.* (1989) determined the concentration of the metals: Cd, Cu, Fe, Mn, Pb and Zn in foodstuff such as spices, dry fruits and nuts. Attention had been drawn by Zhou *et al.* (2000) in their review on toxic metal contents of vegetables in China and remedial measures that could be taken to minimize the contents of toxic metals through farming practices.

The determination of the contents of metals in various food products has been the centre of high priority research projects of such international organizations as FAO, WHO, UN, etc. Toro *et al.* (1994) had summarized the results of an International Atomic Energy Agency (IAEA) coordinated research program involving the determination of toxic heavy metals and other trace metals in foodstuffs from 12 different countries. All the concentration levels of toxic metals in foodstuffs were within the limits specified in the national and international guidelines. Nuclear analytical techniques proved to be sensitive, accurate, and precise for the analysis of this type of matrix. The total diet study is now an integral part of the many government surveillance programmes for monitoring of chemicals in food. Thus, Ysart *et al.* (1999) have reported dietary exposures of 30 metals from the UK and compared the results with those of the previous study and from the investigations of other countries. Dietary exposures were found to be low as compared to provisional tolerable weekly intakes and provisional maximum tolerable daily intakes. To monitor the food supply for toxic and nutritionally important metals, there had been many reports on the total diet surveys conducted in different parts of the world by a number of workers (Cabrera *et al.*, 1995; Dabeka & McKenzie, 1995; *Julshamn et al.,* 1998; Jorhem, 2000; Jorhem & Engman, 2000; Sun *et al.,* 2000). Worthington (2001) had presented an excellent comparison on the nutritional quality of organic versus conventionally grown fruits, vegetables and grains. According to the author organic crops contained significantly more iron, magnesium and phosphorus and comparatively less heavy metals.

Some dietary professionals believe that our metal needs can be met with a well-balanced diet of fruits and vegetables, but recent scientific studies suggest (Ashmead, 1982) that it is very difficult for diet alone, particularly a vegetarian diet, to provide the metals we need to maintain good health. Fresh fruits and vegetables, while high in vitamins, are often low in essential metals. Even vegetables commonly believed to be high in metals such as iron do not contain sufficient concentrations to supply the recommended quantity when consumed in normal-sized portions. In a recent study by Elless *et al.*, (2000) analyzed the amount of metals in a variety of fresh vegetables purchased from a local supermarket. Vegetables such as spinach, broccoli, beets and collard greens were analysed for their metal contents. The results show that these vegetables, even consumed in combination, do not contain sufficient amounts of metals to provide a healthy diet. The most mineral rich vegetables in the group was beet greens, which contains about 85% of the recommended daily intake (RDI) of manganese, and significant amounts of iron, magnesium and calcium. However, beet greens are not a popular vegetable, and must be eaten daily to provide even this partial list of minerals. Spinach contains almost 45% of the RDI of iron, lettuce contains about 70% of the RDI of manganese, and collard greens (another unpopular vegetable) contain almost 60% of the RDI of calcium. None of the vegetables tested contain sufficient amounts of the copper, vanadium, selenium or chromium to be considered sources of these metals. Beets, carrots, green beans and broccoli are not a significant source of metals. According to Elless *et al.,* (2000) if edible plants were enhanced with essential metals they could be used as new source of minerals dietary supplements that would provide metals in a more available form than current inorganically based mineral supplements available in the markets.

Demirbas (2000) has recently reported the accumulation of heavy metals in some edible mushrooms from Turkey. Similarly Gambelli *et al.,* (1999) have identified essential metals in some Italian dairy products. Martin and Llanos, (2001) have presented their results on the proximate composition, minerals and vitamins in selected canned vegetables. Orzaez *et al.,* (2000) have reported the contents of sodium, potassium, calcium and magnesium in breakfast cereals. Soliman and Zikovsky (1999) analyzed a range of metals including bromine, chlorine, rubidium, sulphur, titanium and vanadium, in cereals and vegetables and other food items sold in Canada. Yebra-Biurrun (2001) has presented a detailed review on the concentrations of different metals in seafood. Concentration of metals like, Fe, Cd, Zn, Mn, Cu, Ni, Pb in oysters, clams and some of the bush fruits of Australia had been presented in some of the papers by Peerzada *et al.*, (1993, 1992; and 1990). The studies by Cook *et al.,* (2000); Ranhotra *et al.,* (1998); Guil *et al.* (1998); Sanchez-Castillo *et al.,* (1998); and Glew *et al.,* (1997), analysed the indigenous wild plants (including fruits and vegetables) for their metals contents. Their findings show that these plants are a good source of nutrients including metals, whose contents are comparable with the other commercially cultivated plants.

Data on the nutritional values of East Asian fruits and vegetables and other foods are readily available in the Food Composition Table (Platt, 1962) for use in East Asia. Wong and Koh (1982) have described in detail the contents of metals which were Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and Hg in 17 fruits and 26 vegetables commonly consumed in West Malaysia and Singapore. Their values fall in the normal range reported in the above Food Composition Table despite the fact the origin of the samples were different. Wong et al., (1993) have similarly reported the contents of heavy metals in some of the Chinese herbal plants. The concentration range of the metals was comparable to many of the East Asian fruits and vegetables. However, a few samples were found to contain relatively higher concentrations of the toxic metals such as cadmium, lead and mercury. Hashim and Hamid (1995) have determined metals: Fe, Zn, Cu and Pb in the Malaysian diet (foods and drinks). A normal daily diet was found to contain Fe (19.6 ±6.4 mg), Zn (1.6±0.6 mg), and Cu (1.6±0.6 mg). The nutritive trace metals were found to be lower than the Malaysian RDA for Fe and US RDA for Zn, while Cu was within the recommended range. The study indicated concern regarding the low intake of the essential metals on long-term basis among the female students of UKM. Hoe and Hong (1999) have provided the proximate composition including metals and vitamin contents of 16 fruits and 46 vegetables (leaves, fruits, palm herbs and shoots) of indigenous origin in Sarawak. Indigenous fruits and vegetables are important food sources for rural populations. Nutritious indigenous fruits and vegetables have the potential to be promoted for wider use, domestication and commercialisation.

Seaweeds have also attracted the attention of a number of researchers because they are rich in polysaccharides, minerals and vitamins (Flurence, 1999; Indegard & Minsaas, 1991, Lahaye & Kaeffer,1997; Mabeau and Fleurence, 1993 ; Réuprez & Saura-Calixto,2001). They have been used historically as a source of alginates as an emulsifier in the food industry. Lahaye and Kaeffer (1997) have reviewed the structure, physicochemical and biological properties of seaweeds. Lee *et al.*, (1994) ; Cho *et al.*, (1995); Hou *et al.*, (1997) have reported that seaweed is a source of iodine and other nutrient elements. The recently increased interest in the utilization of edible seaweeds by the general public (Darcy-Vrillon, 1993 ; Koppel, 1997) has, therefore, resulted in cultivation of many seaweed varieties, and their derived products, that are now available from health food stores.

However, just as in vegetables and other natural products, seaweeds also contain both essential and toxic metals, as Sharp *et al.*, (1988) reported the possibility of the presence of toxic metals in them due to the polluted water in which, seaweed crops are harvested. It has been well documented that certain types of marine algae have high affinity for toxic heavy metals (Bryan & Hummerstone, 1973 ; Volesky, 1994; Dickson,1984) has observed that seaweed can be contaminated with radioactive isotopes. Edible seaweed products have been used in many countries, specify Japan, as a food item. Recently, these products have become popular in the food industry because of a number of interesting medical properties that have been associated with certain edible marine algae. Indegaard & Ostgaard (1991); Mabeau & Fleurence (1993) have reported that in Western countries, seaweeds are mainly used as a source of polysaccharide (agar, alginares, carrageernans) for food and pharmaceuticals. Norziah and Ching (2000) studied nutritional composition of edible seaweeds, *Gracilaria changgi* (red algae), culture in Kedah, West cost of Malaysia. The authors, concluded that the red seaweeds contained higher concentrations of fiber and mineral and a moderate concentrations of lipid and protein compared to several other local vegetables.

Thus, the study of some essential and toxic metal levels in vegetables, seaweeds, soil samples, irrigation water and seawater samples in Sabah is much needed to know if these food items are safe for human consumption. Therefore, the main objective of this study was to determine the levels of metals (Cu, Fe, Mn, Zn, Cd, Pb) in vegetables from

Kundasang, Keningau, Penampang, Papar and wholesale market in Kota Kinabalu, and seaweeds from Semporna and Kudat.

The specific objectives of this study were:

1. To compare the metal levels in the same species of vegetables, seaweeds, soil, irrigation water and seawater samples collected from different areas.
2. To evaluate the metal levels in various type of vegetables.
3. To correlate levels of metals from soil, irrigation water to vegetables and from seawater to seaweeds.
4. To measure and show a relationship between the concentrations of the total and water-soluble metals in soil.
5. To determine the bio concentration factor in vegetables and soil.
6. To assess the safe use of these food items as based on the permitted levels of toxic metals by FAO/ WHO and Malaysian Food Act/ Regulation.
7. To provide a baseline data on metal levels in these samples for making a comparison in the future.

CHAPTER 2

LITERATURE REVIEW

2.1. The levels of toxic metals in the environment and food

For some metals, natural and anthropogenic inputs are of the same order (for example, mercury and cadmium), whereas for others (for example, plumbum) inputs which are due to human activities are much more compared to natural inputs (Cleark, 2001). Soil contamination by both the sources are widespread and varies in its level of contamination. Heavy metal contaminations caused by anthropogenic activities are more likely to cause high toxic metal concentration in soil. Contributory factors include mining and smelting of ores; electroplating operations; municipal waste deposits; landfill leakages; applications of fertilizers, fungicides and pesticide; dumping of sewage and sludge from treatments plants and burning of fossil fuels (John & Valaerhoven 1972; Woolson 1973; Boon & Soltanpour 1992; Forstner, 1995; Cobb *et al.*, 2000). In addition to the above activities, industrial waste water is another major cause of contamination, especially in agriculture areas. It is almost impossible to visualize soil and waterways without the presence of trace levels of toxic metals.

Many researchers have found that vegetables are capable of accumulating certain metals from media such as soil, air, irrigation water, nutrient solutions by root of the foliage (Garcia *et al.*, 1981; Khan & Frankland 1983; Xiong 1998; Cobb *et al.*, 2000; Fytianos *et al.*, 2001). Moreover, the entry of metals from the fertilizer into the food chain depends on the amount of fertilizer used in the soil, the water and the ability of particular crops and plants to absorb the metals (Mortved, 1987). Trace quantity of certain heavy metals such as chromium, cobalt, copper, iron, manganese and zinc are essential micronutrients for plant growth (Somers, 1974, Reeves & Baker, 2000; Kahle, 1993; Brady & Weil, 1999; Taiz & Zeiger, 1998) but plants growing in a polluted environment can accumulate metals at high concentrations, causing a serious risk to human health when plant–based food stuffs are consumed (Wenzel & Jackwer 1999; Qian *et al.*, 1996). This is an important exposure pathway for people who consume vegetables grown in heavy metals contaminated soil.

Recently, the safety of food and food products has attracted much attention. Likewise, the contents of metals in food should be carefully checked in order to prevent possible risks. The determination of metals in natural environments has been recognized as an important parameter when environmental and food quality are assessed. The metal contents of vegetables and seaweeds depend on many factors, including the type of soil, water, variety of species, climatic conditions, viticulture practice and pollution. Thus, vegetables and seaweeds are considered as an important economical resource, as they are not only largely harvested but also intensively and largely consumed in the human nutrition. Hence, seaweeds and vegetables are the most suitable food items for the study of metals contamination due to environmental pollution. In addition, soil and water serve as many vital functions in our society, particularly for food production, thus, it is also important to examine their metal levels.

2.2. Terminology of metals

Metals are defined chemically as elements, which conduct electricity, have a metallic lustre, are malleable and ductile, form cations, and have basic oxides. Metals may be defined by the physical properties of the elemental state as element with metallic lustre, the capacity to lose electrons to form positive ions