ASSESSMENT OF HEAVY METALS CONTAMINATION IN VEGETABLES CONSUME IN JOHOR BAHRU

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I dedicate this work

To my dear parents

Puan Roshayati Binti Yaa'cob

Encik Abdul Karim Bin Buang

Whose love, kindness, patience and prayer have brought me this far

To my wife and kids

For their endless laughs and tears

To my siblings and family

Whose presence fills my life with joy

To my friends

For their love, understanding and support through my endeavour

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ABSTRACT

Trace elements such as As, Pb, Hg, Cd, Fe, Ni, Cu, and Zn from organically and inorganically grown Brasssica (*B.*) were determined. In this study, X-ray fluorescence (XRF) technique was used for assessing concentration level of heavy metals (HMs) and its presence from all samples. All samples were obtained from 3 hypermarkets in Johor Bahru. These sampling techniques were known as "Market Basket Study". Approximately, 1 kg of each vegetable was used and was analysed through standard laboratory procedures. The dried samples were grounded into powder form and by using test sieve mechanism, the size of the dried samples was found to be approximately 64 µm. The prepared samples in pellets form were analyzed using Wavelength Dispersive X-Ray Fluorescence (WDXRF) which compared to Standard Reference Material (SRM 1572) Citrus Leaves. The purpose is to detect the presence of trace elements such as As, Cd, Cu, Hg, Fe, Mn, Pb and Zn and their concentration level in vegetable samples. No significant different (p<0.05) of the HMs and mineral contents concentration between organic and inorganic *Brassicas* were observed except for Fe concentration from leaves. The concentration of HMs and trace elements in leaves is higher compare to stem, in the order of Fe > Zn > Mn > Cu > Pb > As > Hg > Cd. The HMs mean concentration of As, Hg, Pb and Cd in Brassicas samples were in the range of 1.94 – 2.55 ppm, 0.05 – 0.07 ppm, 8.33 – 10.91 ppm and 0.02 – 0.03 ppm, respectively. These values were exceeded the permissible level as stated in Food Act 1983 except for Cd, where As (1.00 ppm), Hg (0.05 ppm), Pb (2.00 ppm) and Cd (1.00 ppm).

ABSTRAK

Unsur surihan seperti As, Pb, Hg, Cd, Fe, Ni, Cu and Zn telah ditentukan daripada Brassica yang ditanam secara organik dan bukan organik. Dalam kajian ini, teknik pendaflour sinar-x telah digunakan bagi mengesan kehadiran dan kepekatan logam berat dan mineral daripada semua sampel. Kesemua sampel diperoleh daripada 3 pasaraya di Johor Bahru. Teknik persampelan ini dikenali sebagai Kajian Bakul Pasaran. Kira-kira, 1 kg sayuran telah digunakan dan dianalisis melalui kaedah piawaian makmal. Sampel yang telah dikeringkan telah dikisar menjadi serbuk dan dengan menggunakan mekanisma kaedah tapisan, saiz sampel kering didapati kira-kira 64 μm. Sampel disediakan dalam bentuk pelet dianalisis dengan menggunakan Gelombang Sebaran X-Ray Pendarfluor (WDXRF) yang dibandingkan dengan Bahan Rujukan Piawaian (SRM 1572) Daun Citrus. Tujuannya adalah untuk mengesan kehadiran unsur surih seperti As, Cd, Cu, Hg, Fe, Mn, Pb dan Zn dan tahap kepekatan mereka dalam sampel sayur-sayuran. Tiada perbezaan kepekatan kandungan yang signifikan ($p < 0.05$) bagi logam berat dan kandungan mineral antara Brassicas organik dan bukan organik yag diperhatikan kecuali kepekatan Fe daripada daun. Kepekatan logam berat dan unsur surih dalam daun adalah lebih tinggi berbanding dengan dalam batang, dengan kecenderungan Fe> Zn> Mn> Cu> Pb> As> Hg> Cd. Min kepekatan logam berat bagi As, Hg, Pb dan Cd dalam sampel Brassicas berada diantara julat 1.94-2.55 ppm, 0.05-0.07 ppm, 8.33-10.91 ppm dan 0.02-0.03 ppm, masing-masing. Nilai ini melebihi paras yang dibenarkan seperti yang dinyatakan dalam Akta Makanan 1983 kecuali bagi Cd, di mana As (1.00 ppm), Hg (0.05 ppm), Pb (2.00 ppm) dan Cd (1.00 ppm).

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LIST OF ABBREVIATIONS

DOAM	Department of Agriculture of Malaysia
SOM	Malaysian Organic Scheme
MS	Malaysia Standard
HMs	Heavy metals
IFOAM	International Federation of Organic Agriculture Movement
WHO	World Health Organization
FAO	Food and Agriculture Organization of United Nation
CI	Confidence Interval
FP	Fundamental Parameter
PPB	Part per billion
PPM	Part per million
PPT	Part per trillion
SC	Standardization coupon
SD	Standard deviation
SRM	Standard Reference Material
XRF	X-ray fluorescence
SSD	Source to surface distance
EDI	Estimated daily intake
WDXRF	Wavelength Dispersive X-ray fluorescence
EDXRF	Energy Dispersive X-ray fluorescence
ICP-MS	Inductive Coupled Plasma Mass Spectroscopy
ICP-OES	Inductive Coupled Plasma Optical Electron Spectroscopy
AAS	Atomic absorption Spectroscopy

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CHAPTER 1

INTRODUCTION

This chapter will introduce the bio-accumulation of heavy metals in plants especially vegetables either organically grown or conventionally grown. Additionally, toxicity and permissible limit concentration of heavy metals in food will also be discussed for further understanding.

1.1 Overview

Organic food is perceives by consumers as better taste, safer, more nutritious and less harmful to the environment rather than conventional food or sometimes called by inorganic food. The word "organic" refers to the technique that farmers grow and process agricultural products, such as fruits, vegetables, grains, dairy products and meat. Organic farming applies are designed to encourage soil and water conservation and reduce pollution. For example, rather than using chemical weed killers, organic farming conduct more sophisticated crop rotations and spread peat or manure to keep weeds at bay.

Organically grown vegetables from Malaysia are certified by Department Of Agriculture of Malaysia (DOAM). Rules are varying over region for this certification and with different accreditation body. From 2003 until 2012, almost 84 organic farms have been certified by DOAM which standardized under Malaysian Organic Scheme (SOM) (Handbook of Malaysian Organic Scheme, 2012). This standard mainly follows Malaysian Standard (MS) 1529:2001 *The Production, Processing, Labelling and Marketing of Plant-Based Organically Produced Foods.* While, establishment of MS 1529: 2001 have been guided thoroughly by International

Federation of Organic Agriculture Movements (IFOAM Standard), World Health Organization (WHO) and Food and Agriculture Organization of United Nations (FAO).

Johor Bahru is one of the biggest cities in Peninsular Malaysia which located in State of Johore. Population by 3.4 million of people in 2012, this city is the 5^{th} largest state by land area with a total of 19,210 km^2. State of Johor located in southern region of Malaysia which surrounded by Melaka, Negeri Sembilan and Pahang and it's also a part of border-link between Singapore and Malaysia. In additional, averagely, adult population of Johor Bahru consume 46.5 g per day of green leafy vegetables and this level is considered tolerable enough (Food Consumption among Adult in Johor Bahru, 2003).

Nowadays, with increase awareness and better lifestyle, people choices of food are towards for healthier and additional its safety. Due to this, consumption of organic food such as egg, vegetables, fruit, chicken and meat are increasing gradually in Malaysia. Besides, school education, exaggerate advertising by organic activist and culture-clash have exert this awareness among consumer. In additional, fast forward production and high demand have also leads to lack in conventional food safety. For example, conventional breeding chicken use of unsafe and non-halal vaccine that is unhygienic and not safe for consuming. While, organic chicken breeds and growth in the natural way technique rather than use of substance, vaccine and chemical that helps to reduce chicken maturity age, in order to fulfill customer needs. Role of organic activist that have always promoting benefit of organic meat and organic vegetables, which provide antioxidant and anticancer for those who consume it, almost being part of scandal in food science. Hay (1989) in his conducted survey discovered that organic foods were perceived to be high quality, better at taste, healthier and more nutritious but less appealing than conventional products.

Vegetables known for its constitution as vital part of human diet since its contain of good mineral, vitamin as well as carbohydrate and proteins. Previous study shows that a diet which rich in fruits and vegetables help reducing the risk of

all the major cause of illness and death (Devine *et al*, 1998). In present day, the demand for eating fruits and vegetables has dramatically growing among population from around over the world. Devine et al, 1998 mention that fruits and vegetables provide health promotion because of its contain essential nutrient such as vitamins, minerals, proteins, fibers and bio functional components while also being low in fat, sodium and calories compared to any other foods. World Health Organization (WHO) in their published report on diet, that have recommends intake of minimum of 5 portions of 400 g of fruit and vegetables per person per day but excluding potatoes and other starch tube, will provide more nutritious for body and may help prevention of chronic disease. This recommendation specify at least 2 daily servings of fruits (160 g) and 3 daily servings of vegetables (240 g) with at least one serving of vegetables involving dark green and leafy or orange vegetable (nutrient-rich vegetable) (Striegel-Moore *et al*., 2006). Vegetables is now known as important part of human diet in Malaysia as it consumption increasing gradually every year.

1.2 Relative of soil, antrophogenic effect and growth vegetables with HMs

Ecotoxicological properties of heavy metals (HMs) are generally well known which can cause harmful on human health and on the environment. The HMs chemical risk can be analyzed to help in evaluating ecological situation and to predict the dynamic of future urbanization. R.M Khifli *et al.* (2010) find that human may exposed to As, Pb, Hg and Cd through both naturally occurring source and sources resulting from human industrial and cultural activities. However, exposure to toxic element of HMs for most people is actually through dietary intake (Calderon *et al.* 2003). It is believe that vegetables become contaminated by HMs are growing on soils that contaminated by mining, transportation exhaust, industrial activities and agricultural activities (Li *et al.* 2004)

Increasing consciousness about future sustainable agriculture and hazard free food production has bring organic farming to be a globally emerging alternative farm practice. Besides, public concern regarding the environmental and food safety,

implications of agricultural chemicals was paralleled by growing concern among farmers regarding their effect and that of their families and livestock. Use of inorganic pesticides, waste water and planting vegetables over abundant mine may contribute high level of HMs concentration in edible part of vegetables (Oti W. *et al*, 2012). In order to overcome its detrimental effect, good organically farming have been established so that vegetables are free from any hazardous chemical and HMs toxicity.

Therefore, organic farming has increase gradually due to high demand by consumer. Due to this, almost all hypermarkets in Johor Bahru have taking part in selling organic food in other to cope of this trends and its demand. Safe, better at taste and have been perceives as it provides more nutritious makes organic food is the first people choice even its threefold more expensive than conventional food. For this reason, many researcher (Song et.al, 2009; Islam et. al, 2010) have questioning, is that organic food capable of resist with pollution such as heavy metals which contaminate over atmosphere, soil and water. Again, study by Song Bo *et al.* in 2009 found that there is significant difference of concentration of heavy metal between open-field plants and green-house plant. Thus, this led that environment factor is the main outline of the cautious of heavy metal contamination in food.

1.3 Toxicity of Heavy Metal

A heavy metal is any of a number of higher atomic weight elements, which has the properties of a metallic substance at room temperature. Its density is much greater than water which is at least 5 times. Heavy metal refers to any metallic chemical element that have a relative high toxic density or highly toxic at low concentration. HMs are one of the most serious pollutants in the natural environment due to their toxicity, persistence and ability to accumulate in biota. In small quantities, certain heavy metals are nutritionally essential for a healthy life such as iron, manganese, copper and zinc. These elements, or some form of them, are commonly found naturally in foodstuffs, fruits and vegetables, and in commercially

available multivitamin products. Many of these products are in our homes and add to quality of life when it's properly used.

Cadmium, lead, mercury and silver which are non-essential metals are very toxic even at relatively low concentrations. HMs becomes toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body via food, water, air, or absorption through the skin in agriculture, manufacturing, pharmaceutical, industrial, or residential settings. If heavy metal enters and accumulate in body tissue faster than body's detoxification pathway, a gradual buildup of these toxins will occur. Breathing heavy metal particles, even at levels well below those considered nontoxic, can have serious health effects. Virtually all aspects of animal and human immune system function are compromised by the inhalation of heavy metal particulates. In addition, toxic metals can increase allergic reactions, cause genetic mutation, compete with "good" trace metals for biochemical bond sites, and act as antibiotics, killing both harmful and beneficial bacteria. Much of the damage produced by toxic metals stems from the proliferation of oxidative free radicals they cause.

HMs can also increase the acidity of the blood. The body draws calcium from the bones to help restore the proper blood pH. Further, toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn to the area as a buffer. The calcium coats the inflamed areas in the blood vessels like a bandage, patching up one problem but creating another, namely the hardening of the artery walls and progressive blockage of the arteries.

Previous studies by Harte J et al 1991 indicate that even minute levels of toxic elements have negative health consequences; however, these vary from person to person. Nutritional status, metabolic rate, the integrity of detoxification pathways (ability to detoxify toxic substances), and the mode and degree of heavy metal exposure all affect how an individual responds. Children and the elderly, whose immune systems are either underdeveloped or age-compromised, are more vulnerable to toxicity.

1.4 Problem Statement

HMs such as cadmium, arsenic, mercury and lead has the most potential harms to the human. Known from previous study, both humans and experimental animals indicate a carcinogenic potential for HMs (Islam *et al*, 2010). Earlier research also has demonstrated certain HMs concentration levels in food chain have exceeds permissible limit by World Health Organisation (WHO) and Malaysia Food Regulation 1985 (Ahmad Abas *et al*, 2001; Shahril et al, 2007). For most people, exposures to HMs are from ingestion of food and their risks are of great public concern.

Purchase intention of organic food among Malaysians has increased because it tastes more delicious and most important it have been perceived by consumer its safe rather than conventionally grown food (Nurul Izzah, 2012). Guideline in standard organic farming that has been developed will assure more safety and healthier content without use of pesticide, herbicides, and any inorganic pesticides. However, soil and anthropogenic factor for HMs contaminate in edible part of leafy vegetables have been not considered in many research. A comprehensive research of HMs in food and an assessment of their risk to the population also are lacking.

In this study, the risk of HMs exposure in organic and inorganically grown vegetables can be a reason human get serious health problem. Thus, it's necessary to determine and analyze the cause of high concentration levels of HMs from sampling vegetables.

1.5 General Objectives

In this research, main objectives are to determine concentration of heavy metal in two types of Malaysian grown leafy vegetables, organic and inorganic *Brassicas*. The aim also is to compare the levels of contamination of heavy metals in

vegetables which consumed by population in Johor Bahru. Briefly, specific objectives are listed below:

i. To investigate the presence of heavy metal in organic and conventional growths *Brassica* using Wavelength Dispersive X-Ray Fluorescence method.

ii. To determine and to compare the concentration of heavy metals in organic and conventional inorganic growths of *Brassica.*

iii. To assess and to compare concentration of heavy metal in organic and conventional growths of *Brassica* based on origin.

1.6 Scope of Study

The scope of study was to determine and assessed heavy metal of organic and inorganic vegetables which are favourites among Malaysian. Although there were a lot of aspects were pertaining to this topic, the study also focusing on potential of XRF in detecting heavy metal from food samples. This scope was designed in this way to suit it to several limitations such as time constraint and limited research facilities.

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