**Faculty of Resource Science and Technology**

# Method Development for Heavy Metals Analysis and its Accumulation in Pepper (*Piper nigrum* L.) Cultivation Area, Sarawak, Malaysia

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# Method Development for Heavy Metals Analysis and its Accumulation in Pepper (*Piper nigrum* L.) Cultivation Area, Sarawak, Malaysia

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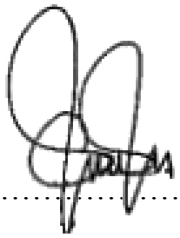
# DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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## ABSTRACT

A method of ICP-MS/MS for the determination of heavy metals in black pepper berries based on microwave-assisted digestion method was established in this study. The method was optimised by optimising gas flow rate, digestion temperature, digestion power, and acid digestion system. The optimisation was successful with satisfactory recoveries between 98.14-114.83%. LOD for As, Pb, Hg, Cd and Sb were established at 0.006, 0.01, 0.003, 0.003 and 0.008 mg/kg respectively. Good linearity was observed with correlation factor (R2) above 0.999 for all the metals of interests. Uncertainties of the method were in the range of 7.21 – 18.29%. The optimised method was then applied to 115 randomly selected imported and domestic pepper berries where 53.91% of the samples analysed were found to contain As (0.01 – 7.00 mg/kg), Pb (0.01 – 3.61 mg/kg) and Cd (0.02 – 1.37 mg/kg). The method is further used to determine uptake of heavy metal in pepper cultivated in Sarawak and heavy metal concentration in compartments of pepper vines. All target elements' concentration in parts of pepper vines, berries and soils collected from the south, middle and north region of Sarawak were found to be below the maximum allowable limit recommended by relevant standard. This shows that the pepper berries collected in Sarawak were completely safe for consumption and soils used in the cultivation area were safe for agriculture use. The value of EF and TF recorded were lower than 1, which shows that there were no hyper accumulations of heavy metals in pepper vines. Non-carcinogenic (ADD, HQ and HI) and carcinogenic (LCR and CRt) calculated shows that there were risk by consumption of pepper presents in local adults. PCA shows clustering between pepper berries and soil samples from the same region, but no clustering involving concentration of heavy metals and its location in pepper leaves and stem.

Statistical analysis (ANOVA) was applied on the samples and there was significant difference between the average concentrations of target elements against area of sampling.

**Keywords**: Heavy metals, black pepper, ICP-MS/MS, method validation, safety level

***Pembangunan Kaedah untuk Analisis Logam Berat dan Penumpuannya dalam Kawasan Penanaman Lada (Piper nigrum L.) Sarawak, Malaysia***

## *ABSTRAK*

*Satu kaedah ICP-MS/MS untuk penentuan logam berat dalam lada hitam berdasarkan kaedah penghadaman yang dibantu gelombang mikro telah dibangunkan dalam kajian ini. Kaedah ini dioptimumkan dengan mengoptimumkan kadar aliran gas, suhu dan kuasa pencernaan, dan sistem asid pencernaan. Kaedah ini telah merekodkan peratusan pemulihan yang memuaskan iaitu 98 - 114.83%. Had pengesanan untuk As, Pb, Hg, Cd and Sb telah ditentukan pada 0.006, 0.01, 0.003, 0.003 dan 0.008 mg/kg masing-masing. Keliniearan yang baik juga telah ditentukan untuk kaedah yang dibangunkan ini dengan faktor korelasi (R2) melebihi 0.999 untuk kesemua logam tersebut. Pengukuran ketidakpastian kaedah adalah berada dalam julat 7.21-18.29%. 115 sampel beri lada import dan domestic telah dipilih secara rawak dan dianalisis menggunakan kaedah yang telah dibangunkan tersebut di mana sejumlah 53.91% sampel yang dianalisis didapati mengandungi As (0.01 – 7.00 mg/kg), Pb (0.01 – 3.61 mg/kg) dan Cd (0.02 – 1.37 mg/kg). Kaedah ini selanjutnya digunakan untuk menentukan pengambilan logam berat dalam lada yang ditanam di Sarawak. Kesemua bahagian pokok lada (daun, batang, tanah dan beri) juga ditentukan kepekatan logamnya. Kesemua kepekatan logam di bahagian pokok lada, buah beri dan tanah didapati berada dibawah had maksimum yang dibenarkan oleh piawaian yang berkaitan. Ini menunjukkan bahawa buah lada yang dikumpul di kawasan penanaman di Sarawak selamat untuk dimakan dan tanah yang digunakan di kawasan penanaman adalah selamat untuk kegunaan pertanian. Nilai EF dan TF direkodkan adalah lebih rendah daripada 1 yang menunjukkan bahawa tiada pengumpulan hiper*

*logam berat dalam pokok lada. Risiko bukan karsinogenik (ADD, HQ dan HI) dan karsinogenik (LCR dan CRt) yang dikira menunjukkan bahawa tiada risiko yang berkaitan dengan pemakanan lada tempatan. Ujian PCA menunjukkan ada pengelompokan diperhatikan antara beri lada dan sampel tanah dari wilayah-wilayah, tetapi tiada pengelompokan yang melibatkan kepekatan logam berat dan lokasinya dalam daun dan batang lada. Analisis statistik (ANOVA) telah dijalankan ke atas sampel. Terdapat perbezaan yang signifikan antara purata kepekatan unsur sasaran dan kawasan persampelan.*

***Kata kunci:*** *Logam berat, lada hitam, ICP-MS/MS, pengesahan kaedah, tahap keselamatan*

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| | |
|---|---|
| AAS | Atomic Absorption Spectrometry |
| ADD | Average Daily Dose |
| AFS | Atomic Fluorescence Spectroscopy |
| ANOVA | Analysis of Variance |
| AT | Average Time |
| Bw | Body Weight (Average) |
| CALEPA | California Environmental Protection Agency |
| CEC | Cation Exchange Capacity |
| CSF | Cancer Slope Factor |
| CWP | Creamy White Pepper |
| DSM | Department of Standard Malaysia |
| ED | Exposure Duration (Average) |
| EF | Enrichment factor |
| EM | Electron Microprobe |
| EXF | Exposure frequency |
| FAO | Food and Agriculture Organization |
| FAQ | Fair Average Quality |
| FP | Flame Photometer |
| GAP | Good Agricultural Practice |
| GFAAS | Graphite Furnace Atomic Absorption Spectroscopy |
| HGAAS | Hydride Generation Atomic Absorption Spectroscopy |

| | |
|---|---|
| HI | Hazard Index |
| HQ | Hazard Quotient |
| IARC | International Agency for Research on Cancer |
| ICP-MS/MS | Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry |
| ICP-OES | Inductively Coupled Plasma Optical Emission Spectrometry |
| IDLH | Immediately Dangerous to Life and Heath |
| INAA | Instrumental Neutron Activation Analysis |
| IPC | International Pepper Committee |
| IR | Ingestion Rate |
| IRIS | Integrated Risk Information System |
| LCR | Lifetime Cancer Risk |
| LOD | Limit of Detection |
| LOI | Loss of Ignition |
| LOQ | Limit of Quantification |
| ML | Maximum Limits |
| MOH | Ministry of Health, Malaysia |
| MPB | Malaysian Pepper Board |
| MPL / MPP | Maximum Permitted Limits / Maximum Permitted Proportion |
| NIOSH | National Institute of Occupational Safety and Health |
| PCA | Principal Component Analysis |
| RfD | Reference Dose |

| | |
|---|---|
| ROS | Relative Oxygen Species |
| RSD | Relative Standard Deviation |
| SD | Standard Deviation |
| SMB | Standard Malaysian Black |
| SOM | Soil Organic Matter |
| SPSS | Statistical Package for Social Science |
| TF | Translocation factor |
| UNEP | United Nations Environment Programme |
| USDA | United States Department of Agriculture |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |
| XRF | X-Ray Flourescence |

# CHAPTER 1

# INTRODUCTION

Food is the most fundamental things in order to provide nutritional support for all living organisms. For humans, foods are often cooked, in which different types of food - i.e. vegetables, meat, fish – are added with seasoning and spices to create pleasant taste and thus more enjoyable to eat. Spices can be seeds, fruit, root bark and other plant substances that are used as flavouring or garnishing but should not be confused with herbs. Apart from its uses as flavouring, they are often used in medicine, cosmetics, and fragrance production. Black pepper is one of the examples of spices that were widely used as unique flavouring, in medicine, cosmetics, and even in fragrance production.

Black pepper, *Piper nigrum L.*, is a flowering vine cultivated for its berries. It is native to the Malabar Coast, India (Verma, 2019) and also extensively cultivated in other tropical regions such as Vietnam, Cambodia, Indonesia, and Malaysia. In Malaysia, the cultivation of black pepper started as early as in the 10$^{th}$ century when the empire extension of South Indian Kings begun. The crop was brought to the Borneo Island (now known as the East Malaysia), in 1840s by Chinese settlers where they actively cultivated black pepper in the areas (Izzah & Asrina, 2019). Now, *P. nigrum* is the most prevalent spice crop grown on agricultural land (Adama et al., 2018). In 2021, black pepper is cultivated on an estimated area of 9,000 hectare in Malaysia by 39,000 farmers, where 98% of the areas of cultivation are in Sarawak. Up to 80% of the pepper cultivation areas are well cultured with high production of pepper (Khew et al., 2022). The pepper plantations produce an average of 25,000 metric tonnes per year, in which a total of 31,600 metric tonnes was exported in 2021 (MPB, 2021). Approximately 70% of the produce was

exported as black pepper and 30% as white pepper. This has placed Malaysia as the fourth out of five IPC (International Pepper Council) countries in black pepper production in 2021 (Kumari, 2022), and the seventh place worldwide in 2019 (FAOSTAT, 2021). Sarawak pepper is globally recognized as premium quality pepper. This recognition comes with the promotion trademark "Hallmark of Quality" (MANRED, 2020). To maintain the premium quality of our black pepper in taste, aroma, size, and colour, it is crucial to guarantee the quality of the product from the food safety point of view, including free from pesticides, microbes, toxins and heavy metals.

Heavy metals are one the concerns for food safety, regulated by the Food Act in Malaysia and the Food and Agriculture Organization (FAO). Heavy metals are present naturally in the environment however their abundance can be elevated *via* anthropogenic activities, including through pesticides leaching into water bodies, air emissions from automobile exhausts, processing of wastes from mining and industries, and through metal smelters. Amongst the known heavy metals, arsenic (As), antimony (Sb), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are related to human poisoning at certain concentrations. These heavy metals exhibit dangerous effects on plants and on human health due to their cumulative toxicity. However, not all metals pose negative effects on humans; some heavy metals such as iron (Fe), zinc (Zn), and copper (Cu) are considered essential and have biological role and importance, whereas Cd, Cr, Hg, As, Pb and Sb do pose negative effects on humans. The central nervous system and kidney can be damaged by prolonged exposure to Hg (Afrifa et al., 2019); Cd affects the respiratory system (pneumonitis), urinary system (proteinuria and kidney stones), reproductive system (such as testicular necrosis and oestrogen-like effects) and skeletal system (loss of bone density and mineralisation) (Suhani et al., 2021); whilst the exposure to Pb, on the other hand,

negatively affect the heart and could lead to heart attacks and strokes in adults (Obeng-Gyasi et al., 2021).

In recent years, there are increasing attention and interest regarding heavy metals contamination on export and import commodities due to strengthening of the food safety regulation and requirements (Rai et al., 2019). This is because heavy metal existence in our daily foods may largely influenced metal intake by the human population, as according to the USDA (2016, Reference No.02030), the mean dietary intake for black and white pepper is 6.9g/person/day whilst the mean oral exposure of black pepper is 3.5g/person/day (Rahman et al., 2018). The presence of heavy metals in black pepper berries poses a great threat to the pepper producers as high concentration of heavy metals would affect the exportation. The exportation is regulated by the increasingly stringent control on the safety of agricultural commodities including pepper by the importers from the European Union (EU), Canada, and the United States. Regulatory authorities of the importing countries have established their own Maximum Permissible Level (MPL) for each identified contaminant. The countries can impose non-tariff barriers on exported berries that the exporter must satisfy to allow their continued export.

Between different cultivars and species, plant would demonstrate varying capabilities in absorbing and accumulating heavy metals (Ismael et al., 2019; Yaashikaa et al., 2022; Yu et al., 2022; Zakaria et al., 2021; Zulkafflee et al., 2022). Usually, heavy metals were absorbed by the plants from the depth of 25 cm under soil surface where roots of most grain crops are distributed (Jiang et al., 2019; Nguyen, 2020). The bioavailability of metals is profoundly governed by various external factors, for instance pH and saturation of the metals in soil (Shi et al., 2021; Wang et al., 2019; Yu et al., 2020). Upon

uptake of the metals, the element will be translocated from roots to shoots and then directed to the grains (Wang et al., 2021), which will be subsequently transferred into the food chain. This is due to the geochemical properties of the metals as they are mostly mobile and readily absorbed and taken up by plant roots before being translocated to aerial parts of the plant (Chen et al., 2021).

## 1.1 Background of the Study

It is crucial to ensure food safety in pepper industry to prevent trade barriers and to maintain the reputation of Sarawak pepper as a pepper of premium quality. In order to do so, a method has to be established to properly identify and quantitate amount of food contaminants in food stuffs, in this case, heavy metals in black pepper berries. The detection and identification of heavy metals in food with high oil content such as pepper berries is a difficult and challenging task since the high and complex intrinsic matrix could hinder the qualification and quantification of the targeted element (Wilschefski & Baxter, 2019). It is well known that pepper berries contain high amounts of fatty acids, fatty acid esters, phytosterols, tocopherols, sugar, polyphenols, theobromine, and caffeine. These matrices, rich in fatty acids, could disrupt the analytical columns, ion sources and detectors of the analytical instruments, and could interfere with the signal.

Despite the aforementioned challenges, improvements have been continuously developed for the qualification and quantification of heavy metals in pepper, for example, through analytical instruments such as graphite furnace atomic absorption spectrometer (GFAAS) and atomic absorption spectrometer (AAS). However, the AAS and GFAAS methods are not specific and insensitive; as shown in the previous studies, the recovery of elements from corn oil using GFAAS method recorded recovery ranging from 90 to 117%,

whilst the Inductively Coupled Plasma (ICP) demonstrated better precision of 93 - 103% (Allen et al., 1998; Velez, 2009). The ICP method is relatively more sensitive than AAS and GFAAS. The ICP method can analyze multiple elements at one time (up to 60 elements simultaneously) in two to six minutes while AAS runs analyses separately for each individual element (Jin et al., 2020). ICP also has longer linear range compared to AAS and GFAAS, where linearity for ICP ranges from 4 to 6 orders of magnitude while AAS and GFAAS range from 2 to 3 orders of magnitude. The ICP experiences less chemical and matrix interference than AAS as a result of high temperature of the plasma (Velez, 2009). The high temperature of the plasma (up to 7,000 $^{o}$C) also helps to excite most chemical elements (Komin et al., 2022). Another difference between AAS and ICP is that AAS analyzed the elements through absorbed light while ICP detects the ions of interest, or targeted ions, themselves. The ions passed through into the mass spectrometer and underwent separation based on their atomic mass-to-charge ratio by a quadrupole. Furthermore, ICP also offers best detection limits, even in the parts-per-trillion range, for most elements (Thermo Elemental, 2001).

Previously, for the detection of heavy metals in black pepper, the methods for sample preparation and analysis of sample took a minimum of one hour, and uses at least 10 mL of strong, undiluted acids (Refer to section 2.10). This is impractical for monitoring programs where large amount of samples were involved. Hence, the development of an analytical method that is sensitive, selective, quicker, cost effective (by using less chemicals and gases), reproducible, and have good recovery are important to ensure reliable analysis of contaminants for enforcement and monitoring programs (Lehotay & Chen, 2018).

The export of pepper berries is vital to the international trade of the country. With the implementation of Food Act (1983) and EC Regulation No 1881/2006 on the maximum permissible proportion of heavy metals in food stuffs, it is important to evaluate the accumulation, distribution and relationships of heavy metals in different plant parts of black pepper to understand the translocation of heavy metals in soil plant system.

Accumulation of heavy metals is a problem of concern as a result of rapid development and increased human activities (Yan et al., 2018; Yunus et al., 2020). The presence of heavy metals in soils creates two problems – firstly, the accumulation of heavy metals would reduce the crop yield resulting from the inhibition of plant transpiration, photosynthesis and oxidation systems (Giannakoula et al., 2021), and secondly, accumulation of heavy metals affects human health due to consumption of heavy metals accumulated (Balali-Mood et al., 2021; Munir et al., 2021). For example, two of the heavy metals with adverse effect to humans are Cd and Pb – the accumulation of excessive Cd in a human body will induce osteoporosis, causing damage to the immune system, kidney and nervous systems (Fatima et al., 2019); and a high level of lead in human body will damage human nervous system and kidney function and at the same time increase blood pressure (Charkiewicz & Backstrand, 2020). Hence, Codex Alimentarius Commission of the joint FAO/WHO has issued a strictly regulated and specified guidelines for the allowable concentration of heavy metals in food stuffs (FAO/WHO, 2004).

It is important to monitor the presence of heavy metals in imported and exported black pepper to ensure the product quality is warrant. Apart from that, plant uptakes heavy metals from soils; the contaminants are then translocated and accumulated in different part of plants (Wang et al., 2016). Singh et al. (2012) studied the distribution pattern of heavy

metals in different vegetables crops which shows that leafy vegetables accumulate higher distribution of metals in the edible parts while fruit type vegetables have fewer metals in fruits than other parts of the vegetable. Mehta et al. (2021) explored the tolerance mechanism and heavy metals detoxification in plants. Hence, by monitoring heavy metals in black pepper planted in agricultural soil contaminated by heavy metals, any relations between concentration of heavy metals in pepper berries and in soils, if any, can be observed.

## 1.2 Objectives of Study

Given the rising concerns over the food safety of pepper products, specifically heavy metals and the need for a more sensitive and selective method for detection of the heavy metals in pepper, the objectives of this study are:

1. To develop the method for simultaneous identification of selected heavy metals in pepper (*P. nigrum*) berries using microwave digestion and Triple Quadrupole ICP-MS with Discrete Dynode Electron Multiplier Detector (ICP-QQQ) for analysis of pepper berries;

2. To determine the heavy metal deposition in different parts of pepper vines along with the extent of heavy metals uptake by pepper influenced by agriculture soils;

3. To determine safety level and concentration of heavy metal in black pepper samples collected in site.

## 1.3 Significance of the study

This study seeks to develop a method that is more reliable and sensitive for analysis of heavy metals, specifically As, Cd, Pb, Hg and Sb present in black pepper berries and to investigate the uptake as well as translocation of these elements in different parts of *P. nigrum* vines which include leaves, stems and berries, along with the soil.

The findings of this study are important as they provide reliable information of heavy metals present in pepper berries and offers insights into the safety of pepper consumption, either carcinogenic or non-carcinogenic risks, associated with the consumption of black pepper berries produced locally in Sarawak. In addition, this study assesses the contamination status of heavy metals in soil of *P. nigrum* cultivation and the uptake behaviour of the elements in plants. This information is essential for the statutory body, authority and stakeholders involved with *P. nigrum* cultivation.

## 1.4 Scope of the Study

The study focuses on optimisation of heavy metals analysis method which uses Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS/MS) and sample preparation using microwave-assisted acid digestion. This study also focuses on six heavy metal parameters: Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg) and Antimony (Sb) as they are regulated by the Malaysian Food Act 1983, and International Standards such as IRIS and CALEPA. Samples used in this study are imported black pepper samples (collected from Indonesia, Vietnam, India, Sri Lanka and Cambodia) domestic black pepper samples (to be exported) and black pepper, along with pepper plant parts collected from farms in Northern, Central and Southern region of Sarawak.

## 1.5 Summary of the Chapter

This chapter highlights backgrounds of black pepper (*P. nigrum*) cultivation in Malaysia, heavy metals and concerns regarding contamination of heavy metals on foodstuffs where maximum limits imposed by importing countries could affect exportation of the commodities. Apart from that, this chapter also highlights background of the study, the objectives of the study and significance of the study, where there is a need on establishing a method for heavy metal detection and quantification in black pepper that could meet the requirements of the importing countries (for the use of enforcement and monitoring) and that could also overcome the disruption of high matrix of pepper. This chapter also highlight the need for assessment of contaminated pepper cultivation area and heavy metals detection in pepper berries on site.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Black Pepper (*Piper nigrum* L.)

*Piper nigrum* L. belonged to the family Piperaceae, and to the genus Piper, which was the economically and ecologically important genus in the family (Sen & Rengaian, 2021). There were about 2,000 species under this genus. The scientific name Piper and the common name 'pepper' originated from the word *pippali*, a Sanskrit term, which meant 'long pepper' (*P. longum*) (Sharmah et al., 2018). Black pepper is a berry-like fruit that produces peppercorns and contained a cluster seed (Sangeetha, 2019). It was the popular choice of spice, and was a very important spice and medicinal crop, especially in India (Aarthi & Kumar, 2019). It was commonly found alongside salt and garlic powder on the dinner table around the world. *P. nigrum* was widely planted and distributed especially in the tropical regions (Abdallah, 2018). It originated from a region southwest of India, and was first cultivated in Malaysia during the 11th century (Izzah & Asrina, 2019). In Sarawak, *P. nigrum* was mainly cultivated on slopes, and farmers were encouraged to use ground cover to avoid erosion (Sulok et al., 2021).

There were various cultivars worldwide which included Cingapura, Equador, Guajarina, Lacara, Kottanadan, Bragantina, Clonada and Uthirankota in Brazil (Barata et al., 2021). Panniyur-1 and Karimunda were among pepper varieties in India (Aarthi & Kumar, 2019). Vin-linh was one of the most popular pepper cultivars in Vietnam (Oanh et al., 2021). Lampung-1, Natar-1 and Natar-2 were the pepper varieties planted in Indonesia (Yudiyanto et al., 2014). In Sarawak, the most widely grown *P. nigrum* cultivar was Kuching, and two other major and improved cultivars were Semenggok Aman and

Semenggok Emas (Izzah & Asrina, 2019) (Figure 2.1).


**Figure 2.1:** Main cultivars of *P. nigrum* (black pepper) in Sarawak (Nang Ori, 2018).

## 2.2 Uses of Pepper

Black pepper has been used for various purposes such as flavouring, pharmaceutical and cosmetics. Historically, in ancient Egypt, black peppercorns were used in mummification rituals of Ramesses II after his death in 1213 BCE (Sen & Rengaian, 2021). It was not known how peppercorns reached the Nile from the South Asia, although black pepper was likely to have been brought by the Romans (Cobb, 2018). During postclassical Europe, peppercorn was so popular and valuable that it was used as collateral or even currency – this is the reason black pepper was known as the black gold (Hu et al., 2019), as it was worth more than gold by weight during that time. Apart from that, black pepper was widely used to cure illnesses such as constipation, insomnia, oral abscesses, sunburn, urinary complaints, liver problems, haemorrhoids, diarrhoea, cholera, arthritis, jaundice and toothaches. In recent years, black pepper is also used as a biocontrol agent (Lau et al., 2020).

Taking the advantage of the heating properties of its essential oil, derived from the outer fruit and its seed, black pepper is widely known among ladies as a self-defence and self- protection weapon in the form of pepper spray. Black pepper extract can also be used to make perfumes, cosmetics, and personal care products. There are also other medicinal properties for example, it is a potential anti-cancer agent (Manayi et al., 2017), antimicrobial agent (Abdallah, 2018), and antiviral agent (Nag & Chowdhury, 2020).

## 2.3 Quality Evaluation of Pepper Spice

Various quality indices are used to indicate the quality of black and white pepper products (Abukawsar et al., 2018). The indices can be grouped into three main categories which are: physical (moisture content, extraneous matter, moulds, indication of infestation, bulk density, and light pepper berries content); chemical and microbiological assessments. Chemical analysis can be classified into extrinsic and intrinsic chemical properties. Extrinsic properties include pesticide residue, mycotoxin content and elemental analysis whilst intrinsic chemical properties refer to piperine content, volatile oils and non-volatile ether extracts, total ash and acid insoluble ash. Other assessment of black and white pepper quality includes sensory properties of pepper such as the flavour, aroma, and texture; quantitative properties such as percentage of sugar, protein, fibre and hidden attributes of peroxides, free fatty acids and enzyme; and also colour uniformity. In Malaysia, the statutory body that regulates quality and food safety of pepper is the Malaysian Pepper Board (MPB). Black pepper consignments are abided by the grading requirements for export, however, for imported pepper, food safety criteria such as percentage of mouldy berries, pesticide content in pepper are requirements at the highest priority (MPB, 2021).

The Malaysian Standard of Pepper categorised black and white peppers into 5

grades, respectively, according to the physicochemical properties (MPB, 2021). Black pepper is divided into Standard Malaysian Black (SMB) Pepper No. 1 (Brown label), Sarawak Special Black (Yellow label), Sarawak Fair Average Quality (FAQ) Black (Black label), Sarawak Field Black (Purple label), and Sarawak Course Field Black (Grey label). Meanwhile, five grades of white pepper are namely Standard Malaysia White Pepper No.1 or Creamy White Pepper (CWP) (Cream label), Sarawak Special White (Green label), Sarawak Fair Average Quality (FAQ) White (Blue label), Sarawak Field White (Orange label), and Sarawak Coarse Field White (Grey label).

## 2.4 Chemical Hazards in Black Pepper Production

Various ranges of chemicals have been used in the pepper production and processing to maintain its quality. Some chemicals, such as heavy metals in fertilizer, which are used for the growth of pepper plant cannot be removed by any subsequent external processes hence, the quantity of heavy metals needs to be monitored and regulated. This will typically be identified through controls in GAP or product testing. Pepper may be subjected to chemical contamination within one or more stages of the supply chain (van Asselt et al., 2018) due to the use of sanitizers, lubricants such as oil and greases, pesticides which are used within the processing facility as well as the manufacturing process. These chemicals can be rigorously controlled. Most of these chemicals do not pose any health hazards; however, some are harmful and able to cause serious health problems. Types of chemical hazards found, other than those used in the processing facilities, include agricultural products such as pesticides, fertilizers, mycotoxins, other field chemicals, toxic elements such as lead and mercury, other heavy metals in food additives such as preservatives, flavour enhancers and colouring agents

(Praveena et al., 2014).

Table 2.1 shows lists of previous studies where hazardous chemicals have been found in pepper.

**Table 2.1:** Hazardous Chemicals Previously Found in Pepper

| Author, year | Types of Hazardous Chemicals Found in Pepper | Average Concentration of Contaminant, mg/kg |
|---|---|---|
| Wan Hamid et al. (2019) | Heavy metals; | |
| | Nickle, Ni | 0.0470 |
| | Lead, Pb | 0.3009 |
| | Copper, Cu | 3.0970 |
| Galani et al. (2021) | Pesticides; | |
| | Captan | 0.2 |
| | Bifentrhin | 0.1 |
| Choudhary et al. (2022) | Contaminant(s); | |
| | Ethylene oxide | |
| | Carbanilide | N/A |
| | 2-amino-5-[(2-carboxyl)vinyl]-Imidazole | |

## 2.5 Background of food safety legislation in Malaysia

Food safety in the world generally and in Malaysia specifically is subjected requires the efforts from all relevant authorities as it concerns the safety and wellbeing of

citizens. In order to improve the food safety, Malaysia is continuously searching for strategies in order to meet with the international requirements by constantly formulating and revising food laws, regulations and standards, promoting certification enforcing laws, and increasing their involvement in international food safety activities (FAO/WHO, 2004).

In Malaysia, the Food Act 1983 is the legislation that was established to guarantee that food is safe at the time of consumption. It is the parent act, where five other legislations were enacted under the power given in section 34 of the Food Act 1983 by the Minister of Health, namely the Food Regulation 1985; Food Regulations 1993 – this version includes the extensions to Tobacco; Control of Tobacco Product Regulations 2004; Food Regulations 2009, which are the standards and guidance for Issuance of Health Certificate for Export of Fish and Fish Product to the European Union; and Food Hygiene Regulations 2009 (Ismail, 2011).

According to the World Health Organization (WHO), in 2010, there were 600 million cases of diseases caused by contaminated food (Chlebicz & Śliżewska, 2018). Foodborne illness is a global issue; every year, millions of people were fatally ill after consuming contaminated food or drinking water, including in a well-developed country (Yusoff et al., 2021). Therefore, constant revision of the Food Act 1983 is conducted by the Food Quality Control Division of the Ministry of Health Malaysia (MOH) to ensure food safety system is firmly regulated.

## 2.6 Heavy Metals

Heavy metals are defined as metals with relatively high densities, atomic weight or atomic numbers. It includes common metals such as iron (Fe), copper (Cu) and tin (Sn), and precious metals such as silver (Ag), gold (Au) and platinum (Pt). Some heavy metals

are regarded as essential nutrients which are needed by the body for biological processes such as iron (Fe), zinc (Zn), copper (Cu) and cobalt (Co) (Kim et al., 2019).

Heavy metals are always taken as highly toxic – some are highly poisonous such as Hg and Cd, while small groups of heavy metals are only toxic when taken or exposed in excess or encountered in certain forms (liquid/gaseous). Some heavy metals take part in important biological activity such as Zn (cell division and growth, among others) and Mg (muscle function and maintaining healthy immune system). Hence, that is the reason that the term 'heavy metals' are regarded as imprecise at best by the International Union of Pure and Applied Chemistry when used to describe elements as the term is often misleading (Pourret, 2018).

Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Antimony (Sb) and Lead (Pb) are the most commonly found heavy metals contaminants in the environment (Masindi & Muedi, 2018). As an essential trace element, Cr plays various important biological roles, such as aids in brain functions, enhancing kinase activity of insulin receptor, and breakdown of fats and carbohydrate (Krikorian et al., 2013). However, when it is present in excess, the element can gather or accumulate at a toxic levels in the plants parts and later transferred to human or animal via food chain, causing fatal diseases (Alfartusie & Mohssan, 2017). Heavy metals including As, Pb, Cd, Sb and Hg at low concentrations could be fatal to human (Rana et al., 2018). Hence, they are generally considered non-essential elements (Nielsen, 2017).

### 2.6.1 Sources of Heavy Metal Emission

Heavy metals are naturally present or are emitted into the environment via anthropogenic causes. The anthropogenic sources (man-made activities) include mining

operations, combustion of fuels, metal-working industries, use of phosphate fertilizers and etc. (Rahman & Singh, 2019). Previous studies reported traces of As, Cd, Cr, Pb, and Hg in water, sediments, snails, and fishes in the Pahang and Kelantan River at above the maximum allowable limits in the organisms. In another study, the sources of these heavy metals are associated with the dredging activities (Manap et al., 2019).

Arsenic (As) exposure can be caused by various routes, such as through contamination of drinking water, industrial and vehicle emissions, and air pollution (Chung et al., 2014). As can be taken up or accumulated by plants, fruit, and soils through weathering of minerals and ores and irrigation with mineralized groundwater (Anawar et al., 2018; S. Verma et al., 2018). Arsenic may cause a significant risk for cancer after exposure through contaminated drinking water, cigarettes, foods (from foods grown in arsenic-contaminated soil and/or irrigated with arsenic-contaminated water), occupational environment and air (Chung et al., 2014).

Lead (Pb) pollution can be caused by various activities. In a study conducted in the Eastern Beibu Gulf of the South China in 2019, the analysis of surface sediments and sediment cores indicated accumulation of Pb originating from both natural rock weathering products and human activities such as oil-gas exploration (Xu et al., 2019). However, in the Southwest Iran, isotopes of Pb were solely attributed to the man-made activities including vehicle emissions and industrial contaminations (Nazarpour et al., 2019). Similar scenario was also recorded in South Korea where high concentrations of Pb were identified in harbours and near industrial facilities (Jeong et al., 2018). The fire of Notre-Dame Cathedral in Paris in April 2019 became one of the sources of pollution due to the Pb-rich dust emitted (Smith et al., 2020). In Peninsular Malaysia, a recent study in the Linggi River

concluded Pb pollution originates from the electronics and electroplating industries (Elias et al., 2018). Natural occurrence of Pb is often associated with radioactive decay of heavier unstable elements into more stable elements (NASA, 2005).

There was also a study suggesting that air pollution can be caused by smoking of tobacco which contains heavy metals, such as Pb and cadmium (Cd) (Matt et al., 2021). Cd contamination may also be caused by natural and anthropogenic activities such as volcanic activities, wreathing, erosion and river transport (WHO, 2010). Cd is non-biodegradable, and its presence in different thropic levels in the food chain raised concern over food safety as bioaccumulation of Cd can disrupts human antioxidant defence system due to oxidative stress (Suhani et al., 2021).

Exposure of Sb in the environment was solely attributed to human activities such as burning of Sb containing spent products (Nishad & Bhaskarapillai, 2021) and mining of Sb (Ye et al., 2018). Sb is widely found in human food chains, water sources, and soil and is one of the air pollutants (Lou et al., 2021). It was recorded that Sb is the most abundant in China than the rest of the world where a previous study noted that drinking water in Hunan, China was detected at 53.6 ± 46.7 μg/L, which exceeded WHO water guidelines (Lai et al., 2022). In recent years, Sb, a carcinogenic element, has gained worldwide concern as Sb pollution can induce significant human toxicity, damaging the liver, heart and nervous system (Ren et al., 2019).

### 2.6.2 Sources of human and plant exposure

Heavy metals can enter the human body through few ways, such as through inhalation of dust and direct consumption of contaminated drinking water, contaminated food or plants grown in metal-contaminated soil. It was reported that Pb, Cd and Cr were

highly concentrated in the plasma of smokers (Alemam et al., 2019). Exposure to As was usually through food and beverages (Cubadda et al., 2017). A report found that an estimated 150 - 240 million of the Southeast Asian population were exposed to water containing As at a concentration higher than the WHO guidelines of 10 µg/L (Uppal et al., 2019). Apart from that, As exposure through inhalation of household dust was also reported in the South West of England (Middleton et al., 2017).

When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues. Studies on leafy plants such as pakchoi, lettuce and spinach planted in metal contaminated soils showed accumulation of the toxic metals in the plants (Eissa & Negim, 2018; Wei et al., 2017). Animals that graze on the contaminated plants and consume water or aquatic organisms that are bred in heavy metal polluted waters will be exposed to metal accumulation in their tissues (Enya et al., 2019) and milk, if lactating (Kabir et al., 2017).

#### 2.6.2.1 Vehicular traffic density

Certain trace elements are essential in plant nutrition and needed in plants biological function in small amounts. However, the plants which grown in the contaminated soil and in polluted environment can absorbed the essential elements to highly dangerous levels (Zwolak et al., 2019) and can cause serious health risk when they are consumed. It is found through studies that elevated lead (Pb) in the urban atmosphere is correlated strongly with the vehicular traffic density (Levin et al., 2021). Pb is not the only element that is emitted through vehicular traffic density – Cd, Cr, Sb, Hg and As were also found to be high in street dust samples in areas with heavy traffic and industrial activities (Natasha et al., 2019; Safiur Rahman et al., 2019; Zhao et al., 2020). The dust could be

later inhaled by human or settled around the nearest vegetation patch, and slowly get accumulated in the living tissues.

#### 2.6.2.2 Water for irrigation

Mining activities and activities from various industries contributed to water pollution by heavy metals. Wastewater containing chemicals cannot be used or reuse especially for irrigation of food crops and for direct consumptions. Uptake and accumulation of metals is directly associated with soil quality, water quality and food safety. Reclaimed or recycled water contamination may be a result of physical contaminations, and if used to irrigate plants, repeated irrigation may leads to build-up or accumulation of the contaminants in the plants (Ungureanu et al., 2020).

The fate and effects of pollutants that is introduced into a particular body of water will depend on several factors, which are the amount of polluting substances emitted, and the hydrological, physical, chemical and biological characteristics of the water body itself. A study conducted in Nigeria specified that Cd and Ni levels were high in vegetables irrigated with water from the highly contaminated Challawa River (Yahaya et al., 2021).

#### 2.6.2.3 Compost and sludge

The presence of organic and inorganic contaminants in compost brought danger to the environment. Heavy metals can be present in composts and sludge due to the presence of coal ashes, manures and litters produced on large-scale industrial feedlots, processed feeds and contaminated soils (Kupper et al., 2014). Compost quality is determined by the amount and type of heavy metals present (Ravindran et al., 2017). The agricultural use of composts and sewage sludge has been restricted mainly due to high heavy metal

concentration The use of sewage sludge can significantly cause accumulation of Pb and Cd in soil in the long run (Musa et al., 2017).

#### 2.6.2.4 Fertilizer application

Intensive agriculture or industrial agriculture activities could lead to a potentially hazardous accumulation of elements in soil and in crops because of excess use of fertilizers and soil amendments (Solgi et al., 2018). Fertilizers are essential to ensure high productivity. However, the fertilizers may contain heavy metals that can contaminate the soil and threaten the well-being of animals and humans (Wei et al., 2020).

Heavy metal contamination in soils by phosphate fertilizers has become a concern as long term application of phosphate fertilizers can lead to high Cd accumulation in soils (Agbenin, 2006). The abundance of heavy metal in phosphate fertilizers depends on the type of rock phosphate used as raw material (Oyedele et al., 2006.).

The Brazilian rock phosphates are low in heavy metals (Faridullah et al., 2017). Investigation into the effects of phosphate fertilizer on agricultural soils finds that heavy metals, such as Cd, might be present in the fertilizers as impurities in natural materials and minerals (Cheraghi et al., 2012). However, Cd concentration in maize amended with phosphate fertilizers increase significantly not only due to the use of fertilizers but also as a result of fungal colonization such as mycorrhizae (Yazici et al., 2021). Other heavy metals such as As, Cr, Pb and Hg are also found to be present in phosphate fertilizers. These elements are relatively less hazardous than Cd as they are not as readily available to the plants (Dharma-wardana, 2018). The application of chicken manure as fertilizers could increase the availability of the DTPA-extractable As and exchangeable As in soils (Wan et al., 2020).

#### 2.6.2.5 Application of Pesticides

Pesticides are used to control insects and diseases in fruits, vegetables and other crops. In agriculture, pesticides which contained metal such as fungicides and insecticides are widely applied (Uwizeyimana et al., 2017). Compounds of Cu have been widely utilized as fungicides to control foliar fungal diseases in citrus plants (Kah et al., 2019), particularly for the production of cocoa (Veronica et al., 2020) and beverage crops (Semu, 2019). However, the overuse of these fungicides can result in the accumulation of Cu in surface soils and become toxic at high levels (Kumar et al., 2021).

### 2.6.3 Toxicity of Related Heavy Metals

#### 2.6.3.1 Arsenic, As

Arsenic is mutagenic, carcinogenic and teratogenic. It is classed as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 2012a) as researchers had found a reliable connection between inorganic As (iAs) exposure in drinking water and the risk of cancer. Cancers that were often associated with iAs includes bladder, liver, lung, prostate, and skin cancers (Zhou & Xi, 2018). However, the mechanism of carcinogenesis induced by exposure to As is not well known. In the study by Demanelis et al. (2019), it was suggested that there is a novel and replicable association between As exposure and DNA methylation at a specific CpG island, a region of the genome that contains a large number of CpG dinucleotide repeats (Ohm, 2019). The changes were epigenetic, where the phenotypic changes that occur were heritable. In order to protect the public against health hazards in food, the Food Act 1983 was passed in Malaysia, commencing in October 1985 (FAO/WHO, 1997). According to the regulation, the maximum permissible level of As in black pepper, in the category of spices other than

curry powder, is stipulated at 5 mg/kg.

#### 2.6.3.2 Cadmium, Cd

Cd is an environmental pollutant that causes health hazard (Xu et al., 2020; Zhao et al., 2017). Exposure to Cd can cause oxidative stress. These stresses were observed in common carp where it induced immunosuppression and glycometabolism disorder (Chen et al., 2019). Apart from that, effect of Cd-induced oxidative stress can also lead to abnormalities in the central nervous system in which its deleterious effects can directly be linked to reactive oxygen species (ROS) generation (Branca et al., 2020).

Cd exposure was known to cause a decrease in reproductive performances such as fertility, abnormal development, prenatal death and sexual dysfunction (Unsal et al., 2020). A study by Zhao et al. (2017) shows that sperms exposed to Cd fora long time were observed to demonstrate decreasing mobility, while those exposed for a shorter time exhibit reduced fertilization rate. Blastocyst formation rate in the early embryonic development was dramatically decreased with increasing cadmium concentration (Zhao et al., 2017).

There are three routes of Cd exposure to humans: oral, inhalation and predominantly cigarette smoke (Unsal et al., 2020). The International Agency for Research on Cancer has named Cd and Cd compounds as carcinogen (IARC, 2012b). The National Institute of Occupational Safety and Health (NIOSH) has also designated Cd as a human carcinogen but has not set a recommended exposure limit (REL). However, NIOSH has indicated that the Immediately Dangerous to Life and Health (IDLH) level for cadmium is 9 µg/L (Faroon et al., 2012). Studies of Cd exposure has shown that Cd levels in smokers are higher than those of non-smokers, and that non-smokers' exposure of Cd is mainly

linked to food intake (Unsal et al., 2020). To protect the public against Cd exposure, the FAO/WHO Expert Committee on Food Additive has specified that 7 µg/L is the tolerable weekly intake level. The Malaysian Food Act, Regulation 38, Fourteenth Schedule states that the permitted proportion of Cd for spices other than curry powder is 1 mg/kg.

#### 2.6.3.3 Chromium, Cr.

Despite safety precautions on Cr intake, Chromium(III) metal and its compounds are not considered a health hazard. Chromium(IV), however, is toxic and has carcinogenic properties where studies have shown that the element can cause lung tumours via inhalation exposure (Rager et al., 2019; Yatera et al., 2018). Cr salts can also induce epithelial allergic reactions in some people such as dermatitis. It is referred to as "chrome ulcers" where it causes ulceration of the skin (Chatterjee et al., 2020). There is an imposed maximum limit for Cr. In the US EPA, the maximum permissible limit of Cr in drinking water is 0.1 mg/kg (ATSDR, 2013). In the Malaysian Food Act 1983, the maximum limit of Cr is set at 1 mg/kg.

#### 2.6.3.4 Mercury, Hg

Toxicity and health hazard of Hg are widely known and must be handled with care. Mercury is toxic as the exposure to Hg can cause damages to the brain, kidney, testicles and liver (Zhou et al., 2019). Symptoms of Hg poisoning depend on the speciation, dosage, route and duration of exposure. The symptoms of Hg poisoning include muscle weakness, poor coordination, numbness to the hand and feet, skin rashes, anxiety, memory problems, trouble in vision, speech and hearing (Kamensky et al., 2019). Minamata disease is one of the diseases caused by methylmercury poisoning (Sakamoto et al., 2018).

To avoid exposure of Hg and safeguard health, 91 countries had ratified the Minamata Convention on Mercury by the United Nation Environment Program (UNEP) in 2013 (Guerrero, 2018). In Malaysia, Hg was banned from cosmetics and medical applications, and its usage in dentistry and food is regulated. In the Malaysian Food Act (1983), the maximum limit (ML) for mercury in spices including pepper is 0.5 mg/kg.

#### 2.6.3.5 Lead, Pb

Pb toxicity is also widely known. Continual Pb exposure may result in deleterious systematic effects such as hypertension, infertility, immunity imbalance, delayed skeletal and dental development, and gastrointestinal effects (Mitra et al., 2017). Pb exposure can be lethal. A study conducted in the United States and Europe showed that adults who grew up exposed to Pb before the Clean Air Act 1970 were less conscientious and more neurotic compared to those born after the act was implemented. Individuals born after the Act was enforced (in which leaded gasoline was banned) were found to be more mature with healthier psychology and personalities (Schwaba et al., 2021).

To protect consumers from Pb exposure through food, the Codex guidelines limits the maximum Pb level in various foods at 2 mg/kg while in natural mineral waters it is set at 0.01 mg/kg. In Malaysia, the maximum permitted proportion of Pb for spices other than curry powder is 2 mg/kg. To protect the public against Pb exposure, further reduction of atmospheric Pb levels is critical (Schwaba et al., 2021). This is agreed by Mitra et al. (2017), where it is suggested that newer strategies for lead risk assessment has to be done as the current maximum limits, that was previously recognized as safe, is now found to be associated with adverse health effects.

#### 2.6.3.6 Antimony, Sb

Antimony exposure can cause pulmonary toxicity in humans, although its carcinogenic impacts to humans have not been thoroughly studied. In rats and mice, however, there were carcinogenic responses due to Sb exposures (Boreiko & Rossman, 2020). Exposure to Sb may take place through inhalation (Boreiko & Rossman, 2020) and food intake (Bolan et al., 2022).

Once Sb and Sb-derived compounds enter the body via direct or indirect exposure, it would react with sulfhydryl leading to cellular hypoxia as a result of enzymatic inhibition and disruption of cellular ionic balance (Bolan et al., 2022). High cytotoxic concentrations of Sb compounds can also induce chromosomes aberrations and micronuclei in bacteria and cultured mammalian cells (Boreiko & Rossman, 2020). Apart from that, Sb is recognized as a genotoxic element (Tao et al., 2021).

There were no evaluations of Sb by the FAO/WHO Joint Expert Committee on Food Additives, hence there were no tolerable limits set for this element (Panhwar et al., 2018). However, Malaysian Food Act 1983 has set a limit of Sb of 1 mg/kg in spices other than curry powder.

## 2.7 Heavy Metals Accumulation in Plants

Recently, the heavy metals accumulations in plants have become environmental concerns. This is because their uptake from contaminated soils enables metals to enter the food chain through consumption and can further pose significant risk to human health (Hembrom et al., 2019; Solgi et al., 2018). A variety of factors are required for plants to absorp, accumulate and distribute heavy metals into different compartments in plants.

These factors include the species of the plant, species of the elements, chemical and bio-availability, redox potential, pH, cation exchange capacity of soils, dissolved oxygen, and temperature (DalCorso et al., 2019).

### 2.7.1 Arsenic, As

Arsenic is generally toxic, not only to plants but to animals and humans. It serves no biological importance to plants and the exposure of As gives adverse effects. However, plants are known to naturally accumulate As, particularly inorganic As (iAs) in different parts of the crops (Mitra et al., 2017). It is an analog of phosphate (P) and competes for the same uptake carriers, thus, accumulation of As would decrease the uptake of phosphate – a compound crucial for metabolic processes (Ackova, 2018). Spinach is shown to accumulate As with increasing As levels in the surrounding (Rafiq et al., 2017). Grasses grown in As-contaminated soils exhibited high As concentration in their shoots (Dradrach et al., 2020).

However, after long term exposure and due to levels of expressions, some plants grow to be more adept at accumulating As than others (Punshon et al., 2017). Arsenic can cause sterility (Punshon et al., 2017). Accumulation of As could also induce oxidative stress and affect excretion of enzymes (Abbas et al., 2018). Apart from that, it will interfere with the intake of P affecting the metabolic processes and biomass production hindering root extension and proliferation (Ackova, 2018).

### 2.7.2 Cadmium, Cd

Cadmium is not an essential element for any biological process in plants (Xu et al., 2019). Nonetheless, it can be easily absorbed by both roots and leaves and tends to be

accumulated in soil organisms (Mortensen et al., 2018; Song et al., 2017). Plants usually employ self-avoidance strategy when it is first exposed to Cd (Song et al., 2017). However, over time, the element is readily taken up through the roots and transported to stems and leaves despite its toxicity (Zong et al., 2017). The absorption and bioaccumulation of Cd may vary between plant species based on their physiologies which lead to differences in their tolerance to the element (Grobelak et al., 2018; Khan et al., 2018).

Accumulation of Cd is visible; the injury caused by Cd is shown by chlorosis, growth inhibition, and death (Varma & Jangra, 2021). Cd could affect chlorophyll synthesis, inhibit the enzymes involved in $CO_2$ fixation and interfere the uptake of other elements (K, Ca, Mg and P) (Ackova, 2018).

### 2.7.3 Chromium, Cr

There is no conclusive evidence where Cr is important for any biological roles in plants. In the initial stage of Cr exposure, plants immediately adopt avoidance strategy, preventing uptake of Cr and its entry through the roots. However, under high concentration of Cr, the element will eventually enter the plant. The tolerance mechanisms and protection against the metal toxicity will then be activated. If the level of Cr accumulated is higher than the tolerance of the plant, the plant begins to suffer the effects of the metal toxicity showing symptoms of injury (Gomes et al., 2017). Similar to other heavy metals, Cr could cause oxidative stress as a result of pigment degradation (essential in photosynthesis). Cr could also cause chlorosis, growth retardation, wilting of shoots and injury to the roots of plants (Varma & Jangra, 2021).

### 2.7.4 Mercury, Hg

Mercury contaminated the soil via application of fertilizers, lime, sludge and manures. Uptake of Hg by the plant roots is largely from the soil, while Hg in above-ground biomass normally comes from atmospheric emission (Zhou et al., 2021). There is an inverse relationship between the concentration of Hg in soil and its uptake in plants governing by factors such as cation-exchange capacity, soil pH, soil aeration and species of plants (Varma & Jangra, 2021). However, the bioaccumulation of Hg may be a reversible process where the plant can accumulate Hg from the atmosphere and transfer the accumulated Hg to the ground, and vice versa (Naharro et al., 2018; Zhou et al., 2021).

Mercury has no beneficiary effects on plants. Exposure of Hg would disrupt cellular function and inhibit growth and development of plants (Varma & Jangra, 2021). However, plant uptake of Hg reduces global atmospheric Hg pool and Hg deposition to global oceans (Zhou et al., 2021). This cycle, also known as vegetative Hg cycle, has significantly reduced the environment impacts of Hg as required by the Minamata Convention (Wang et al., 2019). Further understanding of this cycle is crucial to assess the effectiveness of plants in reducing Hg deposition (Wang et al., 2019; Zhou et al., 2021).

### 2.7.5 Lead, Pb

Some roots and leafy vegetables can naturally accumulate Pb (Zwolak et al., 2019), however, the element plays no essential role in their metabolism (Zulfiqar et al., 2019). Plants can naturally accumulate Pb from soil or through foliar deposits. Pb is predominantly accumulated in roots and is poorly translocated to other parts of the plants (Pachura et al., 2016).

Pb can cause phytotoxicity in plants. Exposure to Pb could cause injuries to the plants at high concentrations (Ackova, 2018). Initial exposure of cell to Pb could cause changes to membrane permeability, hormonal balance, enzyme activities and mineral uptake (Varma & Jangra, 2021). Furthermore, Pb can cause extensive reaction with sulfhydryl group leading to enzyme inhibition and oxidative stress (Ackova, 2018; Varma & Jangra, 2021). A study conducted to determine the defence mechanism of plants against Pb showed that anthocyanins were synthesized to bind to Pb in order to reduce the adverse effects of the element on the plants (Kumar & Prasad, 2018). The most effective way to reduce damage by Pb to plants is to minimize Pb contamination in soils and in the atmosphere (Zulfiqar et al., 2019).

### 2.7.6 Antimony, Sb

Antimony is also not an essential element for plant growth. The uptake and availability of Sb is affected by the water regimes, pH, and organic materials. Upon uptake, Sb is translocated from roots to shoots (Zhu et al., 2020) (Figure 2.2). The translocation and phytotoxicity of Sb in plants are largely controlled by the redox states of the element (Cao et al., 2020). The element can be transferred through the food chain; however, some plants are capable of altering the availability of Sb. For example, vetiver grass was found to be able to convert Sb into precipitates (from Sb(III) to Sb(IV)) (Yu et al., 2020).

There are ways to limit the bioavailability, exposure and contamination of Sb in plants. In the study by Zhang et al., (2021), nitrate ($NO_3^-$) was found to be efficient in reducing the contamination of Sb in plants via reduction in the presence of inhibiting iron and via co-precipitation and oxidation in the presence of iron(II) (Figure 2.3).

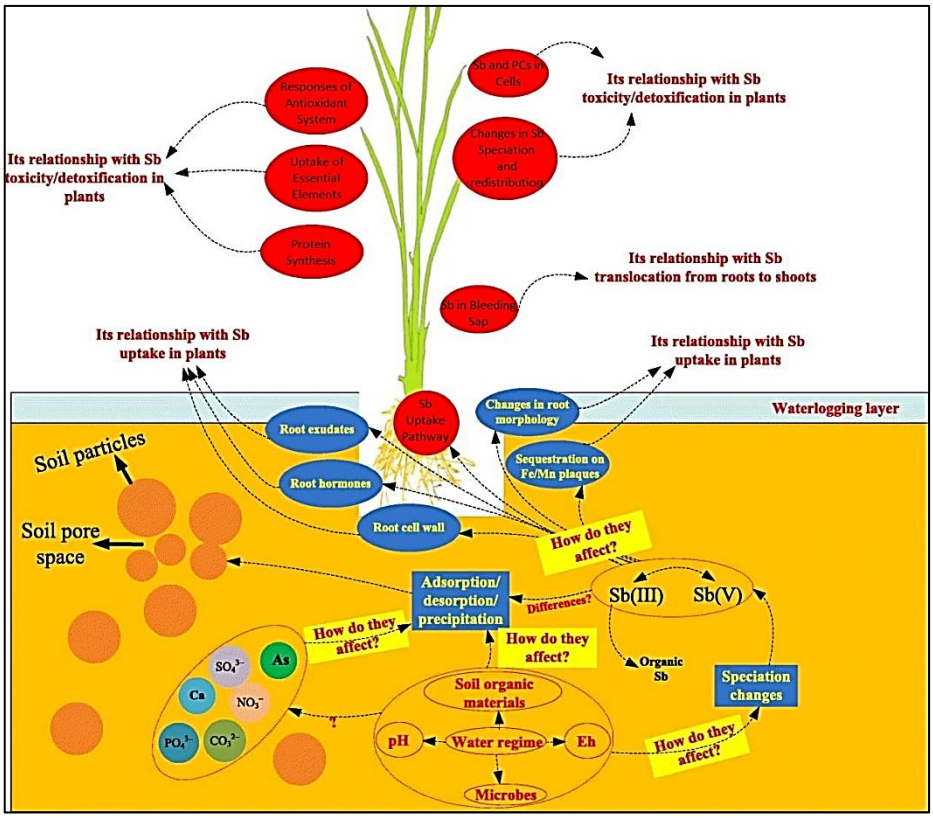


**Figure 2.2:** Uptake pathway of antimonite and their translocation from roots to shoots and redistribution of Sb in plants (Zhu et al., 2020).

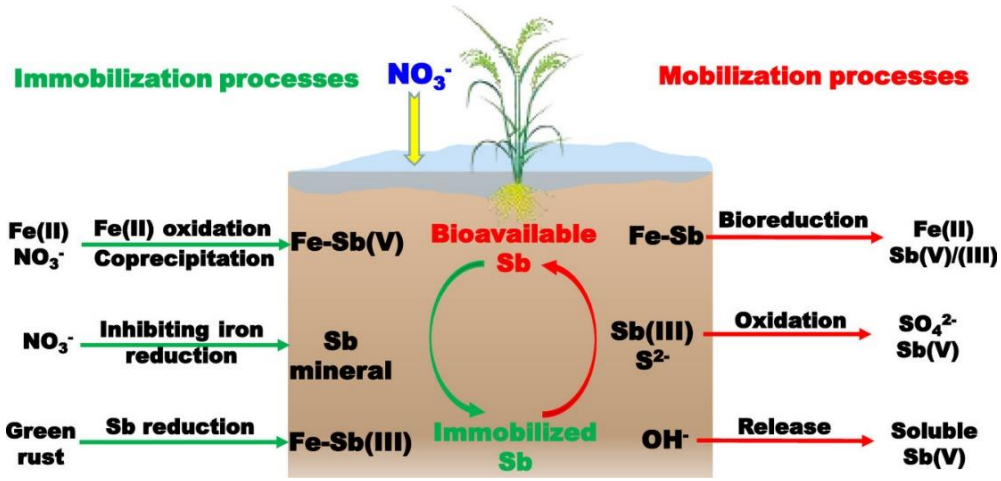


**Figure 2.3:** Conversion of bioavailable Sb to immobilized Sb (Zhang et al., 2021).

## 2.8 Heavy Metal Hyper-accumulation in Plants

Plants routinely absorb metals from soil. However, some plants have the ability to absorb, accumulate and tolerate heavy metals even at a high level, which normally would be toxic to other organisms. Plants that have that ability are termed hyper-accumulators (Sun et al., 2019) and these hyper-accumulators exist all over the plant kingdom (Lima et al., 2018).

In the Brassicaceae family, the genus Alyssum and Thlaspi contains several hyper-accumulators (Manara et al., 2020). The Thlaspi species are recognized for their capability to accumulate Zn, Ni and Cd to an high concentrations in its above-ground (leaves / shoot) biomass, along with high tolerance towards the metals' high concentration (Sytar et al., 2021). Plants that hyper-accumulate toxic metal ions and tolerate the high concentration are believed to use three main modes, which are high metal accumulation rates from soils; efficient translocation of metals from the roots to shoots of plants; and safe storage of heavy metals in appropriate plant parts of the shoot or leaves (Rascio & Navari-Izzo, 2011) to keep the high concentration of the metals from being toxic.

### 2.8.1 Route of Heavy Metal Uptake in Plants

Presence of metals in plant can give rise to significant risk to human health (Wuana & Okieimen, 2011). Absorption of heavy metal by plants can be determined as transfer coefficients which relate between the concentrations of metals in soil to the concentrations in the leaf or shoot of the plants (Adamczyk-Szabela et al., 2021). In order for plants to successfully absorb metals, the elements should first be bioavailable. There are numerous ways in which plants can absorb metals into their compartments, such as from metal articles that are settling on plant surfaces or from the metals in soils absorbed

through their roots (Yue et al., 2019). Most plants, absorption of metal are mainly done through the roots, as foliar absorption are usually stalled by external tissues of epidermal cells (such as cuticle, etc.) (Bonomelli et al., 2020; Zhou et al., 2021).

### 2.8.2 Root Uptake

There are two ways in which the uptake of trace metals by roots can occur, which is through passive or non-metabolic way, and through active or metabolic way. Passive – or non-metabolic – uptake involves the diffusion of ions from the external solution into the root endodermis following a concentration gradient. In contrast, active (or metabolic) uptake requires metabolic energy, and it takes place against a chemical concentration gradient.

Mechanisms of uptake may differ as it is depending on the given element itself. For example, Cr, Sb and Hg are not usually absorbed unless the concentration is high (Gomes et al., 2017; Zhou et al., 2021; Zhu et al., 2020), while As, Cd, and Pb can be absorbed actively (Balafrej et al., 2020; Hippler et al., 2018; Mitra et al., 2017; Mortensen et al., 2018; Roychoudhury, 2020; Song et al., 2017; Zwolak et al., 2019). However, at low metal concentrations, the absorption of trace elements is likely controlled by metabolic processes within the roots (Gupta et al., 2019). A schematic representation of metal transport processes from the roots (below ground) to the shoot (above ground) is described in Figure 2.3:

i. Metal is absorbed at root (below ground),
ii. Absorbed metal moves across cellular membranes into root cells
iii. Metal absorbed into roots is halted in vacuoles of root cell
iv. Intracellular mobile metal crosses cellular membranes into root vascular

tissue (xylem).

v. Absorbed metal is then translocated from the root to aerial tissues above ground (stems and leaves) via xylem.

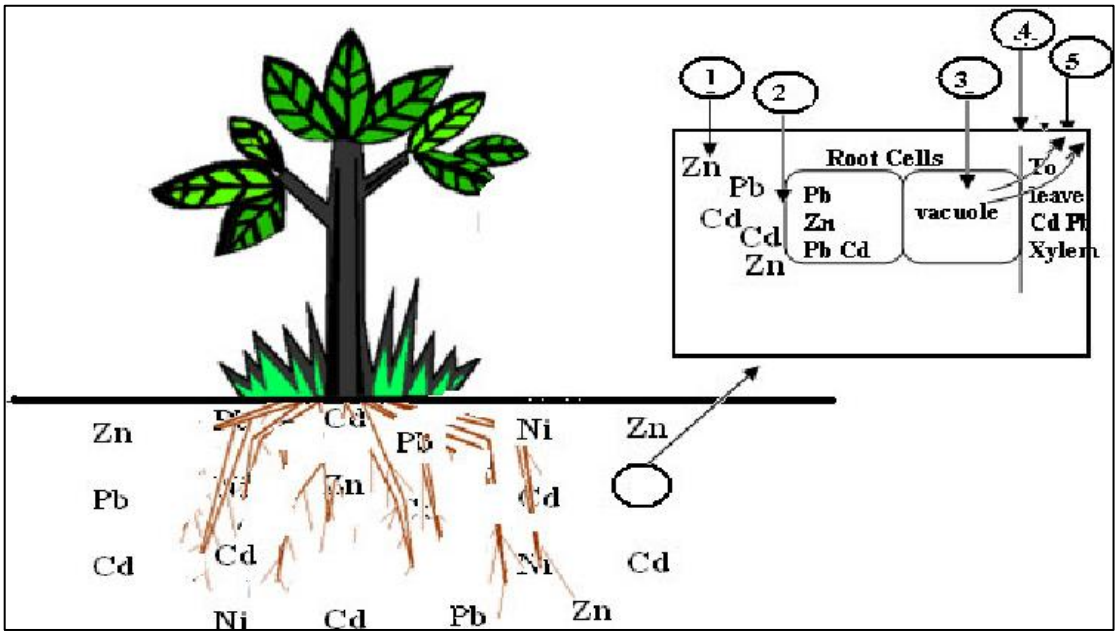


**Figure 2.4:** Metal Uptake and Accumulation in Plants (Verga et al., 2020).

### 2.8.2.1 Foliar Uptake

Metals can be absorbed by leaves from the environment through stomata. Depending on the element and plant species involved, different extents of metals can be absorbed through the leaf cuticle. For example, Cd, Cu and Zn can be absorbed from the cuticle surfaces more readily than Pb (Desai et al., 2019). Pb tends to be accumulated in leaves and stored only in the leaves rather than the whole plant during the vegetation season (Hasmik et al., 2021).

There are believed to be two phases of foliar uptake, namely the non-metabolic and metabolic mechanisms. The major route of entry is the non-metabolic penetration,

which takes place following the concentration gradients. In contrast, metabolic mechanism is for accumulation of element against a concentration gradient. Metals are absorbed by leaves and translocated to other plant tissues where the metals will be stored. Hence, the metals' accessibility in the surrounding and the readiness of the metals to be absorbed pose a significant impact on contamination of plant tissues, making them unsafe for consumption (Awino et al., 2020).

#### 2.8.2.2 Bioavailability of Heavy Metals in Soils

The word bioavailability of metals in soils can be defined as the metal's readiness to be absorbed by living receptors (Petruzzelli et al., 2020). The metals absorbed by plants are only a fraction of the total concentration which is available (Diaconu et al., 2020). Hence, the uptake of bioavailable metals is subsequently determined by the metals present in the soil solution and the total content of metals in the solid phase (Dghaim et al., 2015; Khan et al., 2015; Turkyilmaz et al., 2018). Factors that affect heavy metals' bioavailability includes pH, the presence and concentration of organic and inorganic ligands, root exudates and nutrients in soils (Lv et al., 2019).

## 2.9 Analytical Methods for Soil Analysis

Metals in soils can be determined using the following techniques: Atomic Absorption Spectroscopy (AAS), Atomic Fluorescence Spectroscopy (AFS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Hydride Generation Atomic Absorption Spectroscopy (HGAAS), Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), X−ray fluorescence (XRF), Electron Microprobe (EM), Flame Photometer (FP) and Instrumental Neutron Activation Analysis (INAA) (Ferreira et al., 2018; Mao et al., 2017;

Nevidomskaya et al., 2016; Unsal et al., 2015). These analytical systems could accurately measure the concentration of heavy metals in environmental samples to parts per million (ppm) and parts per billion (ppb) i.e. mg/L, µg $L^{-1}$ and µg $kg^{-1}$ (Diarra & Prasad, 2021; Helaluddin et al., 2016). In order to choose a technique of metal determination, few factors have to be considered, such as the availability of the said instrument of choice, speed of analysis, analyst's technical expertise and the costs of the analysis.

Before determination of any elements or metals, it requires preparation of sample, which is digestion of the said sample using acidic extraction (also known as acidic oxidation digestion) or with target reagents. Digestion is significant to convert all target elements into their inorganic forms to facilitate detection and quantification. These aforementioned analyses measure elements precisely, however, the operations and to maintenance are quite expensive. The instruments are also huge, demanding a large space and needs a fully equipped and staffed laboratory in order to maintain and operate.

### 2.9.1 Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) is an analytical technique that measures the elements' characteristic spectra emitted by the optical spectrometry. Firstly, the samples are nebulized using a nebulizer and the aerosol is transported into the plasma torch. Radiofrequency inductively coupled plasma produced the emission spectra specific to each elements that is crucial for measurement. A grating spectrometer then spread the spectra, and the photosensitive devices observed the intensities of the resulting emission lines (Khan et al., 2021).

For trace element determination using ICP-OES, background correction is required as background is measured adjacent to analyte lines on samples during analysis.

The position selected, either on or both sides of the analytical line, for the background-intensity measurement are determined by the complexity of the spectrum adjacent to the analyte line. Apart from undergoing background correction, analysts may alternatively choose to use multivariate calibration methods (Zivkovic et al., 2018).

### 2.9.2 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) is a technique where concentration of atoms were measured using the absorption of light by free gaseous atoms in a flame or furnace. AAS used the absorption of monochromatic light produced by a cloud of atoms of the analyte metal to quantitate the metal concentration. In AAS, a sample (in liquid form) is pumped into a nebulizer system. The sample then mixes with an oxidant gas which is drawn under pressure into a burner to form an aerosol. Air-acetylene or nitrous-oxide acetylene produced the flame, and the flame operates at a temperature of 2400 $^{o}$C and 2800 $^{o}$C, respectively (Zaib et al., 2015).

The aerosol undergoes evaporation of the solvent and excitation of the gaseous metallic element within the flame. The concentration of the analyte is determined by passing a light beam from a Hollow Cathode Lamp (HCL), where the cathode of the lamp is made of the elements under study, through the flame. The amount of reduction of the light intensity due to absorption (absorbance) by the analyte is detected by a photomultiplier tube attached to the AAS. As mentioned by the Beer-Lambert Law, the absorption is comparative to the concentration of the metal ions (Welz & Vale, 2018).

### 2.9.3 Flame Photometry (FP)

Flame Photometry (FP) is simply an instrumental technique of flame test, where

the coloured glass filter was replaced by monochromator while our eyes are replaced by a photocell detector/readout (Narwal et al., 2004). The burner design is also more modern and sophisticated where the sample is pumped and aspirated continuously into the flame.

Each element emits its own characteristic line spectrum, hence qualitative analysis are performed by observing the emitted wavelengths and comparing these with various standards. The detector has the capability of measuring light intensity, enabling qualitative and quantitative analysis as the intensity of the emitted light increases with concentration. In other words, FP is an atomic technique that measures the wavelength (qualitative analysis) and measures the intensity of light emitted by atoms (in order to quantitate) in a flame resulting from the drop from the excited state, which is due to the absorption of energy from the flame, to lower states. FP uses flame atomic emission and a filter to measure and quantify target elements in samples. The difference between FP and ASS is that no light source is required since the energy imparted to the atoms comes from the flame (Swapna, 2017).

### 2.9.4 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is different than the ICP-OES. Although the first part remains the same where the ionization source fully decomposes a sample into its constituent elements and transforms those elements into ions, ICP-OES allows for detection limit down to part per billion (ppb) or µg/kg, while ICP-MS provides even lower limit, down to part per trillion, or ng/kg. Furthermore, ICP-MS can detect different isotopes of the same elements which can make it useful in fingerprinting or finding geographical origin, and a versatile tool for the isotopic labelling process (Al-Hakkani, 2019).

## 2.10 Previous Research Gap

The previous conducted studies on heavy metals detection and quantification in black pepper, spices and other samples use strong undiluted acids. The methods are also time-consuming, where minimum of one (1) hour is needed for digestion, excluding cooldown time (Table 2.2). Most of the studies, when preparing samples for analysis, will use at least 10 mL of strong acids such as nitric acid and hydrochloric acid (Blagojevic et al., 2015; Kowalska, 2021; Wan Hamid et al., 2019), however, when using microwave or oven assisted digestion, only small amount of acids are required, and digestion can be completed in less time (Kilic & Soylak, 2020; Voica et al., 2012; Thabit et al., 2020).

Previous studies using analysis method that utilized ICP-MS (either ICP-MS or ICP-MS/MS) shown to be able to scan all target elements simultaneously in two to six minutes per sample (Jin et al., 2020) while AAS reads absorbance of only one element in one sample in three to four minutes (Jin et al., 2020; Velez, 2009), which means that analysis using ICP-MS/MS is time saving.

As mentioned previously, there were previously no methods for quantitating heavy metal in black pepper which combined both microwave assisted digestion and ICP-MS/MS. Table 2.2 tabulated previous studies, its sample preparation, analytical instrument, metals under studies and the sample preparation time.

**Table 2.2:** Previous Studies of Heavy Metal Detection in Samples and its Sample Preparation

| Author, year | Sample | Sample preparation | | | Detection / Analysis | | Elements studied |
|---|---|---|---|---|---|---|---|
| | | Digestion procedure / acid used | Condition | Time (min) | Instrument used | Analysis condition | |
| Wan Hamid et al. (2019) | Pepper, fertilizer, water | Aqua regia digestion / 9 mL HCl and 3 mL $HNO_3$ | Temperature 100 $^oC$; Power: NA, | 60 (minimum) | AAS (1 sample = ~12 minutes) | NA | Pb, Ni, Cu |
| Nnari et al. (2020) | Pepper, garlic, uda, salt, ginger, thyme, chilli | Aqua regia digestion / 15 mL $HNO_3$:HCl (1:3) | Temperature 100 $^oC$; Power: NA, | 120 (minimum) | AAS (1 sample = ~20 minutes) | NA | Cu, Cd, Cr, Ni, Pb |
| Kowalska (2021) | Commonly used herbs, spices, tea, coffee | Microwave mineralisation / 10mL $HNO_3$ | Temperature: 209.85$^oC$; Power 1600W | NA | ICPMS (1 sample = ~6 minutes | Plasma gas 18 L/min Cell gas: NA | Hg, Cd, Pb, As |

**Table 2.2** continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kilic & Soylak (2020) | Herbal tea | Microwave assisted digestion, 6mL $HNO_3$ and 2mL $H_2O_2$ | Temperature 225$^{o}$C, Power NA | 30 (including cool time) | ICPMS (1 sample = ~6 minutes | Plasma gas 15L/min; Cell gas: NA | As, Ba, Cd, Co, Cu, Cr, Ni, Pb, Se, V, Zn |
| Thabit et al. (2020) | Wheat, barley grains | Microwave assisted digestion; 5 mL $HNO_3$ and 2mL $H_2O_2$ | Temperature NA; Power 1400W | 45 (including cool time) | ICP-MS/MS (1 sample = ~6 minutes | Plasma gas: 15 mL/min Cell gas: 5 mL/min | Al, As, Cd, Co, Cr, Cu, Fe, Hg, K, Ni, Mg Mn, Mo, Pb, Se, Sr, V, U, Zn |
| Geleta et al. (2023) | Korarima, red pepper, ginger and turmeric | Kjeldahl digestion apparatus / 3 mL $HNO_3$ and 1mL $HClO_4$ | Temperature: 120 $^{o}$C, Power: NA | NA | ICPOES (1 sample = ~6 minutes | Plasma gas 13 L/min Cell gas: NA | Zn, Fe, Mn, Cu, Cr, Cd, Pb, Ni |

## 2.11 Summary of the Chapter

This chapter highlights history of black pepper and its cultivation in Malaysia, the usage of peppers, whether used as flavouring or as raw materials in the cosmetics and perfume production, and the medicinal properties of pepper which includes antiviral, anticancer and antimicrobial agent. This chapter also discussed quality evaluation of pepper, and the presence of hazardous chemicals in pepper. Apart from that, this chapter stated about the background of food safety legislation in Malaysia and in the world.

Heavy metals are also highlighted in this chapter, where the sources of exposure, its toxicity, and its accumulation in plants are discussed. The route of uptake, either through root or foliar and its availability in soils are also highlighted. Lastly, this chapter also highlights analytical methods, instruments, and previous studies involving heavy metals in foodstuffs.

# CHAPTER 3

# MATERIAL AND METHODS

## 3.1 General Experimental Procedures

The study is done following the flow chart as below:

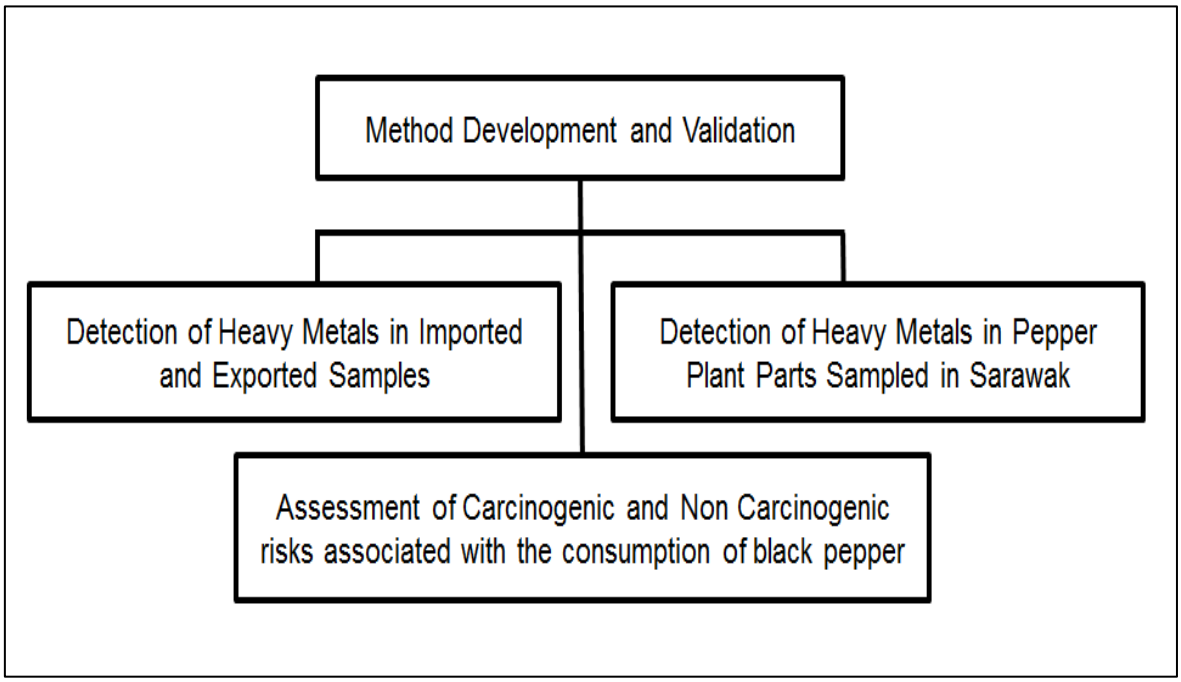


**Figure 3.1:** Experimental Flow Chart

## 3.2 Reagent and Materials

Analytical standards of Cadmium (Cd), Mercury (Hg), Lead (Pb), Arsenic (As) and Antimony (Sb) (purity > 99%) were purchased from Agilent Technologies (Agilent Technologies, Inc., US). Nitric acid ($HNO_3$, trace metal grade 60%, purity >99%), hydrochloric acid (HCl, 37%, EMSURE ® ACS, Reag. Ph Eur grade), sulfuric acid ($H_2SO_4$, 96%, Ultrapur grade) and hydrofluoric acid (HF, 47%, ACS reagent grade) were purchased from Merck (Merck, Germany). Analytical Reagent Grade of hydrogen peroxide

($H_2O_2$, 30%, certified ACS grade) and potassium permanganate ($KMnO_4$, crystalline, Certified ACS grade), were purchased from Fisher Chemical (Thermo Fisher Scientific, US). Heavy metal stock solutions of (100 mg/L) were prepared by dissolving an appropriate amount of analytical standard solution of heavy metal (1000 mg/L) in digested black pepper sample matrix and diluted using ultrapure deionized water (ELGA Water Purification System, which was consisted of ELGA Purelab Option (Model OR008BPM1), Reservoir 25L, and ELGA Purelab Ultra (Model ULXXXANM2). All plastic wares and quartz vessels were soaked in 10% $KMnO_4$, followed by 10% $HNO_3$ for 24 hours before rinsing with ultrapure water.

## 3.3 Method Development and Validation

Method was developed and validated following the flow chart as below:

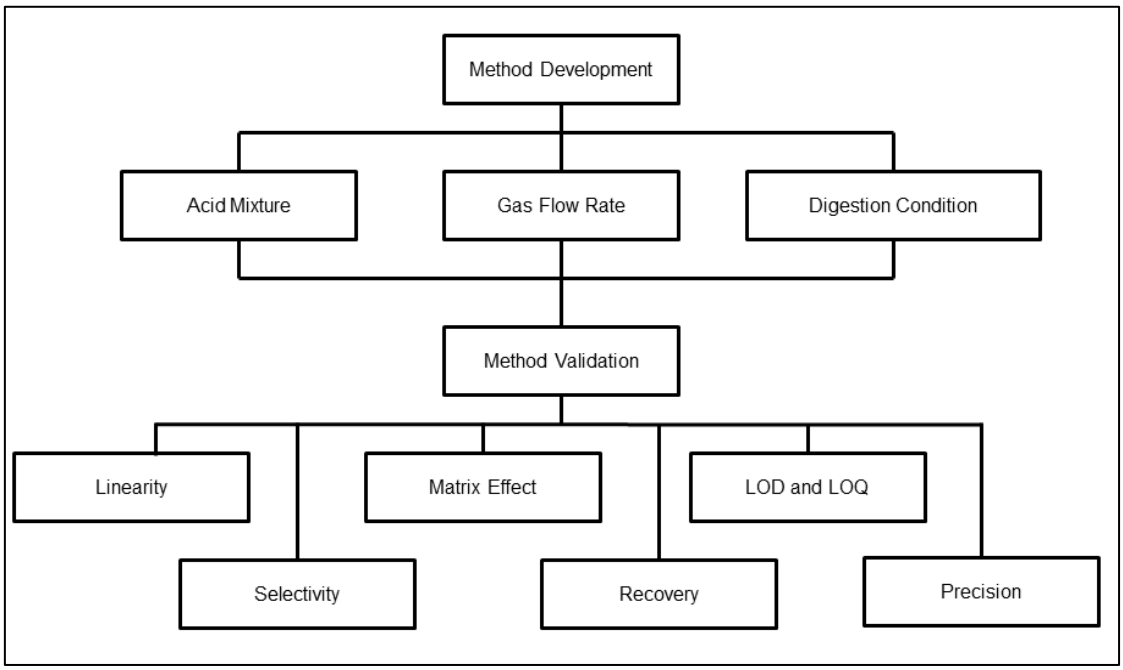


**Figure 3.2:** Method Development, Optimisation and Validation Flow Chart.

### 3.3.1 Samples for Method Development and Optimisation

Pepper berries (2 kg) obtained from Malaysian Pepper Board (MPB) in Kuching, Sarawak were used for the purpose of method development and validation. The standard solutions of 100 mg/L were diluted into a series of standards at 0.005, 0.010, 0.025, 0.25, 0.5 and 1.0 mg/L for calibration curve purposes.

### 3.3.2 Digestion Procedure

Five grams of dried black pepper berries were ground and homogenized using Fritch Pulverisette 2 Mortar Grinder (Rhineland-Palatinate, Germany). A 0.25 g of the homogenized sample was weighed into a 10 mL quartz vessel. The sample was then spiked using 5 µL of 100 mg/L standard solution to yield a concentration of 0.01 mg/kg (as the digested products were made up into 50 mL solution). The finely ground pepper berries were then digested using six different acid systems as follows (Huang et al., 2004; Mohammed et al., 2017; Uddin et al., 2016; Voica et al., 2012);

- 5 mL $HNO_3$ + 1 mL HCl
- 5mL $HNO_3$ + 1 mL $H_2O_2$
- 5mL $HNO_3$ + 1 mL $H_2SO_4$
- 5mL $HNO_3$ + 1 mL HF
- 5 mL $HNO_3$ only
- mL $HNO_3$ only

The digestion was done by using the microwave-assisted digestion method (Milestone Ethos UP Microwave Digestion System, Model ETHOS UP, power input 3500 VA, Frequency Input 50 Hz, purchased from Milestone, Sorisole (BG), Italy). The system

was set at 200 $^{o}$C with a power 1800 W for 45 min, which was based on the manufacturer's recommendation. After the digestion was completed, the digested solution was diluted with 2% $HNO_3$ (60% $HNO_3$ diluted to 2% using ultrapure water) to a final volume of 50 mL and then subjected to analysis using a triple quadrupole ICP-MS. The operating temperature and power of microwave digester system were changed according to the following settings for optimisation by calculating and comparing the recovery performance from the digestion of pepper berries (Alsehli, 2021; Antakli et al., 2018; Damak et al., 2019; Kandil et al., 2020; Volpi, 2022):

i. 150 $^{o}$C, Power; 1000 W

ii. 220 $^{o}$C, Power; 1000 W

iii. 250 $^{o}$C, Power; 1800 W

### 3.3.3 Instrumentation

The optimization of heavy metal analysis was performed using a triple quadrupole ICP-MS (Agilent ICP-MS Model 8800 ICP Triple Quad system, 60 Hz, purchased from Agilent Technologies, Germany). The ICP was set to routine tuning mode with argon (Ar) as the plasma gas and helium (He) as the cell gas.

The optimization of ICP-MS/MS was performed by operating the full scan mode, the MS/MS mode, with different He flow rates of 0 mL/min (No Cell Gas mode), 5 mL/min (Baj et al., 2023; Bradley et al., 2023; Sinkovic et al., 2021; Thabit et al., 2020) and 10 mL/min (Wang et al., 2006). Ar flow rates were constant at 15 L/min (Voica et al., 2012). A solution of pepper matrix at 0.01 mg/kg was analysed and the recovery performance based on the different flow rates of He was determined.

### 3.3.4 Method Validation

The method optimization in this study was evaluated for the followings: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, recovery, matrix effect, and measurement uncertainty according to European Commission GC SANCO (2012). The selectivity was investigated to ensure the co-extract of blank pepper matrix did not interfere with the targeted heavy metal quantification process. The LOD was determined from the signals of 10 blank samples according to Equation 3.1 (Voica et al., 2012) to determine the lowest concentration of an analyte which can be detected with acceptable uncertainty. The LOQ was derived using 10 times the standard deviation of the blank samples which represents the lowest concentration that the analyte could be reliably determined:

$$LOD / LOQ = X \times SD_b + Y_b \qquad \text{Equation 3.1}$$

where,

$SD_b$ = standard deviation of 10 blank samples

$Y_b$ = blank sample signal

X = 3 for LOD; 10 for LOQ

The recovery percentage of metal was calculated to specify the competence of the method developed for quantification of targeted heavy metals in pepper. The method precision, also known as reproducibility, is expressed as the percentage relative standard deviation, as shown in Equation 3.2:

$$Percentage\ Recovery\ (\%) = \frac{C_{measured}}{C_{spiked}} \times 100$$ Equation 3.2

where,

$C_{measured}$ = metal concentration determined experimentally in the spiked sample

$C_{spiked}$ = spiked concentration of metal in the sample

The matrix effect was assessed by associating the slopes of calibration curve of the in matrix with that of the solvent for each targeted element. For ICP-MS, it is recommended to reduce the sample-specific matrix effect to avoid the nebulizer blockage; hence, the total dissolved solids content in the sample is specified at less than 20%. The matrix effect was calculated using equation 3.3 (Wilschefski & Baxter, 2019):

$$Matrix\ effect\ (\%) = 100\ \times \left[1 - \left(\frac{Solvent\ Slope}{Matrix\ Slope}\right)\right]$$ Equation 3.3

The validation was further assessed by calculating measurement uncertainty according to EURACHEM/CITAC and Quantification Uncertainty in Analytical Measurement (Rasul et al., 2018). Ground black pepper, spiked with 0.1 mg/kg of heavy metals, was subjected to different microwave digestion settings, different acid mixtures (Refer to subsection 3.1.3) and analysed using ICP-MS/MS with different helium flow rates in triplicates. The uncertainties of repeatability ($U_{precision}$), recovery ($U_{bias}$) and standard purity ($U_{purity}$) were analysed to derive the combined and expanded uncertainties for respective elements.

## 3.4 Heavy Metal Analysis in Imported Pepper Berries

### 3.4.1 Sampling and Samples

To test the developed and optimized method, imported black pepper berries from India, Vietnam, Sri Lanka Cambodia, and Indonesia, along with domestic samples from different localities in Sarawak were digested using the optimized method and analysed in triplicates. The metal concentrations calculated were compared against the Malaysia Food Act 1983, where the specified MLs for the five heavy metals are As (5 mg/kg), Pb (2 mg/kg), Hg (0.05 mg/kg), Cd (1 mg/kg) and Sb (1 mg/kg). In this study, a total of 115 domestic and international samples (65 domestic samples, and 10 samples from each country mentioned above) were collected by MPB in 2020. MPB is the Import and Export Authority for pepper berries.

## 3.5 Heavy Metals Accumulation in Pepper Plant Parts Cultivated in Sarawak

### 3.5.1 Sampling and Samples

Sampling was done at a total of 160 pepper farms in various parts of Sarawak: the Northern region (Bintulu (20 farms) and Miri (20 farms)), Central region (Sibu (20 farms), Sri Aman (20 farms), Betong (20 farms), and Sarikei (20 farms)) and Southern region (Kuching (20 farms) and Serian (20 farms)). Figure 3.3 shows the location of the study sites. The pepper vines sampled were between 3 - 5 years old and only pepper of Kuching variety was sampled. One vine was randomly selected and labelled in each farm.

The sampling was done immediately before the pepper harvest season and after the end of the fertilization plan. There were 480 samples collected from the three regions consisting of *P. nigrum* leaves, stems and berries between January 2022 and March 2022.

After sampling, the leaves, stems and berries were carefully labelled and transported to the laboratory for subsequent analysis. Prior to heavy metal analysis, the leaves, stems and berries samples were thoroughly washed with tap water and rinsed with distilled water and deionized water. The leaves, stems and berries samples were oven dried at 40 ± 5 $^{o}$C (Memmert Oven, Scwabisch Hall, Germany) for 48 h and stored in plastic bag for heavy metals analysis.

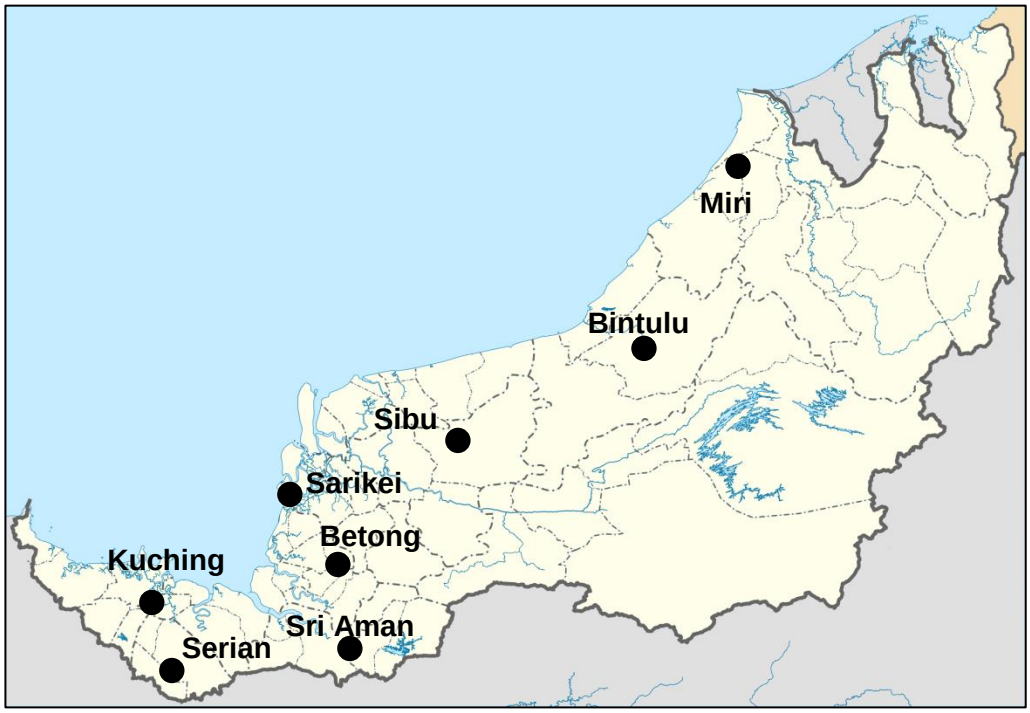


**Figure 3.3:** Location of pepper cultivation areas sampled for heavy metal analysis in Sarawak, Malaysia (Google Maps).

Soil samples were taken using a stainless steel auger of 5 cm in diameter at the depth of 0 - 20 cm where the plant roots have more intense activity of nutrient absorption (Sandeep et al., 2019) between December 2021 and January 2022. Five soil samples were collected at a 10 m radius and homogenized to obtain a 1 kg soil sample. The soil samples were placed in plastic bags and brought back to the laboratory for heavy metals analysis.

The soil samples were then air dried and sieved (size) prior to heavy metals analysis using Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS/MS) method. The pH, organic matter, cation exchange capacity (CEC) and soil particle size were also analyzed.

For pH analysis, 20 g soil samples was weighed into a 100 mL beaker, added with 20 mL deionized water and stirred for 30 min. The mixture was let to stand for 1 h before placing a rinsed and calibrated pH probe in the mixture. In the analysis of organic matter content, loss on ignition (LOI) method was adopted from Sullivan et al. (2019) by measuring sample weight loss on ignition at 360 $^{\circ}$C. Soil Cation Exchange Capacity (CEC) was estimated using the portable X-ray fluorescence (Wan et al., 2020). The soil particle size was determined by sieving the soils through 2 mm, 0.05 mm and 0.01 mm sieves.

### 3.5.2 Determination of Heavy Metals

The oven-dried *P. nigrum* samples, which included the leaves, stems and berries, were weighed (0.25 g) and digested with 3 mL of $HNO_3$ using Milestone Ethos UP Microwave Digester. The digestion was conducted using the optimized and validated method in Chapter 3. The soil samples were prepared by digesting 0.25 g of soil using 5 mL $HNO_3$ and 3 mL HCl for 45 min at 180$^{o}$C (Gebeyehu & Bayissa, 2020). Concentration of As, Cd, Cr, Hg, Pb and Sb in the digested samples were determined using the ICP-MS/MS optimized method (Refer to Chapter 3) by using seven working standard solutions of each metal along with calibration blanks. In order to achieve analytical quality, three replications were carried for each sample and the means were used for statistical analysis.

### 3.5.3 Enrichment Factor (EF) and Translocation Factor (TF)

The enrichment (EF) and translocation factors (TF) were calculated using Equations 3.4 and 3.5 respectively (Zakaria et al., 2021; Zinicovscaia et al., 2020). The EF reveals the absorption of heavy metals from the soil to the pepper berries as the pepper berries are the commonly consumed portion of the pepper plant. The TF, however, refers to the transport of target heavy metals from the soil to different parts of the pepper plant (soil to stem, stem to leaves, and leaves to berries).

$$\text{Enrichment factor (EF)} = \frac{C_p}{C_s} \qquad \text{Equation 3.4}$$

where,

$C_p$ denotes heavy metal concentration in the pepper berries, and

$C_s$ denotes the heavy metal concentration in the soil.

$$\text{Translocation Factor (TF)} = \frac{C_{pp}}{C_s} \qquad \text{Equation 3.5}$$

where,

$C_{pp}$ represents the heavy metal concentration in the plant parts,

$C_s$ represents heavy metal concentration in the soil, root or stem.

## 3.6 Safety Level Assessment (SLA) of Heavy Metals in Sarawak Pepper

### 3.6.1 Average Daily Dose (ADD)

Average daily dose (ADD) is a way to collect information of assumed average dose of certain chemicals consumed per day by a population. It can also be used to measure the oral exposure to heavy metals via pepper consumption for a specific period.

ADD is expressed in mg/kg.day, which denotes the daily dose per unit body weight (Zinicovscaia et al., 2020; Zulkafflee et al., 2022). ADD was calculated using the following Equation 3.6:

$$\text{ADD (kg/person/day)} = \frac{(C_{pepper} \times IR \times ED \times EXF)}{Bw \times AT} \qquad \text{Equation 3.6}$$

where,

$C_{pepper}$ is the average concentration of heavy metals in pepper,

$IR$ is the average pepper ingestion rate,

$EXF$ is exposure frequency,

ED is the average exposure duration, i.e, average lifetime of Malaysians,

Bw is the average body weight of Malaysians, and

AT is the average time of non-carcinogenic (ED x 365 days)

Average concentrations of different heavy metals in pepper were calculated as in Section 3.5.2. The ingestion rate (IR), which is the amount of pepper containing the contaminant that an individual ingests during a specific amount of time (g/day), was estimated at 3.5 g/day (Rahman et al., 2018). The average exposure frequency (EXF), average exposure duration (ED) and average body weight (Bw) values for Malaysians were retrieved from the reports of Department of Statistics Malaysia, hence, EXF is determined at 365 days/year, ED at 74 years and BW at 62.65 kg (Norimah et al., 2008).

### 3.6.2 Hazard Quotient (HQ)

Hazard Quotient, HQ, was determined using Equation 4.4 based on the USEPA (United States Environmental Protection Agency). HQ and HI acts as an indicator of the non-carcinogenic risk for consumption of black pepper. The index is defined as the ratio of

ADD to the reference dose (RfD) while HI is defined as total Hazard Quotient where HQ for each element was summed (Zinicovscaia et al., 2020; Zulkafflee et al., 2022)

$$\mathrm{HQ} = \frac{\mathrm{ADD}}{\mathrm{RfD}} \qquad \text{Equation 3.7}$$

where RfD is the reference maximum allowed human dose of heavy metals via daily exposure (EPA, 2011). The RfD values of targeted heavy metals (Table 3.1), are specified by Integrated Risk Information System, U.S. EPA (IRIS) and World Health Organization (WHO).

$$\mathrm{HI} = \sum HQ \qquad \text{Equation 3.8}$$

**Table 3.1**: The RfD of Targeted Heavy Metals

| Heavy metal | RfD (mg/kg/day) | Source |
|---|---|---|
| **Cd** | $1.00 \times 10^{-3}$ | IRIS |
| **Cr** | 1.5 | IRIS |
| **Hg** | $1.6 \times 10^{-4}$ | WHO |
| **As** | $3.00 \times 10^{-4}$ | IRIS |
| **Pb** | $3.6 \times 10^{-3}$ | WHO |
| **Sb** | $4.00 \times 10^{-4}$ | IRIS |

Note: IRIS – Integrated Risk Information System, U.S.EPA; WHO – World Health Organization

### 3.6.3 Lifetime Cancer Risk (LCR)

Heavy metals are known to be potential carcinogens. Lifetime Cancer Risk (LCR) is evaluated in this study since there is an incremental probability of a person getting cancer during the course of his or her lifetime through exposure to the targeted heavy metals (Zulkafflee et al., 2022). The LCR resulted from the exposure to heavy metals was calculated using Equation 3.9, by multiplying ADD with the cancer slope factor (CSF). The CSF values for each element in this study have been provided by International Agency for Research on Cancer, Integrated Risk Information System and California Environmental Protection Agency (CALEPA), shown in Table 3.2.

**Table 3.2:** Cancer Slope Factor of Targeted Elements

| Element | CSF (mg/kg.day) | References |
|---|---|---|
| **Cd** | 15 | CALEPA |
| **Cr** | 0.5 | CALEPA |
| **Hg** | N/A | N/A |
| **As** | 1.5 | IRIS |
| **Pb** | 0.0085 | IRIS |
| **Sb** | 0.5 | IRIS |

Note: IRIS – International Agency for Research on Cancer, CALEPA – California Environmental Protection Agency

$$\mathrm{LCR} = \mathrm{ADD} \times \mathrm{CSF} \qquad \text{Equation 3.9}$$

The combination effect of multiple carcinogenic elements is represented with the Total Cancer Risk (CRt) where the LCR for each element was summed (Equation 3.10). The International Agency for Research on Cancer (IARC) has classified Cr, Cd and As as carcinogenic elements while Pb, Hg and Sb are categorized as possible carcinogens (IARC,

2012b). For LCR and CRt, the acceptable risks are in the range of $1.0 \times 10^{-6}$ to $1.0 \times 10^{-4}$ (Zulkafflee et al., 2022).

$$\mathrm{CRt} = \sum \mathrm{LCR} \qquad \text{Equation 3.10}$$

### 3.6.4 Statistical Analysis for Heavy Metals Concentration in Sarawak Pepper

Analysis of Variance (ANOVA) was performed to determine significant differences between treatments at 95% confidence level using IBM SPSS (Statistical Package for Social Sciences Version 22.0). Principal Component Analysis (PCA) was used to explore the underlying clustering pattern of heavy metals in black pepper sampled in Sarawak. Heavy metals and sample matrix were standardized and normalized prior to PCA. PCA and normalization of data was done using Agilent Mass Profiler Professional Software Version 12.0 Build 163737.

## 3.7 Summary of the Chapter

This chapter summarised the materials and methods used in the two parts of this study. The first part of the study was method development and validation, where it comprised with the optimisation of sample preparation method and instrumentation method. Validation of the developed method comprised on six parts: linearity, selectivity, LOD and LOQ, precision, recovery and matrix effect. The second part of the study was the implementation of the developed method on import and export pepper samples, and on samples of pepper plant parts (berries, stems and branch). The results then were used to calculate and assess contamination and safety level of heavy metals in pepper berries.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Summary of sample collection

The sample collections for this study were as described in section 3.4.1 and section 3.5.1. The total samples collected were summarised in the following Table 4.1 and Table 4.2.

**Table 4.1:** Summary of Sample Collected for Heavy Metal Analysis in Imported and Exported Pepper Berries

| Country of Origin | Number of Sample | Total Number of Sample |
|---|---|---|
| India | 10 | 115 |
| Indonesia | 10 | |
| Vietnam | 10 | |
| Sri Lanka | 10 | |
| Cambodia | 10 | |
| Malaysia (exported sample) | 65 | |

**Table 4.2:** Summary of Sample Collected for Heavy Metals Accumulation in Pepper Plant Parts Cultivated in Sarawak

| Sampling Area | Number of Sample | Total Number of Sample |
|---|---|---|
| Kuching | 80* | |
| Serian | 80* | |
| Betong | 80* | |
| Sri Aman | 80* | |
| | | 640 |
| Sarikei | 80* | |
| Sibu | 80* | |
| Bintulu | 80* | |
| Miri | 80* | |

NOTE: * 80 samples comprises with 20 samples of pepper leaves, 20 samples of pepper stem, 20 samples of pepper berries and 20 samples of pepper cultivation soil.

## 4.2 Method optimization

### a. Flow Rate

Figure 4.1 shows the recovery percentages of heavy metals using different flow rates of He in ICP-MS. The analysis in the absence of He recorded the lowest recoveries, ranging between 73.12% and 79.99%. When the flow rate was increased to 5 mL/min, the recovery performance (95.10 - 109.88%) was significantly improved. In accordance to the International Organization for Standardization SANCO/12495/2011, a suitable protocol for

quantitative determination of the targeted analyte should result in recovery ranging between 80 - 120% (Holland et al., 2007). He gas flow was further increased to 10 mL/min, yielding the highest recovery percentages between 98.99% and 112.91%. The improved metal recovery with increasing He flow rate is a result of boosted aerosol vaporization of He which contributes to better energy transfer from the plasma to the central channel of ICP and a higher diffusion rate of the vaporized materials. Moreover, He gas serves to minimize elemental fractionation caused by sequential evaporation of volatile elements, reducing diffusion losses in the ICP ( Wang et al., 2006). This observation agrees with the previous findings by Sinkovic et al. (2021) and Thabit et al. (2020), where He flow rates between 5 and 10 mL/min were reported as the best flow rates for efficient recovery of heavy metals from wheat and barley grains and also from common and tartary buckwheat using ICP-MS.

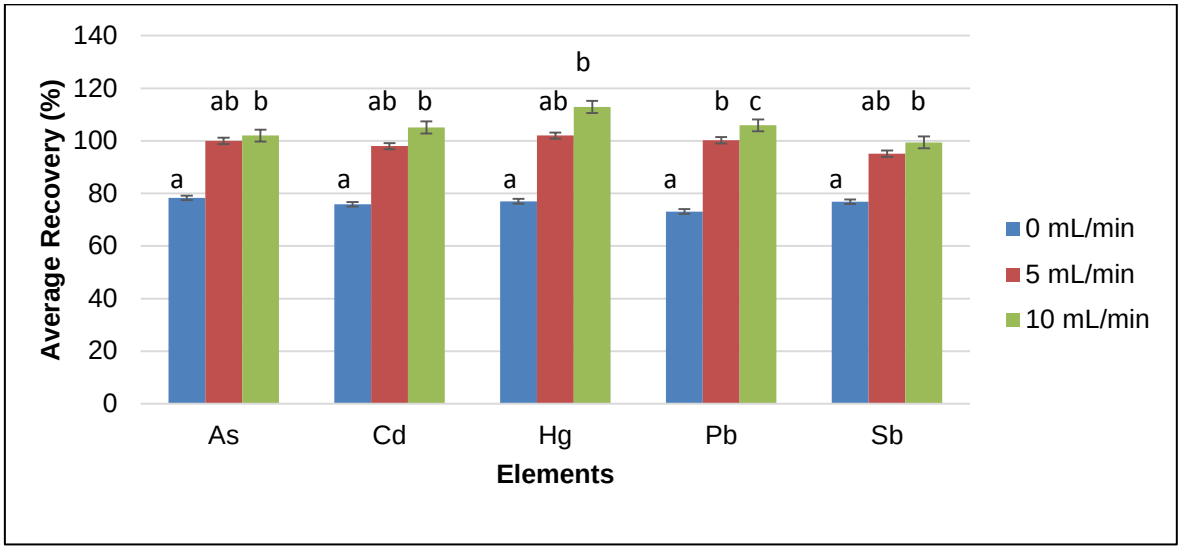


**Figure 4.1:** Recovery percentages of elements using different He gas flow rates. Same letters indicate no significant differences in the recovery at different flow rates for the targeted element ($p > 0.05$).

**b. Acid digestion mixtures**

The recovery of five targeted heavy metals from the spiked pepper samples using six acid mixtures are summarized in Table 4.3. The elements were satisfactorily extracted using 5 mL $HNO_3$ and 3 mL $HNO_3$ attaining recovery percentages in the range of 95 - 105% and 100 – 103%, respectively.

**Table 4.3**: Average Recovery Percentages of Elements Using Different Acidic Mixtures.

| Acid mixtures | Percent recovery (%) (n=10) | | | | | Overall recovery (%) |
|---|---|---|---|---|---|---|
| | As | Cd | Hg | Pb | Sb | |
| 5 mL $HNO_3$ + 1 mL HCl | 122.93± 0.40[c] | 125.92± 0.18[c] | 126.08± 0.02[c] | 127.09± 0.13[c] | 130.48± 0.32[c] | 126.50 ± 2.71[c] |
| 5 mL $HNO_3$ + 1 mL $H_2O_2$ | 79.98 ± 0.36[a] | 77.09 ± 0.11[a] | 76.09 ± 0.11[a] | 77.90 ± 0.23[a] | 76.95 ± 0.19[a] | 77.60 ± 1.47[a] |
| 5 mL $HNO_3$ + 1 mL $H_2SO_4$ | 80.98 ± 0.37[a] | 72.09 ± 0.06[a] | 82.09 ± 0.48[a] | 77.99 ± 2.56[a] | 76.78 ± 0.84[a] | 77.99 ± 3.94[a] |
| 5 mL $HNO_3$ + 1 mL HF | 76.86 ± 0.72[a] | 75.09 ± 0.13[a] | 77.41 ± 0.28[a] | 79.42 ± 0.23[a] | 72.59 ± 0.30[a] | 76.28 ± 2.57[a] |
| 5 mL $HNO_3$ | 101.92 ± 0.09[b] | 99.01 ± 0.01[b] | 100.94 ± 0.20[b] | 103.01 ± 0.20[b] | 104.53 ± 0.10[b] | 101.88 ± 2.09[b] |
| 3 mL $HNO_3$ | 103.39 ± 0.29[b] | 103.99 ± 0.04[b] | 103.99 ± $0.10_b$ | 103.99 ± 0.04[b] | 100.03 ± 0.34[b] | 103.07 ± 1.72[b] |

Note: Same letters indicate no significant differences in the recovery using different acidic mixture for the targeted element ($p > 0.05$).

Nitric acid was observed to be the most efficient digestion agent among the six digestion mixtures; a total of 3 mL $HNO_3$ was observably sufficient to attain complete digestion with excellent recovery (100 – 103%) corroborating the findings by Liu et al. (2020), where heavy metals detected in this study, Zn, Ni and Cu were detected using AAS with recoveries of 98.96-107.82% after digestion using $HNO_3$ on a hot plate. Other acid mixtures yielded recovery less than 80% suggesting that digestion losses might have taken place when metals were oxidized and turned non-electroactive (Somer & Nakişci Ünlü, 2006). Digestion losses might also be caused by the evaporation of volatile compounds in the digested samples (Zioła-Frankowska & Frankowski, 2017), depending by the boiling point of the acid used (Mohammed et al., 2017). As mentioned previously, it is important to use a cost-effective and efficient acid digestion system for routine monitoring of heavy metals in black pepper, especially when a large number of samples are involved.

### c. Operating Conditions of Microwave Digester

The effects of temperature and power of the microwave-assisted acid digestion system on the recovery of heavy metals from black pepper berries are shown in Figure 4.2. The digestion time, amount of $HNO_3$ (3 mL) and sample weight were held constant for this part of optimization. No statistical differences in recovery were identified between digestion conditions of 220 °C, 1000 W (96.88%) and 250 °C, 1800 W (99.57%) although the latter demonstrates a slightly better performance. In contrast, the digestion at 150 ºC at 1000 W demonstrated a poor recovery of 70.56%, below the acceptable recovery value of 80 - 120%. This suggests that the temperature and power used in the digestion at 150$^{o}$C and 1000 W were insufficient to complete the digestion where murky and unclear solutions with the presence of sediment at the bottom of the vessel were observed. The digestion at

250 °C (1800 W) and 220 °C (1000 W) showed comparable recovery efficiencies; since there were no significant differences in the recovery performance, the latter was suggested for the digestion of pepper berries in this work. This result agrees with the findings of Savio et al. (2019) where digestion temperature of 220 $^{o}$C helps in high matrix sample digestion with recovery 80-120%, and also agrees with findings of Alsehli et al. (2021) which shows that the power of 1,000 W is sufficient to maintain desired temperature throughout digestion which leads to complete digestion.

Overall, the optimized microwave-assisted digestion using 3 mL of $HNO_3$ at 220 °C and 1000 W, and He flow rate of 5 mL/min in ICP MS/MS is recommended for the determination of trace metals in the black pepper berries in this study.

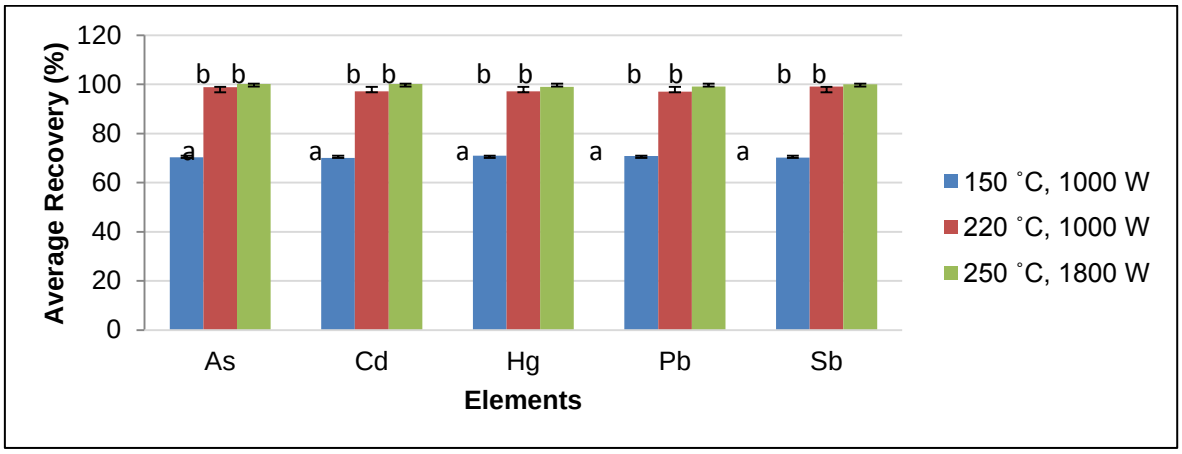


**Figure 4.2:** Average recovery percentages of elements using different temperatures and power setting for the microwave digester. The different letters within a treatment for each element indicate significant differences between the treatment conditions ($P < 0.05$).

### d. Quality Assurance and Method Validation

For the selectivity, the quick scans of each targeted element in both blank pepper matrix and spiked pepper matrix were compared. Figure 4.3 shows the total spectral of

blank pepper matrix and spiked pepper matrix with 1.0 mg/kg of trace elements. With the blank pepper matrix set as the background, all targeted elements were well separated according to their atomic numbers.

The percentage of matrix effect was calculated between −12.01 and 18.48%, well below 20% suggesting that the method is specific and selective with negligible matrix effect (Kim et al., 2023). Arsenic showed the lowest matrix effect, followed by Sb, Hg and Pb (Table 4.4). The matrix effect for Cd fluctuates within replicates suggesting that Cd is more susceptible to matrix effect than other elements. This might be caused by resin-derived organic and inorganics along with other inorganic metals present in the matrix that affects the measurement of Cd by ICP-MS/MS, as shown by the previous studies by Shiel et al. (2009) where Magnesium, Mg (an example of resin-derived inorganic) in sample matrix increases intensities of Cd ion signal which further caused fluctuations in Cd concentration.

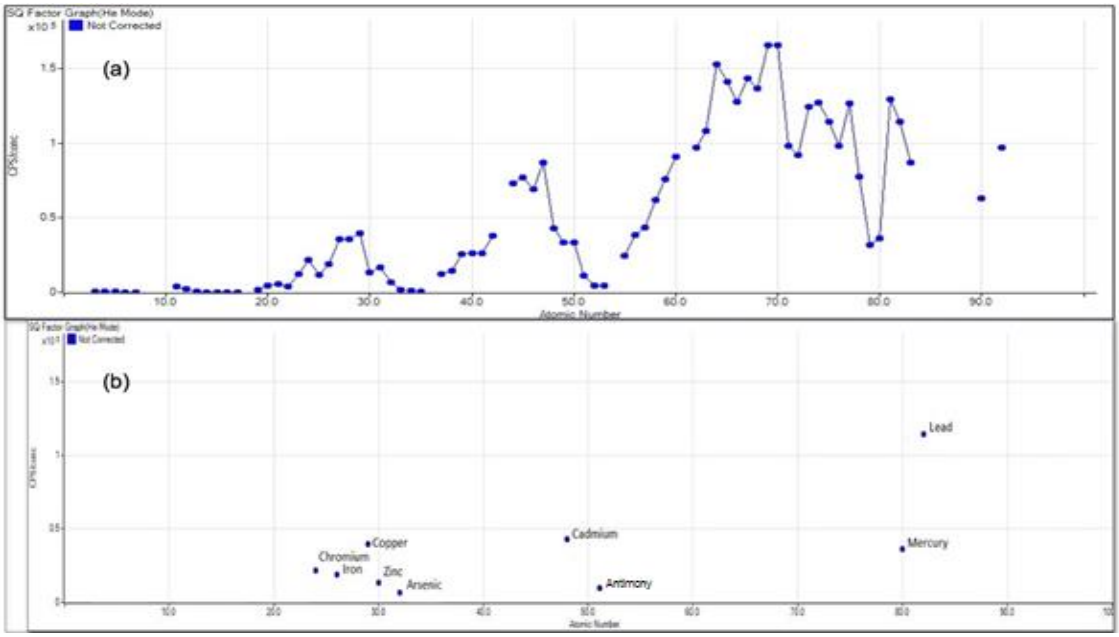


**Figure 4.3:** Spectra of elements according to their atomic numbers in (a) the spiked samples and (b) spiked pepper samples with background of blank pepper matrix

**Table 4.4** Matrix effect of Elements

| Elements | Replicates | Matrix effect (%) |
|---|---|---|
| As | 1 | -7.46895958 |
| | 2 | -5.76020709 |
| | 3 | -2.65922376 |
| Cd | 1 | 18.48277205 |
| | 2 | 6.790861019 |
| | 3 | -12.0175966 |
| Cr | 1 | 0.323518446 |
| | 2 | -0.03676933 |
| | 3 | 0.235665978 |
| Hg | 1 | 2.719161289 |
| | 2 | 3.271066638 |
| | 3 | 0.147371998 |
| Pb | 1 | 4.24378354 |
| | 2 | 4.664270273 |
| | 3 | 5.569072224 |
| Sb | 1 | -4.81934549 |
| | 2 | 1.163064092 |
| | 3 | -5.16118904 |

The calibration curves were established with excellent linearity for all targeted heavy metals ($R^2 \geq 0.9990$), supporting that the analytical signal is a function of the heavy metals' concentration. The LODs for As, Cd, Pb, Hg and Sb were determined between 0.003 and 0.010 mg/kg with corresponding LOQ of 0.032 - 0.096 mg/kg.

Table 4.5 summarizes the method performance (%recovery, %RSD, LOD and LOQ) for respective targeted heavy metals. The LOD is suitable for analysis of pepper berries for compliance with the Food Act 1983 [As (5 mg/kg), Pb (2 mg/kg), Hg (0.05 mg/kg), Cd (1 mg/kg), Sb (1 mg/kg)] and the maximum limit of ASTA (0.01 mg/L) (SANCO/12495/2011). The recovery percentages, recorded between 98.14% and 114.83%, are well within the acceptable range of 80 - 120% (Lugos et al., 2019) indicating that the method established is efficient enough to screen and analyze targeted metals in the digested black pepper berries.

The method precision was evaluated based on the percentage relative standard deviation (%RSD). Samples fortified with the trace metals of 0.01 mg/kg ($n$=3) were analyzed on 3 different days and the %RSD between 0.001 - 0.044 was obtained, indicative of satisfactory reproducibility.

The expanded uncertainty recorded in this study ranges between 7.21% and 18.29% with Sb showing the highest uncertainty and Cd the lowest. In accordance to SANCO/12495/2011 guideline, the uncertainty acceptable criterion is below 50.0% (Pihlstrom et al., 2011). The high uncertainty percentage of Sb might be attributed to high variation of Sb losses due to volatility of Sb compound during the digestion under high temperature (Aracena et al., 2023).

**Table 4.5:** Average Recovery (%), Precision (%RSD), LOD and LOQ of Targeted Elements.

| Element | Average recovery (%) | SD | % RSD | LOD (mg/kg) | LOQ (mg/kg) |
|---|---|---|---|---|---|
| As | 114.83 | 5.02 | 0.044 | 0.006 | 0.058 |
| Cd | 109.11 | 0.11 | 0.001 | 0.003 | 0.032 |
| Hg | 98.14 | 0.20 | 0.002 | 0.003 | 0.032 |
| Pb | 114.07 | 0.07 | 0.001 | 0.010 | 0.096 |
| Sb | 100.04 | 0.10 | 0.001 | 0.008 | 0.082 |

## 4.3 Heavy metals in imported and exported black pepper berries

The optimized and validated method was applied for monitoring of trace metals in 115 imported and domestic pepper berries samples. Table 4.6 summarizes the levels of trace metals present in the samples analysed. The trace elements detected in the samples ranges between 0.01 mg/kg and 6.995 mg/kg. The concentrations of As, Cd and Pb were found above the permissible limits of the Malaysian Food Act (1983) in 17 samples imported from three countries, i.e Cambodia, Sri Lanka and Indonesia. The most prevalent element was Pb which was found above the ML in 10 samples and recorded the highest concentration in sample from Indonesia. This could be due to postharvest factors that have been recorded in previous study where availability of Pb in grinding machines could increase possibility of the metal in pepper (Shango et al., 2021). 4 out of 10 samples from Cambodia exceeded the As permissible limit. Three samples from Sri Lanka were

contaminated with Cd with an average of 1.37 mg/kg. According to the quality monitoring report from the International Pepper Community (IPC), the most commonly detected metals in pepper berries are As and Pb (IPC, 2021).

**Table 4.6:** Comparison of trace metals concentration detected in domestic and imported pepper berries from different countries.

| Country | n | Average concentration (mg/kg) | | | | |
|---|---|---|---|---|---|---|
| | | As | Cd | Hg | Pb | Sb |
| Cambodia | 10 | 7.00 ± 1.38 (4) | ND | ND | 2.35 ± 1.24 (6) | ND |
| India | 10 | 0.01 ± 0.03 (1) | ND | ND | 0.01 ± 0.03 (1) | ND |
| Indonesia | 10 | 0.01 ± 0.01 (1) | ND | ND | 3.61 ± 0.91 (4) | ND |
| Sri Lanka | 10 | ND | 1.37 ± 0.21 (3) | ND | 0.05 ± 0.16 (1) | ND |
| Vietnam | 10 | 0.01 ± 0.03 (1) | ND | ND | 0.04 ± 0.13 (1) | ND |
| Malaysia | 65 | 2.24 ± 1.76 (45) | 0.02 ± 0.12 (2) | ND | 0.53 ± 0.77 (21) | 0.01 ± 0.08 (1) |
| ML (Malaysian Food Act, 1983) | | 5 | 1 | 0.5 | 2 | 1 |

NOTE: mean value ± standard deviation of metals detected in samples, while values in parentheses are numbers of metal positive samples. (ND – Not detected). The concentration is compared to the maximum limits (ML) by the Malaysian Food Act, 1983.

Among the domestic samples, As was the most common element present as it was detected in 69% of the domestic samples. However, the level detected in every sample was well below the national ML of 5 mg/kg. The presence of As in the black pepper berries is likely originated from the soil. Shrivastava et al. (2015) reported that the absorption of As

contaminated water by black pepper vines most likely lead to contamination of the pepper berries. Moreover, As contamination of plants from Bau, Sarawak has been reported by Roney (2004).

## 4.4 Heavy Metal Concentration in Pepper Cultivation Soil

Characteristically, the soil samples collected from *P. nigrum* farms were slightly acidic with pH ranging between 5.00 - 5.50. The soil contained clay, silt and sand in the range of 21.25 - 45.6%, 31 - 42.9% and 53.8 - 68.7%, respectively, thus was classified as clay loam soil. Averagely, the soil organic matters (SOM) ranged between 18.95 - 29.58 g/kg. Overall, the soil is suitable for black pepper cultivation based on Good Agricultural Practice (GAP) recommended by the Department of Standard, Malaysia and Malaysian Pepper Board (DSM, 2005). Figure 4.4 illustrates the soil characteristics collected from different cultivation areas.

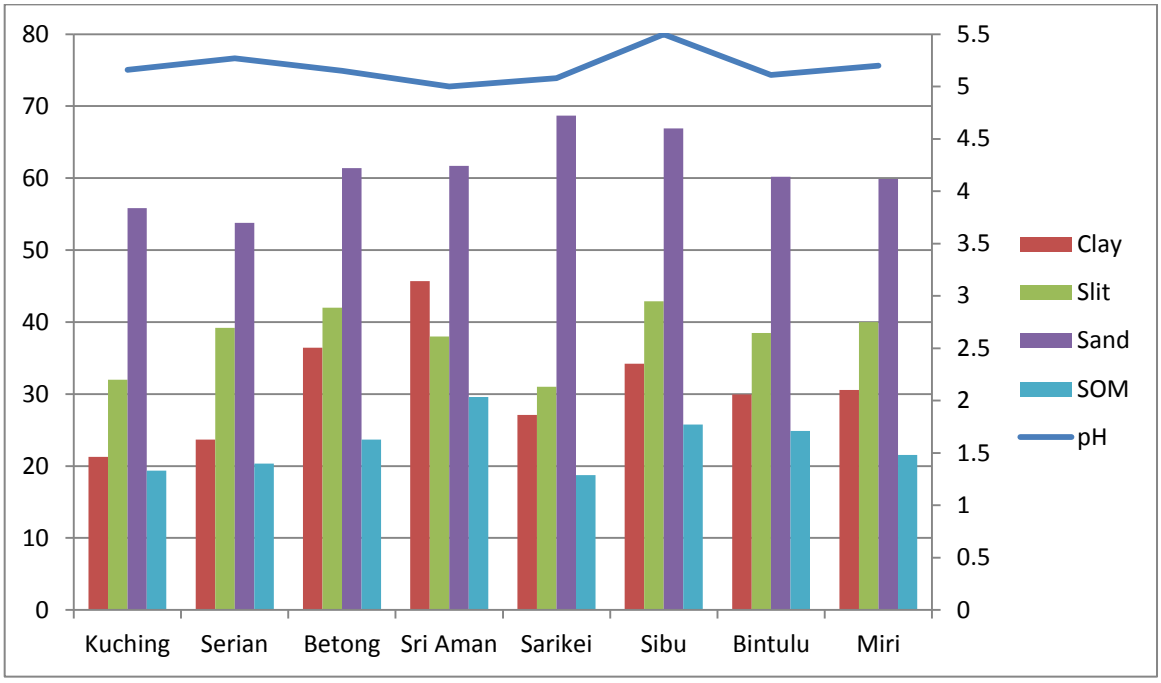


**Figure 4.4:** Soil characteristics sampled from various *P. nigrum* cultivation areas in Sarawak, Malaysia.

Average concentrations of all heavy metals (As, Cd, Cr, Hg, Pb and Sb) in the soil samples from the black pepper farms according to regions are shown in Table 4.7. Among the six elements, average concentration of Sb in soil was the highest ranging between 0.009 to 0.016 mg/kg, followed by Pb (0.010 - 0.015 mg/kg) and Cd (0.004 - 0.014 mg/kg). Overall, the average concentrations of heavy metals across all regions were relatively low (≤ 0.016 mg/kg). The concentration of the target elements in the pepper cultivated soils collected was well below the maximum permissible level of the European Standard Cultivation of Soils (Tóth et al., 2016).

Bintulu exhibits the highest average concentration of Sb (0.016 mg/kg), followed by Sarikei (0.015 mg/kg), and Sri Aman (0.014 mg/kg). The high concentration of Sb is likely a result of natural and anthropological activities, such as weathering of sulfide ores, leaching of mining wastes, smelting, and metallurgical operation (Bolan et al., 2022). The concentration background reference of Sb and Pb in the soil (upper continental crust), was established at 0.31 mg/kg and 17.00 mg/kg, respectively (Wedepohl, 1986; Yap et al., 2022). Our findings show that the Sb and Pb concentrations in the *P. nigrum* cultivated agricultural soil in Sarawak are still lower than the background reference established.

As was only detected in pepper cultivation soils in Sarikei. This could be due to high arsenic content in groundwater in Sarikei Area (Department of Environment Malaysia, 2011) that may have been used for irrigation (Sandil et al., 2021). Furthermore, the contamination of As in soil can also due to application of pesticides, chemical fertilizer and due to the use of farming tractor and machinery (Varol, 2021). Hg, however, is not detected in all *P. nigrum* farm sampled in this study which showed that Hg contamination of pepper cultivation soils in Sarawak is at a minimum level.

**Table 4.7:** Average Concentration of Heavy Metals in Selected Pepper Cultivation Soils in Sarawak, Malaysia

| Plot | Concentration of Heavy metals in pepper cultivation soils (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | As | Cd | Cr | Hg | Pb | Sb |
| Kuching | ND | 0.007±0.000[a] | 0.006±0.001[b] | ND | 0.011±0.000[a] | 0.011±0.002[b] |
| Serian | ND | 0.005±0.000[a] | 0.006±0.001[b] | ND | 0.011±0.001[b] | ND |
| Sri Aman | ND | 0.005±0.001[b] | 0.006±0.001[b] | ND | 0.010±0.000[a] | 0.014±0.002[b] |
| Betong | ND | 0.010±0.002[b] | 0.009±0.001[b] | ND | 0.011±0.002[b] | 0.009±0.001[a] |
| Sarikei | 0.007±0.000[a] | 0.014±0.008[c] | 0.005±0.002[b] | ND | 0.011±0.003[c] | 0.015±0.001[a] |
| Sibu | ND | 0.005±0.001[b] | 0.005±0.001[b] | ND | 0.010±0.000[a] | 0.013±0.002[b] |
| Bintulu | ND | 0.004±0.002[b] | 0.004±0.000[a] | ND | ND | 0.016±0.003[c] |
| Miri | ND | 0.005±0.003[b] | 0.005±0.001[b] | ND | 0.015±0.005[c] | ND |
| Maximum Permissible Level (European Standard) | 50.000 (e) | 20.000 (e) | 200.000 (e) | 2.000 (e) | 200.00 (t) | 10.000 (t) |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper cultivation soils from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p < 0.05$. The maximum permissible level (European Standard) guideline values have been defined on the basis of either ecological risk (e) or health risk (t).

## 4.5 Heavy Metal Concentration in *P. nigrum* Plants

Tables 4.8 – 4.13 tabulate the average metal concentrations extracted from the soil, *P. nigrum* stems, leaves and berries according to regions.

Table 4.8 summarizes the As concentration in different parts of pepper vines, As was not detected in soil samples of all areas of sampling, except Sarikei, where the As was recorded at 0.007 mg/kg. The source of As in the soils of Sarikei could have originated from anthropogenic sources such as farming tractor and machineries, application of chemical fertilizers and pesticides (Varol, 2021) and/or As containing irrigation water (Roney, 2004; Department of Environment Malaysia, 2011). Likewise, As in the stem and leaves of pepper vines was below the detection limit (< 0.006 mg/kg). Based on this result, the As levels in the soil and *P. nigrum* parts are well below the guidelines of 5 mg/kg which means that the pepper berries/products in Sarawak are safe for human consumption. These findings were further confirmed by Malaysia Pepper Board where no consignments were rejected due to As contamination (MPB, 2021).

**Table 4.8**: Concentration of arsenic in different parts of pepper vines collected from different region in Sarawak

| Area | Concentration of Arsenic, As (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | ND | ND | ND | ND |
| Serian | ND | ND | ND | ND |
| Sri Aman | ND | ND | ND | ND |
| Betong | ND | ND | ND | ND |

**Table 4.8** continued

| | | | | |
|---|---|---|---|---|
| Sarikei | 0.007±0.000[a] | ND | ND | ND |
| Sibu | ND | ND | ND | ND |
| Bintulu | ND | ND | ND | ND |
| Miri | ND | ND | ND | ND |
| Maximum Limits (Malaysian Food Act, 1983) for Arsenic, As = 5 mg/kg | | | | |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p < 0.05$.

Table 4.9 summarizes the Cd accumulation in different parts of pepper vines. Cd was undetected in all parts of pepper vines (< 0.003 mg/kg) from various farms in Sarawak, but it was only detected in the soil samples. Although Cd concentration was undetected in the plants, the plants were observed to demonstrate necrosis symptom (Figure 4.5) (Shaari et al., 2022). Toxicity of cadmium could inhibit plant growth by hindering the fixation of carbon and decreasing chlorophyll content thus reducing photosynthetic activity (Haider et al., 2021). The maximum limit of Cd in the Malaysian Food Act 1983 was 1 mg/kg.

In this study, the concentration of Cd in all pepper parts sampled were well below the ML guidelines. Cd present in the soils is also below the background concentrations of 0.36 mg/kg (Arumugam, 2016; Kubier et al., 2019). Cd soil contamination is normally associated with industrial and urbanization activities, however in this study, the risk of contamination of the *P. nigrum* vines is deemed very low (Khan et al., 2018; Niño-Savala et al., 2019; Schaefer et al., 2020).

**Table 4.9:** Concentration of Cadmium, Cd in different parts of pepper vines collected from different regions in Sarawak

| Area | Concentration of Cadmium, Cd (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | 0.007± 0.000[a] | ND | ND | ND |
| Serian | 0.005± 0.000[a] | ND | ND | ND |
| Sri Aman | 0.005± 0.001[b] | ND | ND | ND |
| Betong | 0.010± 0.002[b] | ND | ND | ND |
| Sarikei | 0.014± 0.008[c] | ND | ND | ND |
| Sibu | 0.005± 0.001[b] | ND | ND | ND |
| Bintulu | 0.004± 0.002[b] | ND | ND | ND |
| Miri | 0.005± 0.003[b] | ND | ND | ND |
| Maximum Limits (Malaysian Food Act, 1983) for Cadmium, Cd = 1 mg/kg | | | | |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p < 0.05$.

**Figure 4.5:** Symptom of necrosis on *P. nigrum* leaves

Table 4.10 shows the mean concentration of Cr recorded in different parts of *P. nigrum* vines sampled from different areas in Sarawak. The pepper vines sampled from Sibu contained the highest in Cr in their leaves ($3.309 \times 10^{-3}$ mg/kg) compared to other sampling areas. On average, the Cr accumulated in the pepper stems was the highest at $3.134 \times 10^{-3}$ mg/kg, followed by the leaves at $1.127 \times 10^{-3}$ mg/kg. The concentration of Cr in the pepper berries was well below the maximum permissible level of 1 mg/kg according to the Malaysian Food Act 1983, with an average of $4.991 \times 10^{-4}$ mg/kg.

Our finding which shows that Cr content is the highest in the pepper stem contradicts the findings of Nunes et al. (2018), which reported significantly high Cr detected in the fruits. The redox reaction between chromium(III) and carboxylic functional groups in the plant will encourage the translocation of Cr from the roots to the stem and subsequently accumulated in the leaves and berries (Ao et al., 2022). The abundance of Cr in soil, like Cd, is below the background level of 0.0365 mg/kg (Arumugam, 2016).

**Table 4.10:** Concentration of Chromium, Cr in different parts of pepper vines collected from different regions in Sarawak

| Area | Concentration of Chromium, Cr (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | 0.006 | $1.472 \times 10^{-3}$ | $6.579 \times 10^{-4}$ | $2.070 \times 10^{-4}$ |
| | $\pm 0.001^{b}$ | $\pm 1.648 \times 10^{-4a}$ | $\pm 3.658 \times 10^{-5b}$ | $\pm 1.799 \times 10^{-4a}$ |
| Serian | 0.006 | $4.778 \times 10^{-3}$ | $1.460 \times 10^{-3}$ | $5.576 \times 10^{-4}$ |
| | $\pm 0.001^{b}$ | $\pm 7.260\ 10^{-4c}$ | $\pm 1.745 \times 10^{-4c}$ | $\pm 3.469 \times 10^{-4b}$ |

**Table 4.10** continued

| | | | | |
|---|---|---|---|---|
| Sri Aman | 0.006 | $5.737 \times 10^{-4}$ | $1.228 \times 10^{-4}$ | $3.574 \times 10^{-4}$ |
| | $\pm 0.001^{b}$ | $\pm 8.726 \times 10^{-5a}$ | $\pm 6.873 \times 10^{-6a}$ | $\pm 1.235 \times 10^{-4a}$ |
| Betong | 0.009 | $5.601 \times 10^{-3}$ | $2.942 \times 10^{-4}$ | $9.391 \times 10^{-5}$ |
| | $\pm 0.001^{b}$ | $\pm 3.042 \times 10^{-4b}$ | $\pm 3.727 \times 10^{-5b}$ | $\pm 7.677 \times 10^{-5c}$ |
| Sarikei | 0.005 | $1.464 \times 10^{-3}$ | $9.453 \times 10^{-4}$ | $2.068 \times 10^{-4}$ |
| | $\pm 0.002^{b}$ | $\pm 2.164 \times 10^{-4b}$ | $\pm 9.446 \times 10^{-4c}$ | $\pm 2.004 \times 10^{-4a}$ |
| Sibu | 0.005 | $3.352 \times 10^{-3}$ | $3.309 \times 10^{-3}$ | $7.889 \times 10^{-4}$ |
| | $\pm 0.001^{b}$ | $\pm 5.811 \times 10^{-4c}$ | $\pm 3.180 \times 10^{-4c}$ | $\pm 4.779 \times 10^{-4b}$ |
| Bintulu | 0.004 | $3.553 \times 10^{-3}$ | $1.227 \times 10^{-3}$ | $9.825 \times 10^{-4}$ |
| | $\pm 0.001^{a}$ | $\pm 3.683 \times 10^{-4b}$ | $\pm 3.873 \times 10^{-4c}$ | $\pm 5.169 \times 10^{-4b}$ |
| Miri | 0.005 | $4.116 \times 10^{-3}$ | $1.000 \times 10^{-3}$ | $7.988 \times 10^{-4}$ |
| | $\pm 0.001^{b}$ | $\pm 8.406 \times 10^{-4c}$ | $\pm 1.626 \times 10^{-4c}$ | $\pm 5.825 \times 10^{-4b}$ |

Maximum Limits (Malaysian Food Act, 1983) for Chromium, Cr = 1 mg/kg

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p < 0.05$.

In contrast, Table 4.11 shows that the Hg was not detected (< 0.03 mg/kg) in various parts of the pepper vines from different regions throughout Sarawak. Therefore, it is confirmed safe for consumption with the Hg level well below the permissible level (0.5 mg/kg) according to the Food Act 1983.

**Table 4.11:** Concentration of Mercury, Hg in different parts of pepper vines collected from different region in Sarawak

| Area | Concentration of Mercury, Hg (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | ND | ND | ND | ND |
| Serian | ND | ND | ND | ND |
| Sri Aman | ND | ND | ND | ND |
| Betong | ND | ND | ND | ND |
| Sarikei | ND | ND | ND | ND |
| Sibu | ND | ND | ND | ND |
| Bintulu | ND | ND | ND | ND |
| Miri | ND | ND | ND | ND |
| Maximum Limits (Malaysian Food Act, 1983) for Mercury, Hg = 0.5 mg/kg | | | | |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p<0.05$.

Table 4.12 demonstrates the mean and standard deviation of Sb concentration detected in various parts of pepper plants sampled from different areas of Sarawak. In this study, there were no detectable amount of Sb in pepper stem, leaves and berries. Based on the results, the level of Sb in berries was considered low with Sb concentration less than the detectable concentration of 0.008 mg/kg and less than 1 mg/kg, the guidelines by Malaysian Food Act 1983. Similarly, the Sb concentrations detected in stems and leaves were also low, at a concentration < 1 mg/kg. The highest concentration of Sb in stem was recorded in Kuching at $5.396 \times 10^{-4}$, the highest concentration of Sb in leaves and berries

were recorded in Sri Aman at 4.877 x $10^{-4}$ and 1.486 x $10^{-4}$ respectively. As in the case of Cr and Cd, Sb was also known to occur naturally with background level established at 0.31 mg/kg, hence, the uptake of Sb in plant parts are also expected (Arumugam, 2016)

**Table 4.12:** Concentration of Antimony, Sb in different parts of pepper vines collected from different regions in Sarawak

| Area | Concentration of Antimony, Sb (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | 0.011± 0.002[b] | ND | ND | ND |
| Serian | ND | ND | ND | ND |
| Sri Aman | 0.014± 0.002[b] | ND | ND | ND |
| Betong | 0.009± 0.001[a] | ND | ND | ND |
| Sarikei | 0.015± 0.001[a] | ND | ND | ND |
| Sibu | 0.013± 0.002[b] | ND | ND | ND |
| Bintulu | 0.016± 0.003[c] | ND | ND | ND |
| Miri | 0.004± 0.001[a] | ND | ND | ND |
| Maximum Limits (Malaysian Food Act, 1983) for Antimony, Sb = 1 mg/kg | | | | |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p<0.05$.

Similar to Hg, all parts of pepper vines collected from different regions recorded Pb concentration below detectable concentration of 0.01 mg/kg (Table 4.9), hence, meeting the MLs established for Pb in the Malaysian Food Act 1983 which is 2.00 mg/kg. However, there were studies reported high Pb accumulation in food crops in China (Zhuang et al., 2009). The different accounts of Pb accumulation may be caused by

different agricultural practices and geographical factors. Overall, the average concentration of all pepper berries collected in all study regions were less than MLs specified in Malaysian Food Act 1983 suggesting that the pepper berries sampled were safe for consumption.

**Table 4.13:** Concentration of Lead, Pb in different parts of pepper vines collected from different regions in Sarawak

| Area | Concentration of Lead, Pb (mg/kg) | | | |
|---|---|---|---|---|
| | Soil | Stem | Leaves | Berries |
| Kuching | 0.011± 0.000[a] | ND | ND | ND |
| Serian | 0.011± 0.001[b] | ND | ND | ND |
| Sri Aman | 0.010± 0.000[a] | ND | ND | ND |
| Betong | 0.011± 0.002[b] | ND | ND | ND |
| Sarikei | 0.011± 0.003[c] | ND | ND | ND |
| Sibu | 0.010± 0.000[a] | ND | ND | ND |
| Bintulu | ND | ND | ND | ND |
| Miri | 0.015± 0.005[c] | ND | ND | ND |
| Maximum Limits (Malaysian Food Act, 1983) for Lead, Pb = 2 mg/kg | | | | |

Note: Results are presented as Mean ± SD for concentration of heavy metals in pepper parts from different areas in Sarawak. Values with different superscripts refer to statistical significance at $p < 0.05$.

### 4.5.1 Enrichment Factor (EF) and Translocation Factor (TF)

Heavy metals pollution in the environment can have potential toxicity on human health. Contamination by heavy metals generally occurs through root uptake from the soil (Shahid et al., 2017) and/or by leaf absorption of heavy metals from the polluted air (Altaf

et al., 2021). Enrichment Factor (EF) in this study was used to estimate the absorption mechanism of heavy metals in the *P. nigrum* plant. Table 4.14 shows the Enrichment Factor (EF) of metals from the samples collected from different regions in Sarawak. Referring to Tables 4.7 – 4.12, all elements were detected in the soil samples except Hg. The EF for all metals was less than 1.

According to Zulkafflee et al. (2021), if the enrichment value is greater than 1, it indicates that the plant is a hyper accumulator plants with high tendency for metal uptake. Our results infer that *P. nigrum* plant does accumulate heavy metals significantly. The absorption of heavy metals from the soil is influenced by the plant variety, concentration of metals in soil and bioavailability of metals (Chen et al., 2018; Zwolak et al., 2019).

**Table 4.14:** Enrichment Factors (EF) for Heavy Metals in Pepper Berries Collected From Different Regions in Sarawak.

| Area | $EF_{As}$ | $EF_{Cd}$ | $EF_{Cr}$ | $EF_{Hg}$ | $EF_{Pb}$ | $EF_{Sb}$ |
|---|---|---|---|---|---|---|
| **Kuching** | NA | 0.00 | 0.038 | NA | 0.00 | 0.00 |
| **Serian** | NA | 0.00 | 0.093 | NA | 0.00 | NA |
| **Sri Aman** | NA | 0.00 | 0.059 | NA | 0.00 | 0.00 |
| **Betong** | NA | 0.00 | 0.010 | NA | 0.00 | 0.00 |
| **Sarikei** | 0.00 | 0.00 | 0.043 | NA | 0.00 | 0.00 |
| **Sibu** | NA | 0.00 | 0.174 | NA | 0.00 | 0.00 |
| **Bintulu** | NA | 0.00 | 0.237 | NA | NA | 0.00 |
| **Miri** | NA | 0.00 | 0.166 | NA | 0.00 | NA |
| **AVERAGE** | 0.00 | 0.00 | 0.102 | NA | 0.00 | 0.00 |

The Translocation Factor (TF) was also compared for all metals. All elements, except Cr, were undetected in *P. nigrum* indicating minimal risk of translocation. Cr recorded TF in an increasing trend of stem to leaves (0.405) < soil to stem (0.567) < leaves to berries (0.748). TF < 1 shows that the pepper berries from Sarawak were safe for consumption with no risk of contamination. A higher TF value indicates higher mobility or availability of the metals in the plants (Zulkafflee et al., 2021).

In a study in Peninsular Malaysia, the TF values of As, Cd, Cr and Pb from rice paddy stem to the rice grain were greater than 1 (Zulkafflee et al., 2021). In a separate report, Tripathi et al. (2021) showed TF > 1 for As, Cd and Cr from the roots to the shoot and from the shoot to leaves for native medicinal plants in India.

## 4.6 Safety Level Assessment of Heavy Metals in Black Pepper

In the present study, the consumption of black pepper is considered as one of the main routes of exposure of heavy metals in human. To determine the health risk assessment of consumption of black pepper contaminated with heavy metals, the average daily dose (ADD) was calculated. ADD was used to determine the average daily intake of the target elements into the body through consumption of black pepper for adults in Malaysia.

The overall results showed that the ADD value of each metal did not exceed the safe intake limit stated in the Malaysian Food Act, 1983 (Table 4.15).

**Table 4.15**: ADD Values of Heavy Metals and Maximum Permitted Proportion (MPP) Levels in The Malaysian Food Act, 1983

| Heavy Metal | ADD | MPP (mg/kg) (Malaysian Food Act, 1983) |
|---|---|---|
| **As** | 0.00 | 5 |
| **Cd** | 0.00 | 1 |
| **Cr** | $2.788 \times 10^{-5}$ | N/A |
| **Hg** | 0.00 | 0.5 |
| **Pb** | 0.00 | 2 |
| **Sb** | 0.00 | 1 |

The hazard quotient (HQ) can be calculated to assess the non-carcinogenic health risk. The HQ values for all targeted elements were found to be less than 1 (HQ < 1). This indicates that there are no potential non-carcinogenic risks in consuming black pepper berries from Sarawak. In addition, Hazard Index (HI) can be determined to evaluate the non-carcinogenic health risks for black pepper consumers. The HI values were calculated as a combined exposure to heavy metals per areas based on a typical pepper consumption pattern, where pepper is mostly consumed as a spice or in Ayuverdic medicines. In this study, all HI values were less than 1, further supporting the low risks associated to black pepper consumption (Zulkafflee et al., 2021). In contrast, the high HI reported for rice grain (HI >1) could be due to higher ingestion rates of rice, which is at 600g/day compared to ingestion rate of black pepper, which is at 3.5g/day (Rahman et al., 2018; Zulkafflee et al., 2021).

Lifetime Cancer Risk (LCR) and Total Cancer Risk (CRt) were also calculated. The acceptable limit for LCR is < 1 x $10^{-4}$ while the acceptable CRt range recommended by USEPA is between 1 x $10^{-6}$ and 1 x $10^{-4}$. In this study, the LCR for all elements were below the acceptable limits at 0.00 which indicates that there is no potential carcinogenic risk through pepper consumption. This result is similar compared to previous finding conducted in China in 2018 on dietary Cd exposure where LCR calculated were within acceptable value of <$10^{-4}$ (Qing et al., 2020). The LCR for As, Cd, Hg and Pb in this study is also similar with previous findings conducted in Poland in 2021 where the LCR of the metals in spices (including black pepper) is recorded at 1.29 x $10^{-5}$ (max) (Kowalska, 2021). Table 4.16 summarizes the LCR values for the elements involved.

Figure 4.6 shows the CRt from combined exposure to the multiple carcinogenic agents present in black pepper collected from different areas in Sarawak. The CRt calculated in this study is also safely within the acceptable range (<1x$10^{-4}$), where the CRt of pepper planted in Bintulu was the highest followed by Miri, Sibu, Serian, Betong, Sarikei, Kuching, and Sri Aman. Although the CRt calculated is within acceptable range, the value calculated reveals a potential carcinogenic risk, although relatively low, due to black pepper consumption. High CRt is of concern because it shows that the *P. nigrum* vines in Sarawak have potential heavy metal contamination, which could be attributed to metal-contaminated soils (agricultural areas with toxic elements) (Kowalska, 2021) and / or irrigated with contaminated water. These contaminated black pepper could introduce the heavy metals into humans through their diet. Even though the LCR and CRt values in this study were relatively low, which indicates low health risk, precautions should always be taken to ensure pepper berries collected from these regions undergo proper postharvest processing to guarantee food safety prior to export or consumption.

**Table 4.16**: ADD, CSF and LCR values for targeted elements.

| Elements | ADD | CSF | LCR |
|---|---|---|---|
| **As** | 0.00 | 1.5000 | 0.00 |
| **Cd** | 0.00 | 15.000 | 0.00 |
| **Cr** | 0.00 | 0.5000 | 0.00 |
| **Hg** | 0.00 | N/A | N/A |
| **Pb** | 0.00 | 0.0085 | 0.00 |
| **Sb** | 0.00 | 0.5000 | 0.00 |

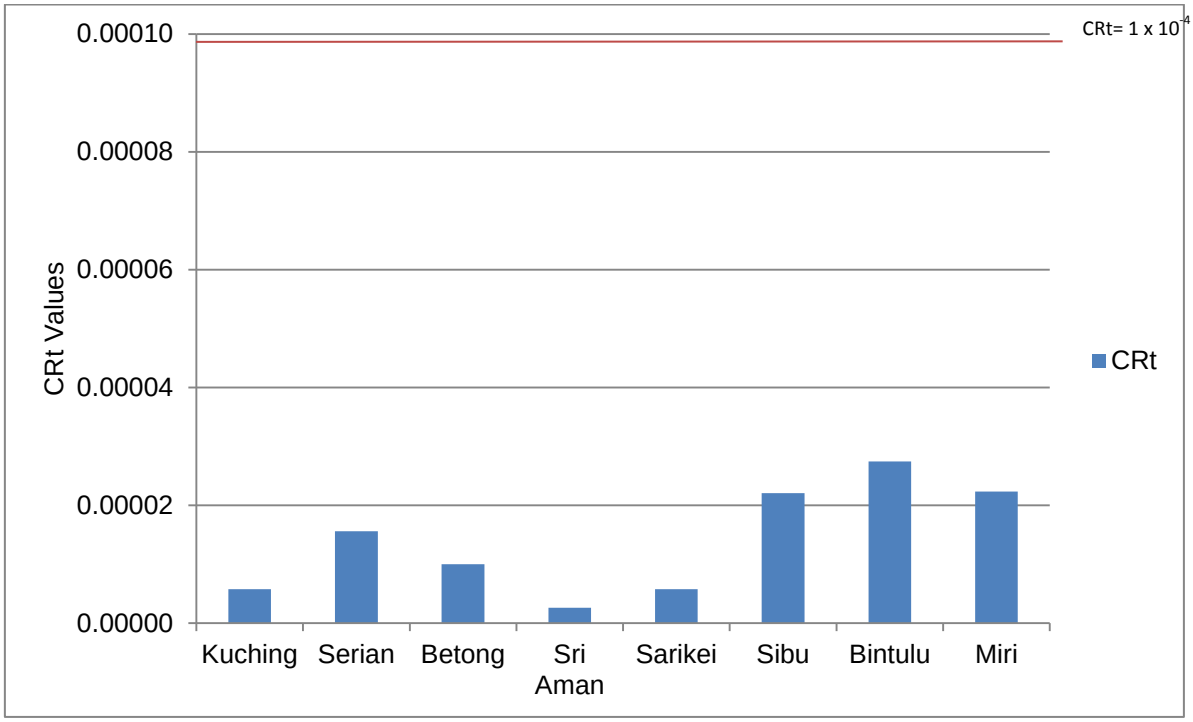


**Figure 4.6:** Total Cancer Risk (CRt) from Combined Exposure of Multiple Carcinogenic Present in Black Pepper Sampled from Eight Different Areas of Sarawak. Red line indicates CRt = 1 x $10^{-4}$ which is the maximum acceptable value recommended by the USEPA.

## 4.7 Statistical Analysis For Heavy Metals in Pepper Planted in Sarawak

### 4.7.1 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is applied to obtain the underlying clustering pattern of the data. Figures 4.7 - 4.10 show the PCA score plots for the heavy metals present in *P. nigrum* according to its plant parts. The total variance for PC1 and PC2 (in this order) for different samples are as follows: 45.15% and 33.52% for the black pepper berries; 45.97% and 22.98% for the pepper leaves; 29.09% and 15.76% for the pepper stem and 44.30% and 32.08% for the heavy metals found in soil samples from the *P. nigrum* farms.

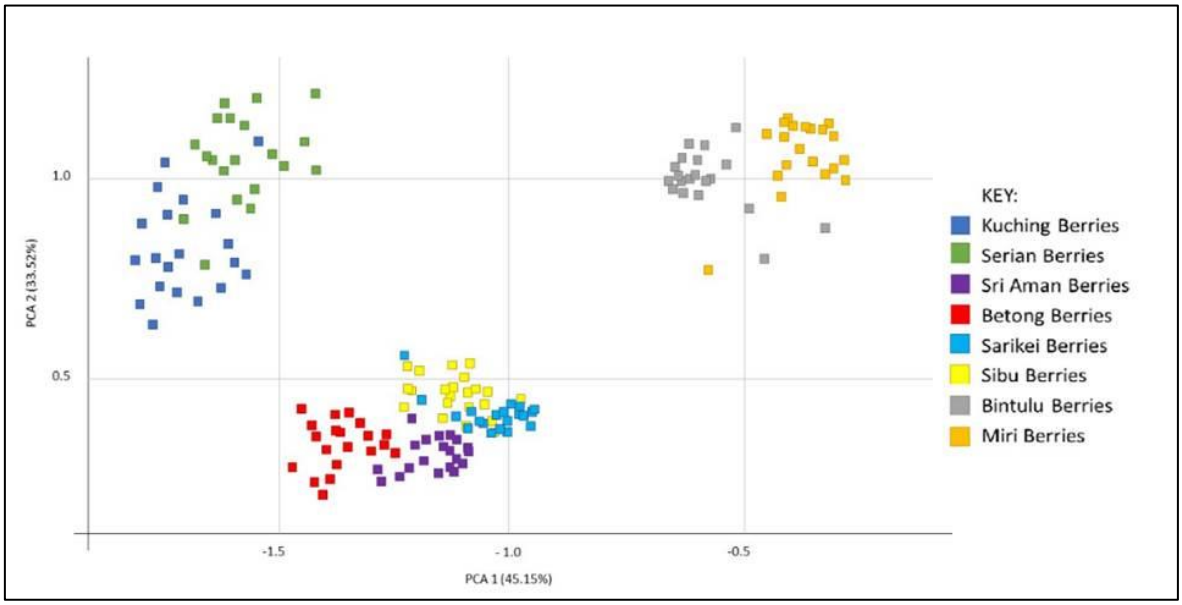


**Figure 4.7:** PCA scores plot for heavy metals present in the black pepper berries sampled from Sarawak cultivated areas.

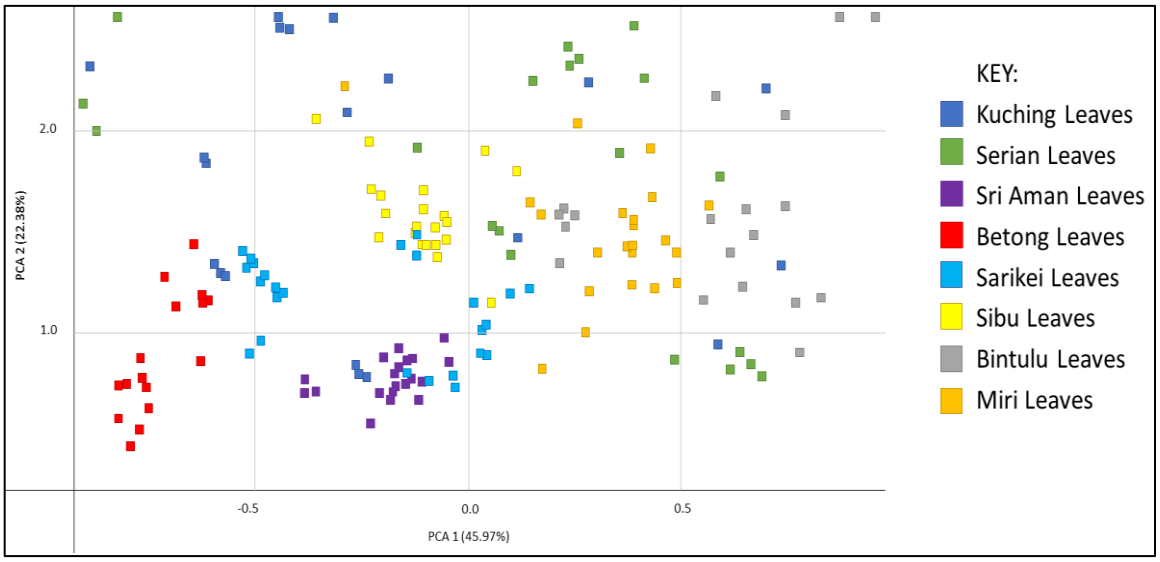


**Figure 4.8:** PCA scores plot for heavy metals present in the *P. nigrum* leaves sampled from Sarawak cultivated areas.

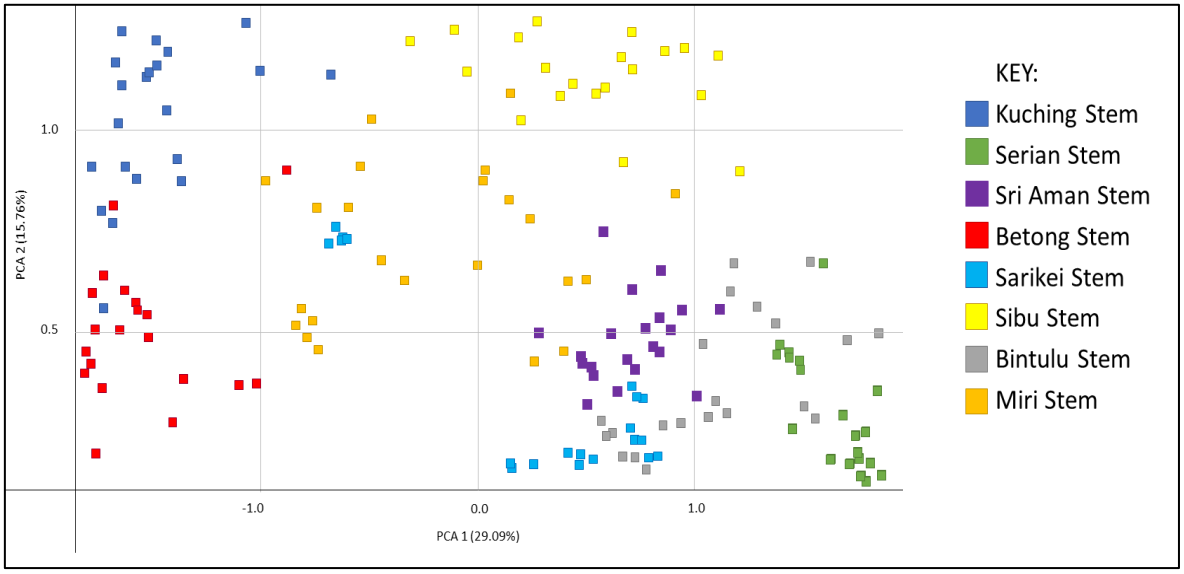


**Figure 4.9:** PCA scores plot for heavy metals in the *P. nigrum* stems sampled from Sarawak cultivated areas..

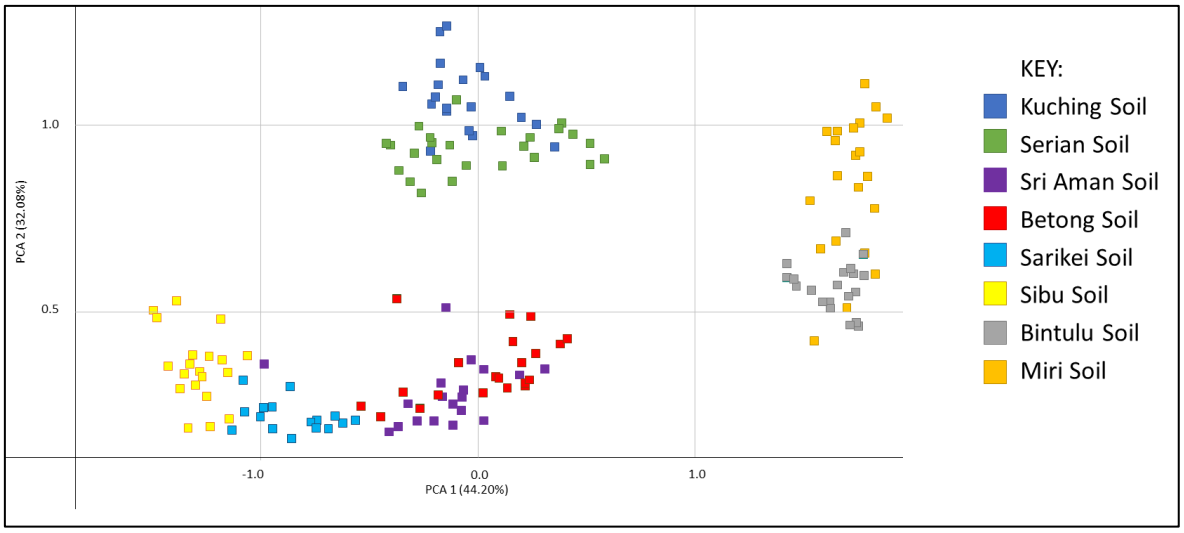


**Figure 4.10:** PCA scores plot of heavy metals present in the *P. nigrum* planting soils sampled from Sarawak cultivated areas.

There were no observable clustering patterns observed in the PCA scores plot for the *P. nigrum* stems and leaves (Figure 4.8 – 4.9). In contrast, the PCA scores plot of berries and soils (Figure 4.7 and 4.10) were found to be clustered according to their farms - the Northern region (Miri and Bintulu), the Center region (Betong, Sri Aman, Sarikei and Sibu) and the Southern region (Kuching and Serian).

Clustering pattern of heavy metals in the pepper berries nearly seems to mirror clustering pattern of heavy metals in the pepper planting soils. This indicates that pepper berries were contaminated with heavy metals in similar fashion as the soil (Zeiss et al., 2018), such as through similar agricultural practices between regions, leading to soil contamination. Based on the clustering pattern, it can be seen that the pepper berries planted in different areas can be significantly distinguished (Clemente et al., 2018) from one region to another region brought by the significant differences between average concentration of Pb, Cr, Cd and Sb in each region.

The non-clustering pattern observed in the leaves and stem samples, irregardless of the farm location or region, suggesting that the heavy metal accumulation/contamination in the stem and leaves were random. Random contamination of heavy metals on the stem and leaves may occur by atmospheric deposition, industrial emission, and erosion (Li et al., 2022; WHO, 2007). Heavy metals can also disperse with the help of wind from initial point of origin to as near as 4 kilometers downwind (Li et al., 2022; Mokhtari et al., 2018), while distribution of heavy metals from industrial wastewater was affected by water flow redistribution in rivers (Li et al., 2022).

### 4.7.2 ANOVA

One-Way ANOVA was conducted using IBM SPSS ver 22 software to determine the significant difference between the metals in the pepper berries. There were three sampling zones in our study, consisting of eight sampling locations (Kuching, Serian, Betong, Sri Aman, Sarikei, Sibu, Miri and Bintulu). Statistical analysis indicates significant differences between the metals in the pepper berries from different regions. The post-hoc test showed that the average As concentration in the pepper berries from Sri Aman and Miri was significantly different from those collected from other regions. The average Hg concentration in the samples from Miri was statistically different from other areas, while As was statistically similar for all areas. However, the average concentration of Cd, Cr, Pb and Sb are significantly different in every sampling area.

## 4.8 Summary of the Chapter

As a summary, this chapter highlight the development of detection of heavy metal in black pepper using ICP-MS/MS where the sample was prepared using microwave-assisted acid digestion. The developed method was then used to detect and quantitate heavy metals in

imported and exported black pepper. The method was also used to quantitate heavy metals in *P. nigrum* parts (stem, leaves and berries), where the concentration were further analysed and compared. Assessment of health risk was also calculated in this chapter, where the EF, TF, ADD, HQ, HI, LCR and CRt were quantified and compared with Malaysian Food Act 1983. Overall, there were no hyper accumulations of heavy metals, and no carcinogenic or non-carcinogenic risk in consuming *P. nigrum* planted in Sarawak, Malaysia. Apart from that, statistical analysis such as principal component analysis (PCA) and ANOVA test were also conducted where clustering pattern between the pepper berries and soil samples from the same region were revealed; however, there was no clustering observed involving concentration of heavy metals and its location in the pepper leaves and stem. For ANOVA test, there were significant differences between average concentrations of target elements against area of sampling by using the ANOVA and Tukey-B post-hoc tests.

# CHAPTER 5

# CONCLUSIONS AND RECOMMENDATION

This study had successfully established an optimised microwave-assisted digestion and ICP-MS/MS method for determination of selected heavy metals in black pepper berries. The optimised method used helium as cell gas with flow rate of 5 ml/min, with microwave digestion temperature and power was optimised as 220 $^{o}$C and 1,000 W respectively. The acid digestion systems were also optimised where only 3 mL was needed to fully digest black pepper samples. The method is reliable with recoveries between 98.14-114.83%, LODs recorded for As, Pb, Hg, Cd and Sb was established in the range of 0.003-0.01 mg/kg, R2 were above 0.999 for all metals of interest, and expanded uncertainties were in the range of 7.21 – 18.29%. This cost-effective method will be valuable in the pepper industry, especially for import and export regulatory and statutory bodies where continuous monitoring of imported and exported pepper needed to be done to ensure food safety. This method can also be used by pepper producers where pepper quality control will be conducted routinely.

Quantitation on concentrations of all target elements in different parts of the pepper vines sampled from the South, Central and Northern part of Sarawak found all metals of interest to be below the maximum allowable limit recommended by the Malaysian Food Act 1983. Calculations of non-carcinogenic (ADD, HQ and HI) and carcinogenic (LCR and CRt) risks were also conducted in pepper berries sampled in Sarawak, and there were no risks found due to pepper consumption in Malaysian adults. These results show that the pepper berries collected in cultivation areas in Sarawak are completely safe for human consumption. Furthermore, all metal contents in the soils were

below maximum allowable limit recommended by the relevant standards, indicating that the soils are safe for agriculture uses.

However, analysis of heavy metals in 115 imported and domestic pepper berries samples showed that 53.91% of the samples was found to contain or was contaminated with As (0.01 – 7.00 mg/kg), Pb (0.01 – 3.61 mg/kg) and Cd (0.02 – 1.37 mg/kg). Hence, further studies are recommended to extend to different types of pepper products/berries (whole, cracked, and/or ground pepper) available in the market in order to evaluate the safety of the commercial consumer products. Besides heavy metals, the commercially available pepper products should also be analysed for other contaminants such as pesticide residues, mycotoxins and microbiological contamination to enable a more thorough risk assessment related to pepper consumption.

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# APPENDICES

## APPENDIX 1

## ICP-MS/MS OPERATING CONDITION

| Parameter | Tune Setting |
|---|---|
| Scan Type | MS/MS |
| Plasma Mode | High Matrix HMI |
| Lenses Extract 1 | 0.00V |
| Lenses Extract 2 | -200.00V |
| Omega Bias | -100.00V |
| Omega Lens | 5.80V |
| Q1 Entrance | -19.00V |
| Q1 Exit | -3.00V |
| Cell Focus | -5.00V |
| Cell Entrance | -40.00V |
| Cell Exit | -50.00V |
| Deflect | 8.40V |
| Plate Bias | -50.00V |
| Q1 Mass Gain | 155.00 |
| Q1 Mass Offset | 128.00 |
| Q1 Axis Gain | 1.0050 |
| Q1 Axis Offset | 0.00 |
| Q1 Bias | -4.00V |
| Q1 Prefilter Bias | -30.00V |
| Q1 Postfilter Bias | -14.00V |

| | |
|---|---|
| Q1 Ion Guide: SLS Factor | 0.40 |
| Q1 Ion Guide: SLG Factor | 0.90 |
| Octopole Bias | -8.00V |
| Octopole RF | 160.00V |
| Energy Discrimination | 5.00V |
| Q2 Mass Gain | 118 |
| Q2 Mass Offset | 127 |
| Q2 Axis Gain | 0.9960 |
| Q2 Axis Offset | 0.08 |
| Q2 Bias | -3.00V |
| Torch Axis H | -0.5mm |
| Torch Axis V | 0.6mm |
| EM Discriminator | 4.7mV |
| Analog HV | 1822V |
| Pulse HV | 1091V |

**APPENDIX 2**

## METHOD DEVELOPMENT

### AVERAGE RECOVERY FOR GAS FLOW RATE

| | No Gas | Medium Flow | High Flow |
|---|---|---|---|
| As | 78.3425 | 99.9981 | 101.9998 |
| Cd | 75.912 | 97.9912 | 105.0928 |
| Hg | 77.0091 | 102.0006 | 112.9129 |
| Pb | 73.1211 | 100.2334 | 105.8988 |
| Sb | 76.87687 | 95.0991 | 99.4356 |

### AVERAGE RECOVERY FOR ACID MIXTURE

| Acid Mixture | Average | | | | | Average Recovery (%) | Stdev |
|---|---|---|---|---|---|---|---|
| | As | Cd | Hg | Pb | Sb | | |
| 5mL HNO3 + 1mL HCl | 122.93 | 125.92 | 126.09 | 127.09 | 130.48 | 126.50 | 2.71 |
| 5mL HNO3 + 1mL H2O2 | 79.98 | 77.09 | 76.09 | 77.90 | 76.95 | 77.60 | 1.48 |
| 5mL HNO3 + 1mL H2SO4 | 80.98 | 72.10 | 82.10 | 77.99 | 76.78 | 77.99 | 3.94 |
| 5mL HNO3 + 1mL HF | 76.87 | 75.09 | 77.41 | 79.42 | 72.59 | 76.28 | 2.57 |
| 5mL HNO3 only | 101.93 | 99.01 | 100.94 | 103.01 | 104.53 | 101.88 | 2.09 |
| 3mL HNO3 | 103.39 | 103.99 | 103.99 | 103.99 | 100.03 | 103.08 | 1.72 |

### AVERAGE RECOVERY FOR TEMPERATURE AND POWER.

| | 150 $^{o}$C , 1000W | 220 $^{o}$C, 1000W | 250 $^{o}$C, 1800W |
|---|---|---|---|
| As | 70.60 | 98.91 | 100.10 |
| Cd | 70.03 | 97.33 | 100.10 |
| Hg | 71.00 | 95.89 | 99.01 |
| Pb | 70.86 | 96.88 | 99.60 |
| Sb | 70.31 | 95.39 | 99.05 |
| Average | 70.56 | 96.88 | 99.57 |
| Stdev | 0.39 | 1.37 | 0.53 |

**APPENDIX 3**

## HEAVY METALS CONCENTRATION IN IMPORTED PEPPER

| Sample | ARSENIC | | | | | CADMIUM | | | | | MERCURY | | | | | LEAD | | | | | ANTIMONY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cambodia | India | Indonesia | Sri Lanka | Vietnam | Cambodia | India | Indonesia | Sri Lanka | Vietnam | Cambodia | India | Indonesia | Sri Lanka | Vietnam | Cambodia | India | Indonesia | Sri Lanka | Vietnam | Cambodia | India | Indonesia | Sri Lanka | Vietnam |
| 1 | 4.98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 4.32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0 | 0 | 0 | 0 | 0 | 2.22 | 0 | 3.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 5.38 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 | 1.31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 7.88 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.91 | 0 | 0 | 0 | 0 | 0 | 0 | 3.00 | 0 | 2.00 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 7.15 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.98 | 0 | 0 | 0 | 0 | 0 | 0 | 2.29 | 0.1 | 1.58 | 0 | 0.4 | 0 | 0 | 0 | 0 | 0 |
| 6 | 4.59 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 1.00 | 0 | 0 | 0 | 0 | 0 | 0 | 2.09 | 0 | 3.22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 4.59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.96 | 0 | 0 | 0 | 0 | 0 | 0 | 2.45 | 0 | 1.99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 7.57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.99 | 0 | 0 | 0 | 0 | 0 | 0 | 2.06 | 0 | 1.96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 4.58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 4.86 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.94 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Average | 5.59 | 0.01 | 0.00 | 0 | 0.01 | 0 | 0 | 0 | 1.10 | 0 | 0 | 0 | 0 | 0 | 0 | 1.41 | 0.01 | 2.59 | 0.05 | 0.04 | 0 | 0 | 0 | 0 | 0 |
| STDEV | 1.38 | 0.03 | 0.00 | 0.00 | 0.03 | 0 | 0 | 0 | 0.21 | 0 | 0 | 0 | 0 | 0 | 0 | 1.24 | 0.03 | 0.91 | 0.16 | 0.13 | 0 | 0 | 0 | 0 | 0 |

**Highlighted concentration is the concentration of metal above MFA 1983 limit**

**APPENDIX 4**

## HEAVY METAL IN EXPORTED PEPPER BERRIES

| Sample Number | Arsenic | Cadmium | Mercury | Lead | Antimony |
|---|---|---|---|---|---|
| 1 | 1.98 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 4.11 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3 | 1.65 | 0.00 | 0.00 | 1.55 | 0.00 |
| 4 | 1.22 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | 4.22 | 0.00 | 0.00 | 1.85 | 0.00 |
| 6 | 1.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| 7 | 1.58 | 0.00 | 0.00 | 1.52 | 0.00 |
| 8 | 1.92 | 0.00 | 0.00 | 1.59 | 0.00 |
| 9 | 1.15 | 0.00 | 0.00 | 1.54 | 0.00 |
| 10 | 4.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11 | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | 4.55 | 0.00 | 0.00 | 0.00 | 0.00 |
| 13 | 4.55 | 0.00 | 0.00 | 1.89 | 0.00 |
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 | 4.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16 | 4.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| 17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20 | 0.00 | 0.00 | 0.00 | 1.65 | 0.00 |
| 21 | 0.00 | 0.00 | 0.00 | 1.58 | 0.00 |
| 22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 24 | 2.66 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 | 2.15 | 0.00 | 0.00 | 1.90 | 0.00 |
| 26 | 1.44 | 0.00 | 0.00 | 0.00 | 0.00 |
| 27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 28 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.65 |
| 30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.00 | 0.00 | 1.60 | 0.00 |
| 32 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 34 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | |
|---|---|---|---|---|---|
| 38 | 0.00 | 0.90 | 0.00 | 0.00 | 0.00 |
| 39 | 4.55 | 0.40 | 0.00 | 1.69 | 0.00 |
| 40 | 2.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| 41 | 3.96 | 0.00 | 0.00 | 1.69 | 0.00 |
| 42 | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 43 | 3.45 | 0.00 | 0.00 | 0.00 | 0.00 |
| 44 | 3.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| 45 | 3.45 | 0.00 | 0.00 | 0.00 | 0.00 |
| 46 | 3.11 | 0.00 | 0.00 | 0.00 | 0.00 |
| 47 | 2.19 | 0.00 | 0.00 | 0.00 | 0.00 |
| 48 | 2.11 | 0.00 | 0.00 | 1.55 | 0.00 |
| 49 | 4.66 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 | 2.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| 51 | 2.65 | 0.00 | 0.00 | 1.83 | 0.00 |
| 52 | 2.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 53 | 3.49 | 0.00 | 0.00 | 1.58 | 0.00 |
| 54 | 3.90 | 0.00 | 0.00 | 1.34 | 0.00 |
| 55 | 4.53 | 0.00 | 0.00 | 1.17 | 0.00 |
| 56 | 4.56 | 0.00 | 0.00 | 1.89 | 0.00 |
| 57 | 3.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| 58 | 3.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| 59 | 3.12 | 0.00 | 0.00 | 0.00 | 0.00 |
| 60 | 3.15 | 0.00 | 0.00 | 1.59 | 0.00 |
| 61 | 2.56 | 0.00 | 0.00 | 1.86 | 0.00 |
| 62 | 4.70 | 0.00 | 0.00 | 0.00 | 0.00 |
| 63 | 4.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| 64 | 4.58 | 0.00 | 0.00 | 1.58 | 0.00 |
| 65 | 4.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| AVERAGE | 2.24 | 0.02 | 0.00 | 0.53 | 0.01 |
| STDEV | 1.76 | 0.12 | 0.00 | 0.77 | 0.08 |

**Highlighted concentration is the concentration of metal above MFA 1983 limit**

**APPENDIX 5**

## SOIL ANALYSIS

### pH

| Sample No | pH | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUCHING | SERIAN | BETONG | SRI AMAN | SARIKEI | SIBU | BINTULU | MIRI |
| 1 | 5.12 | 5.17 | 5.17 | 4.99 | 4.90 | 5.75 | 5.15 | 5.12 |
| 2 | 5.05 | 5.15 | 5.22 | 4.95 | 4.90 | 5.70 | 5.05 | 5.38 |
| 3 | 5.55 | 5.11 | 5.03 | 5.00 | 4.85 | 5.78 | 5.11 | 5.32 |
| 4 | 5.17 | 5.02 | 5.11 | 5.01 | 4.98 | 5.17 | 5.18 | 5.32 |
| 5 | 5.02 | 5.30 | 5.12 | 5.05 | 4.95 | 5.18 | 5.10 | 5.28 |
| 6 | 5.16 | 5.16 | 5.33 | 5.09 | 5.20 | 5.08 | 5.02 | 5.22 |
| 7 | 5.15 | 5.16 | 5.12 | 4.91 | 5.25 | 5.58 | 5.06 | 5.11 |
| 8 | 5.14 | 5.56 | 5.16 | 5.00 | 5.15 | 5.51 | 5.08 | 5.16 |
| 9 | 5.14 | 5.11 | 5.17 | 5.01 | 5.28 | 5.19 | 5.07 | 5.24 |
| 10 | 5.13 | 5.17 | 5.22 | 5.02 | 5.19 | 5.58 | 5.11 | 5.05 |
| 11 | 5.12 | 5.22 | 5.12 | 5.02 | 4.99 | 5.51 | 5.14 | 5.30 |
| 12 | 5.11 | 5.07 | 5.14 | 5.03 | 4.96 | 5.85 | 5.15 | 5.30 |
| 13 | 5.10 | 5.13 | 5.12 | 5.03 | 4.90 | 5.44 | 5.11 | 5.25 |
| 14 | 5.09 | 5.88 | 5.22 | 5.00 | 5.25 | 5.47 | 5.02 | 5.35 |
| 15 | 5.09 | 5.13 | 5.04 | 4.95 | 5.21 | 5.59 | 5.07 | 4.95 |
| 16 | 5.58 | 5.59 | 5.12 | 5.05 | 5.18 | 5.65 | 5.22 | 4.95 |
| 17 | 5.07 | 5.54 | 5.12 | 5.05 | 5.27 | 5.57 | 5.26 | 5.28 |
| 18 | 5.31 | 5.09 | 5.10 | 4.82 | 5.29 | 5.34 | 5.30 | 4.92 |
| 19 | 5.05 | 5.66 | 5.15 | 5.05 | 4.90 | 5.60 | 4.95 | 5.22 |
| 20 | 5.05 | 5.18 | 5.22 | 4.97 | 5.00 | 5.46 | 5.05 | 5.28 |
| Average | 5.16 | 5.27 | 5.15 | 5.00 | 5.08 | 5.50 | 5.11 | 5.20 |

**CLAY**

| Sample No | Clay | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUCHING | SERIAN | BETONG | SRI AMAN | SARIKEI | SIBU | BINTULU | MIRI |
| 1 | 20.95 | 22.99 | 36.55 | 45.68 | 26.99 | 34.50 | 30.00 | 29.99 |
| 2 | 20.99 | 22.99 | 36.15 | 45.66 | 26.99 | 34.50 | 30.00 | 29.99 |
| 3 | 20.98 | 22.99 | 36.00 | 45.95 | 27.01 | 34.55 | 29.95 | 29.99 |
| 4 | 21.15 | 23.04 | 37.15 | 45.66 | 27.10 | 34.45 | 29.95 | 30.01 |
| 5 | 21.11 | 23.75 | 37.65 | 45.66 | 27.10 | 34.20 | 29.50 | 30.51 |
| 6 | 21.60 | 23.65 | 37.95 | 45.66 | 27.10 | 34.20 | 30.00 | 32.01 |
| 7 | 21.17 | 23.66 | 36.00 | 45.85 | 27.15 | 34.25 | 29.85 | 30.45 |
| 8 | 21.21 | 23.45 | 36.45 | 45.85 | 27.10 | 34.20 | 29.87 | 30.18 |
| 9 | 21.25 | 24.00 | 36.55 | 45.75 | 26.99 | 34.20 | 29.95 | 31.87 |
| 10 | 21.28 | 24.00 | 36.95 | 45.55 | 26.99 | 34.20 | 29.95 | 30.40 |
| 11 | 21.32 | 23.95 | 36.92 | 45.50 | 27.30 | 34.45 | 30.05 | 29.80 |
| 12 | 21.25 | 23.95 | 36.44 | 45.55 | 27.22 | 34.05 | 30.00 | 29.80 |
| 13 | 21.33 | 22.99 | 35.95 | 45.65 | 27.10 | 34.05 | 29.91 | 31.00 |
| 14 | 21.20 | 23.45 | 35.90 | 45.55 | 27.10 | 34.45 | 29.93 | 31.00 |
| 15 | 21.22 | 23.66 | 36.05 | 45.56 | 27.10 | 34.45 | 29.99 | 30.50 |
| 16 | 21.59 | 23.48 | 36.44 | 45.65 | 27.31 | 34.15 | 29.97 | 29.50 |
| 17 | 21.22 | 24.08 | 36.25 | 45.68 | 27.05 | 34.15 | 29.97 | 29.50 |
| 18 | 21.34 | 24.22 | 35.95 | 45.65 | 27.05 | 34.15 | 30.00 | 31.50 |
| 19 | 21.58 | 24.55 | 35.85 | 45.69 | 27.05 | 33.20 | 30.00 | 31.50 |
| 20 | 21.26 | 24.35 | 35.85 | 45.65 | 27.00 | 34.25 | 29.96 | 31.50 |
| Average | 21.25 | 23.66 | 36.45 | 45.67 | 27.09 | 34.23 | 29.94 | 30.55 |

**SLIT**

| Sample No | Slit | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUCHING | SERIAN | BETONG | SRI AMAN | SARIKEI | SIBU | BINTULU | MIRI |
| 1 | 32.01 | 38.90 | 43.00 | 37.70 | 32.40 | 41.99 | 39.06 | 40.01 |
| 2 | 31.99 | 38.90 | 43.00 | 38.50 | 31.05 | 40.96 | 38.56 | 40.25 |
| 3 | 32.00 | 38.90 | 43.00 | 37.70 | 29.50 | 42.66 | 38.96 | 40.25 |
| 4 | 32.01 | 39.10 | 41.50 | 39.45 | 29.50 | 43.27 | 39.01 | 40.26 |
| 5 | 32.01 | 39.10 | 41.50 | 39.45 | 30.00 | 43.34 | 38.25 | 40.96 |
| 6 | 32.01 | 39.00 | 42.00 | 37.65 | 30.45 | 43.40 | 38.96 | 40.36 |
| 7 | 32.00 | 38.90 | 42.00 | 37.25 | 30.30 | 43.05 | 38.05 | 40.37 |
| 8 | 32.00 | 38.80 | 41.50 | 37.25 | 31.30 | 43.30 | 39.00 | 40.26 |
| 9 | 32.00 | 39.40 | 41.50 | 37.70 | 34.00 | 43.56 | 38.96 | 40.25 |
| 10 | 32.01 | 39.40 | 41.90 | 37.70 | 32.40 | 45.40 | 39.67 | 40.59 |
| 11 | 31.99 | 39.40 | 41.80 | 38.50 | 30.40 | 43.59 | 39.00 | 40.00 |
| 12 | 31.99 | 39.00 | 41.50 | 37.50 | 31.70 | 43.67 | 39.01 | 39.96 |
| 13 | 31.99 | 39.00 | 41.70 | 37.65 | 32.05 | 40.60 | 38.55 | 39.46 |
| 14 | 32.00 | 39.40 | 42.00 | 38.00 | 29.95 | 42.00 | 38.50 | 39.96 |
| 15 | 32.00 | 39.45 | 42.00 | 38.00 | 30.40 | 41.11 | 38.05 | 39.66 |
| 16 | 31.99 | 39.45 | 42.15 | 39.50 | 30.50 | 42.00 | 38.15 | 39.46 |
| 17 | 31.99 | 39.40 | 42.15 | 37.45 | 30.10 | 43.66 | 38.45 | 39.67 |
| 18 | 31.99 | 39.50 | 41.85 | 38.05 | 30.55 | 44.00 | 38.45 | 39.66 |
| 19 | 31.99 | 39.50 | 42.00 | 37.50 | 31.45 | 43.96 | 38.66 | 39.48 |
| 20 | 32.03 | 39.50 | 41.95 | 37.50 | 32.00 | 42.52 | 34.72 | 39.18 |
| Average | 32.00 | 39.20 | 42.00 | 38.00 | 31.00 | 42.90 | 38.50 | 40.00 |

**SAND**

| Sample No | Sand | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUCHING | SERIAN | BETONG | SRI AMAN | SARIKEI | SIBU | BINTULU | MIRI |
| 1 | 55.89 | 52.33 | 68.77 | 60.70 | 69.63 | 66.92 | 60.21 | 59.85 |
| 2 | 58.46 | 55.66 | 62.85 | 60.25 | 69.77 | 66.45 | 60.13 | 60.00 |
| 3 | 55.56 | 56.62 | 67.69 | 60.37 | 66.63 | 66.22 | 60.01 | 58.45 |
| 4 | 58.26 | 50.01 | 61.75 | 60.59 | 69.96 | 68.00 | 60.03 | 58.81 |
| 5 | 54.45 | 54.45 | 64.45 | 61.25 | 66.63 | 65.55 | 60.07 | 58.65 |
| 6 | 54.59 | 57.24 | 60.89 | 61.59 | 68.77 | 68.00 | 60.22 | 58.66 |
| 7 | 54.12 | 54.45 | 60.34 | 64.96 | 68.96 | 66.75 | 60.04 | 61.13 |
| 8 | 54.63 | 50.00 | 54.47 | 61.27 | 67.79 | 66.45 | 60.11 | 61.59 |
| 9 | 54.23 | 57.24 | 53.95 | 62.34 | 69.63 | 66.90 | 61.02 | 60.61 |
| 10 | 54.24 | 54.45 | 53.44 | 62.33 | 68.77 | 66.65 | 60.01 | 58.14 |
| 11 | 54.12 | 50.00 | 52.92 | 63.33 | 66.63 | 69.00 | 60.03 | 60.12 |
| 12 | 54.30 | 57.24 | 62.40 | 62.33 | 68.77 | 66.32 | 60.01 | 58.47 |
| 13 | 57.24 | 54.45 | 61.53 | 61.25 | 66.63 | 66.05 | 60.06 | 58.66 |
| 14 | 55.96 | 50.00 | 61.37 | 61.25 | 68.77 | 66.45 | 60.02 | 60.95 |
| 15 | 58.97 | 50.00 | 60.85 | 61.70 | 69.96 | 68.97 | 60.07 | 60.00 |
| 16 | 55.26 | 57.24 | 68.58 | 60.25 | 69.96 | 65.99 | 60.03 | 60.00 |
| 17 | 57.96 | 52.25 | 66.63 | 60.23 | 68.77 | 65.75 | 60.32 | 60.55 |
| 18 | 55.95 | 52.26 | 63.33 | 62.66 | 69.63 | 68.97 | 60.03 | 60.90 |
| 19 | 57.66 | 55.15 | 63.25 | 62.66 | 68.77 | 65.45 | 60.09 | 60.78 |
| 20 | 55.20 | 54.99 | 58.16 | 62.71 | 69.63 | 67.18 | 61.07 | 61.52 |
| Average | 55.85 | 53.80 | 61.38 | 61.70 | 68.70 | 66.90 | 60.18 | 59.89 |

## SOIL ORGANIC MATERIAL (SOM)

| Sample No | SOM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUCHING | SERIAN | BETONG | SRI AMAN | SARIKEI | SIBU | BINTULU | MIRI |
| 1 | 19.00 | 20.03 | 23.56 | 29.93 | 19.01 | 25.57 | 23.65 | 21.00 |
| 2 | 19.96 | 20.55 | 23.63 | 25.66 | 18.63 | 25.67 | 25.50 | 21.65 |
| 3 | 20.15 | 20.03 | 23.66 | 26.66 | 18.75 | 26.76 | 24.90 | 21.67 |
| 4 | 20.00 | 20.45 | 23.47 | 25.15 | 19.01 | 23.75 | 25.50 | 21.95 |
| 5 | 18.83 | 20.55 | 23.93 | 30.00 | 18.66 | 23.65 | 25.50 | 21.34 |
| 6 | 19.00 | 20.35 | 23.96 | 30.15 | 19.01 | 23.66 | 24.60 | 21.35 |
| 7 | 18.33 | 20.03 | 23.96 | 30.55 | 19.01 | 26.08 | 24.85 | 23.11 |
| 8 | 18.37 | 20.55 | 24.00 | 30.15 | 19.54 | 26.22 | 25.50 | 23.45 |
| 9 | 19.95 | 20.03 | 23.65 | 30.15 | 18.63 | 25.66 | 24.85 | 23.78 |
| 10 | 18.25 | 20.55 | 24.66 | 30.15 | 19.01 | 26.45 | 24.85 | 21.85 |
| 11 | 19.66 | 20.45 | 23.56 | 30.15 | 18.65 | 26.14 | 24.86 | 21.54 |
| 12 | 19.75 | 20.55 | 22.95 | 30.15 | 18.65 | 27.00 | 23.65 | 21.67 |
| 13 | 19.78 | 20.45 | 22.92 | 30.15 | 18.63 | 26.83 | 24.90 | 20.05 |
| 14 | 18.11 | 20.55 | 23.66 | 31.25 | 18.04 | 26.49 | 24.88 | 21.23 |
| 15 | 19.95 | 20.03 | 23.46 | 30.15 | 18.63 | 26.66 | 25.26 | 21.15 |
| 16 | 20.00 | 20.45 | 22.67 | 30.34 | 19.01 | 26.49 | 25.50 | 21.15 |
| 17 | 19.49 | 20.03 | 23.65 | 30.37 | 17.55 | 25.56 | 24.85 | 20.94 |
| 18 | 19.45 | 20.45 | 23.96 | 30.15 | 18.63 | 25.66 | 23.65 | 20.15 |
| 19 | 19.67 | 20.54 | 23.99 | 30.15 | 19.01 | 25.91 | 25.50 | 20.25 |
| 20 | 19.84 | 20.03 | 23.75 | 30.15 | 19.01 | 25.41 | 24.85 | 21.55 |
| Average | 19.37 | 20.33 | 23.65 | 29.58 | 18.75 | 25.78 | 24.88 | 21.54 |

**APPENDIX 6**

# HEAVY METALS IN PEPPER PLANT PARTS

## KUCHING

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Kuching | 1 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.003 | 0.007 | 0.005 | 0.000 | 0.011 | 0.010 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.003 | 0.007 | 0.005 | 0.000 | 0.011 | 0.010 |
| | 3 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.004 | 0.000 | 0.011 | 0.010 |
| | 4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.005 | 0.000 | 0.011 | 0.006 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.004 | 0.000 | 0.012 | 0.012 |
| | 6 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.009 | 0.000 | 0.012 | 0.012 |
| | 7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.002 | 0.007 | 0.005 | 0.000 | 0.012 | 0.012 |
| | 8 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.004 | 0.000 | 0.012 | 0.012 |
| | 9 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.003 | 0.007 | 0.005 | 0.000 | 0.011 | 0.011 |
| | 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.003 | 0.007 | 0.003 | 0.000 | 0.012 | 0.012 |
| | 11 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.001 | 0.002 | 0.007 | 0.006 | 0.000 | 0.011 | 0.011 |
| | 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.001 | 0.001 | 0.007 | 0.005 | 0.000 | 0.012 | 0.012 |
| | 13 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.006 | 0.000 | 0.013 | 0.013 |
| | 14 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.002 | 0.007 | 0.005 | 0.000 | 0.012 | 0.012 |
| | 15 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.000 | 0.001 | 0.007 | 0.005 | 0.000 | 0.011 | 0.011 |
| | 16 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.007 | 0.006 | 0.000 | 0.011 | 0.011 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.000 | 0.001 | 0.007 | 0.009 | 0.000 | 0.011 | 0.011 |
| | 18 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.007 | 0.005 | 0.000 | 0.012 | 0.020 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.007 | 0.008 | 0.000 | 0.012 | 0.012 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.001 | 0.000 | 0.001 | 0.007 | 0.005 | 0.000 | 0.012 | 0.012 |
| Average | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.002 | 0.007 | 0.006 | 0.000 | 0.011 | 0.011 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.002 |

## HEAVY METALS IN PEPPER PLANT PARTS

## SERIAN

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Serian | 1 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.004 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.006 | 0.000 | 0.000 | 0.001 | 0.002 | 0.006 | 0.006 | 0.000 | 0.009 | 0.005 |
| | 3 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.006 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.005 | 0.000 | 0.011 | 0.005 |
| | 4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.006 | 0.000 | 0.012 | 0.005 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.001 | 0.002 | 0.005 | 0.005 | 0.000 | 0.010 | 0.000 |
| | 6 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.002 | 0.005 | 0.006 | 0.000 | 0.010 | 0.000 |
| | 7 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.010 | 0.000 |
| | 8 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.010 | 0.005 |
| | 9 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.010 | 0.000 |
| | 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.006 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.011 | 0.000 |
| | 11 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.010 | 0.000 |
| | 12 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.008 | 0.000 | 0.010 | 0.000 |
| | 13 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.002 | 0.005 | 0.006 | 0.000 | 0.012 | 0.001 |
| | 14 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.011 | 0.001 |
| | 15 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.011 | 0.001 |
| | 16 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.002 | 0.006 | 0.007 | 0.000 | 0.012 | 0.000 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.011 | 0.000 |
| | 18 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.002 | 0.005 | 0.006 | 0.000 | 0.011 | 0.000 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.000 | 0.000 | 0.001 | 0.002 | 0.006 | 0.006 | 0.000 | 0.012 | 0.000 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.000 | 0.001 | 0.002 | 0.005 | 0.006 | 0.000 | 0.012 | 0.006 |
| Average | | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.011 | 0.002 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.002 |

## HEAVY METALS IN PEPPER PLANT PARTS

## BETONG

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Betong | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.005 | 0.000 | 0.003 | 0.000 | 0.001 | 0.011 | 0.009 | 0.000 | 0.010 | 0.009 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.002 | 0.001 | 0.011 | 0.008 | 0.000 | 0.010 | 0.010 |
| | 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.001 | 0.010 | 0.008 | 0.000 | 0.010 | 0.009 |
| | 4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.004 | 0.000 | 0.001 | 0.008 | 0.008 | 0.000 | 0.010 | 0.009 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.001 | 0.008 | 0.007 | 0.000 | 0.010 | 0.009 |
| | 6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.003 | 0.001 | 0.002 | 0.008 | 0.008 | 0.000 | 0.010 | 0.009 |
| | 7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.004 | 0.000 | 0.001 | 0.014 | 0.008 | 0.000 | 0.019 | 0.009 |
| | 8 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.006 | 0.000 | 0.003 | 0.000 | 0.002 | 0.014 | 0.009 | 0.000 | 0.010 | 0.010 |
| | 9 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.001 | 0.011 | 0.009 | 0.000 | 0.010 | 0.010 |
| | 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.003 | 0.000 | 0.001 | 0.009 | 0.009 | 0.000 | 0.011 | 0.007 |
| | 11 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.003 | 0.001 | 0.002 | 0.008 | 0.010 | 0.000 | 0.010 | 0.009 |
| | 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.006 | 0.000 | 0.004 | 0.001 | 0.002 | 0.008 | 0.010 | 0.000 | 0.011 | 0.009 |
| | 13 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.005 | 0.000 | 0.003 | 0.000 | 0.002 | 0.008 | 0.010 | 0.000 | 0.010 | 0.010 |
| | 14 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.002 | 0.001 | 0.008 | 0.010 | 0.000 | 0.010 | 0.011 |
| | 15 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.006 | 0.000 | 0.003 | 0.000 | 0.001 | 0.014 | 0.010 | 0.000 | 0.010 | 0.010 |
| | 16 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.005 | 0.000 | 0.003 | 0.001 | 0.001 | 0.011 | 0.011 | 0.000 | 0.010 | 0.009 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.002 | 0.008 | 0.009 | 0.000 | 0.010 | 0.009 |
| | 18 | 0.000 | 0.000 | 0.000 | 0.019 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.001 | 0.011 | 0.009 | 0.000 | 0.010 | 0.010 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.003 | 0.000 | 0.002 | 0.008 | 0.009 | 0.000 | 0.010 | 0.009 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.005 | 0.000 | 0.004 | 0.000 | 0.001 | 0.008 | 0.008 | 0.000 | 0.010 | 0.010 |
| Average | | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.006 | 0.000 | 0.004 | 0.000 | 0.001 | 0.010 | 0.009 | 0.000 | 0.011 | 0.009 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | 0.001 | 0.000 | 0.002 | 0.001 |

# HEAVY METALS IN PEPPER PLANT PARTS

## SRI AMAN

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Sri Aman | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.002 | 0.006 | 0.006 | 0.000 | 0.010 | 0.016 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.006 | 0.007 | 0.000 | 0.010 | 0.016 |
| | 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.002 | 0.005 | 0.007 | 0.000 | 0.010 | 0.016 |
| | 4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.007 | 0.000 | 0.010 | 0.016 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.010 | 0.016 |
| | 6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.002 | 0.005 | 0.007 | 0.000 | 0.010 | 0.015 |
| | 7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.010 | 0.015 |
| | 8 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.006 | 0.008 | 0.000 | 0.010 | 0.014 |
| | 9 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.008 | 0.000 | 0.010 | 0.010 |
| | 10 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.007 | 0.000 | 0.010 | 0.010 |
| | 11 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.017 |
| | 12 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.016 |
| | 13 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.016 |
| | 14 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.012 |
| | 15 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.012 |
| | 16 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.012 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.014 |
| | 18 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.008 | 0.000 | 0.010 | 0.013 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.020 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.001 | 0.002 | 0.005 | 0.009 | 0.000 | 0.010 | 0.013 |
| Average | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.005 | 0.006 | 0.000 | 0.010 | 0.014 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.002 |

# HEAVY METALS IN PEPPER PLANT PARTS

## SARIKEI

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Sarikei | 1 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.001 | 0.000 | 0.001 | 0.001 | 0.008 | 0.016 | 0.005 | 0.000 | 0.016 | 0.016 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.001 | 0.000 | 0.001 | 0.002 | 0.008 | 0.015 | 0.006 | 0.000 | 0.010 | 0.015 |
| | 3 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.011 | 0.006 | 0.000 | 0.017 | 0.016 |
| | 4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.006 | 0.000 | 0.016 | 0.015 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.002 | 0.000 | 0.020 | 0.016 |
| | 6 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.001 | 0.000 | 0.009 | 0.015 |
| | 7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.020 | 0.001 | 0.000 | 0.009 | 0.018 |
| | 8 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.003 | 0.002 | 0.002 | 0.000 | 0.001 | 0.001 | 0.007 | 0.021 | 0.005 | 0.000 | 0.010 | 0.015 |
| | 9 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.042 | 0.005 | 0.000 | 0.010 | 0.015 |
| | 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.005 | 0.000 | 0.009 | 0.013 |
| | 11 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.006 | 0.012 | 0.006 | 0.000 | 0.009 | 0.016 |
| | 12 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.005 | 0.000 | 0.010 | 0.016 |
| | 13 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.005 | 0.000 | 0.010 | 0.016 |
| | 14 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.002 | 0.000 | 0.001 | 0.001 | 0.008 | 0.012 | 0.005 | 0.000 | 0.009 | 0.015 |
| | 15 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.007 | 0.020 | 0.005 | 0.000 | 0.010 | 0.016 |
| | 16 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.002 | 0.009 | 0.000 | 0.010 | 0.014 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.007 | 0.012 | 0.009 | 0.000 | 0.009 | 0.016 |
| | 18 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.008 | 0.008 | 0.005 | 0.000 | 0.010 | 0.016 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.000 | 0.001 | 0.000 | 0.008 | 0.011 | 0.002 | 0.000 | 0.010 | 0.015 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.000 | 0.001 | 0.001 | 0.008 | 0.012 | 0.004 | 0.000 | 0.010 | 0.015 |
| Average | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.001 | 0.000 | 0.001 | 0.000 | 0.007 | 0.014 | 0.005 | 0.000 | 0.011 | 0.015 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.008 | 0.002 | 0.000 | 0.003 | 0.001 |

# HEAVY METALS IN PEPPER PLANT PARTS

## SIBU

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Sibu | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.007 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.014 |
| | 2 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.017 |
| | 3 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.004 | 0.000 | 0.010 | 0.013 |
| | 4 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.001 | 0.000 | 0.001 | 0.008 | 0.003 | 0.000 | 0.009 | 0.012 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.009 | 0.012 |
| | 6 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.012 |
| | 7 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.006 | 0.006 | 0.000 | 0.010 | 0.013 |
| | 8 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.001 | 0.000 | 0.001 | 0.004 | 0.006 | 0.000 | 0.010 | 0.013 |
| | 9 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.013 |
| | 10 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.010 | 0.013 |
| | 11 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.002 | 0.000 | 0.010 | 0.011 |
| | 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.001 | 0.000 | 0.001 | 0.004 | 0.003 | 0.000 | 0.009 | 0.012 |
| | 13 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.003 | 0.000 | 0.010 | 0.018 |
| | 14 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.007 | 0.005 | 0.000 | 0.010 | 0.013 |
| | 15 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.010 | 0.003 | 0.000 | 0.010 | 0.019 |
| | 16 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.007 | 0.000 | 0.010 | 0.018 |
| | 17 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.000 | 0.000 | 0.001 | 0.006 | 0.004 | 0.000 | 0.010 | 0.011 |
| | 18 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.008 | 0.000 | 0.011 | 0.012 |
| | 19 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.000 | 0.000 | 0.002 | 0.005 | 0.005 | 0.000 | 0.011 | 0.013 |
| | 20 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.001 | 0.000 | 0.001 | 0.006 | 0.005 | 0.000 | 0.011 | 0.011 |
| Average | | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 | 0.000 | 0.001 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.010 | 0.013 |
| STDEV | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.002 |

## HEAVY METALS IN PEPPER PLANT PARTS

## BINTULU

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Bintulu | 1 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.010 | 0.001 | 0.002 | 0.007 | 0.006 | 0.000 | 0.009 | 0.012 |
| | 2 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.009 | 0.001 | 0.002 | 0.006 | 0.004 | 0.000 | 0.009 | 0.013 |
| | 3 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.003 | 0.000 | 0.009 | 0.001 | 0.002 | 0.006 | 0.004 | 0.000 | 0.009 | 0.012 |
| | 4 | 0.000 | 0.000 | 0.002 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.010 | 0.000 | 0.001 | 0.003 | 0.004 | 0.000 | 0.009 | 0.013 |
| | 5 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.004 | 0.000 | 0.010 | 0.000 | 0.002 | 0.005 | 0.003 | 0.000 | 0.009 | 0.012 |
| | 6 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.009 | 0.001 | 0.002 | 0.004 | 0.004 | 0.000 | 0.009 | 0.015 |
| | 7 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.009 | 0.011 |
| | 8 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.004 | 0.003 | 0.000 | 0.009 | 0.017 |
| | 9 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.002 | 0.000 | 0.009 | 0.000 | 0.001 | 0.009 | 0.005 | 0.000 | 0.009 | 0.018 |
| | 10 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.003 | 0.000 | 0.009 | 0.000 | 0.001 | 0.005 | 0.004 | 0.000 | 0.009 | 0.018 |
| | 11 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.003 | 0.000 | 0.009 | 0.000 | 0.001 | 0.003 | 0.004 | 0.000 | 0.009 | 0.017 |
| | 12 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.008 | 0.000 | 0.002 | 0.003 | 0.003 | 0.000 | 0.009 | 0.016 |
| | 13 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.002 | 0.007 | 0.004 | 0.000 | 0.009 | 0.017 |
| | 14 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.009 | 0.000 | 0.002 | 0.001 | 0.004 | 0.000 | 0.009 | 0.018 |
| | 15 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.002 | 0.001 | 0.003 | 0.000 | 0.009 | 0.019 |
| | 16 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.007 | 0.005 | 0.000 | 0.009 | 0.019 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.002 | 0.003 | 0.005 | 0.000 | 0.010 | 0.020 |
| | 18 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.001 | 0.006 | 0.000 | 0.010 | 0.020 |
| | 19 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.004 | 0.000 | 0.009 | 0.001 | 0.001 | 0.002 | 0.003 | 0.000 | 0.010 | 0.015 |
| | 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.000 | 0.000 | 0.000 | 0.002 | 0.002 | 0.004 | 0.000 | 0.010 | 0.001 | 0.002 | 0.003 | 0.003 | 0.000 | 0.009 | 0.016 |
| Average | | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.004 | 0.004 | 0.000 | 0.009 | 0.016 |
| STDEV | | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.000 | 0.000 | 0.003 |

# HEAVY METALS IN PEPPER PLANT PARTS

## MIRI

| | | Berries | | | | | | Leaves | | | | | | Stem | | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Farm | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Miri | 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.005 | 0.000 | 0.010 | 0.000 | 0.002 | 0.005 | 0.005 | 0.000 | 0.020 | 0.005 |
| | 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.011 | 0.001 | 0.002 | 0.005 | 0.006 | 0.000 | 0.020 | 0.005 |
| | 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.004 | 0.000 | 0.012 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.020 | 0.005 |
| | 4 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.011 | 0.000 | 0.001 | 0.004 | 0.006 | 0.000 | 0.019 | 0.005 |
| | 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.010 | 0.001 | 0.001 | 0.004 | 0.002 | 0.000 | 0.019 | 0.004 |
| | 6 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.009 | 0.001 | 0.001 | 0.005 | 0.006 | 0.000 | 0.017 | 0.005 |
| | 7 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.005 | 0.000 | 0.009 | 0.001 | 0.001 | 0.005 | 0.006 | 0.000 | 0.020 | 0.004 |
| | 8 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.004 | 0.000 | 0.010 | 0.001 | 0.001 | 0.005 | 0.006 | 0.000 | 0.020 | 0.004 |
| | 9 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.020 | 0.004 |
| | 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.006 | 0.000 | 0.011 | 0.000 | 0.001 | 0.005 | 0.006 | 0.000 | 0.019 | 0.004 |
| | 11 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.005 | 0.000 | 0.008 | 0.000 | 0.001 | 0.004 | 0.005 | 0.000 | 0.020 | 0.004 |
| | 12 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.007 | 0.000 | 0.002 | 0.004 | 0.005 | 0.000 | 0.013 | 0.004 |
| | 13 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.008 | 0.000 | 0.002 | 0.002 | 0.004 | 0.000 | 0.010 | 0.004 |
| | 14 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.003 | 0.000 | 0.008 | 0.000 | 0.001 | 0.004 | 0.005 | 0.000 | 0.010 | 0.005 |
| | 15 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.003 | 0.000 | 0.008 | 0.000 | 0.001 | 0.009 | 0.005 | 0.000 | 0.010 | 0.004 |
| | 16 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.011 | 0.000 | 0.001 | 0.009 | 0.003 | 0.000 | 0.010 | 0.005 |
| | 17 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.008 | 0.000 | 0.001 | 0.014 | 0.000 | 0.000 | 0.010 | 0.004 |
| | 18 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.009 | 0.000 | 0.002 | 0.002 | 0.006 | 0.000 | 0.010 | 0.004 |
| | 19 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.008 | 0.000 | 0.002 | 0.003 | 0.004 | 0.000 | 0.010 | 0.004 |
| | 20 | 0.000 | 0.000 | 0.002 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.000 | 0.010 | 0.000 | 0.002 | 0.005 | 0.007 | 0.000 | 0.010 | 0.004 |
| Average | | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.004 | 0.000 | 0.009 | 0.000 | 0.001 | 0.005 | 0.005 | 0.000 | 0.015 | 0.004 |
| STDEV | | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.003 | 0.001 | 0.000 | 0.005 | 0.001 |

**APPENDIX 8**

## ENRICHMENT AND TRANSLOCATION FACTOR

| | Enrichment Factor (EF) | | | | | |
|---|---|---|---|---|---|---|
| | As | Cd | Cr | Hg | Pb | Sb |
| Kuching | 0.000 | 0.000 | 0.038 | 0.000 | 0.000 | 0.000 |
| Serian | 0.000 | 0.000 | 0.093 | 0.000 | 0.000 | 0.000 |
| Sri Aman | 0.000 | 0.000 | 0.059 | 0.000 | 0.000 | 0.000 |
| Betong | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 | 0.000 |
| Sarikei | 0.000 | 0.000 | 0.043 | 0.000 | 0.000 | 0.000 |
| Sibu | 0.000 | 0.000 | 0.174 | 0.000 | 0.000 | 0.000 |
| Bintulu | 0.000 | 0.000 | 0.237 | 0.000 | 0.000 | 0.000 |
| Miri | 0.000 | 0.000 | 0.166 | 0.000 | 0.000 | 0.000 |
| AVERAGE | 0.000 | 0.000 | 0.102 | 0.000 | 0.000 | 0.000 |

## TRANSLOCATION FACTOR

| AREA | Stem / Soil | | | | | | Leaves / Stem | | | | | | Berries / Leaves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb | As | Cd | Cr | Hg | Pb | Sb |
| Kuching | 0.000 | 0.000 | 0.267 | NA | 0.000 | 0.000 | NA | NA | 0.447 | NA | NA | NA | NA | NA | 0.315 | NA | NA | NA |
| Serian | 0.000 | 0.000 | 0.794 | NA | 0.000 | 0.000 | NA | NA | 0.306 | NA | NA | NA | NA | NA | 0.382 | NA | NA | NA |
| Sri Aman | 0.000 | 0.000 | 0.094 | NA | 0.000 | 0.000 | NA | NA | 0.214 | NA | NA | NA | NA | NA | 2.909 | NA | NA | NA |
| Betong | 0.000 | 0.000 | 0.624 | NA | 0.000 | 0.000 | NA | NA | 0.053 | NA | NA | NA | NA | NA | 0.319 | NA | NA | NA |
| Sarikei | 0.000 | 0.000 | 0.304 | NA | 0.000 | 0.000 | NA | NA | 0.646 | NA | NA | NA | NA | NA | 0.219 | NA | NA | NA |
| Sibu | 0.000 | 0.000 | 0.739 | NA | 0.000 | 0.000 | NA | NA | 0.987 | NA | NA | NA | NA | NA | 0.238 | NA | NA | NA |
| Bintulu | 0.000 | 0.000 | 0.857 | NA | 0.000 | 0.000 | NA | NA | 0.345 | NA | NA | NA | NA | NA | 0.801 | NA | NA | NA |
| Miri | 0.000 | 0.000 | 0.854 | NA | 0.000 | 0.000 | NA | NA | 0.243 | NA | NA | NA | NA | NA | 0.799 | NA | NA | NA |
| Average | 0.000 | 0.000 | 0.567 | NA | 0.000 | 0.000 | NA | NA | 0.405 | NA | NA | NA | NA | NA | 0.748 | NA | NA | NA |

**APPENDIX 9**

## SAFETY LEVEL ASSESSMENT

### ADD

| Areas | As | Cd | Cr | Hg | Pb | Sb |
|---|---|---|---|---|---|---|
| **Kuching** | 0.00E+00 | 0.00E+00 | 2.07E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Serian** | 0.00E+00 | 0.00E+00 | 5.58E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Betong** | 0.00E+00 | 0.00E+00 | 3.57E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Sri Aman** | 0.00E+00 | 0.00E+00 | 9.39E-05 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Sarikei** | 0.00E+00 | 0.00E+00 | 2.07E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Sibu** | 0.00E+00 | 0.00E+00 | 7.89E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Bintulu** | 0.00E+00 | 0.00E+00 | 9.82E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Miri** | 0.00E+00 | 0.00E+00 | 7.99E-04 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **Average** | **0.000E+00** | **0.000E+00** | **4.991E-04** | **0.000E+00** | **0.000E+00** | **0.000E+00** |
| **ADD** | **0.000E+00** | **0.000E+00** | **2.788E-05** | **0.000E+00** | **0.000E+00** | **0.000E+00** |

### HQ

| Areas | As | Cd | Cr | Hg | Pb | Sb |
|---|---|---|---|---|---|---|
| ADD | 0.00E+00 | 0.00E+00 | 2.79E-05 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| RFD | 3.00E-04 | 1.00E-03 | 1.50E+00 | 1.60E-04 | 3.60E-03 | 4.00E-04 |
| **HQ** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** |

### HI

| Areas | HI |
|---|---|
| **Kuching** | **2.93E-07** |
| **Serian** | **0.00E+00** |
| **Betong** | **6.45E-07** |
| **Sri Aman** | **0.00E+00** |
| **Sarikei** | **2.46E-06** |
| **Sibu** | **0.00E+00** |
| **Bintulu** | **3.07E-06** |
| **Miri** | **0.00E+00** |

### LCR

| Areas | As | Cd | Cr | Hg | Pb | Sb |
|---|---|---|---|---|---|---|
| ADD | 0.00E+00 | 0.00E+00 | 2.79E-05 | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| CSF | 1.50E+00 | 1.50E+01 | 5.00E-01 | NA | 8.50E-03 | 5.00E-01 |
| **LCR** | **0.000E+00** | **0.000E+00** | **1.394E-05** | **NA** | **0.000E+00** | **0.000E+00** |

### CRT

| Areas | Kuching | Serian | Betong | Sri Aman | Sarikei | Sibu | Bintulu | Miri |
|---|---|---|---|---|---|---|---|---|
| CRt | 5.78E-06 | 1.56 E-05 | 9.98 E-06 | 2.62 E-06 | 5.78 E-06 | 2.20 E-05 | 2.74 E-05 | 2.23 E-05 |