UNIVERSITI PUTRA MALAYSIA

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UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2023/2024

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BY

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Thesis submitted in fulfilment of the requirement for the degree of Bachelor of Science in Environmental and Occupational Health with Honours from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ACKNOWLEDGEMENTS

This research project would not have been completed without the assistance from several parties. Upon completion of this research project, I would like to express my highest gratitude to the parties that had provided me with assistance and encouragement during the research.

Firstly, I would like to convey the highest appreciation to my supervisor, Dr. Vivien How for her advice, supervision, encouragement and guidance throughout this research project. Without her supervision and valuable comments, this project may not have been completed. I am deeply grateful for the time and energy dedicated to helping complete this research.

Special thanks to Baba Eco Group for funding for the research. Their commitment to environmental sustainability and dedication to promoting scientific exploration have been instrumental in the successful execution of this study. Besides, I would like to thank my co-supervisor, Dr. Zurahanim Fasha Anual and Medical Laboratory Technologist (MLT), Miss Cathrinena Robun from the Institute for Medical Research, National Institutes of Health for the technical assistance rendered and advice given. I also would like to express my gratitude to all the respondents who volunteered to participate in this study and for their cooperation throughout the data collection process.

Furthermore, I would like to thank my parents for always providing assistance, support, reassurance and motivation during the progress of this research. Without their support and encouragement, I would not have the motivation to continue this research. Moreover, without their financial and emotional support, this research would not have been conducted as smoothly.

Finally, I would like to express my gratitude to my peers and team mates who had aided me during the research. The valuable insights and suggestions given by them were truly valuable and indeed had aided me tremendously in refining and completing my research. I am deeply thankful to all parties and whoever contributed to the success of this research. A million thanks to everyone that had assisted me along the way.

ABSTRACT

TRACE ELEMENTS IN THE NAILS OF FARMERS EXPOSED TO SYNTHETIC PESTICIDES AND CHEMICAL FERTILIZERS IN WEST COAST OF PENINSULAR MALAYSIA

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Introduction: The widespread use of synthetic pesticides and chemical fertilizers in agriculture, which helps to reduce the adverse impact of weeds and pests while enhancing crop yields is attributed to the advancement of agricultural production. This is particularly concerning as farmers may be exposed to these chemicals while handling crops or tending to animals, leading to potential health risks. The use of synthetic pesticides and chemical fertilizers in both environmental and occupational settings for an extended period has been shown to result in deficiency or elevated levels of these elements. **Objective**: This study aims to compare the levels of trace elements zinc (Zn), manganese (Mn), lead (Pb), and ammonia (NH_3) in nail samples among conventional and organic farmers. **Methodology**: Respondents were first interviewed face-to-face using questionnaires to gather socio-demographic and occupational background information. Nail samples were collected from 109 respondents in six areas of Peninsular Malaysia for trace element analyses using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Health risk was assessed based on the usage of synthetic pesticides and chemical fertilizers containing trace elements (Zn, Mn, Pb, NH_3). **Result & Discussion:** It was observed that the levels of Zn, Mn and NH_3 in the nail samples were significantly higher in conventional farmers compared to organic farmers ($p<0.05$). There were significant associations between the health risks of using synthetic pesticides and chemical fertilizers containing Zn, Mn and NH_3 with the level of these elements among respondents ($p<0.05$). By using the method Stepwise of Ordinal Least Square Regression Test, age as the individual factor and the type of eye protection as the occupational factor significantly contributed to 15.6% and 11.0 % of the variance in the level of trace element Zinc respectively among the conventional farmers. While 7.9% of the variance in the level of trace element Manganese among the conventional farmers can be significantly explained by individual factors of the year of working (*p*-value= 0.033). Besides, the occupational factor of the type of spraying machine significantly contributed to 13.5% of the variance in the level of trace element Ammonia among the conventional farmers (*p*-value= 0.009). 10.9% of the variance in the level of trace element Zinc among the organic farmers can be explained by the occupational factor (type of foot protection) and it was proven that there was a significant linear relationship between these two variables (*p*-value=0.046). In addition, an individual factor of gender significantly contributed to 12.8% of the variance in the level of trace element Manganese among the organic farmers (*p*-value=0.013). Lastly, 9.6% of the variance in the level of ammonia among the organic farmers can be explained by the individual factor of taking alcohol habit and it was shown that there was a significant linear relationship between both variables (*p*-value= 0.044). **Conclusion**: This study suggests that chronic exposure to synthetic pesticides and chemical fertilizers results in an increased concentration of trace elements (Zn, Mn, and NH_3) in the human body.

Keywords: *Occupational exposure; Agroecosystem; Pesticides risks; Health implications*

ABSTRAK

MINERAL MIKRO DALAM KUKU PETANI YANG TERDEDAH KEPADA RACON PERSOAK SINTETIK DAN BAJA KIMIA DI PANTAI BARAT SEMENANJUNG MALAYSIA

WONG WEI VEN

Pengenalan: Penggunaan racun perosak sintetik dan baja kimia secara meluas dalam pertanian, yang membantu mengurangkan kesan buruk rumpai dan perosak sambil meningkatkan hasil tanaman adalah disebabkan oleh kemajuan pengeluaran pertanian. Ini amat membimbangkan kerana petani mungkin terdedah kepada bahan kimia ini semasa mengendalikan tanaman atau menjaga haiwan, yang membawa kepada potensi risiko kesihatan. Penggunaan racun perosak sintetik dan baja kimia dalam alam sekitar dan pekerjaan untuk tempoh yang panjang telah ditunjukkan untuk mengakibatkan kekurangan atau peningkatan tahap mineral mikro ini (Watts, 1990). **Objektif:** Kajian ini bertujuan untuk membandingkan tahap unsur surih zink (Zn), mangan (Mn), plumbum (Pb), dan ammonia (NH_3) dalam sampel kuku dalam kalangan petani konvensional dan organik. **Metodologi**: Responden pertama kali ditemu bual secara bersemuka menggunakan soal selidik untuk mengumpul maklumat latar belakang sosio dan pekerjaan. Sampel kuku telah dikumpul daripada 109 responden di enam kawasan di Semenanjung Malaysia untuk analisis unsur surih menggunakan Spektrometri Jisim Plasma Berganding Induktif (ICP-MS). Risiko kesihatan dinilai berdasarkan penggunaan racun perosak sintetik dan baja kimia yang mengandungi mineral mikro (Zn, Mn, Pb, NH_3)**. Keputusan & Perbincangan:** Diperhatikan bahawa paras Zn, Mn dan NH_3 dalam sampel kuku adalah lebih tinggi secara signifikan dalam petani konvensional berbanding petani organik ($p<0.05$). Terdapat perkaitan yang signifikan antara risiko kesihatan penggunaan racun perosak sintetik dan baja kimia yang mengandungi Zn, Mn dan NH_3 dengan tahap mineral mikro ini dalam kalangan responden ($p<0.05$). Dengan menggunakan kaedah Stepwise dari Ordinal Least Square Regression Test, faktor umur individu dan faktor pekerjaan jenis perlindungan mata menyumbang secara signifikan kepada 15.6% dan 11.0% daripada varians dalam tahap mineral mikro Zink masing-masing dalam kalangan petani konvensional (nilai-$p<$ 0.05). Manakala, 7.9% varians dalam tahap mineral mikro Mangan dalam kalangan petani konvensional boleh dijelaskan dengan ketara oleh faktor individu tahun bekerja (nilai-$p=$ 0.003). Selain itu, faktor pekerjaan jenis mesin penyembur menyumbang secara signifikan kepada 13.5% varians dalam tahap mineral mikro Ammonia dalam kalangan petani konvensional (nilai-$p=$ 0.009). 10.9% varians tahap mineral mikro Zink dalam kalangan petani organik boleh dijelaskan oleh faktor pekerjaan (jenis pelindung kaki) dan terbukti bahawa terdapat hubungan linear yang signifikan antara kedua-dua pembolehubah ini (nilai-$p=$ 0.046). Di samping itu, faktor individu jantina menyumbang secara signifikan kepada 12.8% varians dalam tahap mineral mikro Mangan dalam kalangan petani organik (nilai-$p=$ 0.013). Akhir sekali, 9.6% varians dalam tahap ammonia di kalangan petani organik boleh dijelaskan oleh faktor individu mengambil tabiat alkohol dan menunjukkan bahawa terdapat hubungan linear yang signifikan antara kedua-dua pembolehubah (nilai-p = 0.044). **Kesimpulan**: Kajian ini mencadangkan bahawa pendedahan kronik kepada racun perosak sintetik dan baja kimia mengakibatkan peningkatan kepekatan mineral mikro (Zn, Mn, dan NH_3) dalam tubuh manusia.

Kata kunci: *Pendedahan pekerjaan; Agroekosistem; Risiko racun perosak; Implikasi Kesihatan*

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LIST OF ABBREVIATIONS

DOA	Department of Occupational Safety and Health
CHRA	Chemical Health Risk Assessment
USEPA	United States Environmental Protection Agency
USECHH	Use and Standard of Exposure to Chemical Hazardous to Health
DOA	Department of Agriculture
FAO	Food and Agriculture Organization
FSS	Food Standard Scotland
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
CICAD	Concise International Chemical Assessment Document
SPSS	Statistical Product and Service Solutions
PPE	Personal Protective Equipment

CHAPTER 1

INTRODUCTION

1.1 Research Background

According to Portal MyHealth Ministry of Health, trace elements play a vital role in the formation of biological structures (MOH, 2016). In the field of analytical chemistry, a trace element is defined as an element with an average concentration of fewer than 100 parts per million (ppm) or less than 100 micrograms per gram when measured by atomic count (MOH, 2016). Whereas, in biochemistry, for an organism's proper growth, development, and physiology, it is necessary to obtain certain dietary elements that are essential in minute quantities (MOH, 2016). In the human body, trace elements are referred to as essential components that are integral to enzymes, hormones, and cellular structures (MOH, 2016). Zinc (Zn), Manganese (Mn), Chromium (Cr), Copper (Cu), Iron (Fe), Cobalt (Co), Selenium (Se), Molybdenum (Mo) are a few examples of the trace elements present in the human body (MOH, 2016). For example, trace elements primarily serve as catalysts within enzyme systems, with certain metallic ions like iron and copper taking part in oxidation-reduction reactions crucial for energy metabolism (National Research Council (US) Committee on Diet and Health, 1989).

The evolution and progress of agriculture in Malaysia have been crucial in shaping societies, economies, and ecosystems throughout history. Hence, the widespread use of synthetic pesticides and chemical fertilizers in agriculture, which helps to reduce the adverse impact of weeds and pests while enhancing crop yields can be attributed to the advancement of agricultural production. According to the Department of Agriculture, pesticide refers to any substance used to manage, deter, eradicate, weaken, repel, alleviate, or reduce pests (DOA, 2018). The use of pesticides in the agricultural sector is one of the methods to address plant pest problems in Good Agricultural Practices (GAP) since the conventional approach to vegetable cultivation is ineffective in eradicating pest infection (DOA, 2018). According to Article 2 (1) of the FAO legislative guide, fertilizer is defined as a substance used to induce a chemical alteration in the soil, serving to supply plants with nutrients or aid in their growth, or as a substance applied directly to a plant to enhance its nutritional status (FAO, 2019). When using synthetic pesticides and chemical fertilizers, these chemical remnants could potentially spread throughout the environment and persist via the air, water, soil, and other components of the ecosystem. Some of these chemicals would remain in surface water and groundwater, posing a potential risk of exposure to farmers who work on the farm. This is particularly concerning as farmers may be exposed to these chemicals while handling crops or tending to animals, leading to potential health risks. Additionally, these chemicals may also find their way into local water sources, such as rivers and lakes, which can impact the health of wildlife and the surrounding ecosystem. The use of synthetic pesticides and chemical fertilizers in both environmental and occupational settings for an extended period has been shown to affect trace element levels such as antagonistic effects in the human body, resulting in deficiency or elevated of these elements (Watts, 1990). Deviation from normal levels of these elements may disrupt metabolic processes in the body, potentially resulting in a range of health issues.

1.2 Problem Statement

According to the Ministry of Agriculture and Food Security, Malaysia is now at 41st position on the Global Security Food Index (GSFI) 2022 (MAFS, 2023). Malaysia's food production is insufficient to meet the needs of its population and industrial sector, with a reliance on imports for essential food products. Hence, to both enhance crop productivity and ensure the quality and safety of food, farmers may consider utilizing pesticides and fertilizers in their cultivation processes. Agriculture in Malaysia can be categorized into two distinct approaches: organic, which emphasizes the utilization of natural and agroecological methods, and conventional farming. Organic farming focuses on the cultivation of crops without the need for synthetic chemicals, placing a strong emphasis on enhancing soil fertility and promoting biodiversity within a sustainable ecosystem. In contrast, conventional agriculture is heavily reliant on the utilization of synthetic pesticides and chemical fertilizers to maximize crop production, despite the potential negative impacts on the environment. In other words, organic farmers prioritize the utilization of eco-friendly resources to ensure the long-term environmental sustainability of their farms while achieving sufficient crop yields simultaneously. Conversely, conventional farmers heavily depend on synthetic chemicals and energy inputs, which contributes to the gradual degradation of biodiversity within their agricultural lands. As farming practices vary, farmers may face various health risks due to the different types of pesticides and fertilizers they use in their fields, highlighting the need to understand these potential effects on both farmers' health and the environment.

Like numerous emerging economies, a significant portion of pesticide users in these regions lack knowledge regarding the various types of pesticides, their mode of action, potential risks, and the necessary safety precautions (Moustafa MSA, 2017). Although some of them were informed about the potential adverse effects of pesticides on human health, the implementation of preventive measures to reduce exposure was a seldom-seen practice (Moustafa MSA, 2017). This issue should be a concern as individuals involved in pesticide and fertilizer application, whether they are farm owners or workers, are often exposed to synthetic pesticides and chemical fertilizers across different phases of the process, such as storage, mixing, preparation, and the actual application (Suratman et al., 2015). Besides, farmers are susceptible to pesticide exposure through a variety of pathways, including dermal contact, ingestion, inhalation, and more as well as its ability to bioaccumulate in the human body (Ho et al., 2018). A lack of knowledge, a low-risk perception, and limited awareness of the proper use of synthetic pesticides and chemical fertilizers increase the likelihood of farmers being exposed to these substances. Farmers are unaware and unfamiliar with the health risks of the synthetic pesticides and chemical fertilizers that are being used, especially small-scale vegetables and nursery farmers. Past research concerning the knowledge, attitude, and practice (KAP) regarding pesticide exposure among farmers in Kelantan, Malaysia revealed that most farmers exhibited a low level of concern regarding the use of pesticides, as well as the associated health hazards and effects. (Jambari et al., 2020).

Trace minerals are needed by the body in minimal amounts, yet they are crucial for various biological functions such as catalysts for enzyme actions and forming body structures. Manganese (Mn), zinc (Zn), ammonia (NH_3) and lead (Pb) are the four trace elements that are assessed in this study.

Manganese (Mn) contributes to the body's formation of connective tissue, bones, blood clotting factors, and sex hormones. Additionally, it participates in the metabolism of fats and carbohydrates, aids in the absorption of calcium, and regulates blood sugar. A deficiency in this mineral is linked to a range of health consequences, including overall growth inhibition, birth defects, decreased fertility, compromised bone development, and disruptions in the metabolism of lipids, proteins, and carbohydrates (Keen et al., 1999). Prolonged human exposure to manganese primarily leads to nervous system impacts and respiratory issues, including a higher likelihood of experiencing coughing, bronchitis, breathing difficulties during physical activity, and an elevated vulnerability to infectious lung diseases (EPA, 2016).

Zinc (Zn) is highly engaged in the processes of protein, lipid, nucleic acid metabolism, and gene transcription (McClung, 2019). Low levels of zinc in the body are accountable for a range of physiological disorders, including apoptosis, organ damage, DNA injuries, and oxidative harm to cellular components caused by reactive oxygen species (ROS) (Hussain et al., 2022). An excessive intake of zinc can disrupt the absorption or intake of copper and iron, probably by competing for binding sites within the cells of the intestinal mucosa. Additionally, stomach acid can dissolve metallic zinc, generating zinc chloride, which is a corrosive substance that can harm the lining of the stomach (T3DB, 2014).

Ammonia (NH_3) in the human body primarily serves as a waste product that needs to be removed. It helps the body eliminate excess nitrogen, which is a byproduct of protein metabolism, maintaining the body's acid-base balance and small amounts of ammonia can be used in the synthesis of neurotransmitters in the brain, such as glutamate. According to the Agency for Toxic Substances and Disease Registry, frequent or continuous exposure to ammonia can lead to chronic irritation of the respiratory tract (ATSDR, 2017).

Lead (Pb) is a toxic non-essential trace element, and it does not have a biological function in the human body. In fact, lead exposure can be extremely harmful to human health. Lead is stored in the teeth and bones of the body, where it gradually accumulates over time (WHO, 2023). Excessive lead exposure can result in severe harm to the brain and central nervous system, potentially leading to conditions like coma, seizures, and, in extreme cases, fatality (WHO, 2023).

The extent of trace elements, such as zinc (Zn), manganese (Mn), lead (Pb), and ammonia (NH_3), in synthetic pesticides and chemical fertilizers, can vary based on several factors, including the specific formulation of the pesticide or fertilizer, the raw materials used in their production, and the manufacturing processes. Table 1 summarizes the sources of trace elements in both synthetic pesticides and chemical fertilizers.

Table 1.1: Summary of sources of trace elements in both synthetic pesticides and chemical fertilizers

Trace Elements	**Synthetic Pesticides**	**Chemical Fertilizers**
Zinc (Zn)	may present as impurities from the manufacturing process or as intentional additives (Rashid et al., 2023).	added to chemical fertilizers as micronutrients. They play essential roles in plant growth, and their levels are carefully controlled to ensure optimal plant nutrition (Ahmed et al., 2024).
Manganese (Mn)	May present as impurities from the manufacturing process or as intentional additives (Rashid et al., 2023).	added to chemical fertilizers as micronutrients. They play essential roles in plant growth, and their levels are carefully controlled to ensure optimal plant nutrition (Alejandro et al., 2020).

Trace Elements	Synthetic Pesticides	Chemical Fertilizers
Lead (Pb)	Come from pollution of pesticide formulations during the manufacturing process involving petroleum materials, industrial waste, or the introduction of chemical nanoparticles in pesticide production (Dewil et al., 2022).	Come from pollution of fertilizer formulations during the manufacturing process involving petroleum materials, industrial waste, or the introduction of chemical nanoparticles in chemical fertilizer production (Dewil et al., 2022).
Ammonia (NH_3)	Present as an active ingredient in pesticides, undergoes gradual decomposition into ammonia, carbon dioxide, and water when in the form of ammonium bicarbonate (USEPA, n.d.).	Ammonia in the form of ammonium compounds. Ammonium nitrate, for example, is a common nitrogen fertilizer.

It is crucial to maintain a balance of trace elements within the specified limits to ensure optimal bodily function. For example, disruption in the balance, either through excessive or deficient levels of these trace elements, can contribute to the pathophysiology of numerous brain disorders (Bayir, 2015). Nevertheless, exposure to pesticides and fertilizers can lead to a depletion of essential trace elements in the human body due to multiple underlying factors such as the antagonistic impact of the heavy metals in pesticides and fertilizers (Abdul Hamid et al., 2017). Farmers who are exposed to pesticides tend to have more damage to their DNA and the extent of this damage is influenced by both the conditions of their exposure to pesticides and their genetic makeup (Kaur & Kaur, 2018). DNA damage and repair mechanisms could potentially be influenced by alterations in trace elements, such as selenium and zinc (Wang et al., 2023). The absorption of lead significantly affects the zinc levels in the body, leading to consequences such as DNA damage, abnormalities in blood cells, and oxidative stress resulting from exposure to lead among occupationally exposed workers (Wani et al., 2019). Although a previous study (Wąsowicz et al., 2001) has revealed that occupational exposure to cadmium or lead can disrupt the body's antioxidant capacity, leading to alterations in the concentration of trace elements and the activity of certain enzymes, the exact mechanisms underlying the interaction between toxic heavy metals and trace elements remain unclear.

In this study, vegetable and nursery farmers are selected as the research subjects. These farming communities are exposed to various highly hazardous pesticides (HHPs) based on the specific farming practices and geographical locations of their farming activities. To date, there has been limited research studying the effects of trace elements in synthetic pesticides and chemical fertilizers that potentially affect metabolic disorders among farming communities.

The variability in the presence of biomarkers for trace elements is quite pronounced. In prior research, trace elements have predominantly been analysed in blood, urine, hair, and nail samples, and in certain cases, bone tissues are being used to study heavy metals (He, 2010). Research has clarified that blood and urine samples are primarily suitable for assessing short-term effects since homeostasis tends to rectify any elemental imbalances in these substances over time (Longnecker et al., 1993). Hairs are composed of the same keratinous tissue as nails, but hair analysis can be influenced by cosmetic procedures like dyeing, bleaching, and permanent waving, as these treatments can alter the content of trace elements in the hair (Jurado et al., 1997). Therefore, in this study, nail sampling acts as an alternative to analyse the trace elements of the human body. Considering the evidence of pre-existing metabolic health risks induced by obesogenic effects stemming from the use of synthetic pesticides among conventional farmers, the question arises as to what extent could the use of synthetic pesticides and chemical fertilizers potentially affect the presence of trace elements in the nails and, in turn, impact the metabolic health risks among conventional farmers.

1.3 Study Justification

Due to emerging trends in globalization, enhancements in working conditions are inherently encompassed within the aspects of quality management and product quality. Nowadays, enhancements to agricultural working conditions can be substantially achieved practically and cost-effectively by implementing safety and health measures. Focusing on occupational safety and health not only contributes to increased labour productivity but also fosters healthier workplace relationships. However, a lack of effective policy formulation and supervision has resulted in the growth or expansion of Malaysia's agriculture over the past 50 years has had negative consequences for both the environment and the goal of sustainable development. With recent challenges such as deteriorating soil quality, climate change, insufficient energy, and a shortage of labour, the existing agricultural model is no longer sufficient to meet the requirements of ensuring future food security and sustainable agricultural development.

In order to improve the working conditions of agricultural farmers, there is a need to identify the potential enduring health impacts resulting from the use of synthetic pesticides and chemical fertilizers. This will be achieved through a comprehensive analysis of trace elements present in the human body that may be linked to exposure to these agricultural chemicals. By determining and understanding the specific trace elements associated with synthetic pesticide and chemical fertilizer exposure, the research aims to contribute valuable insights toward the development of appropriate preventive measures and robust safety protocols. The ultimate goal is to mitigate the potential risks posed by these agrochemicals to human health in the long term, providing a foundation for more informed and proactive approaches to agricultural practices.

Blood and urine concentrations are indicative of exposure events that have taken place in a relatively recent timeframe (Ilhan et al., 2004). In contrast, nails, especially toenails, offer a comparatively long exposure over an extended or prolonged duration. Hence, it can be said that chronic effects of synthetic pesticides and chemical fertilizers are hard to detect if they are analysed in blood and urine. Most of the time, the use of urine, blood, and serum samples has been suggested to more accurately reflect the effects of exposure because they directly interact with the body (Abdul Hamid et al., 2017). Nevertheless, the accuracy of reflection depends on the substance being measured. For example, blood and serum can give real-time information about various biomarkers, making them more precise and accurate for assessing current health status and recent exposure. However, for some substances, urine might be a more accurate indicator of exposure, while for others, other matrices like hair or nails could be more appropriate for long-term assessments.

In contrast, hair and nails can be non-invasively obtained, providing a comprehensive overview of element concentrations in an organism. Notably, these materials are independent of the influence of metabolic processes and homeostatic mechanisms. Additionally, they have easy transportability and storage without the risk of degradation and contamination (Fukushima et al., 2009). The longer detection window in nail samples is useful for studying the cumulative effects of chronic exposure among farmers and provides valuable information about the potential impacts of these synthetic chemicals on human health. This data will be utilized as a reference to compare it with established standards.

Nails, particularly from the big toe (hallux), providing enough mass for trace element analysis and their faster growth rate compared to the other toenails, have been recommended as suitable biomarkers for trace element intake (Pessan & Buzalaf, 2011). Toenails have been proven to exhibit lower susceptibility to external contaminants when contrasted with fingernails (Pessan & Buzalaf, 2011). The endogenous trace element composition of nails is believed to reflect the metabolic milieu during their formation (Elekdag-Turk et al., 2019). The concentration of trace elements in nail samples represents the average level of fluoride intake and plasma concentration over an extended period (Elekdag-Turk et al., 2019).

This research is oriented towards the implementation of policies promoting sustainable agricultural health and safety. The objective is to enhance the working and living conditions of small-scale farmers while preventing occupational accidents and diseases in the agricultural sector. The anticipated results of this study aim to play a crucial role in the creation of sustainable training tools for agricultural health, along with the development of capacity-building initiatives and support mechanisms tailored specifically for small-scale farmers within Malaysia.

1.4 Research Questions

a) What is the background of health risks found in synthetic pesticides and chemical fertilizers used by conventional vegetable and nursery farmers?

b) What is the difference in trace elements (Zn, Mn, Pb, NH_3) in nails between organic and conventional farmers?

c) What is the association between the health risk of synthetic pesticides and chemical fertilizers used with the level of trace elements (Zn, Mn, Pb, NH_3) among small-scale vegetable and nursery farmers?

d) What is the relationship between socio-demographic background, farming activities and practices with the level of trace elements (Zn, Mn, Pb, NH_3) among small-scale vegetable and nursery farmers?

1.5 Study Objectives

1.5.1 General Objective

To compare the trace elements (Zn, Mn, Pb, NH_3) contained in nails among conventional and organic farmers in small-scale vegetable and nursery farms.

1.5.2 Specific Objectives

a) To determine the socio-demographic, farming activities and practice of small-scale vegetable and nursery farmers.

b) To determine the health risk of the types of synthetic pesticides and chemical fertilizers used among small-scale vegetable and nursery farmers.

c) To determine the level of trace elements (Zn, Mn, Pb and NH_3) contained in nails among small-scale vegetable and nursery farmers.

d) To compare the difference of trace elements (Zn, Mn, Pb and NH_3) contained in nails among small-scale vegetable and nursery farmers.

e) To associate the health risk of synthetic pesticides and chemical fertilizers used and the level of trace elements (Zn, Mn, Pb and NH_3) among small-scale vegetable and nursery farmers.

f) To identify the relationship between the trace elements (Zn, Mn, Pb and NH_3), socio-demographic background, farming activities and practices among small-scale vegetable and nursery farmers.

1.6 Research Hypothesis

a) There is a significant difference in trace elements (Zn, Mn, Pb and NH_3) contained in nails among small-scale vegetable and nursery farmers.

b) There is a significant association between the health risk of synthetic pesticides and chemical fertilizers used and the level of trace elements (Zn, Mn, Pb and NH_3) among small-scale vegetable and nursery farmers.

c) There is a significant linear relationship between the trace elements (Zn, Mn, Pb and NH_3), socio-demographic background, farming activities and practices among small-scale vegetable and nursery farmers.

1.7 Study Variables

I. **Independent variable:** The level of trace element (Zn, Mn, Pb and NH_3) contained in nails among the organic and conventional farmers in West Coast of Peninsular Malaysia.

II. **Dependent variable:** Health risk of the types of synthetic pesticide and chemical fertilizers used.

1.8 Definition of Terms

Research Title: **Trace Elements** in the Nails of Farmers Exposed to **Synthetic Pesticides and Chemical Fertilizers** in West Coast of Peninsular Malaysia.

i. Term: **Synthetic Pesticides and Chemical Fertilizers.**

- **Conceptual definition:** Synthetic pesticides contain conventional active ingredients that are generally produced synthetically, which help to prevent, mitigate, destroy, or repel any pest; or that act as a plant growth regulator, desiccant, defoliant or nitrogen stabilizer (USEPA, 2022). Chemical fertilizer means any substance containing one or more of the basic elements used as a fertilizer (The Chemical Fertilizers Control Act, 1980).

- **Operational definition:** The health risk posed by synthetic pesticides and chemical fertilizers will be identified and characterized using Pesticide and Fertilizer Health Risk Assessment based on the health hazard of the synthetic pesticides and chemical fertilizers and the level of exposure experienced by the study respondents.

ii. Term: **Trace elements.**

- **Conceptual definition:** Trace element is a dietary element in very minute quantities required for the growth, development and physiology of the organism (MOH, 2016).
- **Operational definition:** The level of trace elements in the respondents' body are identified by collecting their nail samples and analyzed using Inductively coupled plasma mass spectrometry (ICP-MS).

1.9 Conceptual Framework

Figure 1.1 illustrates the conceptual framework of the study by initially characterizing the health risks of farmers. This is achieved through an exploration of the background of health hazards associated with the types of pesticides and fertilizers used for pest control activities and growth promotion, as well as the farmers' exposure to these substances. It is then followed by a comparison of trace element levels (Zn, Mn, Pb, and NH_3) found in the nails of organic and conventional farmers within small-scale nursery and vegetable farms.

Farmers commonly utilize two types of pesticides for pest control: biological (natural) and synthetic. Biopesticides are derived from natural sources, including animals, plants, bacteria, and specific minerals. In contrast, synthetic pesticides are composed of artificial chemicals employed to manage and control pests. The primary categories of synthetic pesticides include organochlorines, organophosphates, carbamates, and pyrethroids. To comprehend the health hazards associated with these pesticides, the study employed the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). This classification system was utilized to categorize the health hazards of the pesticides based on their ingredients and the criteria for hazard classification. The pesticide health risk assessment is then employed to characterize the potential adverse health risks posed by synthetic pesticides and chemical fertilizers to both organic and conventional farmers, based on their respective levels of exposure.

Although synthetic pesticides and chemical fertilizers give benefits for crop production, their extensive use can lead to severe consequences due to their characteristics such as bioaccumulation and high persistence in nature. Numerous pesticides have contributed to direct or indirect pollution of the air, water, soil, and the broader ecosystem, posing significant health hazards to living beings (Sharma et al., 2019). Considering this exposure route, adults' skin is considered a primary pathway for pesticide exposure (How et al., 2015). Moreover, pesticide compounds are expected to accumulate in the body over time, and chronic health effects may manifest over the course of the year through low cumulative exposure while working in the farmland.

Non-invasive samples are generally preferred for exposure monitoring whenever possible such as hair and nail samples. These samples enable the identification of organic pesticides from various classes, including both parent compounds and their metabolites, as well as the measurement of the level of trace elements. Furthermore, prior research has demonstrated that these non-invasive samples offer information representative of the chronic level of exposure (Iglesias-González et al., 2020). Hence, in this context, nail samples were collected. Risk factors encompass parameters that may influence the exposure level of the study population. Consequently, socio-demographic variables and occupational factors were assessed within the study population.

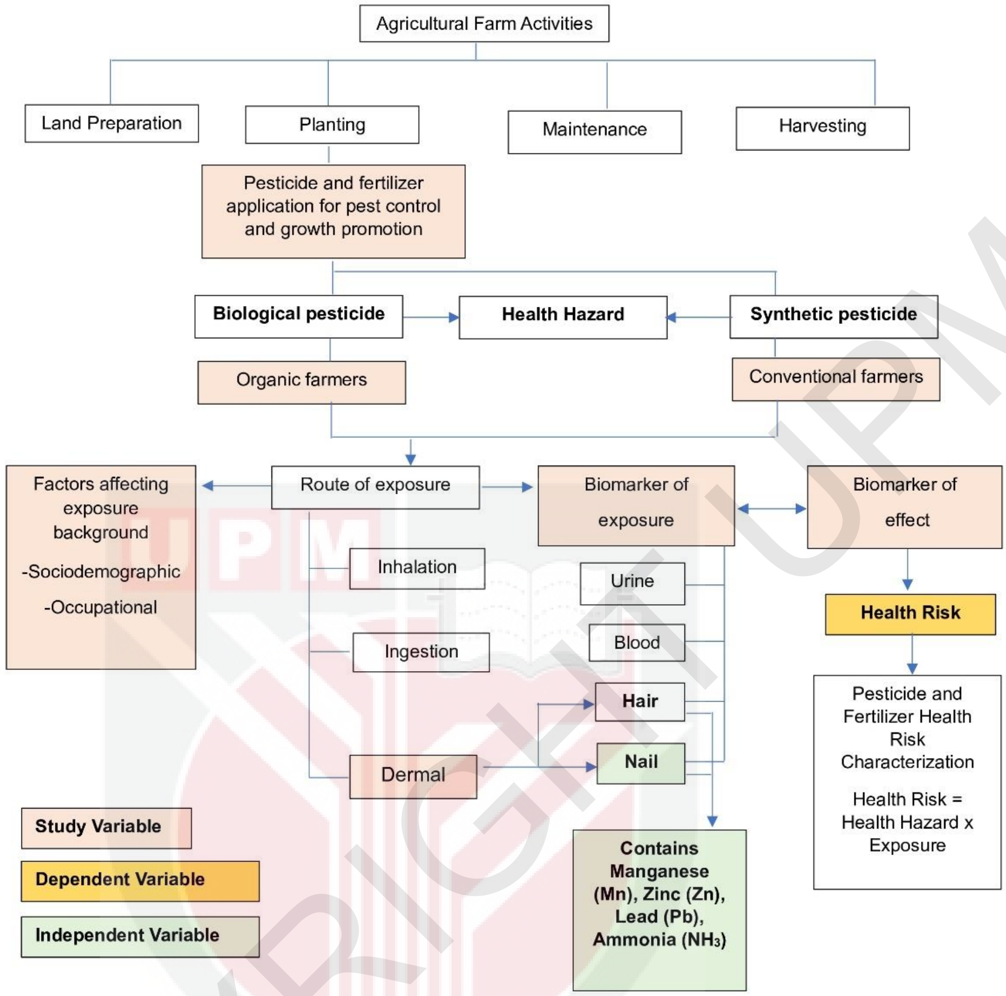

Figure 1.1: Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Synthetic Pesticides

According to the Pesticide Act 1974, synthetic pesticide refers to any substance that contains an active ingredient, or any mixture that contains any one or more of the active ingredients as one of its constituents. It is designed for preventing, eradicating, deterring, or reducing the impact of pests, or serving as a plant growth regulator, defoliant, or desiccant as well as acting as a nitrogen stabilizer (USEPA, 2023). It is primarily used in agriculture sectors in Malaysia, especially on vegetable and fruit farms, paddy, rubber as well as palm oil plantation (Bakar, 2009). They are utilized to safeguard plants against pests, weeds, or diseases, and to shield humans from vector-borne diseases like malaria, dengue fever, and schistosomiasis (Nicolopoulou-Stamati et al., 2016).

Typical examples of pesticides include insecticides, fungicides, herbicides, rodenticides, nematicides and plant growth regulators, which the agent exhibits primary effectiveness against insects, fungi, weeds, rodents, and nematodes respectively (Garcia et al., 2012). The commonly used pesticides in agriculture in Malaysia include organophosphates, pyrethroids, glyphosate, and 2,4-D-dimethylammonium but there are certain pesticides with stringent regulations governing their use, including paraquat (classified as WHO class II), monocrotophos (classified as WHO class 1a), chlorpyrifos (classified as WHO class 1b), and methamidophos (classified as WHO class 1b). Moreover, highly toxic pesticides like endosulfan (classified as WHO class II) and methomyl (classified as WHO class Ib) have been prohibited (Kamaruzaman, 2020).

The heightened usage of pesticides can be attributed to the intensification of the agricultural sector (Jambari et al., 2020). According to the Statista Research Department (2022), about 36,000 metric tons of pesticides were applied in Malaysia for agricultural use in 2020 and the sales worth of manufactured pesticides and agrochemical products reached 2.68 billion Malaysian ringgit in 2021. Elevated concentrations of pesticide residues have the potential to be harmful to the extent that they can lead to persistent cancer, harm the nervous and reproductive systems, result in birth defects, and significantly disturb the immune system (Jayaraj et al., 2016).

2.2 Overview of Chemical Fertilizers

According to Chandini et al. (2019), chemical fertilizer refers to any substance that is made of synthetic origin and is used to provide one or more essential plant nutrients to provide specific nutrients to plants and fix nutrient deficiency problems in plants. Chemical fertilizers can be industrially produced and consist of the three primary major nutrients, which are nitrogen (N), phosphorous (P) or potassium (K) and minor elements such as zinc, manganese, ferum and many more. Chemical fertilizers are used to promote and enhance the growth of plants by supplying additional nutrients or alternating the physical structure or fertility of the soil. Hence, when the crops and plants grow vigorously, higher yields are produced. This is important in developing countries or less developed countries to meet food demands and improve crop profitability.

While chemical fertilizers play a significant role in ensuring an adequate food supply for the global population, excessive usage of these fertilizers is giving rise to significant issues that affect both the current and future generations, especially the impacts towards the environment and human health (Chandini et al., 2019). According to Statista Research Department (2023), there were approximately 7.58 million metric tons of fertilizer were manufactured in Malaysia in 2022, and it was an increase from about 5.84 million metric tons compared to the previous year. Jais (2012), on the other hand, suggested that chemical fertilizers are used by 90% of the cropping system in Malaysia and this resulted in consumers' concern about the health risks due to the overuse of chemical fertilizer. Rajan et al. (2021) also stated fertilizers are commonly used in agricultural regions across Malaysia to boost the growth of crops for giving higher yields. Nonetheless, the frequent use of chemical fertilizers has been demonstrated to raise the levels of metals in the soils as a result of impurities and pre-existing contamination in the chemical fertilizers (Rajan et al., 2021). According to Savci (2012), the utilization of inorganic fertilizers can be beneficial for crop growth, but when used excessively, it may lead to the introduction of potential sources of heavy metals like arsenic, cadmium, copper, lead, mercury, nickel, and zinc into the environment.

2.3 Occupational Pesticide and Fertilizer Exposure and Route of Transmission

Individuals are often in contact with synthetic pesticides and chemical fertilizers during various stages, including manufacturing, transportation, preparation, and utilization in their work environment, causing occupational exposure to synthetic pesticides and chemical fertilizers. The levels of exposure are different from one other, and it is based on the route (inhalation or contact with skin), intensity (the level of pesticide application), frequency (how often it occurs), magnitude (how long the exposure lasts) of the human exposure to the synthetic pesticides and chemical fertilizers. Safety practices such as the use of personal protective equipment and the chemical and toxicological properties of the specific pesticides and fertilizers being used also affect the degree of human exposure to synthetic pesticides and chemical fertilizers. According to Ye (2013), in work environments, individuals who have direct and repeated contact with pesticides represent the highest-risk groups for exposure. Furthermore, he also added that it is important to note that occupational pesticide exposure is prevalent among agricultural workers and their family members as incidences such as unintentional pesticide spills, seepages, improper equipment handling, and violations of safety regulations occur in the workplace. In contrast to environmental exposures, which generally involve relatively low levels of exposure, occupational exposure to pesticides often entails relatively high doses, whether it's an acute or chronic exposure (Damalas, 2011).

In agricultural occupations, approximately 10% of total pesticide exposure typically takes place through the respiratory route, while the remaining exposure occurs through either dermal absorption or ingestion (Dowling & Seiber, 2002).

Dermal absorption happens when pesticides come into contact with the skin directly or through exposure to clothing and tools that have been contaminated with pesticide residues (Sanborn et al., 2002). Farmers can have direct exposure to residues on foliage, soil, and groundwater through dermal pathways (Khan, 2012). Dermal exposure can also be significant factors in causing systemic inflammation or sensitization when individuals experience high-level pesticide exposures in the workplace (Maestrelli et al., 2009). As shown in Figure 2.1, the dermal exposure pathway is influenced by the use of pesticides on treated farms, where a combination of pesticides seeps into the soil and enters groundwater through runoff and spray drift. Pesticides can be absorbed through direct contact with the air, contact with contaminated surfaces, or by skin exposure to the substance (How et al., 2014). Notably, the skin's metabolic processes can transform the trace element compounds from lipophilic to more hydrophilic, facilitating their penetration through the skin.

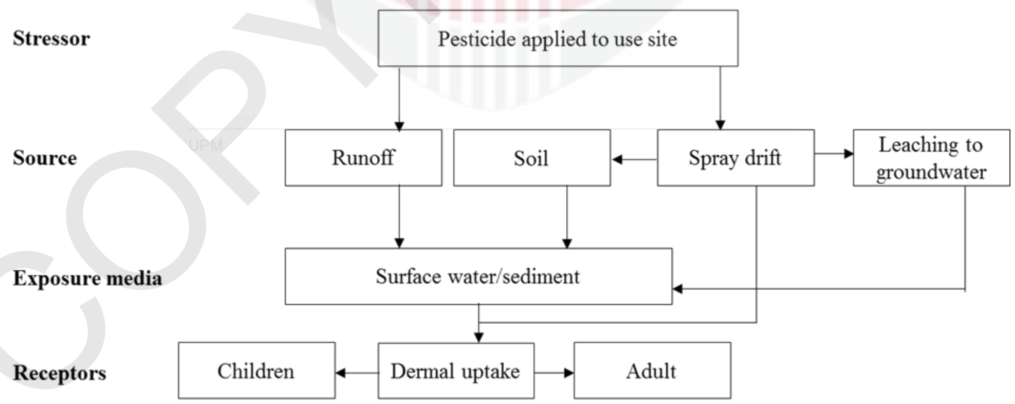

Figure 2.1: Dermal Exposure Pathway from Pesticide Treated Farm

(Adapted from Brady, 2011)

The superficial surface area of an adult human's skin is approximately 1.73 square meters, making it the primary route of exposure to environmental contaminants (Baharuddin et al., 2011; Hashmi and Khan, 2011). Dermal exposure can occur at various times, including during the storage, handling, transportation, and disposal of pesticides and their by-products. The condition of the workers' protective gear may also act as an additional pathway for exposure (How et al., 2014).

2.4 Trace Element Contained in Synthetic Pesticide and Chemical Fertilizers and Its Health Effect

Trace elements refer to elements found in agroecosystems at low concentrations, typically less than one milligram per kilogram (mg kg^{-1}) (He et al., 2005). Various nutrients, such as boron (B), molybdenum (Mo), manganese (Mn), copper (Cu), zinc (Zn), and iron (Fe), are provided to plants as trace elements. The interaction between these trace elements and essential elements is crucial for ensuring the sustained and robust growth of plants. He also claimed that, with the exception of Boron, these elements are categorized as heavy metals and can become detrimental to plants when present in elevated concentrations (He et al., 2005). Furthermore, certain other trace elements, including cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), mercury (Hg), and arsenic (As), possess harmful effects on living organisms and are frequently classified as contaminants. The presence of trace elements in an agroecosystem can be originated from the natural composition of soil parent materials or be introduced through human activities. Contamination of soil with heavy metals and toxic elements, originating from both natural sources and specific human activities, typically take place in localized areas, facilitating easier detection. However, a broader scale of contamination can arise from the persistent application of metal-enriched synthetic pesticides, chemical fertilizers, and organic amendments like sewage sludge and wastewater. The increased risk of large-scale contamination downplays the significance of adopting sustainable agricultural practices and robust environmental management strategies to safeguard ecosystems and human health from the potential consequences of prolonged exposure to these elements.

Various chemical processes play a crucial role in altering trace elements within soils. Among these, precipitation–dissolution, adsorption–desorption, and complexation stand out as the most significant processes, influencing the bioavailability and mobility of trace elements in soils (He et.al, 2005). It's noteworthy that both deficiency and toxicity of trace elements can take place in agroecosystems, underscoring the intricate balance required for optimal plant growth and environmental health. Presence of heavy metals in the crops and soils encompass contributions from commercial fertilizers, liming materials, agrochemicals, sewage sludges, and various wastes utilized as soil amendments, as well as atmospheric deposition and irrigation waters (Senesil et al., 1999). Soils exposed to continuous and frequent applications of organic manures, fungicides, and pesticides displayed elevated concentrations of extractable metals, leading to subsequent increases in heavy metal concentrations in runoff (Daniel et al., 1998).

The exposure of agricultural workers to synthetic pesticides and chemical fertilizer is linked to negative health consequences especially health effects. Heavy metals, including Mercury, Lead, Cadmium, and Uranium, have been identified in fertilizers (Kumar & Dev, 2017). This presence poses a potential risk of disruptions in kidney, lung, and liver functions, as well as an elevated risk of cancer (Kumar & Dev, 2017). Manganese is often utilized in a wide variety of other products, including the fertilizer and the primary health issues observed in individuals exposed to elevated levels of manganese often associated with the nervous system (ATSDR, 2014). These effects on health encompass alterations in behaviour and other symptoms in the nervous system, leading to movements that can exhibit reduced speed and coordination (ATSDR, 2014). Three pesticide active ingredients fall under the category of zinc salts, which are zinc chloride, zinc oxide, and zinc sulfate monohydrate (or zinc sulfate) and they are used as herbicides, however, pesticide with zinc chloride and zinc sulfate can pose health effects to humans such as eye irritation and skin corrosion (USEPA, 1992). Heavy metals, such as cadmium (Cd) and lead (Pb) from the farming chemicals in plant matter, can accumulate in the body and the food chain, serving as precursors for the development of diseases (Oyugi et al., 2021).

Table 2.1: Environmental persistence of trace elements contained in synthetic pesticide and chemical fertilizer

Trace Element	Environmental Persistence/ source
Zinc (Zn)	The biological half-lives of Zn in each region studied were in the range of 16-43 days; the longest was observed in the amygdaloid nuclei (Takeda, Sawashita & Okada, 2012)
Manganese (Mn)	The biological half-lives of Mn in each region were 51-74 days; the longest were those in the hypothalamic nuclei and thalamus (Takeda, Sawashita & Okada, 2012)
Lead (Pb)	The half-life of blood lead in humans is approximately 40 days, indicating a relatively short period of retention in the bloodstream (Wani et.al, 2015).
Ammonia (NH_3)	The best estimate of the half-life of ammonia is a few days (ASTDR, 2004).

2.5 Chemical Health Risk Assessment of Farmers Using Pesticides and Fertilizers

In this research, population's vulnerability to both environmental and occupational exposure was evaluated to various pesticides and fertilizers within the same agricultural community. This was accomplished through a chemical health risk assessment, as depicted in Figure 2.2.

To meet the assessment criteria, a four-step approach is considered.

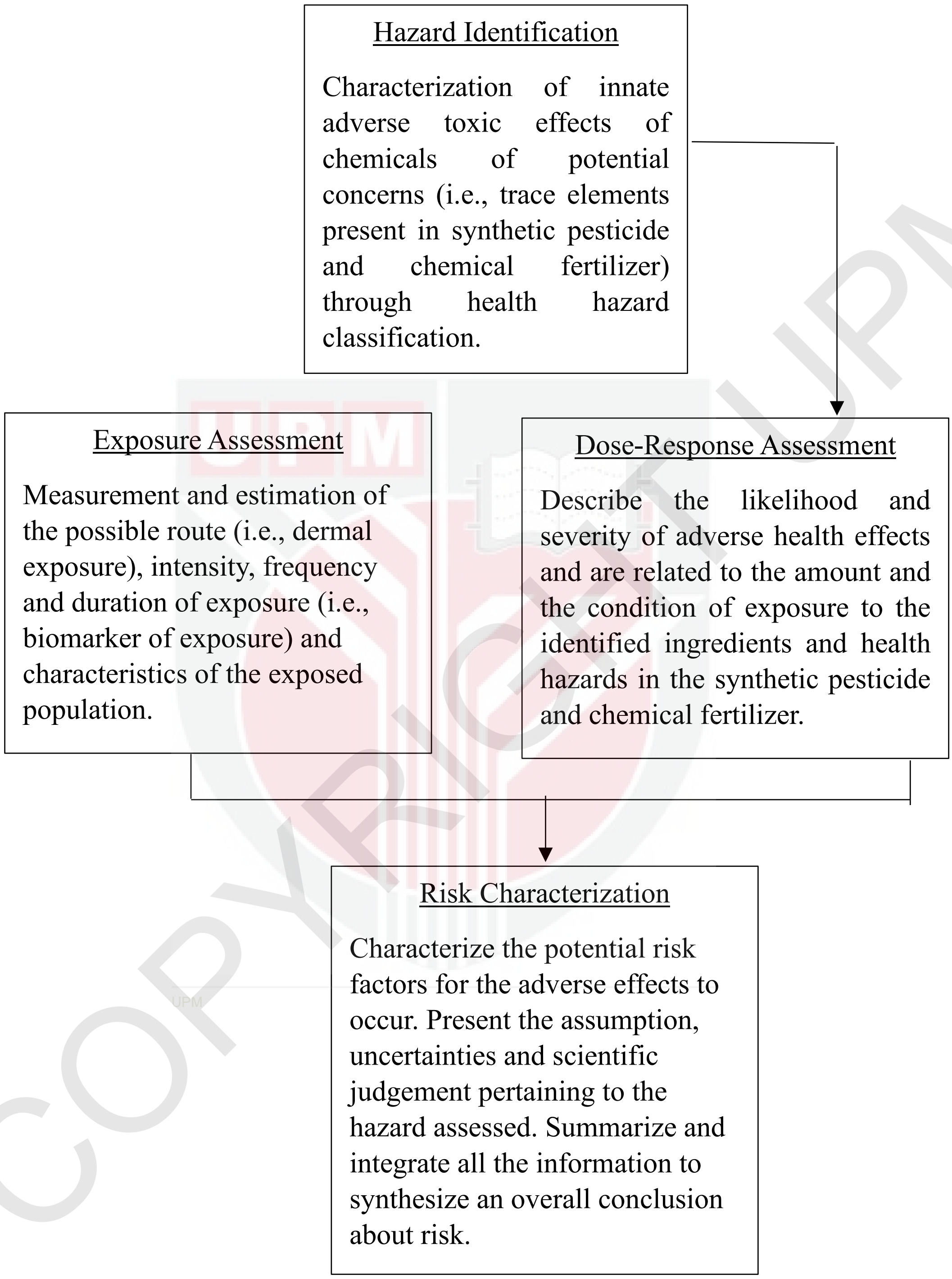

Figure 2.2: Chemical Health Risk Assessment for Pesticides and Fertilizers Components

This assessment involves:

i) Identifying groups of individuals who is at risk and may be exposed to pesticides (potentially exposed populations).

ii) Recognizing the potential pathways and routes through which exposure can occur (potential exposure pathways).

iii) Calculating the intakes or potential doses resulting from this exposure (intakes/potential doses from uptake exposure).

iv) Assessing potential health effects arising from the presence of trace elements in the pesticides and fertilizers (potential health effects).

v) Characterizing the potential risk factors associated with pesticide and fertilizer exposure.

2.6 Biomonitoring of Trace Element Contained in Pesticide and Fertilizer Exposure

Human Biomonitoring (HB) is a valuable tool for assessing the extent to which chemical substances have entered our bodies, providing a means to quantify human exposure to pollutants as well as playing a crucial role in identifying either an excess or deficiency of essential elements (Bocato et al., 2019). It involves in a systematic, continuous, or repetitive practice of examining biological samples allows for the prompt application of findings related to pollutant concentration or specific non-adverse biological effects (Waseem & Arshad, 2016). In Human Biomonitoring (HB) studies focused on pollutants, the measurement of substance levels in body fluids (such as blood, urine, and breast milk) or tissues (like hair, nails, or teeth) assists in identifying potential health risks or associated adverse effects by comparing it with the reference level (Bocato et.al, 2019). To assess and characterize the potential health risks, monitoring doses (exposure biomonitoring) is complemented by studying along with its biological effects (effect biomonitoring) (Budnik & Baur, 2009). As suggested in this context, the pathway for biological measurements is adapted, as shown in Figure 2.3.

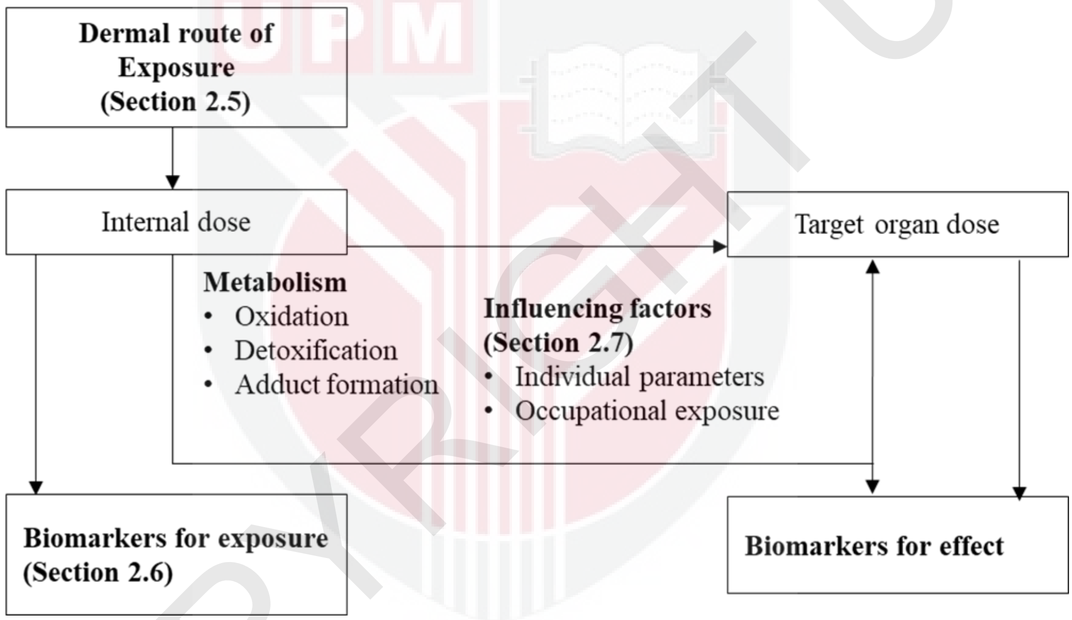

Figure 2.3: Pathways for Biological Measurements of Trace Element Contained in Pesticide Exposure

(Adapted from Kapka-Skrzypczak et al., 2011)

A biomarker of exposure serves as a tool for evaluating exposure by estimating the internal dose, representing the quantity of a chemical present within the body. It gives the data on the specific chemical exposure of individuals, tracks changes in levels over time, and exhibits variation among different populations ("Biomarkers of Exposure", 2012).

Biomonitoring, which involves analysing biomarkers of exposure in biological matrices, which makes it as one of the most prevalent methods for evaluating exposure. For pesticide exposure assessment, the choice of specimens for analysis can vary depending on the context. Blood is commonly act as a biomarker to identify persistent compounds like organochlorines, while urine is the preferred specimen for analysing metabolites of various pesticides such as organophosphates and pyrethroids (Kokkinaki, 2014). In numerous studies involving trace elements, whether as essential dietary nutrients such as Selenium (Se) or toxic substances such as Lead (Pb), the nail specimen is frequently collected and stored prospectively for months or even years. It is then retrieved and analysed in epidemiological studies, examining the concentrations of trace elements concerning one or more clinical endpoints (Xun et al., 2010). This is because the primary composition of human nails consists predominantly of keratin-rich proteins. These proteins integrate trace elements in correlation with dietary intakes and other exposures through diverse mechanisms, including protein synthesis and chemical binding with sulfhydryl groups (He, 2011). While there are variations in the availability of biomarkers for different trace elements, nails serve as a time-integrated measure of body intake. Specifically, toenail clippings, due to their relatively slow growth rate, may offer insights into a more extended exposure time frame (He, 2011).

Nails have several advantages as a biomarker for tracking the levels of trace elements. Its sampling method is comparatively easy, and unlike fluids, they do not require refrigeration before transport for analysis. Other from the technical considerations regarding the samples collection, transport and storage, human factors such as ethical and safety issues must be taken into account during sampling. Therefore, non-invasive samples such as nails are typically preferred whenever feasible as the selection of the matrix is crucial and could significantly affect the quality of exposure assessment and data interpretation (Hardy et al., 2021).

2.7 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

There are various methods that can be used for the analysis of trace elements, including atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). Nevertheless, AAS is limited to determining a single element per run due to its narrow linear range, while ICP-AES does not allow for the removal of spectral interference. In contrast, inductively coupled plasma mass spectrometry (ICP-MS) has gained widespread acceptance over the past two decades. Compared to traditional inorganic analytical techniques, ICP-MS offers several advantages, such as a larger linear range, low detection limit and a very high level of interference control (Huang et al., 2006). It has been extensively utilized for the analysis of essential elements and well-recognized potentially toxic metals (Pappas et al., 2006). Therefore, the analysis of trace elements in the nail samples is often conducted using inductively coupled plasma mass spectrometry (ICP-MS). It is a form of mass spectrometry utilizing inductively coupled plasma for sample ionization. The process includes atomizing the sample, generating atomic and small polyatomic ions, which are then detected. In simpler terms, it entails breaking down a sample into its elemental components and converting them into ions. The most significant advantage of using ICP-MS is its multi-element capability, which allows multiple elements to be analyzed concurrently in a single analysis. ICP-MS provides the opportunity for very high number of samples to be produced in the laboratory, facilitated by the combination of brief analysis periods and straightforward sample preparation (Wilschefski & Baxter, 2019).

2.8 Risk Characterization

Risk characterization represents the concluding stage in the evaluation of human health risks associated with synthetic pesticides and chemical fertilizers. In this context, it involves the summarization and integration of health hazard, dose-response, and exposure assessments to provide an all-encompassing description of the overall conclusion of risk associated with a synthetic pesticide and chemical fertilizer, typically based on the formula,

$$Synthetic\ Pesticide\ and\ Chemical\ Fertilizer\ Health\ Risk$$
$$= Health\ Hazard\ x\ Exposure\ Level$$

Given that risk is a function of both hazard and exposure, risk characterization involves the integration of synthetic pesticide and chemical fertilizers' toxicity and exposure data to forecast the likelihood and severity of adverse human health effects. While toxicity and exposure data are examined independently, the assessments derived from each are subsequently combined to characterize the risk and determine the significance of the risk. This process takes into account the factors that could influence alterations in risk characterization.

i. Age

There is substantial evidence indicating a correlation between age and trace element contamination (Nyeste et al., 2019). Research has emphasized that trace element contamination notably rises in individuals over the age of 30 (Fry et al., 2021).

ii. Body Mass Index (BMI)

Chronic oxidative stress is identified as the primary mechanism responsible for obesity-related co-morbidities (Vincent et al., 2007). The heightened oxidative stress resulting from obesity can lead to persistent metabolic health implications (Gandhi and Kaur, 2012). Consequently, individuals with obesity exhibit a notable reduction in their overall antioxidant levels, accompanied by an increase in trace element contamination within the body. To mitigate the risk of chronic metabolic health issues among obese individuals, weight reduction and the maintenance of balanced energy intake are recommended (Gandhi and Kaur, 2012).

iii. Smoking Habit

Tobacco smoking exerts an influence on the concentrations of various elements within specific organs. It is recognized that cigarette smoking can serve as a significant source of exposure to harmful trace elements, including but not limited to Al, As, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Po-210, Se, and Zn, not only for the smokers themselves but also for nonsmokers through passive smoke exposure (Chiba & Masironi, 1992). Additionally, other studies have proposed that the median concentrations of trace elements are notably higher in households where smoking is prevalent (Kastury et al., 2020).

iv. Duration of exposure

The duration of exposure serves as a surrogate marker for pesticide exposure, especially among individuals involved in handling and spraying pesticides (Nuralain et al., 2017). It is evident that the length of time individuals is exposed to pesticides has a substantial impact on the development of chronic health risks among pesticide applicators (How et al., 2022). Notably, farmers who have been exposed to pesticides for a period exceeding 10 to 20 years face an elevated risk of chronic health issues (Siti Mariam & How, 2017).

v. Personal Protective Equipment (PPE)

Personal protective equipment (PPE) plays a crucial role in acting as a protective barrier to prevent cross-contamination resulting from the absorption of pesticides through the skin and respiratory system (Feola and Binder, 2010). Research indicates that the proper utilization of PPE is effective in lowering the occurrence of adverse health outcomes (Nuralain et al., 2017). Nevertheless, it's important to note that PPE is often not regularly replaced or cleaned, and working without suitable protective gear, such as bare hands, is linked to an elevated risk of chronic health issues (How et al., 2013).

vi. Pesticide Storage and Disposal Practices

As the majority of local agricultural laborers in Malaysia are smallholders who manage their family-subsistence farms, the enforcement of national legislation, standards, and training can be challenging, as it may conflict with traditional agricultural practices and norms. In 2004, the Department of Agriculture (DOA) introduced the "Pesticide Container Recycling Program" with the aim of ensuring the proper disposal of empty pesticide containers to protect the environment, in accordance with Good Agricultural Practices (GAP). However, it's important to note that despite these efforts, the storage and disposal practices of pesticides by farmers still show a significant risk of accidental pesticide exposure (How et al., 2014).

2.9 Pesticide usage Related Acts, Regulations and Policies

The Department of Agriculture Malaysia (DOA) states the need for a premarket evaluation of pesticide products and food, focusing on fundamental toxicological data. However, this assessment is considered insufficient due to the reported health effects arising from exposure to combinations of pesticides, known as mixtures, such as when using more than one type of pesticide (Alavanja & Bonner, 2012). This insufficiency may be attributed to toxicology tests primarily involving short-term exposure of single active ingredients to inbred animal strains, without accounting for the human body's mechanisms when chronically exposed to a mix of pesticides (How et al., 2013). Animal studies aid in addressing specific gaps in knowledge and drawing the inference of potential health hazards to humans, but there are uncertainties associated with extrapolating the results from animal subjects to humans. Consequently, the government has initiated various measures to ensure the responsible use of pesticides. Among the enacted legislation aimed at regulating pesticide use are:

i. Pesticides Act 1974, to impose penalties for all offences, control the pesticides' importation, control possession or unapproved use of pesticides, enforce the pesticide disposal charges and so on.

ii. Environmental Quality Act 1974, to control the discharge of pesticide waste from factories into the environment in terms of volume, composition or manner so as not to cause adverse effects on human health and the environment.

iii. Occupational Safety and Health Act 1994, to promote and stimulate the provision of safety and health to those who handle pesticides at the workplace.

iv. Food Act 1983 (Act 281), The Food Act addresses the maximum residue limits (MRLs) of pesticides in food and sets standards to safeguard food safety.

v. National Pesticide Policy (NPP), Malaysia's NPP outlines the country's strategy for the responsible use of pesticides, with a focus on minimizing environmental impact and protecting human health.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter explains the overall research methodology, which includes study design, study location, study duration, study sample, study instrument, quality control, statistical analysis and ethical consideration. Each component was in the purpose of answering the research objectives and at the same time align with the research interest, which is to determine the level of trace elements (Zn, Mn, Pb and NH_3) among the organic and conventional farmers from the small-scale nursery and vegetable farms in West Coast of Peninsular Malaysia.

3.2 Study Design

The study design used for this research is a cross-sectional study. The objective of this study is to compare trace elements (Zn, Mn, Pb, NH_3) contained in nails among conventional and organic farmers in small-scale vegetable and nursery farms in West Coast of Peninsular Malaysia.

The independent variable of this study is the level of trace elements (Zn, Mn, Pb, NH_3) contained in nails among conventional and organic farmers in small-scale vegetable and nursery farms in West Coast of Peninsular Malaysia. While, the dependent variable of this study is the health risk of the types of synthetic pesticide and chemical fertilizers used.

3.3 Study Location

Since there are two types of small-scale farmers participating in this study, which are vegetable farmers and nursery farmers, several study locations were covered to ensure both the small-scale organic and conventional farm activities are included.

Study Location 1 [Highland Agricultural Farmland]

Cameron Highlands is the agriculture production centre in Peninsular Malaysia and is suitable for temperate agriculture crops such as tea, vegetables, fruits and flowers. The total land area used for agriculture is 7,785 ha.

Study Location 2 [Lowland Agricultural Farmland]

Lowland agricultural practices are different from highland agricultural farmland due to the variation in climate and pest life cycle. Lowland small-scale vegetable and nursery farms in Peninsular Malaysia (Johor, Malacca, Selangor, Perak and Penang) were randomly targeted and approached.

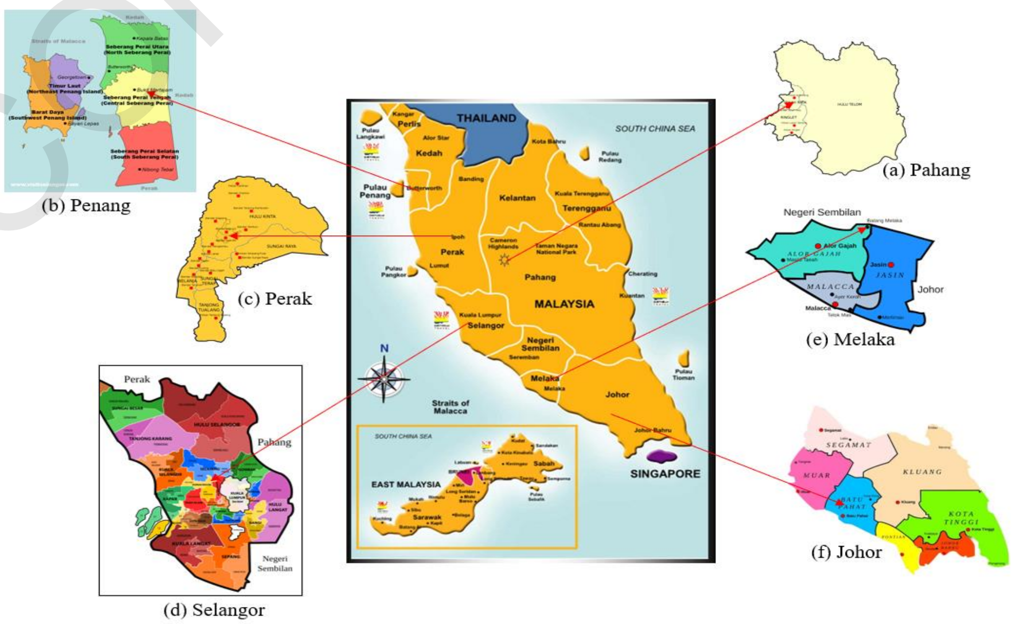

Figure 3.1: Location of sampling in the West Coast of Peninsular Malaysia, (a) Pahang, (b) Penang, (c) Perak, (d) Selangor, (e) Melaka, (f) Johor

3.4 Study Duration

This study was conducted between March 2023 and December 2023.

3.5 Study Population

In this study, a total of 109 smallholder vegetable and nursery farmers from conventional and organic farming groups, meeting the inclusion criteria, were recruited. Consequently, 58 farmers were chosen as representatives of the conventional farming groups and 51 farmers were chosen as representatives of the organic farming groups. As detailed below, two phases of selection criteria were used to establish the study population frame.

3.5.1 Pre-selection Sampling Frame

Both conventional and organic farmers were required to meet the inclusion criteria as outlined in Figure 3.2 and Figure 3.3 during the initial phase of pre-screening the sampling frame. The inclusion and exclusion criteria are provided below.

Inclusion criteria for conventional farmers (Vegetable Farms and Nursery Farms)

- Both gender at the age 18 – 60 years old
- Has been involved in conventional farming at the studied location for at least 12 months
- Living in the agricultural farming communities for the past 12 months
- Handling conventional farming activity, including using synthetic pesticides and fertilizers in their routine farming activity

Inclusion criteria for organic farmers (Vegetable Farms and Nursery Farms)

- •Both gender at the age 18 – 60 years old
- Has been involved in organic farming at the studied location for at least 12 months
- Living in the agricultural farming communities for the past 12 months
- Handling organic or natural or agroecological farming activity and has not been using any synthetic agrochemicals for the past 12 months

Exclusion Criteria for both organic and conventional farmers

- Had medical conditions, such as anaemia (Rice, 1977), nephritic problems (Fourman, 1966) and hepatic diseases (Meng et al., 2013)
- Had a history of exposure to cytotoxic therapeutic drugs (e.g. chemotherapy) and radiation (Blagosklonny, 2005)

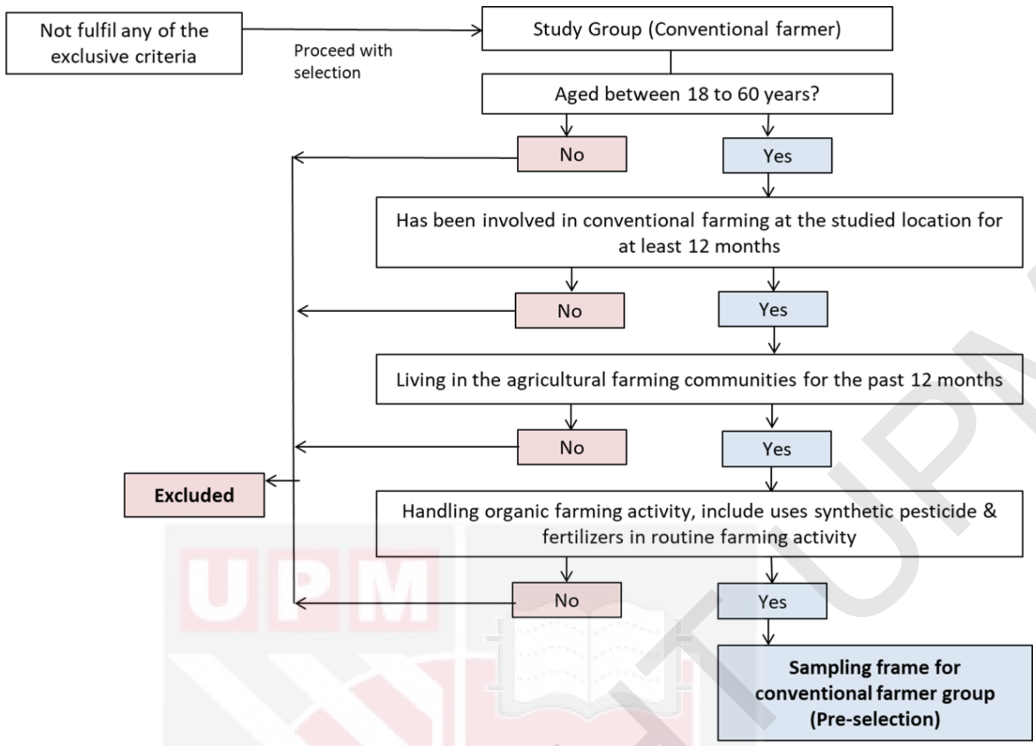

Figure 3.2: Flow Chart for Pre-selection Conventional Farmer

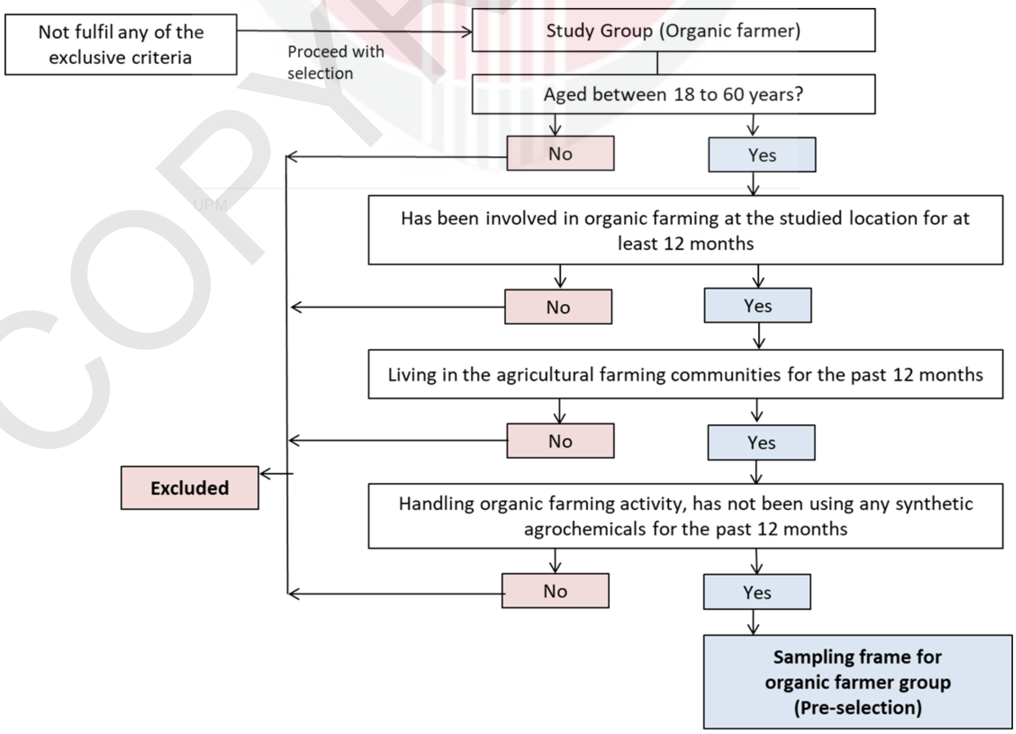

Figure 3.2: Flow Chart for Pre-selection Organic Farmer

3.5.2 Final Selection of Sampling Frame

After the pre-selection of the conventional farmer and organic farmers, phone interview sessions with each of the farmers were conducted to ascertain their eligibility for nail sampling in this study. Only those farmers who satisfied the inclusion criteria for nail samples participated in this research, as depicted in Figure 3.4.

Inclusion Criteria for both (organic and conventional) farmers of nail samples

- No dermatological disease, trauma or injury affecting the nails.
- No nail polish or other chemicals on nails for at least three months before the date of nail sample collection.
- The respondents were notified about the study and advised not to cut their nails for four weeks before sample collection. Respondents with nail polish or other chemicals on their nails waited for three months and then one extra month for nails to grow before sample collection.

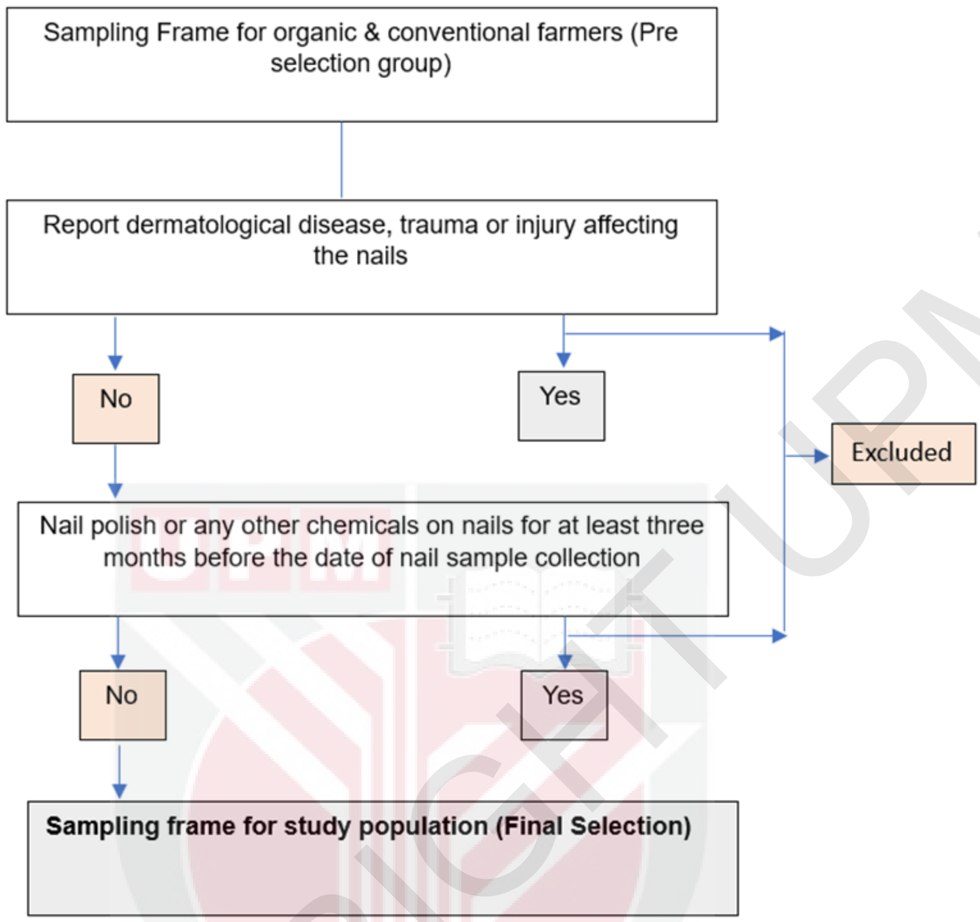

Figure 3.4: Flow Chart for Final-selection Study Population

3.6 Study Sample and Sample Size

Highland and lowland vegetable and nursery farm sites were determined through random cluster sampling of selected state areas (Cameron Highlands, Penang, Perak, Selangor, Melaka and Johor). Respondents meeting the inclusion criteria were selected using stratified random sampling. Those willing to be part of the study, as indicated in the consent letter given to respondents, were then chosen through simple random sampling methods. Before selection as study participants, respondents were required to fulfil both inclusive and exclusive criteria.

This estimation was calculated with a reasonable level within 5% of the true prevalence with 95% confidence. The formula for estimating the size for group-comparison (two-group) design using the combined (or pooled) standard deviation ($2\sigma^2$) for the two groups adopted from Lemeshow et al. (1990) is used as follows:

$$n = \frac{2\sigma^2\left[Z_{1-\alpha/2} + Z_{1-\beta}\right]^2}{(\mu 1 - \mu 2)^2}$$

Where,

σ = Estimated standard deviation (assumed to be equal to each group)

μ^1 = Estimated mean (larger)

μ^2 = Estimated mean (smaller)

$Z_{1-\alpha}$ = Standardized value for the confidential interval, 95% CI=1.96

$Z_{1-\beta}$ = Standardized value for power, 80% of power = 0.84

The sample size calculation was identified based on the study's objective. The sample size for the study's main objective was calculated using data from a prior survey by Husin et al (1999). Based on all objectives, the sample size was 52 farmers for one group, with 20% of non-respondents' rates included.

$$n = \frac{2 \times (1127.64)^2\,[1.96 + 0.84]^2}{(5160.75 - 4479.20)^2}$$

Therefore, n = 43 + 9 = 52 respondents. For the two groups of respondents, n= 2×52 = 104 respondents.

3.7 Study Flow and Study Instrument

As outlined in Figure 3.5, this study comprises three sections of field data collection and laboratory analysis. Each section was conducted among both organic and conventional farmers.

To determine sociodemographic and occupational background	
A	Face to Face Interview Questionnaire

To determine the health risks of the synthetic pesticide and chemical fertilizer	
B	Chemical Health Risk Assessment

To evaluate trace element levels contained in nail sample	
C	Nail sampling

Figure 3.5: Summary of Data Collection

A flowchart illustrating on-site data collection procedures among the study population was presented in Figure 3.6. Samples collected from Part C were forwarded to the laboratory for in-depth analysis.

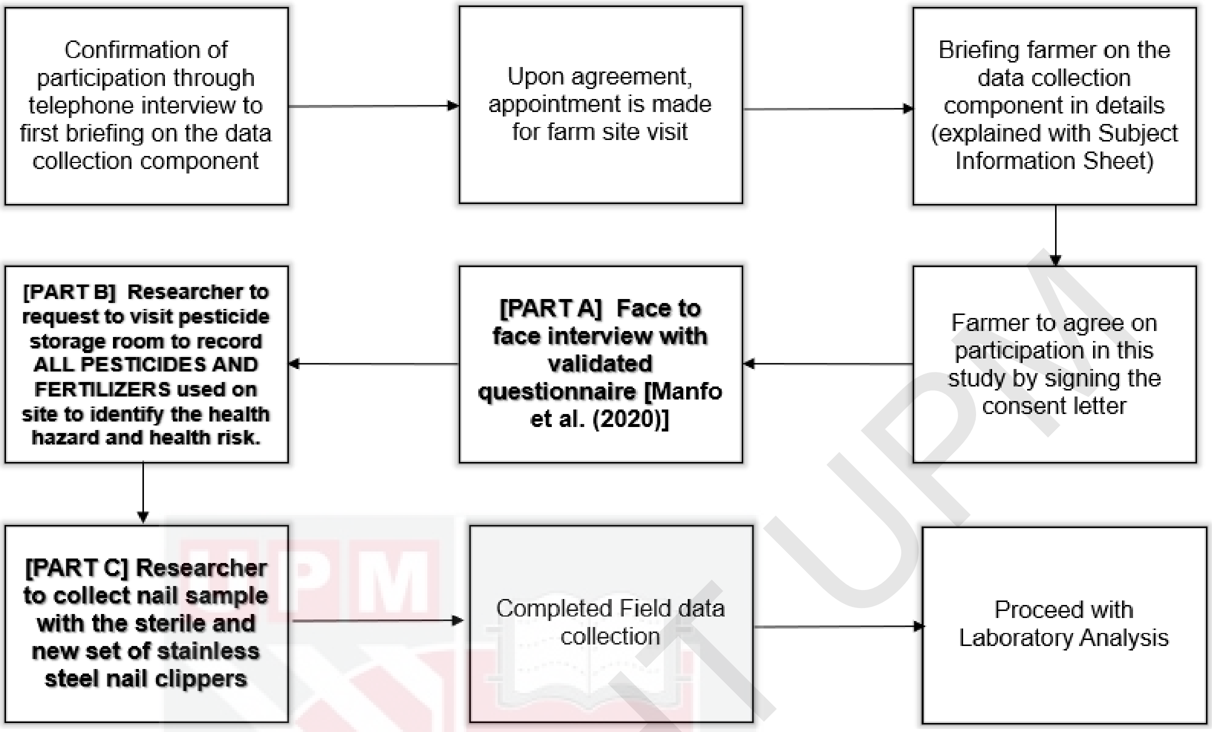

Figure 3.6: Flow Chart for Data Collection Procedures

3.7.1 Questionnaire

A validated questionnaire (**Refer to Appendix I**) adapted from Manfo et al. (2020) was used to assess the study population's background information, which includes five sections. An interview was conducted with the farmers face-to-face to obtain the information.

Section A: Personal Information

Section B: Lifestyle Information

Section C: Workplace Background

Section D: Information on the Use of Synthetic Pesticides and Chemical Fertilizers

Section E: Information on Use of Personal Protective Equipment (PPE)

3.7.2 Chemical Health Risk Assessment of Farmers Using Pesticides and Fertilizers

A pesticide and fertilizer health hazard checklist (Refer to Appendix II) adapted from the "A Manual of Recommended Practice on Assessment of the Health Risks Arising from the Chemicals Hazardous to Health at the Workplace 2018 (3rd Edition)" was used by the researcher to evaluate the potential health risks from the synthetic pesticides and chemical fertilizers used at the farm. The health hazards based on the Globally Harmonized System (GHS) for Hazard Classification and Labelling was assigned to each pesticide based on the pesticide label, or the Safety Data Sheet (SDS) obtained at the farm site. The health risk assessment method, endorsed by the US Environmental Protection Agency (USEPA), is used and designed to evaluate human health in relation to various exposures. The health risk of synthetic pesticides and chemical fertilizers was characterized based on the health hazards and the level of exposure among the study respondents. This section was carried out by a certified chemical health risk assessor registered under the Department of Occupational Safety and Health (DOSH), Ministry of Human Resources. The degree of hazard, which is the hazardous properties of the trace elements present in the synthetic pesticides and chemical fertilizers (Zn, Mn, Pb and NH_3) as well as the extent and degree of contact were determined. The level of risk for exposure was determined based on the information on the hazardous properties, observation on the extent of contact and duration of exposure as shown in table 3.2.

Table 3.1: Exposure Rating

		MAGNITUDE RATING (MR)				
		1	**2**	**3**	**4**	**5**
FREQUENCY - DURATION RATING (FDR)	**1**	1	2	2	2	3
	2	2	2	3	3	4
	3	2	3	3	4	4
	4	2	3	4	4	5
	5	3	4	4	5	5

Source: CHRA manual, 3rd edition (DOSH, 2013)

Table 3.2: Level of Risk Determination

		EXPOSURE RATING (ER)				
		1	**2**	**3**	**4**	**5**
HAZARD RATING (HR)	**1**	**RR=1**	**RR=2**	**RR=3**	**RR=4**	**RR=5**
	2	**RR=2**	**RR=4**	**RR=6**	**RR=8**	**RR=10**
	3	**RR=3**	**RR=6**	**RR=9**	**RR=12**	**RR=15**
	4	**RR=4**	**RR=8**	**RR=12**	**RR=16**	**RR=20**
	5	**RR=5**	**RR=10**	**RR=15**	**RR=20**	**RR=25**

RR= Risk Rating
Low risk: RR=1 to RR=4 ; Moderate risk: RR= 5 to RR=12 ; High risk: RR=15 to RR=25
Source: CHRA manual, 3rd edition (DOSH, 2013)

3.7.3 Nail Sample Collection

At the onset of sample collection, respondents were instructed to allow their fingers and toenails to grow for four to six weeks, respectively. On the sampling day, each respondent was supplied with new and sterile stainless steel nail clippers for hygiene purposes. The protocol for nail sample collection has adhered to the standard guidelines recommended by Elekdag-Turk et al. (2019).

Nail samples, for both toenail and finger samples, were collected in 5g samples. Permission to cut respondents' toenails and fingernails was requested before sample collection. Using each participant's nail clipper, the participants were allowed to clip their nail's free end. The clipped nails were stored separately in labelled sterile zip-locked plastic bags. All samples were stored at room temperature until their shipment to the designated laboratory for trace element analysis.

3.8 Laboratory Analysis

The Analytical Methodology to Evaluate Trace Elements in Nails is based on research by Przybylowicz et al. (2012) on their work on "Examination of the distribution of trace elements in hair, fingernails and toenails as alternative biological materials".

3.8.1 Experimental Procedure – Chemicals and Materials

All trace element measurements were carried out using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; NexION 350S, Perkin Elmer, USA). Argon with a purity of 99.999% was utilized throughout the work. 1% nitric acid (HNO_3) and Ultra-Pure water was used for various tasks, including cleaning. Acetone was used for washing nail samples while 65% nitric acid and 30% hydrogen peroxide were utilized in the digestion process. Internal standard mix, Multi-element Calibration Standard 3, 65% Nitric acid, Mercury and Gold were involved in the process of making standard solution, quality control sample as well as internal standard.

3.8.2 Experimental Procedure – Preparations of Samples

Each of the nail samples was placed into a 15 ml centrifuge tube using forceps. The forceps were wiped with 1% nitric acid (HNO_3) each time after coming into contact with the samples to prevent contamination with subsequent samples. It's important to avoid touching the samples with hands, even when wearing gloves, to prevent contamination. The body of the centrifuge tube was labelled with a pencil to ensure the labelling remained intact during the washing process, while the cap of the centrifuge tube was labelled with a marker pen.

During the sample washing process, the nail samples were alternately soaked in acetone and Ultra-Pure Water (UPW) as shown in figure 3.7. This washing procedure was done after the nail samples had undergone sonification, a process using ultrasound energy to agitate particles on the nail samples. Acetone was employed to remove dirt from the samples, while UPW was used to eliminate the acetone. Following the washing process, the samples were dried in a desiccator at 70°C overnight and stored in a zipped bag at room temperature for further analysis.

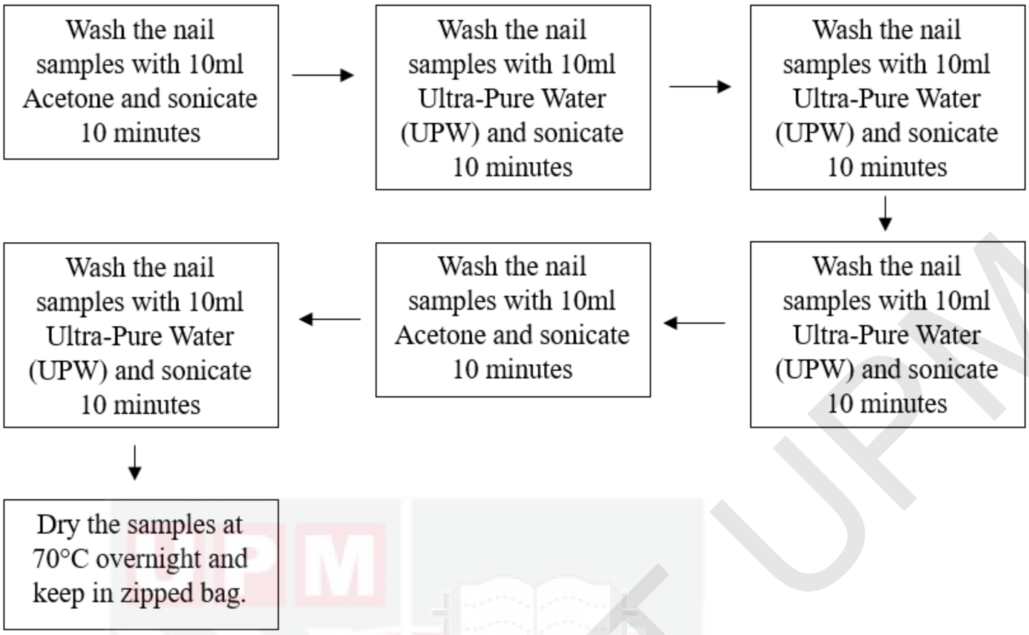

Figure 3.7: Procedure of Washing Nail Samples

Digestion of nail samples was conducted after the washing process. Initially, nail samples were weighed directly into the vial and the weight was recorded. A total of 2.25 ml of 65% nitric acid (HNO3) and 0.75 ml of 30% hydrogen peroxide (H2O2) were added into the vials containing nail samples. The acids were added drop by drop to wet the samples directly to the inner wall of the insert, and the solution was gently swirled to homogenize the samples with acids. The vials were covered, put into the holders and set aside to allow the reaction to occur for about 30 minutes. After 30 minutes, the base load consisting of 10ml Ultra-pure water (UPW) was added to the TFM vessel and the holder with vials was put into the TFM vessel. The samples were digested using Milestone Ethos Easy Microwave Digestion System at 1800W for 15 minutes at 180°C, 1800W for 10 minutes at 180°C and allowed to cool for 15 minutes. The digested solution was transferred into the 15ml centrifuge tube. The quartz vial was rinsed with UPW and transferred into the same 15ml tube. This step was repeated

3 times until the final volume reached 12ml. The 15ml tube was inverted a few times to mix the sample. The samples were then filtered using a 0.45 µm PTFE filter into a new clean 15 ml centrifuge tube. The cover of the centrifuge tubes was sealed using parafilm and the samples were stored at 4°c until further analysis.

3.9 Quality Control

3.9.1 On-Site Data Collection

A pilot study was conducted to test the logistics and identify potential deficiencies in the study design. This pre-testing study covered all three data collection sections, as shown in Figure 3.4. The research protocols and quality control measures for each section were identified and analysed. To ensure the reliability and validity of the collected samples throughout the study, the following steps were followed:

i. Chain-of-custody

A Chain-of-custody procedure was utilized for sample and evidence management to ensure that procedures were being followed, the research team employed tags or labels, field notes, picture capture, and any other recorded information for accuracy.

ii. Traceability of Sample

When relevant samples were collected, the researcher filled out a checklist table (Refer to Appendix III). This was done to ensure that biological samples (nail samples) could be traced through field records from the researcher who collected the samples or made the measurements.

iii. Variability and Reproducibility of Samples

Samples were duplicated, involving the collection of replicated samples simultaneously to minimize differences. Nail and hair samples were collected and replicated into two zip-lock sterile bags. Before being submitted for laboratory analysis, these samples were preserved in the same manner. To avoid potential variations in biological samples during sampling procedures, duplicated samples were collected to ensure that the range of obtained results was around the mean values within the population.

iv. Sample Collection Precautions

Precautionary steps were emphasized to prevent cross-contamination, especially during the collection of biological samples. Detailed precautions regarding sample handling, mixing, preservation, storage, and transport are discussed in the section 3.9.4.

3.9.2 Quality Control for Questionnaire

The validated questionnaire (Refer to Appendix I) was prepared for respondents during the pilot study. A group of research assistants was trained specifically to conduct a survey questionnaire among the study population. This was done to reduce the conditions of inter-observer variability during a face-to-face interview. Respondents were interviewed by these trained research assistants to ensure the content and validity of the questionnaire. Consequently, the contents of the item questions were scrutinized to ensure that they matched the actual situation being studied and had the ability to predict a hypothesized outcome.

After the pre-testing assessment, the variables in the questionnaires were selected to test for their reliability and internal consistency. A test-retest reliability of the questionnaire for the farmer was estimated by performing the same questionnaire with the same respondents at after 1 week.

During the data collection day, study respondents were briefed on the purpose of the study with the aid of a subject information sheet (Refer to Appendix IV). Those who returned the written consent (Refer to Appendix IV) following the detailed briefing session were recruited as respondents. The face-to-face interview was done to ensure the validity of questions, particularly to avoid misinterpretation of the question items for those who were illiterate.

3.9.3 Quality Control for Chemical Health Hazard Checklist

A validated Chemical Health Hazard Checklist (Refer to Appendix II) was used to examine the background of pesticide health hazards. To ensure the reliability and validity of the health hazard classification, a certified and registered chemical health risk assessor (Refer to Appendix VI) by the Department of Occupational Safety and Health (DOSH), Ministry of Human Resources Malaysia, was appointed to conduct the pesticide health hazard identification. The chemical health risk assessor is registered under the Occupational Safety and Health (Use and Standards of Exposure of Chemicals Hazardous to Health) Regulations 2000, or in short, OSH (USECHH) Regulations 2000.

3.9.4 Quality Control for Nail Sampling

Human nails are primarily composed of keratin-rich proteins. Nail samples are frequently collected and preserved in advance for extended periods, sometimes for months or years, before being retrieved and analyzed in epidemiological research aimed at exploring the correlation between concentrations of trace elements and one or more clinical endpoints. Quality control for nail sampling is based on He (2011) in the analysis titled "Trace Elements in Nails as Biomarkers in Clinical Research."

3.9.4.1 Sample Collection Precautions

Participants were instructed not to trim their nails for a couple of weeks or longer to accumulate a larger nail mass. Nails were collected by clipping with a stainless-steel clipper from the two great toes and tiny toes from the feet and thumbs or other fingers from the hands. Participants were requested to obtain as many nails as possible, and clippings should have been from both hands and feet.

3.9.4.2 Sample Preservation, Storage and Transport

Nail samples were transported to the Toxicology Laboratory, Environmental Health Research Centre, Institute for Medical Research (IMR), with the labelled specimens placed in a sealed zip-closable bag. The specimens were stored in zip-closable bags at room temperature throughout the storage and transportation.

3.9.4.3 Laboratory Analysis

Glassware underwent a process of soaking in 65% nitric acid, followed by rinsing with ultrapure water, and subsequent air drying before use. The analysis of heavy metals was conducted using an ICP-MS NexION 350S (Perkin Elmer, USA) which consists of a nebulizer, cyclonic spray chamber, and an autosampler. The internal standards selected were Iridium (Ir) for Pb and rhodium (Rh) for Zn, Mn and NH_3, and each test batch was assessed through an internal quality approach. The batches were validated to ensure compliance with predefined internal quality controls. Every analysis included a blank, internal standard in samples and duplicate analysis of samples to mitigate batch-specific errors. The calibration curves created for the determination of Zn, Mn, NH_3, and Pb exhibited high linearity ($r^2 > 0.999$). Quantitative analysis using external calibration standards derived from a 10 mg/mL Reference Standard, ICP-MS Multi-Element Calibration Standard 3 in 1% (v/v) HNO_3 acid at concentrations of 1.25, 2.5, 5.0, and 20.0 mg/L for Zn, Mn, NH_3 and Pb. In instances where the sample concentration exceeded the calibration standards range, dilution was carried out, and the samples were re-analysed. The laboratory reagent blank aimed to identify potential contamination and carry-overs resulting from the sample preparation procedure. Additionally, a rinse blank was utilized to clear the instrument uptake system and nebulizer between standards, check solutions, and samples, minimizing memory interference. For the initial and periodic validation of calibration standards or stock standard solutions, standard solutions and quality control samples were essential for verifying the performance of the instrument. They were prepared daily before the calibration of the instrument. The precision of the method involved spiking three replicates of samples with a Zn, Mn, Pb and NH_3 standard (low, medium, high). The concentration of trace elements was then compared before and after the addition. The recovery values for these elements fell within the acceptable range of 85% to 115%.

3.9.5 Biological Sample (Nail) Disposal

All collected biological samples were disposed of immediately at the end of the study. The nail samples were disposed of following the Clinical Waste Management System in Malaysia, where the samples were placed into a yellow bag and temporarily stored at the Scheduled Waste Section of the Faculty of Medicine and Health Sciences, UPM. The disposal method involved an incineration process, converting the clinical waste into ash for landfill.

3.10 Statistical Analysis

Since this is a quantitative study, all variables were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 24.0. The level of significance (α) was set at α = 0.05. In this study, statistical analysis aimed to achieve a significance level of 0.05 for a two-sided test, where the absolute value of the test statistic (|z|) must be greater than or equal to the critical value of 1.96. The data was analysed based on the study objectives and the normality background of each data set.

Table 3.3: Types of statistical analysis used

Study Objective	Statistical Test
To determine the socio-demographic background and farming activities and practice of among small-scale vegetable and nursery farmers	Univariate analysis (Frequency, percentage, mean ± Standard deviation)
To determine the health risk of the types of synthetic pesticide and chemical fertilizers used among small-scale vegetable and nursery farmers	Univariate analysis (Frequency, percentage, mean ± Standard deviation)
To determine the level of trace elements (Zn, Cu, Cr, Mn) contained in nails among small-scale vegetable and nursery farmers	Univariate analysis (mean ± Standard deviation)
To compare the difference of trace elements (Zn, Cu, Cr, Mn) contained in nails among conventional and organic farmers in small-scale vegetable and nursery farms	Bivariate analysis Independent t-test & Mann Whitney U-Test
To associate the health risk of synthetic pesticide and chemical fertilizers used and the level of trace elements (Zn, Mn, Pb, NH_3) among small-scale vegetable and nursery farmers	Bivariate analysis Pearson correlation test & Spearman correlation test
To determine the relationship between the trace elements (Zn, Mn, Pb, NH_3), socio-demographic background, farming practices and activities among small-scale vegetable and nursery farmers	Bivariate analysis Ordinary least squares regression test (OLS)

3.11 Ethical Consideration

The principal investigator's primary responsibility in human subjects' research is to ensure that the rights and welfare of the participants are protected. Hence, this research was submitted to Ethic Consideration ensure all the procedures concerned are well addressed. The research proposal was submitted to be reviewed by the Ethics Committee for Research Involving Human Subjects University Putra Malaysia (UPM) to obtain approval involving human samples for the research (JKEUPM-2023-187). A detailed explanation was given to the respondents of the study before answering the questionnaire (Refer to Appendix I) and collecting nail samples from the respondents during the research. Sufficient time was allocated for them to participate in the research upon going through the subject information sheet (Refer to Appendix IV). Next, the respondents who agreed to participate in the research were given a consent form (Refer to Appendix IV) to obtain their signature for agreement of the study's ethical purposes. Data confidentiality was strictly controlled and privately disclosed as only the researchers can retrieve it.

CHAPTER 4

RESULTS

4.1 Response Rate

This study achieved a response rate of 100%. Based on the sample size calculation, it was determined that a minimum of 104 participants should have been recruited for this study. Nevertheless, a total of 109 participants were recruited by the researcher to ensure the delivery of reliable and robust findings.

$$Response\ Rate = \frac{Respondents\ completed\ all\ data\ collection\ section}{Respondents\ involved\ in\ data\ collection}\ x\ 100 \quad - Equation\ 4.1$$

4.2 Normality Test

Table 4.1 shows the normality of the individual risk factor and the level of trace elements (Zn, Mn, Pb and NH_3) by using Kolmogorov–Smirnov test. The age and BMI distributions of both organic and conventional farmers were found to be normally distributed, where p= 0.061, 0.193, 0.200 and 0.057 (>0.05) respectively. The distribution of trace element (Mn, NH_3 and Pb) among the organic farmers and conventional farmers were not normally distributed where the Kolmogorov-Smirnov level was <0.05, except for the level of zinc among the organic and conventional farmers and the level of ammonia among the organic farmers were normally distributed, p= 0.200 (>0.05) and p=0.182 (>0.05) respectively. Likewise, based on table 4.2, the association between the level of trace element (Mn, NH_3 and Pb) and the health risk of synthetic pesticides and chemical fertilizers (Mn, NH_3 and Pb) among organic and conventional farmers were also not normally distributed as the significance level was <0.05 for all three. Only the level of trace element (Zn) and the health risk of synthetic pesticides and chemical fertilizers (Zn) among organic and conventional farmers was normally distributed, p=0.146 (<0.05). In addition to the utilisation of the p-value as an indicator of the normality of the study variable, the ratio of skewness to its standard error is also employed as a means to assess the normality. If the ratio is less than -2 or greater than +2, normality is not feasible. Conversely, a ratio within the range of +/- 2 is regarded as normal.

Table 4.1: The normality test of the individual risk factors and the level of trace elements among the organic and conventional farmers

Variables	Statistics	Skewness	Standard Error	Ratio of Skewness/Standard Error	p-value	Normality
Organic farmers (n=51)						
Age (year)	44.3137	0.044	0.333	0.132	0.061*	Normal
BMI (kg/m^2)	25.7209	1.185	0.333	3.559	0.200*	Normal
Year of Working	11.8235	1.381	0.333	4.147	0.004	Not normal
Level of Zinc (mg/kg)	71.9942	-0.074	0.388	-0.191	0.200*	Normal
Level of Manganese (mg/kg)	0.6003	0.671	0.343	1.956	0.037	Not normal
Level of Lead (mg/kg)	0.5621	0.843	0.333	2.531	0.015	Not normal
Level of Ammonia (mg/kg)	0.0490	0.496	0.361	1.374	0.182*	Normal

Conventional farmers (n=58)						
Age (year)	43.0517	0.393	0.314	1.252	0.193*	Normal
BMI (kg/m^2)	26.1614	0.586	0.314	1.866	0.057*	Normal
Year of Working	16.9138	1.000	0.314	3.185	0.009	Not normal
Level of Zinc (mg/kg)	100.8460	0.123	0.319	0.386	0.200*	Normal
Level of Manganese (mg/kg)	1.7467	1.213	0.314	3.863	<0.001	Not normal
Level of Lead (mg/kg)	0.7962	0.799	0.316	2.528	0.004	Not normal
Level of Ammonia (mg/kg)	0.0785	0.369	0.337	1.095	0.003	Not normal

* Normality is not violated at p>0.05

Table 4.2: The normality test of the level of trace elements and the level of health risk of synthetic pesticide and chemical fertilizers among the organic and conventional farmers

Variables	Statistics	Skewness	Standard Error	Ratio of Skewness/Standard Error	p-value	Normality
Low Risk (n=39)						
Level of Zinc (mg/kg)	73.1473	-0.163	0.378	-0.431	0.146*	Normal
Moderate Risk (n=54)						
Level of Zinc (mg/kg)	101.0818	0.102	0.325	0.314	0.154*	Normal
Low Risk (n=50)						
Level of Manganese (mg/kg)	0.5904	0.726	0.337	2.154	0.044	Not normal
Moderate Risk (n=25)						

Level of Manganese (mg/kg)	1.2929	0.913	0.464	1.985	<0.001	Not normal
High Risk (n=21)						
Level of Manganese (mg/kg)	2.2027	0.941	0.421	2.235	0.001	Not normal
Low Risk (n=51)						
Level of Lead (mg/kg)	0.5621	0.843	0.333	2.532	0.008	Not normal
Moderate Risk (n=6)						
Level of Lead (mg/kg)	0.6214	0.017	0.845	0.020	0.050*	Normal
High Risk (n=51)						
Level of Lead (mg/kg)	0.8167	0.713	0.333	2.141	0.001	Not normal
Low Risk (n=45)						
Level of Ammonia (mg/kg)	0.0492	0.474	0.354	1.339	0.152*	Normal

Moderate Risk (n=48)						
Level of Ammonia (mg/kg)	0.0795	0.334	0.343	0.974	0.003	Not normal

* Normality is not violated at p>0.05

4.3 Sociodemographic Characteristics Among Study Respondents

Table 4.3 describes the sociodemographic characteristics of the respondents. All respondents were Malaysian and they were divided into two groups, which are organic farmers with a mean age of 44.31 ± 15.47 years old and conventional farmers with a mean age of 43.05 ± 10.90 years old. Meanwhile, the majority of the organic farmers and conventional farmers comprised of male (68.6% and 72.4% respectively), and with a mean BMI of 25.72 ± 5.31 and 26.16 ± 4.82 respectively. The mean years of working for conventional farmers was 16.91 ± 11.65 years, while organic farmers had an average of 11.82 ± 10.78 years of experience. As for smoking habit, 68.6% of organic farmers and 65.5 % of conventional farmers were smokers. In terms of taking alcohol habit, the greatest proportion of the organic farmers were non-drinker (49.0%), followed by drinking alcohol once a week (45.1%) and lastly drinking alcohol 3 times a month (5.9%). While for conventional farmers, 41.4% of them were non-drinker, followed by drinking alcohol once a week (24.1%), drinking alcohol 3 times a month (22.4%) and lastly drinking alcohol 3 to 4 times a week (12.1 %).

Table 4.3: Sociodemographic characteristics among both organic and conventional farmers (N=109)

Socio-demographics characteristics	Organic Farmers (n=51)		Conventional Farmers (n=58)	
	Frequency (n%)	Mean (SD)	Frequency (n%)	Mean (SD)
Gender				
Female	16 (31.4%)	-	16 (27.6%)	-
Male	35 (68.6%)		42 (72.4%)	
Age		44.31 (15.47)		43.05 (10.90)
BMI	-	25.72 (5.31)	-	26.16 (4.82)
Year of Working		11.82 (10.78)		16.91 (11.65)
Smoking Habit				
Yes	35(68.6%)	-	38 (65.5%)	-
No	16 (31.4%)		20 (34.5%)	
Alcohol Habit				
No Alcohol	25 (49.0%)	-	24 (41.4%)	-
Once a week	23 (45.1%)		14 (24.1%)	
3 to 4 times a week	0 (0.0%)		7 (12.1%)	
3 times a month	3(5.9%)	-	13 (22.4%)	-
1 to 2 times a year	0 (0.0%)		0 (0.0%)	

4.4 Farming Activities and Practices Among Study Respondents

Table 4.4 describes the farming activities and practices of the respondents. 66.7 % of organic farmers are exposed to synthetic pesticides and chemical fertilizers once per year or less that. Of the conventional farmers, 67.2% of them exposed to synthetic pesticides and chemical fertilizers greater than once time per week. In terms of the duration of the synthetic pesticides and chemical fertilizers used, 64.7% of the organic farmers used the pesticides less than 1 hour, while 63.8% of the conventional farmers used pesticides less than 4 hours but greater than 2 hours. Besides, 64.7% of organic farmers did not experience chemical drift and spillage. In the other hand, 65.5% of the conventional experienced both chemical drift and spillage. Meanwhile, more than half of organic farmers (62.7%) did not use any spraying machine but for conventional farmers, most of them (70.7 %) used type B machines. A large portion of organic farmers and conventional farmers did not have any eye protection while using synthetic pesticides and chemical fertilizers, which are 96.1% and 96.6% respectively. In terms of respiratory protection, 90.2% of organic farmers and 79.3% of conventional farmers used incorrect respiratory protection. For hand protection, the majority of the organic farmers and conventional farmers also did not have any of them, which are 82.4% and 81% respectively. 96.1% of the organic farmers and 96.6% of the conventional farmers are also not protected by correct body protection. For foot protection, 78.4% of organic farmers used incorrect foot protection but 74.1% of conventional farmers had the correct foot protection. Figure 4.1 shows the pictures of types of spraying machines that are commonly used by farming communities while figure 4.2– 4.6 are the pictures of protective personal equipment (PPE) for illustration.

Figure 4.1: Types of Spraying Machine

Figure 4.2: Types of Correct Eye Protection

Incorrect respiratory protection	 **Surgical Face Mask**  **Cloth Face Mask**
Partial respiratory protection	  **Half Facepiece Disposable Filtering Respirator**

Full respiratory protection	  **Half/ Full Facepiece Reusable/ Elastomeric Respirator**

Figure 4.3: Types of Respiratory Protection

Improper hand protection	 **Cotton Knitted Gloves**
Proper hand protection	 **Cotton/ Canva PVC Polka Dot Glove with Knitted Wrist**  **Nitrile Disposable Gloves**
Complete hand protection	  **Nitrile/ Neoprene Latex Chemical Resistant Gloves**

Figure 4.4: Types of Hand Protection

Minimal body protection	 **Cotton Work Shirts and Pants**
Partial body protection	 **Chemical Resistant Protective Apron**
Full body protection	 **Chemical Resistant Protective Coverall**

Figure 4.5: Types of Body Protection

 High Cut PVC Steel Cap Boots	 Low Cut Safety Shoes
 Anti-Slip Rubber Shoes	 High Cut Safety Boots

Figure 4.6: Types of Correct Foot Protection

Table 4.4: The farming activities and practices among both organic and conventional farmers (N=109)

Farming Activities and Practices	Organic Farmers (n=51)	Conventional Farmers (n=58)
	Frequency (n%)	Frequency (n%)
Frequency of Synthetic Pesticide and Chemical Fertilizer Used		
Exposure once per year or less	34 (66.7%)	1 (1.7%)
Exposure greater than one time per month	7 (13.7 %)	4 (6.9 %)
Exposure greater than one time per week	4 (7.8%)	39 (67.2%)
Exposure more than one time per shift per day	6 (11.8 %)	14 (24.1%)
Duration of Synthetic Pesticide and Chemical Fertilizer Used		
Less than 1 hour	33 (64.7%)	1 (1.7%)
Less than 2 hours but greater than 1 hour	15 (29.4%)	20 (34.5%)
Less than 4 hours but greater than 2 hours	2 (3.9%)	37 (63.8%)

Less than 7 hours but greater than 4 hours	1 (2.0%)	0 (0.0%)
More than or equal to 7 hours	0 (0.0%)	0 (0.0%)
Scenario While Spraying Synthetic Pesticides and Chemical Fertilizer		
Not applicable	33 (64.7%)	1 (1.7%)
Chemical drift	9 (17.6%)	5 (8.6%)
Chemical Spillage	6 (11.8%)	14 (24.1%)
Chemical drift and spillage	3 (5.9 %)	38 (65.5%)
Type of Spraying Machine		
Not using chemical	32 (62.7%)	1 (1.7%)
Hand held sprayer	2 (3.9%)	3 (5.2%)
Knapsack hand sprayer	7 (13.7%)	41 (70.7%)
Backpack sprayer	10 (19.6%)	13 (22.4%)
Knapsack power blower/ sprayer	0 (0.0%)	0 (0.0%)

Type of Eye Protection		
No eye protection	49 (96.1%)	56 (96.6%)
Correct eye protection	2 (3.9%)	2 (3.4%)
Type of Respiratory Protection		
Incorrect respiratory protection	46 (90.2%)	46 (79.3%)
Partial protection	3 (5.9%)	10 (17.2%)
Full protection	2 (3.9%)	2 (3.4%)
Type of Hand Protection		
No hand protection	42 (82.4%)	47 (81%)
Improper hand protection	2 (3.9%)	1 (1.7%)
Proper hand protection	6 (11.8%)	10 (17.2%)
Complete hand protection	1 (2.0%)	0 (0.0%)
Type of Body Protection		
Minimal body protection	49 (96.1%)	56 (96.6%)

Partial body protection	0 (0.0%)	0 (0.0%)
Full body protection	2 (3.9%)	2 (3.4%)
Type of Foot Protection		
Incorrect Foot Protection	40 (78.4%)	15 (25.9%)
Correct Foot Protection	11 (21.6%)	43 (74.1%)

4.5 Health Risks of Synthetic Pesticide and Chemical Fertilizers Among Organic Farmers and Conventional Farmers

In this study, 100% of organic farmers were assessed as having a low-risk level for all types of trace elements (Zinc, Manganese, Lead, and Ammonia). This low-risk classification was due to their exposure rating towards synthetic pesticides and chemical fertilizers, which consistently received the lowest rating of 1. For zinc (Zn) and ammonia (NH_3), which are present in synthetic pesticides and chemical fertilizers, exposure ratings of 2, 3, and 4 led to a significant portion of conventional farmers falling into the moderate risk category. In contrast, only 3.4% of conventional farmers were classified as low risk due to their consistent exposure rating of 1. When it comes to manganese (Mn), an exposure rating of 4 resulted in 53.4% of conventional farmers being categorized as high-risk. Exposure ratings of 2 and 3 caused 43.1% of conventional farmers to fall into the moderate risk category. Lastly, for lead (Pb), exposure ratings of 2, 3, and 4 substantially increased the percentage of conventional farmers classified as high risk, reaching 96.5%. The lowest exposure rating (1) only resulted in two conventional farmers (3.4%) being categorized as moderate risk.

Table 4.5: Health risk of synthetic pesticide and chemical fertilizer among both organic and conventional farmers (N=109)

Trace Element	Health Hazard	Exposure Rating	Health Risk		
			Level of risk	Organic Farmers, n (%)	Conventional Farmers, n (%)
Zn	Specific Target Organ Toxicity – Single Exposure Category 3 (STOT SE Cat.3) HR3	1	Low	51 (100%)	2 (3.4%)
			Moderate	0 (0.0%)	0 (0.0%)
			High	0 (0.0%)	0 (0.0%)
		2	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	4 (6.9%)
			High	0 (0.0%)	0 (0.0%)
		3	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	21 (36.2%)
			High	0 (0.0%)	0 (0.0%)

		4	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	31 (53.4%)
			High	0 (0.0%)	0 (0.0%)
Mn	Specific target organ toxicity - Repeated exposure - Category 1 (respiratory system, nervous system) STOT RE Cat.1 HR4	1	Low	51 (100%)	2 (3.4%)
			Moderate	0 (0.0%)	0 (0.0%)
			High	0 (0.0%)	0 (0.0%)
		2	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	4 (6.9%)
			High	0 (0.0%)	0 (0.0%)
		3	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	21 (36.2%)
			High	0 (0.0%)	0 (0.0%)
		4	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	0 (0.0%)

			High	0 (0.0%)	31 (53.4%)
Pb	Reproductive Toxicity Category 1A HR5	1	Low	51 (100%)	0 (0.0%)
			Moderate	0 (0.0%)	2 (3.4%)
			High	0 (0.0%)	0 (0.0%)
		2	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	4 (6.9%)
			High	0 (0.0%)	0 (0.0%)
		3	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	0 (0.0%)
			High	0 (0.0%)	21 (36.2%)
		4	Low	0 (0.0%)	0 (0.0%)
			Moderate	0 (0.0%)	0 (0.0%)
			High	0 (0.0%)	31 (53.4%)
NH_3	Acute Toxicity Category 3	1	Low	51 (100%)	2 (3.4%)

HR3		Moderate	0 (0.0%)	0 (0.0%)
		High	0 (0.0%)	0 (0.0%)
	2	Low	0 (0.0%)	0 (0.0%)
		Moderate	0 (0.0%)	4 (6.9%)
		High	0 (0.0%)	0 (0.0%)
	3	Low	0 (0.0%)	0 (0.0%)
		Moderate	0 (0.0%)	21 (36.2%)
		High	0 (0.0%)	0 (0.0%)
	4	Low	0 (0.0%)	0 (0.0%)
		Moderate	0 (0.0%)	31 (53.4%)
		High	0 (0.0%)	0 (0.0%)

Health Risk = Hazard Rating (HR) x Exposure Rating (ER)
HR3=Hazard Rating at 3, ER =1 (Exposure Rating at 1); ER =2 (Exposure Rating at 2); ER =3 (Exposure Rating at 3); ER =4 (Exposure Rating at 4)
HR4=Hazard Rating at 4, ER =1 (Exposure Rating at 1); ER =2 (Exposure Rating at 2); ER =3 (Exposure Rating at 3); ER =4 (Exposure Rating at 4)
HR5=Hazard Rating at 5, ER =1 (Exposure Rating at 1); ER =2 (Exposure Rating at 2); ER =3 (Exposure Rating at 3); ER =4 (Exposure Rating at 4)

4.6 The Level of Trace Elements (Zn, Mn, Pb and NH_3) of the Respondents

Table 4.6 shows the descriptive statistics of the trace elements (Zn, Mn, Pb and NH_3) of all the respondents. Since there was only the level of zinc, which was normally distributed, as stated in Table 4.1, the mean level of the Zn was 89.3674 ± 39.5861 mg/kg, while the median level of Mn, Pb and NH_3 were 0.6975 mg/kg, 0.5529 mg/kg and 0.0530 mg/kg respectively.

Table 4.6: The level of trace elements (Zn, Mn, Pb and NH_3) in nail sample among study respondents

	Zinc (Zn)	Manganese (Mn)	Lead (Pb)	Ammonia (NH_3)
Mean (mg/kg)	89.3674	1.2276	0.6856	0.0648
Median (mg/kg)	88.5791	0.6975	0.5529	0.0530
Mode (mg/kg)	12.6951	0.0086	0.0129	0.0003
Standard Deviation	39.5861	1.3732	0.4816	0.0506
Variance	1567.058	1.8857	0.232	0.003
Minimum	12.6951	0.0086	0.0129	0.0003
Maximum	178.8238	6.5557	2.3565	0.1995

4.7: Comparison Between the Level of Trace Elements (Zn, Mn, Pb and NH_3) Among the Organic Farmers and Conventional Farmers

By using the Independent T-Test, the level of Zinc element (Zn) in nail samples was proved significantly different at a 95% confidence level among organic farmers and conventional farmers, which is $p<0.05$ ($t= -3.717$, $p<0.001$). The level of Manganese (Mn) in nail samples among organic farmers and conventional farmers also was significant different using the Mann Whitney U test, which is $p<0.05$ ($z= -1.498$, $p<0.001$). Besides, the conventional farmers had a higher median score of the level of Ammonia element (NH_3) in nail samples than the organic farmers and this difference was found to be significant, where $z= -2.050$, $p=0.040$ (<0.05). The level of Lead (Pb) in nail samples among conventional farmers [0.6576 (IQR 0.8040)] mg/kg also had greater median scores as compared to the organic farmers [0.4639 (IQR 0.4735)] mg/kg, but it is not statistically significant, where $p=0.134$. The results are shown in the Table 4.7.

Table 4.7: Comparison between the level of trace elements (Zn, Mn, NH_3 and Pb) in nail samples among the organic farmers and conventional farmers

Trace element	Organic	Conventional	z-value [a] / t-value [b]	p-value
	Median (IQR)[a] / Mean (SD)[b]			
Mn[a]	0.4924 (0.5039)	1.050 (2.457)	-3.669	<0.001**
Pb[a]	0.4639 (0.4735)	0.6576 (0.8040)	-1.498	0.134
NH_3[a]	0.0460 (0.0492)	0.0649 (0.1115)	-2.050	0.040*
Zn[b]	71.99 (35.63)	100.85 (38.12)	-3.717	<0.001**

*p-value is significant at 0.05 level; ** p-value is significant at 0.001 level
a= Mann-Whitney U Test b= Independent sample T-Test

4.8: The Association Between the Health Risk of Synthetic Pesticides and Chemical Fertilizers (Zn, Mn, Pb and NH_3) and the Level of Trace Elements (Zn, Mn, Pb and NH_3) of the Organic and Conventional Farmers

Based on table 4.8, a significant association between the health risk of synthetic pesticides and chemical fertilizers (Zn) and the level of zinc element of the organic and conventional farmers in the nail samples, where the bivariate correlation between these two variables was positive and fair, r (93) = 0.350, $p<0.001$. Besides, the bivariate correlation between the health risk of synthetic pesticides and chemical fertilizers (Mn) and the level of manganese element of the organic and conventional farmers in the nail samples was positive and fair, r (106) = 0.444, $p<0.001$, indicating there was a statistically significant association between these two variables. Furthermore, the association between the health risk of synthetic pesticides and chemical fertilizers (NH_3) and the level of ammonia element of the organic and conventional farmers in the nail samples was also significant, where it was a positive and poor association, r (93) =0.216, p= 0.038. Only the health risk of using synthetic pesticides and chemical fertilizers containing Pb was found to not be significantly associated with the level of Pb in the nail samples among the respondents. The results are shown in the Table 4.8.

Table 4.8: Association between the health risk of synthetic pesticides and chemical fertilizers (Zn, Mn, Pb and NH_3) and the level of trace elements (Zn, Mn, Pb and NH_3) of the organic and conventional farmers in nail samples

Trace element	n	Health Risk Correlation Coefficient (R-value)	p-value
Zn	93	0.350	<0.001**[a]
Mn	106	0.444	<0.001**[b]
Pb	108	0.146	0.132[b]
NH_3	93	0.216	0.038*[b]

** p-value is significant at 0.001 level; * p-value is significant at 0.05 level

[a] Pearson's Correlation

[b] Spearman's rank Correlation

4.9: The Relationship Between the Trace Elements (Zn, Mn, Pb and NH_3) and the Individual and Occupational Risk Factors Among the Organic and Conventional Farmers

The relationship between the level of trace elements in the nail samples with the individual and occupational risk factors were analysed using Ordinal Least Square regression test. Based on table 4.9, 25.9% of the variance in the level of trace element Zinc among conventional farmers can be explained by individual factors including gender, age, BMI, smoking habit, alcohol habit and year of working by using Enter Method and there is significant linear relationship between the individual factors and the level of trace element Zinc among the conventional farmers since p value= 0.018 (<0.05). However, by using method Stepwise, it shows that individual factor of age was significantly contributing to 15.6% of the variance in the level of trace element Zinc among the conventional farmers with the p-value of 0.003 (<0.05) while occupational factor of type of eye protection was significantly contributing to 11.0% of the variance in the level of trace element Zinc among the conventional farmers with the p-value of 0.013(<0.05). While, table 4.10 describes that 7.9% of the variance in the level of trace element Manganese among the conventional farmers can be significantly explained by individual factor of year of working with the p-value of 0.033 (<0.05) by using method Stepwise. Besides, the occupational factor of type of spraying machine was significantly contributing to 13.5% of the variance in the level of trace element Ammonia among the conventional farmers since p-value= 0.009 (<0.05) by using method Stepwise. Based on table 4.13, with the method Stepwise, 10.9% of the variance in the level of trace element Zinc among the organic farmers can be explained by the occupational factor (type of foot protection) and it was proven that there was a significant linear relationship between these two variables since p-value=0.046 (<0.05). In addition, individual factor of gender was significantly contributing to 12.8% of the variance in the level of trace element Manganese among the organic farmers as the p-value=0.013 (<0.05) by using method Stepwise. Lastly, 9.6% of the variance in the level of ammonia among the organic farmers can be explained by the individual factor of taking alcohol habit and it was shown that there was a significant linear relationship between both variables as p-value= 0.044 (<0.05) by using method Stepwise.

Table 4.9: The trace element Zinc and its risk factors among conventional farmers (Method= Enter)

	Risk Factors	b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	-44.406	-3.478	0.156	0.259 (0.018) *
	Age	0.783	1.439	0.001	
	BMI	1.502	1.369	0.177	
	Smoking Habit (0=No, 1=Yes)	9.098	0.813	0.420	
	Alcohol Habit (0= No Alcohol, 1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)	4.079	0.973	0.335	
	Year of Working	-0.174	-0.340	0.735	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week,	-26.548	-0.688	0.495	0.234 (0.154)

4= Exposure more than one time per shift per day)			
Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	-16.356	-0.349	0.729
Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	57.840	1.995	0.052
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	-44.935	-1.721	0.092
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	189.580	2.707	0.010

Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	110.213	1.964	0.056
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-7.734	-0.571	0.571
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection, 2= Full body protection)	-109.525	-2.204	0.033
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-15.090	-0.301	0.765

*p-value is significant at 0.05 level

Method= Enter

[Individual Factor] Zinc (Nail)= 55.927 – 44.406(Gender) + 0.783(Age) + 1.502(BMI) + 9.098(Smoking Habit) + 4.079(Alcohol Habit) - 0.174(Year of Working)

[Occupational Factor] Zinc (Nail)= 102.818 – 26.548(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) – 16.356(Duration of Pesticide Used) + 57.840(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) – 44.935 (Type of Spraying Machine) + 189.580 (Type of Eye Protection) +110.213 (Type of Respiratory Protection) – 7.734 (Type of Hand Protection) – 109.525 (Type of Body protection) – 15.090 (Type of Foot Protection)

Method= Stepwise

[Individual Factor] Zinc (Nail)= 125.533 – 33.719(Age) [p=0.003]

[Occupational Factor] Zinc (Nail)= 98.437 + 67.449 (Type of Eye Protection) [p=0.013]

Table 4.10: The trace element Manganese and its risk factors among conventional farmers (Method= Enter)

Risk Factors		b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	-0.004	-.008	0.994	0.144 (0.221)
	Age	0.014	0.573	0.569	
	BMI	-0.040	-0.848	0.400	
	Smoking Habit (0=No, 1=Yes)	0.766	1.476	0.146	
	Alcohol Habit (0= No Alcohol,	-0.212	-1.097	0.278	

	1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)				
	Year of Working	0.030	1.266	0.400	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)	-0.206	0.180	0.858	0.088 (0.857)
	Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	1.061	0.659	0.513	
	Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage,	0.293	0.421	0.676	

4= Chemical drift and spillage)			
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	-0.054	-0.076	0.940
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	1.475	0.706	0.484
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	-1.294	-0.602	0.550
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	0.479	0.777	0.441
Type of Body Protection (0= Incorrect body protection,	0.660	0.410	0.684

	1= Partial body protection, 2= Full body protection)			
	Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-2.113	-1.330	0.190

Method= Enter

[Individual Factor] Manganese (Nail)= 1.660 – 0.004(Gender) + 0.014(Age) – 0.040(BMI) + 0.766(Smoking Habit) - 0.212(Alcohol Habit) + 0.030(Year of Working)

[Occupational Factor] Manganese (Nail)= - 0.993 + 0.206(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) + 1.061(Duration of Pesticide Used) - 0.293(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) – 0.054 (Type of Spraying Machine) + 1.475 (Type of Eye Protection) – 1.294 (Type of Respiratory Protection) + 0.479 (Type of Hand Protection) + 0.660 (Type of Body protection) – 2.113 (Type of Foot Protection)

Method= Stepwise

[Individual Factor] Manganese (Nail)= 1.070 + 0.040(Year of Working) [p=0.033]

Table 4.11: The trace element lead and its risk factors among conventional farmers (Method= Enter)

	Risk Factors	b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	-0.004	0.017	0.994	0.139 (0.253)
	Age	0.018	1.925	0.060	
	BMI	0.000	-.023	0.982	
	Smoking Habit (0=No, 1=Yes)	0.251	1.374	0.176	
	Alcohol Habit (0= No Alcohol, 1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)	-0.063	-0.928	0.358	
	Year of Working	-0.019	-2.169	0.035	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week,	-0.523	-1.416	0.163	0.232 (0.150)

4= Exposure more than one time per shift per day)			
Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	0.057	-0.110	0.913
Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	0.000	-0.002	0.999
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	0.687	2.996	0.004
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	0.817	1.212	0.232

Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	-0.013	-0.019	0.985
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-0.109	-0.547	0.587
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection, 2= Full body protection)	-0.427	0.823	0.415
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-0.177	-0.346	0.731

Method= Enter

[Individual Factor] Lead (Nail)= 0.357 + 0.004(Gender) + 0.018(Age) – 0.000(BMI) + 0.251(Smoking Habit) – 0.063(Alcohol Habit) – 0.019(Year of Working)

[Occupational Factor] Lead (Nail)= 0.936 – 0.523(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) + 0.057(Duration of Pesticide Used) - 0.000(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 0.687 (Type of Spraying Machine) + 0.817 (Type of Eye Protection)- 0.013 (Type of Respiratory Protection) - 0.109 (Type of Hand Protection) + 0.427 (Type of Body protection) - 0.177 (Type of Foot Protection)

Table 4.12: The trace element Ammonia and its risk factors among conventional farmers (Method= Enter)

Risk Factors		b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	-0.004	0.236	0.815	0.092 (0.630)
	Age	0.006	-0.354	0.725	
	BMI	-0.002	-0.942	0.352	
	Smoking Habit (0=No, 1=Yes)	-0.015	-0.730	0.469	
	Alcohol Habit (0= No Alcohol, 1= Once a week,	0.007	0.897	0.375	

	2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)				
	Year of Working	0.001	1.219	0.230	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)	-0.036	- 0.900	0.475	0.179 (0.480)
	Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	0.017	- 0.302	0.374	
	Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	-0.005	-0.203	0.840	

Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	0.046	1.891	0.066
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	0.050	0.700	0.488
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	0.065	0.882	0.383
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-0.012	-0.562	0.577
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection,	-0.033	-0.592	0.557

2= Full body protection)			
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	0.005	0.087	0.931

Method= Enter

{Individual Factor} Ammonia (Nail)= 0.113 + 0.006(Gender) + 0.000(Age) – 0.002(BMI) – 0.015(Smoking Habit) + 0.007(Alcohol Habit) + 0.001(Year of Working)

{Occupational Factor} Ammonia (Nail)= 0.048 – 0.036(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) + 0.017(Duration of Pesticide Used) - 0.005(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 0.046(Type of Spraying Machine) + 0.050 (Type of Eye Protection) +0.065 (Type of Respiratory Protection) - 0.012 (Type of Hand Protection) – 0.033 (Type of Body protection) + 0.005 (Type of Foot Protection)

Method=Stepwise

{Occupational Factor} Ammonia (Nail)= 0.002+ 0.035(Type of Spraying Machine) [p=0.009]

Table 4.13: The trace element Zinc and its risk factors among organic farmers (Method= Enter)

	Risk Factors	b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	6.270	0.416	0.680	0.094 (0.790)
	Age	0.400	0.726	0.473	
	BMI	0.978	0.834	0.411	
	Smoking Habit (0=No, 1=Yes)	-8.838	-0.568	0.574	
	Alcohol Habit (0= No Alcohol, 1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)	-3.784	-0.473	0.640	
	Year of Working	-0.629	-0.787	0.437	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month,	-8.164	-0.430	0.671	0.166 (0.789)

3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)			
Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	-6.816	-0.320	0.751
Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	-0.633	-0.044	0.966
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	8.231	0.603	0.551
Type of Eye Protection (0= No eye protection,	-11.536	-0.226	0.823

1= Correct eye protection)			
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	9.242	0.274	0.786
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-6.457	-0.369	0.715
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection, 2= Full body protection)	-3.341	-0.059	0.953
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-19.859	-0.316	0.754

Method= Enter

[Individual Factor] Zinc (Nail)= 35.365 + 6.270(Gender) + 0.400(Age) + 0.978(BMI) – 8.838(Smoking Habit) – 3.784(Alcohol Habit) - 0.629(Year of Working)

[Occupational Factor] Zinc (Nail)= 94.701 – 8.164(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) – 6.816(Duration of Pesticide Used) - 0.633(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 8.231(Type of Spraying Machine) -11.536 (Type of Eye Protection) + 9.242(Type of Respiratory Protection) – 6.456 (Type of Hand Protection) -3.341 (Type of Body protection) – 19.859 (Type of Foot Protection)

[Occupational Factor] Zinc (Nail)= 79.530 – 25.347 (Type of Foot Protection) [p=0.046]

Table 4.14: The trace element Manganese and its risk factors among organic farmers (Method= Enter)

Risk Factors		b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	0.290	2.229	0.031	0.227 (0.087)
	Age	0.004	0.867	0.391	
	BMI	-0.010	-1.062	0.294	
	Smoking Habit (0=No, 1=Yes)	0.004	0.032	0.975	
	Alcohol Habit	0.066	0.920	0.363	

	(0= No Alcohol, 1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)				
	Year of Working	0.002	0.341	0.735	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)	0.232	1.920	0.062	0.163 (0.599)
	Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	-0.014	-0.072	0.943	
	Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift,	-0.114	-0.832	0.411	

3= Chemical Spillage, 4= Chemical drift and spillage)			
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	0.008	0.066	0.948
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	0.345	0.691	0.494
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	-0.280	-1.062	0.295
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-0.187	-1.086	0.284
Type of Body Protection	0.099	0.297	0.768

	(0= Incorrect body protection, 1= Partial body protection, 2= Full body protection)			
	Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-0.218	-0.695	0.491

Method= Enter

[Individual Factor] Manganese (Nail)= 0.422 + 0.290(Gender) + 0.004(Age) – 0.010(BMI) + 0.004(Smoking Habit) – 0.066(Alcohol Habit) + 0.002(Year of Working)

[Occupational Factor] Manganese (Nail)= 0.543 + 0.232(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) -0.014(Duration of Pesticide Used) - 0.114(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 0.008 (Type of Spraying Machine) + 0.345 (Type of Eye Protection) - 0.280 (Type of Respiratory Protection) - 0.187(Type of Hand Protection) + 0.099 (Type of Body protection) - 0.218 (Type of Foot Protection)

Method= Stepwise

[Individual Factor] Manganese (Nail)= 0.403 + 0.287(Gender) [p=0.013]

Table 4.15: The trace element Lead and its risk factors among organic farmers (Method= Enter)

	Risk Factors	b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	0.020	0.184	0.855	0.039 (0.935)
	Age	0.002	0.614	0.542	
	BMI	-0.003	-0.362	0.719	
	Smoking Habit (0=No, 1=Yes)	0.093	0.836	0.408	
	Alcohol Habit (0= No Alcohol, 1= Once a week, 2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)	-0.035	-0.568	0.573	
	Year of Working	-0.004	-0.749	0.458	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month,	-0.068	-0.700	0.488	0.107 (0.832)

3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)			
Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	-0.035	-0.216	0.830
Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	-0.075	-0.676	0.503
Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	0.100	1.083	0.285
Type of Eye Protection (0= No eye protection,	-0.180	-0.446	0.658

1= Correct eye protection)			
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	-0.160	-0.748	0.459
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	0.029	0.207	0.837
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection, 2= Full body protection)	0.220	0.813	0.421
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-0.108	-0.423	0.675

Method= Enter

[Individual Factor] Lead (Nail)= 0.563 + 0.020(Gender) + 0.002(Age) – 0.003(BMI) + 0.093(Smoking Habit) - 0.035(Alcohol Habit) - 0.004(Year of Working)

[Occupational Factor] Lead (Nail)= 0.778 - 0.068(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) - 0.035(Duration of Pesticide Used) - 0.075(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 0.100 (Type of Spraying Machine) - 0.180 (Type of Eye Protection) - 0.160 (Type of Respiratory Protection) + 0.029 (Type of Hand Protection) + 0.220 (Type of Body protection) - 0.108 (Type of Foot Protection)

Table 4.16: The trace element Ammonia and its risk factors among organic farmers (Method= Enter)

Risk Factors		b (Unstandardized B)	t-value	p-value	R^2 (p-value)
Individual Factors	Gender	0.010	0.784	0.438	0.180 (0.276)
	Age	0.000	-1.007	0.321	
	BMI	-0.001	-0.609	0.547	
	Smoking Habit (0=No, 1=Yes)	-0.019	-1.533	0.134	
	Alcohol Habit (0= No Alcohol, 1= Once a week,	-0.014	2.171	0.037	

	2= 3 to 4 times a week, 3= 3 times a month, 4= 1 to 2 times a year)				
	Year of Working	7.820 E-5	0.116	0.908	
Occupational Factors	Frequency of Synthetic Pesticide or Chemical Fertilizer Used (1= Exposure once per year or less, 2= Exposure greater than one time per month, 3= Exposure greater than one time per week, 4= Exposure more than one time per shift per day)	0.021	1.974	0.057	0.175 (0.636)
	Duration of Pesticide or Chemical Fertilizer Used (1= Less than 1 hour, 2= Less than 2 hours but greater than 1 hour, 3= Less than 4 hours but greater than 2 hours, 4= Less than 7 hours but greater than 4 hours, 5= More than or equal to 7 hours)	0.000	-0.024	0.981	
	Scenario While Spraying Pesticides (1= Not applicable, 2= Chemical drift, 3= Chemical Spillage, 4= Chemical drift and spillage)	-0.022	-1.794	0.082	

Type of Spraying Machine (0= Not using chemical, 1= Hand held sprayer, 2= Knapsack hand sprayer, 3= Backpack sprayer, 4= Knapsack power blower/ sprayer)	0.010	0.962	0.343
Type of Eye Protection (0= No eye protection, 1= Correct eye protection)	0.061	1.390	0.174
Type of Respiratory Protection (0= Incorrect respiratory protection, 1= Partial protection, 2= Full protection)	-0.023	-0.772	0.446
Type of Hand Protection (0= No hand protection, 1= Improper hand protection, 2= Proper hand protection, 3= Complete hand protection)	-0.027	-1.837	0.075
Type of Body Protection (0= Incorrect body protection, 1= Partial body protection,	0.015	0.382	0.705

2= Full body protection)			
Type of Foot Protection (0= Incorrect Foot Protection, 1= Correct Foot Protection)	-0.010	-0.325	0.747

Method= Enter

[Individual Factor] Ammonia (Nail)= 0.072 + 0.010(Gender) + 0.000(Age) – 0.001(BMI) – 0.019(Smoking Habit) + 0.014(Alcohol Habit) + 7.820 E^{-5}(Year of Working)

[Occupational Factor] Ammonia (Nail)= 0.052 + 0.021(Frequency of Synthetic Pesticide or Chemical Fertilizer Used) + 0.000(Duration of Pesticide Used) - 0.022(Scenario While Spraying Synthetic Pesticide and Chemical Fertilizer) + 0.010 (Type of Spraying Machine) + 0.061 (Type of Eye Protection) - 0.023 (Type of Respiratory Protection) - 0.027 (Type of Hand Protection) + 0.015 (Type of Body protection) - 0.010 (Type of Foot Protection)

Method= Stepwise

[Individual Factor] Ammonia (Nail)= 0.041 + 0.012(Alcohol Habit) [p=0.044]

CHAPTER 5

DISCUSSION

5.1 The Socio- and Occupational Background of Farming Communities in Malaysia

In this study, organic farmers had a mean age of 44.31 ± 15.47 years, while conventional farmers had a mean age of 43.05 ± 10.90 years. A study in Thailand indicated a higher likelihood of pesticide exposure and an increased risk of pesticide contamination with advancing age among farmers (Thongkum et al., 2022). Another study also stated that the farming population, especially those aged 50 and above, experiences disproportionately high rates of mortality and morbidity (Amshoff & Reed, 2005).

Moreover, the majority of both organic and conventional farmers were males, constituting 68.6% and 72.4%, respectively. While there is a lower proportion of female farmers in this research, a study involving female agricultural workers showed a higher likelihood of incidence of ovarian and pancreatic cancer, acute myeloid leukemia, and breast cancer (de-Assis et al., 2021). This could be attributed to their frequent agricultural exposure to pesticides, increasing their health risks.

The mean BMI of organic and conventional farmers were 25.72 ± 5.31 and 26.16 ± 4.82, respectively. Falling within this range is considered normal if it is below 25 and overweight if it is above 25 in Malaysia. A study in Brazil showed that pesticide exposure was positively correlated with body mass index and associated comorbidities

(Skonieski et al., 2023). Thus, indicating a higher BMI enhances the probability of farmers experiencing agricultural exposure to pesticides and fertilizers.

The mean years of working for conventional farmers and organic farmers were 16.91 ± 11.65 years and 11.82 ± 10.78 years, respectively. A longer working duration helps to reflect the effect of prolonged exposure of agricultural farmers to pesticides and fertilizers, thereby evaluating the health risks of the farmers.

Regarding smoking habits, 68.6% of organic farmers and 65.5 % of conventional farmers were smokers. According to Lukyn et al. (2020), smokers experience a twofold higher exposure to pesticides than non-smokers, as smoking may damage the body's metabolism of pesticides. 49% of organic farmers and 41.4% of conventional farmers were non-alcoholic drinkers. A study showed that low alcohol intake in agricultural workers in rural areas is less likely to have health impacts, such as developing cancer (de-Assis et al., 2021), resulting in lower health risks.

Occupational factors, such as wearing personal protective equipment (PPE) on various body parts, including respiratory, eyes, mouth, hands, body, and feet, were also investigated in this study. Given the different routes of pesticide exposure, such as dermal contact, inhalation, and oral ingestion, it is necessary for agricultural workers to wear appropriate PPE to protect themselves from exposure (Damalas & Koutroubas, 2020). Okoffo et al. (2016) also claimed that the lack of awareness and knowledge of using PPE has increased the susceptibility of farmers to pesticide-related health risks, including body aches, skin and eye irritation, dermatitis, cancer, endocrine disruptions, and more.

5.2 Health Risks of Synthetic Pesticide and Chemical Fertilizer Among Organic and Conventional Farmers

The use of synthetic pesticides and chemical fertilizers not only brings a devastating effect on the environment but also the population's health. To date, only a handful of studies have published data that determine where the health risks of using synthetic pesticides and chemical fertilizers among organic and conventional farmers as well as their level of trace elements (Zn, Mn, Pb, NH_3), specifically in Malaysia. The behaviour of using synthetic pesticides and chemical fertilizers along with its uncertainties makes it critical for the government to plan future initiatives that strike the balance between public and environmental health. Therefore, it is of utmost importance for the level of trace elements (Zn, Mn, Pb, NH_3) among the organic and conventional farmers to be investigated to guide this effort.

This study indicated that the health risks for all the organic farmers were found to be low after undergoing the chemical health risk assessment (CHRA) which integrated the health hazards associated with synthetic pesticides and chemical fertilizers, along with the level of occupational exposure for the farmers. This method of assessing the health risk posed by synthetic pesticides and chemical fertilizers on farmers has not been used in other studies, particularly in Malaysia. A previous study indicated that organic agriculture not only enhances human health through aspects like consuming organic food but also benefits farming workers. For instance, Mie et al. (2017) demonstrated that organic agriculture reduces the occupational exposure of farm workers to pesticides, but also reduces drift exposures of rural populations. He also claimed that many of the pesticides approved for use in organic agriculture pose relatively low toxicological concerns for consumers, which is attributed to factors such as the absence of identified toxicity in certain pesticides such as spearmint oil, quartz and sand, their classification as part of a normal diet or human nutrients such as iron, potassium bicarbonate, rapeseed oil or their approval exclusively for use in insect traps (Mie et.al, 2017).

Even though this study is by far the first to use the CHRA method to estimate the health risks associated with pesticides used among farmers, other studies have indicated that exposure to these substances does pose health risks for agricultural workers. For example, humans face a threat from the elevated accumulation of heavy metals in soil and surroundings resulting from the repeated application of zinc fertilizer (Jiao et al., 2012). While this study shows a majority of the conventional farmers (96.5%) who utilize synthetic pesticides or chemical fertilizers containing Zn were characterized under moderate risk after assessing their level of exposure and hazard rating of Zn.

This study also reveals that 53.4% of conventional farmers faced high risks, while 43.1% of them experienced moderate risks when using synthetic pesticides and chemical fertilizers containing Mn. More than half of the farmers fell under high risks, primarily attributed to the higher hazard rating (4) of Mn compared to Zn and NH_3, which have a hazard rating of 3. Whereas, another study has suggested that occupational workers, such as mechanics, employees in the gasoline industry, and those involved in pesticide manufacturing and spraying, such as agricultural workers, are at a high risk of being exposed to significant amounts of manganese through their skin, which increases their health risk (Williams et al., 2012).

Due to the high hazard rating (5) of Pb, 89.6% of conventional farmers were categorized as being at high risk, as exposure to Pb is known to cause damage to the brain and nervous system in humans (CDC, 2022). The toxic effects of heavy metals, such as cadmium, lead, and copper, resulting from pesticide exposure, adversely affect human health, inducing health risks such as carcinogenic effects, and in cases of high exposure, may even lead to death (Alengebawy et al., 2021).

Among conventional farmers who utilize synthetic pesticides and chemical fertilizers containing NH_3, the percentage of farmers classified as having a moderate risk level was identical (96.5%) to that of Zn, as both substances have equivalent levels of exposure and hazard rating. The study also found that agricultural workers using soil fertilizer face the risk of ammonia exposure, and increased levels of exposure can significantly increase the likelihood of health risks, including skin, mouth, throat, lungs, and eyes irritation and burns (CDC, 2019).

5.3 The Level of Trace Elements (Zn, Mn, Pb and NH_3) of the Respondents

From this study, the mean level of zinc in nail samples among the 109 study respondents was 89.3674 ± 39.5861 mg/kg. According to a study by Roohani et al. (2013), the normal range for zinc levels in the human body is between 2.8 mg/kg and 40 mg/kg. This indicates that the overall mean zinc level among the respondents was high and exceed than the normal range. The minimum zinc level observed in the respondents was 12.6951 mg/kg, which still falls within the aforementioned normal range. The maximum zinc level detected in the respondents was 178.8238 mg/kg, far exceeding the upper limit of the normal range.

Furthermore, the median level of manganese in the nail samples among the 109 study respondents was 0.6975 mg/kg. The study revealed that the normal concentration of manganese in the human body typically ranges from 0.004 mg/kg to 0.015 mg/kg; however, it is important to note that these levels may vary depending on specific body parts (ATSDR, 2012). For instance, the typical Mn concentrations in human tissues are 1 mg/kg in the bone, 1.04 mg/kg in the pancreas, and 0.98 mg/kg in the kidney cortex (Myking et al., 2002). In this study, the median manganese level is outside the previously mentioned normal range. The minimum observed level of manganese in the respondents was 0.0086 mg/kg, still within the proposed range. In contrast, the maximum detected level of manganese in the respondents was 6.5557 mg/kg, much exceeding the upper limit of the normal range.

While this study reveals that the median level of lead in the nail samples among the 109 respondents was 0.5529 mg/kg, it is important to note that a lead level of 0.35 mg/kg or above is considered elevated in adults (CDC, 2023). However, exposure to lead is universally recognized to have detrimental effects at any level; in other words, there is no safe level for lead (WHO, 2023). The results shown in this study exceed the recommended levels, and humans should not have high levels of lead in the body, as it can lead to harmful effects. Besides, our result was higher than the study conducted by Mortada et al in Egypt and Ghazali et al in MADA, Kedah. The minimum level of lead observed in the respondents was 0.0129 mg/kg, while the maximum level was 2.3565 mg/kg.

In addition, this study revealed that the median level of ammonia in the nail samples among the 109 respondents was 0.0530 mg/kg. Another study has indicated a safe range for ammonia in the human body, which is reported to be between 0.187 mg/kg to 0.852 mg/kg (Brannelly et al., 2016). The results presented in this study were considerably lower than the suggested range. This variance could be attributed to the short biological half-life of ammonia in the human body, lasting only a few days

(ATSDR, 2014). Consequently, the concentration of ammonia in nail samples may be challenging to detect, as nails are more adept at reflecting long-term exposure compared to blood and urine. The respondents exhibited a minimum ammonia level of 0.0003 mg/kg, indicating a very low concentration. In contrast, the maximum detected ammonia level in the respondents was 0.1995 mg/kg, remaining within the suggested normal range.

5.4 Comparison Between the Level of Trace Elements (Zn, Mn, Pb, NH_3) Among the Organic and Conventional Farmers

Numerous studies conducted over the past two decades have compared trace mineral concentrations in crops between organic and conventional farming systems (Barański et al., 2014). However, there is comparatively less documentation on safety and health concerns related to agricultural farmers, especially in Malaysia. However, this is the first study to compare the trace elements in nail samples among farming communities.

5.4.1 Trace element (Zn) in nails among farming communities

This study shows that there is a significant difference between the level of zinc element in the nail samples among the organic and conventional farmers, where $p<0.001$ by using an Independent T-test. In some countries such as Africa, Zn-enriched fertilizers were used and applied to cereal crops to alleviate human dietary Zn deficiency (Joy et al., 2015). Applying zinc fertilizer to the soil has proved to be an effective strategy for enhancing both crop yield and the accumulation of zinc in wheat grains (Liu et al., 2020). Hence, repeated application of Zn fertilizer among conventional farmers potentially leads to a significantly higher level of zinc element among conventional farmers as zinc can penetrate the whole epidermis (Agren, 1990). The zinc fertilizer causes severe eye irritation and poses toxicity risks with prolonged exposure through inhalation and skin contact during the manual spraying process (Bodar et al., 2005). In a prior study conducted in Southern Brazil, the levels of arsenic, nickel, zinc, manganese, and copper elements were examined among 54 farmers and 108 healthy unexposed individuals. The results for each sample were determined using dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) and it indicates that the level of heavy metals in farmers were two to four times higher than those in controls (Rocha et al., 2014). This difference was observed for all tested heavy metals and was statistically significant ($p < 0.05$) (Rocha et al., 2014).

5.4.2 Trace element (Mn) in nails among farming communities

Besides, this study shows a significant difference between the level of Manganese element in the nail samples among the organic and conventional farmers, where $p<0.001$ by using Mann-Whitney U Test. Individuals residing in areas with natural manganese ore deposits or those where materials containing manganese, such as pesticides and batteries, are utilized, may experience heightened levels of manganese exposure (CICAD, 1999). Although limited information is available concerning human dermal exposure to manganese, adverse neurological and respiratory effects associated with manganese exposure are known to occur, particularly in occupational settings (CICAD, 1999). A study evaluating the probability of agricultural workers in Idaho encountering low levels of organic manganese and the potential for such exposure to result in adverse health effects discovered that exposure to manganese among agricultural farmers is primarily attributed to aerial spraying and ground treatments involving manganese-containing pesticides (Porter, 2015). Dermal exposure to manganese is typically not considered a significant source of exposure for the general population as limited evidence supporting substantial skin absorption through dermal contact with manganese (ATSDR, 2012). To support the significant result from this study, indicating that conventional farmers using synthetic pesticides and chemical fertilizers containing manganese are more prone to higher levels of manganese, the study in Southern Brazil shows a significant difference ($p<0.05$) in the level of manganese between the farmers and healthy unexposed individuals (Rocha et al., 2014).

5.4.3 Trace element (NH_3) in nails among farming communities

In addition, there is also a significant difference between the level of ammonia in the nail samples among the conventional and organic farmers, where $p=0.04$ (<0.05) by using Mann-Whitney U test. Although there is no research studying the level of ammonia level in agricultural farmers, but previous study shows workers at a urea fertilizer factory in Bangladesh are subjected to personal exposures to ammonia in high level and acute effects were determined (Rahman et al., 2007). As ammonia is a type of common fertilizer due to its capability to replenish nitrogen in the soil, it is greatly used by the conventional farmers to improve agricultural productivity and reduce input expenses. Hence, this increases the probability of personal exposure of conventional farmers to ammonia and enhance the level of ammonia in the body as shown in the result. This is because exposure to ammonia can occur through direct skin contact with substances containing ammonia such as ammonia, other than via inhalation, diet and drinking water (EPA, 2016). A study found that emissions from fertilizer factories, a source of ammonia, can affect the health of both workers and citizens, with respondents reporting at least two symptoms related to ammonia effects, with dizziness and cough being the most frequently mentioned symptoms (Faisyah et al., 2020). Therefore, agricultural farmers working with ammonia-containing fertilizers are also at a high risk of experiencing elevated levels of trace elements in their bodies due to frequent exposure.

5.4.4 Trace element (Pb) in nails among farming communities

Furthermore, in this study, there is no significant difference between the level of lead element in the nail samples among the organic and conventional farmers, where p=0.134 (>0.05). Some fertilizers unintentionally contain non-essential heavy metal contaminants, including arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni), as these metals are inadvertently added alongside desired 'micronutrients' sourced from industrial wastes or by-products like electric arc furnace dust (US EPA, 1999). A study in British Columbia, Canada concluded that there is no significant evidence suggesting that tree planters working with fertilizer are at an increased risk of exposure to arsenic, lead, cadmium, chromium, and nickel compared to those who do not use fertilizer (Ng et al., 2011). Another study in British, Columbia by Davies & Stjernberg (2009) also concluded that no significant evidence from air, blood, or skin samples to substantiate the hypothesis that handling fertilizers containing heavy metals during seedling planting led to increased exposure to heavy metals. Generally, the levels of exposure to metals were low, and any exceptions did not seem to be associated with contact with fertilizer, instead, they could potentially be attributed to other factors such as prior work experience or cigarette smoking (Davies & Stjernberg, 2009).

5.5 Association between the health risk of synthetic pesticides and chemical fertilizers (Zn, Mn, Pb and NH_3) and the level of trace elements (Zn, Mn, Pb and NH_3) in nail samples among the respondents

Apart from comparing the difference in the trace element levels in nail samples among organic and conventional farmers, the study aimed to determine the association between the health risks associated with using synthetic pesticides and chemical fertilizers and the trace element levels of the study respondents. This was done to identify potential correlations between these two variables. Nevertheless, there is no existing study that has investigated and discussed the correlation between the health risks associated with the use of synthetic pesticides and chemical fertilizers and the levels of trace elements among farmers, especially in Malaysia. Therefore, the new findings from this study provide a reference or baseline data for future research in this field.

A significant correlation was observed between the health risk associated with synthetic pesticides and chemical fertilizers (Zn) and the zinc element levels among both organic and conventional farmers by using the Pearson Correlation Test. The bivariate correlation between these two variables was found to be positive and fair, with $r(93) = 0.350$, $p < 0.001$. Many studies have primarily demonstrated the association between trace element concentrations in soil and the application of pesticides and fertilizers (He, 2005). Soil contamination with heavy metals and toxic elements may result from either inherent factors in soil parent materials or inputs through human activities, including the repeated use of metal-enriched chemicals, fertilizers, and organic amendments (He, 2005). This is because the application of zinc to soil is a promising strategy to enhance zinc uptake in populations experiencing zinc deficiency (Yaseen & Hussain, 2021). Nevertheless, this study places greater emphasis on the impact on agricultural farmers who are more likely to come into contact with pesticides and fertilizers, where farmers are routinely exposed to higher levels of pesticides and fertilizer, typically surpassing those experienced by consumers (Damalas & Koutroubas, 2016). This can be due to the potential for significant eye, inhalation and dermal exposure among mixers, loaders and applicators of Zn containing pesticide and fertilizers (USEPA, 1992).

Besides, the bivariate correlation between the health risk associated with synthetic pesticides and chemical fertilizers (Mn) and the manganese element levels among organic and conventional farmers revealed a positive and fair relationship, with a statistically significant association, $r(106) = 0.444$, $p < 0.001$. Recent studies underscore the importance of microbial interactions in improving manganese uptake in plants, providing a more environmentally sustainable method to tackle manganese deficiencies among human (Khoshru et al., 2023). However, the excessive application of chemical fertilizers containing manganese can result in adverse outcomes for soil and environmental health, including soil and water pollution (Khoshru et al., 2023). Hence, the findings from this study provide additional information on the effects of pesticides and fertilizers containing manganese on users, specifically agricultural farmers. This is particularly significant as there are limited studies that correlate the association between the health risk of using synthetic pesticides and fertilizers with the levels of trace elements among conventional and organic farmers. A study by Morales-Pérez et al (2021) emphasized that manganese is frequently found in the environment as a byproduct of human activities, such as the use of pesticides or fertilizers, and despite evidence associating elevated manganese concentrations with various diseases in humans, it is not typically taken into account in risk assessments. Hence, it further stresses the importance of decreasing the use of synthetic pesticides and fertilizers containing manganese, as it results in a higher concentration of manganese in the human body, as demonstrated by the significant positive and fair relationship revealed in this study.

Furthermore, the correlation between the health risk associated with synthetic pesticides and chemical fertilizers (NH_3) and the ammonia element levels among organic and conventional farmers was also significant, indicating a positive and poor association, with r (93) = 0.216, p = 0.038. Farmers commonly use pesticides and fertilizers containing ammonia to boost crop productivity. Therefore, most studies are more likely to focus on discussing trace element levels in the soil rather than in humans, often overlooking the associated health risks for humans, especially farmers and their association. For instance, a study claimed that the process of fertilization can enhance the availability of trace elements such as ammonia, posing a hazard to human when present in elevated concentrations (Winckel, 2021). Winckel (2021) also stated that the population of agricultural workers is significantly more impacted by the risk of ammonia in the chemical fertilizers compared to individuals in other occupations. However, a poor association was identified between the health risk associated with the use of synthetic pesticides and chemical fertilizers containing ammonia and the ammonia levels among the respondents. This could be attributed to the short biological half-life of ammonia, resulting in the detection of low ammonia levels among the respondents. This makes it challenging to establish a direct correlation between the health risk associated with ammonia pesticides and fertilizers and the ammonia levels among the respondents, given that ammonia can be rapidly eliminated from the body.

On the other hand, there is positive and poor association between the health risk of using synthetic pesticides and chemical fertilizers containing lead with the level of lead element among the respondents, but it is not significant proven. A study by Wei & Yang (2010) claimed that farmers with 20–30 years of exposure to pesticides and fertilizers are likely to experience an increase in the levels of heavy metals such as lead and arsenic in their bodies. The insignificant result may be attributed to the exposure of lead among organic farmers from other sources, even though they do not use pesticides and fertilizers containing lead. For example, Food Standards Scotland (2023) stated that the discarded or fly-tipped materials, such as car and electric fence batteries, old paint as well as bonfire ash can cause exposure of lead and pose a real risk to both livestock and farmers. Once lead is introduced into the environment from wastewater originates from various sources, including metal plating, tanneries, oil refining, and mining, it disseminates through soil and water streams, accumulating in the body via the food chain, posing a significant threat to human health (Ali et al., 2022).

Additionally, this study demonstrates that the lead (Pb) concentration in crops cultivated using raw wastewater is significantly higher than in crops grown with treated wastewater, surpassing the acceptable limit (Ali et al., 2022). Besides, a prior study from Akthar et al (2022), the change to wastewater application due to a lack of fresh water supplies, resulted in a buildup of Pb in soil. Therefore, the water used by the farmers may expose them to lead, potentially explaining the insignificant result, despite organic farmers being categorized as being at low risk.

5.6 The relationship between trace elements (Zn, Mn, Pb and NH_3) with the individual and occupational risk factors

In this research, the relationship between trace elements and individual and occupational risk factors was studied to identify additional factors that may influence the observed differences in results, beyond the use of synthetic pesticides and chemical fertilizers. From this study, age of the conventional workers significantly contributing to 15.6% of the variance in the level of trace element Zinc with the p-value of 0.003 (<0.05). A study by Rodushkin & Axelsson (2000) showed that a few elements such as Zn show a correlation with age in both hair and nail samples. The zinc content in the body gradually rises with age and subsequently stabilizes at a consistent level (Rodushkin & Axelsson, 2000). They also claimed that while there is compelling evidence suggesting that variables such as age might influence element concentrations in hair and nails, a more extensive statistical dataset would be required to validate these observations (Rodushkin & Axelsson, 2000) since their study is conducted during year 2000. Additionally, a prior study also concluded that there were significant correlations between heavy metals and subjects' age and working period of farmers (Ghazali et al., 2012). Besides, 7.9% of the variance in the level of trace element Manganese among the conventional farmers can be significantly explained by individual factor of year of working with the p-value of 0.033 (<0.05) by using method Stepwise. The manganese level of the farmers from a study is shown to have significant positive and poor association with working duration (Abdul Hamid et al, 2017), where $p<0.05$. Besides, a similar study on welders who are exposed to Manganese also concluded that the manganese concentration in welders exhibited a gradual increase of 1.5% with each year of accumulated work experience (Ramzani et al., 2022). The correlation coefficients from a study also demonstrated an increase in manganese levels with the extended latency time after the cessation of exposure and higher work seniority (Lucchini et al., 1995).

On the other hand, the occupational factor of the type of eye protection used by the conventional farmers was significantly contributing to 11.0% of the variance in the level of trace element Zinc with a p-value of 0.013(<0.05). 10.9% of the variance in the level of trace element Zinc among the organic farmers can be significantly explained by the occupation factor of type of foot protection with the p-value of 0.046 (<0.05). The types of eye protection and foot protection are divided into two types, which are correct and incorrect protection. According to Abdul Hamid et al (2017), Personal Protective Equipment (PPE) practices appeared to have the most significant impact on the rate of exposure, aside from the exposure period. The maintenance and inspection of the PPE before and after the use of PPEs also important in reducing the exposure of workers (Dosemeci et al., 2002). PPEs used must be thoroughly cleaned and kept away from pesticides and fertilizers to minimize the potential for excessive exposure (Abdul Hamid et al, 2017). Improper use of PPEs has been proven to elevate the risk of exposure (Zariyantey et al., 2014). For example, the levels of zinc in farmers were significantly associated with PPE practices, indicated by a moderate correlation ($p < 0.05$) between zinc levels and PPE score (Abdul Hamid et al, 2017).

In addition, occupational factor of type of spraying machine was significantly contributing to 13.5% of the variance in the level of trace element Ammonia among the conventional farmers since p-value= 0.009 (<0.05). The previous study by Abdul Hamid et al (2017) indicated that the methods of spraying and the manner in which synthetic pesticides and chemical fertilizers are handled impact the exposure rate. A study from Porter (2015) also concluded that types of spraying method such as aerial spraying and ground treatments have the potential to lead to worker exposures through distinct pathways. Farmers engaged in manual labour in pesticide-treated areas may experience significant exposure through spraying of pesticides and fertilizers directly, drift from adjacent areas, or contact with pesticide residues on crops or soil (Damalas & Koutroubas, 2016). For instance, farmers in India have taken measures to reduce exposure by utilizing spraying machines, including the use of drone machines for pesticide application (Yallappa et al., 2017)

Besides, individual factor of gender was significantly contributing to 12.8% of the variance in the level of trace element Manganese among the organic farmers as the p-value=0.013 (<0.05). The previous study from Abdul Hamid et al (2017) stated that the variations in trace element levels between men and women can potentially be attributed to differences in metabolism, physiology, knowledge levels, practices, awareness, and the handling and use of pesticides and fertilizers. Study from Chojnacka et al (2010) also claimed that men were more likely to be responsible for making decisions about the selection and application of pesticides and fertilizers, as well as for carrying out the actual spraying activities. Women typically have a higher percentage of body fat, making them more prone to storing pollutants that can accumulate in fat tissue (Ilang-Ilang Quijano, 2022). Additionally, women possess a greater amount of hormonally sensitive tissues, rendering them more susceptible to pesticides, particularly those with hormonal activity or known to disrupt the endocrine system (Ilang-Ilang Quijano, 2022).

Furthermore, 9.6% of the variance in the level of ammonia among the organic farmers can be explained by the individual factor of taking alcohol habit and it was shown that there was a significant linear relationship between both variables as p-value= 0.044 (<0.05). A study from Dhanarisi et al (2018) also concluded that ethanol in alcohol significantly contributes to the risk of pesticide poisoning and is frequently co-ingested in cases of self-poisoning involving pesticides. Apart from that, there is research indicates that it is crucial, to abstain from consuming alcohol for at least 24 hours before handling pesticides and other hazardous agricultural chemicals to prevent poisoning (Brand et al., 2023). The alcohol has the ability to undermine the skin's protective barrier against toxic chemicals, which enhances the skin's permeability, increase the probability for greater absorption of chemicals into the body (Brand et al., 2023). Hence, the extent of chemical absorption increases with higher alcohol consumption (Brand et al., 2023).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This cross-sectional study was conducted to compare the level of trace elements (Zn, Mn, Pb and NH_3) in the nail samples among the conventional farmers and organic farmers within the small-scale nursery and vegetable farms in selected states of West Coast of Peninsular Malaysia. This study identified significantly different levels of these three trace elements, which are Zn, Mn and NH_3 in the nail samples among conventional and organic farmers. The levels of Zn, Mn, and NH_3 were found to be significantly higher in conventional farmers compared to organic farmers. In addition, all organic farmers experienced low risks associated with the use of synthetic pesticides and chemical fertilizers containing Zn, Mn, Pb, and NH_3. On the other hand, the majority of conventional farmers were exposed to a high risk of using synthetic pesticides and chemical fertilizers containing Pb, a moderate risk of using synthetic pesticides and chemical fertilizers containing Zn and NH_3, and almost an equal proportion faced moderate and high risks of using synthetic pesticides and chemical fertilizers containing Mn.

The results indicated a significant correlation between the health risks of using synthetic pesticides and chemical fertilizers containing Zn, Mn, and NH_3 with the levels of these elements in the nail samples among the study respondents. However, the health risk associated with synthetic pesticides and chemical fertilizers containing Pb showed no significant association with the level of Pb in the nail samples among the study respondents.

Furthermore, individual factors of age and occupational factors of the type of eye protection demonstrated a significant linear relationship with the level of Zn in the nail samples among conventional farmers. Additionally, individual factor of year of working also contributed to a significant relationship with the level of manganese among the conventional farmers. The occupational factor of foot protection also exhibited a significant linear relationship with the level of Zn among organic farmers. Significant linear relationships were found between the occupational factor of the type of spraying machine with the level of NH_3 among conventional farmers, as well as the individual factor of alcohol consumption with the level of NH_3 among organic farmers. Gender was also identified to have a significant linear relationship with the level of Mn among organic farmers. However, the level of Pb was found not to have a significant linear relationship with any of the individual factors and occupational factors among conventional and organic farmers in this study.

6.2 Study Strength

This study is the first to report the difference in the level of trace elements (Zn, Mn, Pb and NH_3) in the nail samples among the conventional and organic farmers within small-scale nursery and vegetable farms in selected states of West Coast of Peninsular Malaysia.To acquire the analytical performance data of the measurements, quality control was implemented for during the sampling and analysis process. For instance, the questionnaire utilized in this study underwent a validation process to ensure robust internal consistency. Pretesting of the questions was conducted iteratively until the desired outcomes were achieved.

The findings of this study offer benchmark information to assess the impact of synthetic pesticides and chemical fertilizers on the levels of trace elements (Zn, Mn, Pb and NH_3) among farmers. This early information addresses current needs and problems, aiding government stakeholders in planning and implementing future interventions for agricultural farmers. The results also contribute to enhancing occupational policies and programs, promoting a more holistic and cost-effective approach to addressing issues related to the use of synthetic pesticides and chemical fertilizers.

In addition, it emphasizes the necessity of future initiatives to strike a balance between occupational and environmental health as this study demonstrates that the use of synthetic pesticides and chemical fertilizers not only causes soil and water contamination but also has an impact on occupational workers. Furthermore, the study has the potential to enhance workers' awareness and knowledge of the issues associated with synthetic pesticides and chemical fertilizers through the questionnaire survey conducted during the data collection stage. This, in turn, can contribute to the prevention of occupational accidents and diseases, thereby reinforcing the pursuit of sustainable agricultural health.

6.3 Study Limitation

There are unavoidable limitations in this study stemming from the chosen research methods and time constraints. Firstly, the results for several respondents regarding the levels of trace elements were below the limit of detection, leading to their exclusion from this study. Therefore, the overall results from the respondents did not meet the required sample size, potentially leading to insufficient statistical power to detect differences between the groups. Additionally, the study had a limited number of respondents, as it was conducted only in selected states due to time constraints. Consequently, the study's results may not be fully representative of the entire population of agricultural farmers in the West Coast of Peninsular Malaysia. Furthermore, this study solely assessed the four trace elements present in synthetic pesticides and chemical fertilizers and their direct correlations with the levels among the respondents. The interrelationship and correlation between these trace elements were not investigated, despite the potential for additive, synergistic, and antagonistic effects between them. Moreover, the collected nail samples were not distinguished between toenail and fingernail samples. It is worth noting that previous studies recommend this separation, as toenails, with their larger size, are considered to provide a more extensive sample and reflect exposures from a more distant past due to their slower growth rate, while it is hard to distinguish between endogenous and exogenous contamination in the fingernail. Another limitation is the exclusion of dietary nutrition as one of the studied risk factors in this research, despite its significant role as a pathway influencing the levels of trace elements in the human body.

6.4 Recommendation

The findings of this study offer benchmark information to assess the impact of synthetic pesticides and chemical fertilizers on the levels of trace elements (Zn, Mn, Pb and NH3) among farmers. It has proven that the utilization of synthetic pesticides and chemical fertilizers has heightened the health risk among farmers, consequently increasing their levels of trace elements such as Zn, Mn, and NH_3 in the body. Therefore, future studies could explore additional trace elements in the synthetic pesticides and chemical fertilizers, such as Copper, Chromium and many more, to determine if they follow similar routes of transmission as this study, leading to increased levels of these elements in the human body. At the same time, studies can explore the potential for additive, synergistic, and antagonistic effects between the trace elements to uncover a more comprehensive understanding of their interrelationships. Besides, it is crucial to investigate this issue in the Malaysian context rather than focusing on a specific state or area. This approach aims to enhance the generalizability of the findings to broader populations and to explore how geographical location and weather conditions may impact the results. Re-conducting this study can facilitate the comparison of findings, enabling the assessment of the effectiveness of specific interventions and ensuring continuous improvement.

To address the aforementioned limitations, future studies should incorporate dietary nutrition as one of the investigated risk factors. This inclusion will contribute to building a more comprehensive model elucidating the impact of synthetic pesticides and chemical fertilizers containing trace elements on the levels of these elements in the human body. Furthermore, future studies also should separate the nail samples into fingernails and toenails during the sampling process as their difference in growth rates can affect the representation of exposures over time. Hence, more accurate and precise results can be obtained to have a better understanding of how trace elements accumulate and manifest in different timeframes.

Effective intervention programs and targeted policies have the potential to elevate occupational knowledge among agricultural farmers, fostering heightened awareness and consciousness regarding the use of synthetic pesticides and chemical fertilizers. Seizing this opportunity, relevant authorities could disseminate the information and findings of this study through engaging mediums like infographics. This strategic approach could aim to advocate for the adoption of organic pesticides and fertilizers while concurrently educating farmers on the correct and proper use of appropriate Personal Protective Equipment (PPE). By leveraging these educational tools, authorities can significantly contribute to promoting a safer and more informed agricultural community. Instead of solely placing the primary responsibility on pesticide and fertilizer users, the scientific community, manufacturers, merchants, and legislators collectively play a vital role in addressing the health issues associated with pesticides and fertilizers, working towards achieving sustainable agricultural health.

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APPENDICES

Appendix I: Questionnaire (English and Malay Version)

UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

FACULTY OF MEDICINE AND HEALTH SCIENCES

QUESTIONNAIRE:

"TRACE ELEMENTS IN THE NAILS OF FARMERS EXPOSED TO SYNTHETIC PESTICIDES AND CHEMICAL FERTILIZERS IN WEST COAST OF PENINSULAR MALAYSIA"

It is a pleasure to have you chosen as a respondent for this research. The objective of this research is to compare the level of trace elements (zinc, manganese, ammonia, lead) in nails among small-scale vegetable and nursery farmers in West Coast of Peninsular Malaysia. Please answer the questions as accurately, honestly, and completely as possible. All the answers are strictly confidential and for research purposes only. Thank you for your participation in this research. / *Sukacita kerana anda telah dipilih sebagai responden untuk penyelidikan ini. Objektif kajian ini adalah untuk membandingkan tahap mineral mikro (zink, mangan, ammonia, plumbum) dalam paku dalam kalangan petani sayur-sayuran dan tapak semaian skala kecil di Pantai Barat Semenanjung Malaysia. Sila jawab soalan setepat, jujur, dan selengkap mungkin. Semua jawapan adalah sulit dan untuk tujuan penyelidikan sahaja. Terima kasih atas penyertaan anda dalam penyelidikan ini.*

Name of Researcher: Wong Wei Ven

B. Sc. (Environmental & Occupational Health)

Instructions / ***Arahan***

This questionnaire has 12 printed pages including the front page. Soal selidik ini mempunyai 12 halaman bercetak termasuk muka depan.

The survey form consists of / Borang kaji selidik ini terdiri daripada:

Part A: Personal Information/ ***Maklumat Peribadi***

Part B: Lifestyle Information/ ***Maklumat Gaya Hidup***

Part C: Workplace Background/ ***Latar Belakang Tempat Kerja***

Part D: Information on the Use of Synthetic Pesticides and Chemical Fertilizers/ *Maklumat tentang Penggunaan Racun Perosak Sintetik dan Baja Kimia*

Part E: Information on Use of Personal Protective Equipment (PPE)*/ Maklumat mengenai Penggunaan Peralatan Pelindung Diri*

Please answer ALL questions. Thank you for your cooperation. / *Sila jawab SEMUA soalan. Terima kasih atas kerjasama yang diberikan.*

Part A: Personal Information/ *Maklumat Peribadi*

Please fill in the blanks and mark (/) in the box below/ *Sila isi tempat kosong dan tandakan (/) pada kotak di bawah.*

1. Respondent ID/ *ID responden*

    ____

2. Name of Respondent/ *Nama responden*

    ____

3. Gender/ *Jantina*

☐ Male/ *Lelaki*

☐ Female / *Perempuan*

4. Age/ *Umur*

____

5. BMI (Body Mass Index)/ *(Indeks Jisim badan)*

____

6. Phone number/ *Nombor telefon*

____

Part B: Lifestyle Information/ *Maklumat Gaya Hidup*

1. Smoking habit/ *Tabiat merokok*

☐ Yes/ *Ya*

☐ No/ *Tidak*

☐ Ex-smoker/ *Bekas perokok*

☐ Social smoker/ *Perokok social*

2. Alcohol consumption/ *Pengambilan alkohol*

☐ Yes/ *Ya*

☐ No/ *Tidak*

3. Frequency of consuming alcohol/ *Kekerapan mengambil alkohol*

☐ Once a day/ *Sehari sekali*

☐ More than once a day/ *Lebih daripada sehari sekali*

☐ Once a week/ *Seminggu sekali*

☐ More than once a week/ *Lebih daripada seminggu sekali*

☐ Occasionally/ *Kadang*

☐ Not drinking coffee/ *Tidak minum kopi*

Part C: Workplace Background/ Latar Belakang Tempat Kerja

1. Type of Nursery/ *Jenis tapak semaian*

☐ Conventional Nursery/ *Tapak semaian konvensional*

☐ Organic Nursery/ *Tapak semaian organik*

2. How many years on the current farm? / *Berapakah tahun berada di ladang semasa?*

____

3. How many years of using pesticide/how many years have you ever used pesticide before you transformed into an organic nursery? / *Berapakah tahun menggunakan racun perosak / berapakah tahun anda pernah menggunakan racun perosak sebelum anda berubah menjadi tapak semaian organik?*

    ____

4. Job description/ *Deskripsi kerja*

    ____

Part D: Information on the Use of Synthetic Pesticides and Chemical Fertilizers/ *Maklumat tentang Penggunaan Racun Perosak Sintetik dan Baja Kimia*

1. Frequency of using synthetic pesticide and chemical fertilizer in a day/ *Kekerapan menggunakan racun perosak sintetik dan baja kimia dalam sehari*

☐ Exposure one or more time per shift or per day/ *Pendedahan satu kali atau lebih setiap syif atau setiap hari*

☐ Exposure greater than one time per week/ *Pendedahan lebih daripada seminggu sekali*

☐ Exposure greater than one time per month/ *Pendedahan lebih daripada sebulan sekali*

☐ Exposure once per year or less/ *Pendedahan setahun sekali atau kurang*

2. Duration of exposure per shift/ *Tempoh pendedahan setiap syif*

☐ More than or equal to 7 hours/ *Lebih daripada atau sama dengan 7 jam*

☐ 4 hours to less than 7 hours/ 4 *jam hingga kurang daripada 7 jam*

☐ 2 to less than 4 hours/ *2 hingga kurang daripada 4 jam*

☐ 1 to less than 2 hours/ *1 hingga kurang daripada 2 jam*

☐ Less than 1 hour/ *Kurang daripada 1 jam*

3. Which of the following scenarios/situations happened while you spray pesticide? / *Antara senario/situasi berikut, yang manakah berlaku semasa anda menyembur racun perosak?*

☐ Detectable odor of pesticides/ *Bau racun perosak yang dapat dikesan*

☐ Working cloth is wet with pesticide drift/ *Kain kerja basah dengan hanyut racun perosak*

☐ Not applicable/ *Tidak berkenaan*

4. Type of sprayer use during pesticide spraying/ *Jenis penggunaan penyembur semasa penyemburan racun perosak*

☐ A

☐ B

☐ C

☐ D

☐ E

☐ F

☐ Not using pesticide/ *Tidak menggunakan racun perosak*

Part E: Information on Use of Personal Protective Equipment (PPE)/ *Maklumat mengenai Penggunaan Peralatan Pelindung Diri*

1. Type of PPE (foot protection)/ *Jenis PPE (perlindungan kaki)*

☐ A

☐ B

☐ C

☐ D

☐ Slipper/ *Selipar*

☐ Sport shoes/ *Kasut sukan*

☐ Barefoot/ *Tidak memakai kasut*

☐ Other/ *Lain-lain*: ____________________

2. Type of PPE (body protection)/ *Jenis PPE (Perlindungan badan)*

☐ A

☐ B

☐ C

☐ D

3. Type of PPE (respiratory protection)/ *Jenis PPE (Perlindungan pernafasan)*

☐ A

☐ B

☐ C

☐ D

☐ E

☐ F

☐ No wearing any protection/ *Tidak memakai sebarang perlindungan*

4. Type of PPE (eye protection)/ *Jenis PPE (Perlindungan mata)*

☐ A

☐ B

☐ C

☐ D

☐ No eye protection/ *Tiada perlindungan mata*

5. Type of PPE (hand protection)/ *Jenis PPE (Perlindungan tangan)*

☐ A

☐ B

☐ C

☐ D

☐ E

☐ No hand protection/ *Tiada perlindungan tangan*

Appendix II: Chemical Health Hazard Checklist

Table I: Farm-Site Information

1. Type of Farm (Organic/Conventional)		**2. Type of Farm Activities**	
3. Farm Location		**4. Date of Assessment**	
5. Working Hours			

Table II: List of Pesticide(s) and Fertilizer(s) Used

No	Name of chemical	Hazardous Ingredient	Physical form	Source of Information	Hazard Classification	H-code	Inhalation (Y/N)	Dermal (Y/N)	Ingestion (Y/N)
1									
2									
3									
4									
5									
6									
7									

GUIDE TO CLASSIFY HEALTH HAZARD Based on "*A Manual of Recommended Practice on Assessment of the Health Risks Arising from the Chemicals Hazardous to Health at the Workplace 2018 (3rd Edition)*".

Hazard Classification	H-code: Hazard Statement
Acute toxicity category 4	**H332:** Harmful if inhaled
Acute toxicity category 4	**H312:** Harmful if in contact with skin
Acute toxicity category 4	**H302:** Harmful if swallowed
Acute toxicity category 3	**H331:** Toxic if inhaled
Acute toxicity category 2	**H330:** Fatal if inhaled
Acute toxicity category 3	**H331:** Toxic if inhaled
Acute toxicity category 3	**H311:** Toxic if in contact with skin
Acute toxicity category 3	**H301:** Toxic if swallowed
Acute toxicity category 2	**H330:** Fatal if inhaled
Acute toxicity category 1	**H330:** Fatal if inhaled
Acute toxicity category 2	**H330:** Fatal if inhaled
Acute toxicity category 1	**H310:** Fatal if in contact with skin
Acute toxicity category 2	**H300:** Fatal if swallowed
Specific target organ toxicity – repeated exposure category 2	**H373:** May cause damage to organs through prolonged or repeated exposure
Skin corrosion or irritation category 1B	**H314:** Causes severe skin burns and eye damage
Skin corrosion or irritation category 1A	**H314:** Causes severe skin burns and eye damage
Serious eye damage or eye irritation category 2	**H319:** Causes serious eye irritation
Specific target organ toxicity – single exposure category 3	**H335:** May cause respiratory irritation
Skin corrosion or irritation category 2	**H315:** Causes skin irritation
Specific target organ toxicity – single exposure category 1	**H370:** Causes damage to organs *(or state all organs effected, if known) (state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Serious eye damage or eye irritation category 1	**H318:** Causes serious eye damage
Respiratory sensitisation category 1	**H334**: May cause allergic or asthma symptoms or breathing difficulties if inhaled
Skin sensitisation category 1	**H317:** May cause allergic skin reaction
Specific target organ toxicity – repeated exposure category 2	**H373:** May cause damage to organs *(or state all organs effected, if known) through prolonged or repeated exposure (state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Specific target organ toxicity – repeated	**H372:** Causes damage to organs (*or state all organs effected, if known) through prolonged*

Hazard Classification	H-code: Hazard Statement
exposure category 1	*or repeated exposure (state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Effect on or via lactation	**H362:** May cause harm to breast-fed children
Aspiration hazard category 1	**H304:** May be fatal if swallowed and enters airways
Specific target organ toxicity- single exposure category 3	**H336:** May cause drowsiness or dizziness
Specific target organ toxicity – single exposure category 2	**H371:** May cause damage to organs *(or state all organs effected, if known) (state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Carcinogenicity category 1A	**H350:** May cause cancer *(state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Carcinogenicity category 1B	**H350:** May cause cancer *(state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Carcinogenicity category 2	**H351:** Suspected of causing cancer *(state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Germ cell mutagenicity category 1B	**H340:** May cause genetic defects (state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)
Germ cell mutagenicity category 2	**H341:** Suspected of causing genetic defects *(state route of exposure, if it is conclusively proven that no other routes of exposure cause the hazard)*
Reproductive toxicity category 1A	**H360F:** May damage fertility **H360D:** May damage the unborn child
Reproductive toxicity category 1B	**H360F:** May damage fertility **H360D:** May damage the unborn child
Reproductive toxicity category 2	**H361f:** Suspected of damaging fertility **H361d:** Suspected of damaging the unborn child
Reproductive toxicity category 1A	**H360FD:** May damage fertility. May damage the unborn child. H360FD: May damage fertility. May damage the unborn child.

Appendix III: Fieldwork Checklist Table

Location :

Date :

Kindly refer to the instruction before you start the data collection:

1. Researcher is required to give brief explanation as stated in the **Subject Information Sheet** to the respondent before they could participate in the study
2. Only respondent who return with signed **consent letter** should they participate in the study
3. Tick (/) only to the column which is relevant to your section
4. Respondent's details should keep private & confidential for research ethical purpose.
5. Those who sampled human biological samples are **COMPULSORY** to wear disposable gloves prior sample collection

Respondent ID	Consent letter	Questionnaire	Nail sample

Appendix IV: Subject Information Sheet and Consent Form

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE : Trace Elements in the Nails and Hair of Farmers Exposed to Synthetic Pesticides and Chemicals Fertilizers

2. INTRODUCTION:

Trace elements are essential components of human biological structures. They're involved in most of the biochemical processes in the human body contain. The development of agricultural production has caused the broad application of synthetic pesticides and chemical fertilizers. Residuals of these chemicals would circulate through the atmosphere, water, soil and biosphere. Worst still, some are vestigial in surface water and groundwater, where farmers are at immediate risk of exposure to these chemicals based on their nature of work on the farm. Prolonged exposure to synthetic pesticides and chemical fertilizers through environmental and occupational settings has been shown to lower the levels of trace elements in the human body as an antagonistic effect. The deficiency of these elements could disturb some metabolic processes in the body, eventually leading to various health problems.

3. WHAT WILL YOU HAVE TO DO?

This study involve simple random sampling based on the list of sampling frame. Those who fulfil the inclusive criteria would need to involve in three (3) parts of data collection as below,

Station 1: Face-to-face Questionnaires using structured questionnaires that farmers will be conducted. The information to be asked during the face-to-face interview are as follows: (Estimate duration: 5 -10 minutes)

- Section A: Personal Information
- Section B: Lifestyle Information
- Section C: Workplace Background
- Section D: Information on the Use of Synthetic Pesticides and Fertilizers
- Section E: Information on Use of Personal Protective Equipment (PPE)

Station 2: The researcher will check an on-site pesticide health hazard checklist. Participants would be required to gather all the pesticide-used bottles for the purpose of record keeping by researcher. The researcher will take photos of each pesticide bottle gathered during this process.
(Estimate duration: 5 -10 minutes)

Station 3: Hair and nail sample (finger and toenail) samples will be collected, and each participant will be provided with stainless-steel scissors and stainless-steel nail clippers for hygiene purposes.

Hair samples (~20-30 strands) will be collected from the occipital region, within 2 mm from the scalp and approximately 3 cm in length, using stainless-steel scissors during the field sampling. Nail samples, for both toenail and finger samples, will be collected in 5g samples by asking permission to cut the participant's nails with clean stainless-steel nail clippers. (Estimate duration: 5 -10 minutes)

The expected duration for overall data collection is thirty (30) minutes. All data collection methods will be conducted in the field on the same day. The participants will receive RM20 as a token of appreciation for participating in the study. Each participant will also receive a new set of stainless-steel scissors and stainless-steel nail clippers as a bring-home gift following the hair and nail sample collection. Participation is voluntary, and participants may withdraw anytime without penalty or loss of benefit to which they are entitled.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

A total of 100 farmers would be recruited in this study. The researcher would check two (2) phases of inclusive and exclusive criteria to validate your participation in this study. During the first phase of selection, both organic and conventional vegetable and nursery farmers to fulfil the following requirements,

<u>Inclusive criteria for conventional farmers</u>

- Both gender, at the age of 18 – 60 years old
- Has been involved in conventional farming at the studied location for at least 12 months
- Living in the agricultural farming communities for the past 12 months
- Handling conventional farming activity, including using synthetic pesticides and fertilizers in their routine farming activity

<u>Inclusive criteria for organic farmers</u>

- Both gender, at the age of 18 – 60 years old
- Has been involved in organic farming at the studied location for at least 12 months
- Living in the agricultural farming communities for the past 12 months
- Handling organic/natural/agroecological farming activity has not been using any synthetic agrochemicals for the past 12 months

However, a participant who is included in any of the criteria below would be excluded from participating in this study,

<u>Exclusive Criteria for farming communities</u>

- Had medical conditions, e.g. anaemia, nephritic problems and hepatic diseases
- Had a history of exposure to cytotoxic therapeutic drugs (e.g. chemotherapy) and radiation

During the second phase of selection, participants who have fulfiled the first phase of selection would be undergone a second time of screening to ensure they fulfil the following criteria to proceed with final participation in this study,

<u>Inclusive Criteria for farmers of nail samples</u>

- No dermatological disease, trauma or injury affecting the nails.
- No nail polish or other chemicals on nails for at least three months before the date of nail sample collection.
- The participants will be notified about the study in advance and advised not to cut their nails for four weeks before sample collection. Participants who had nail polish or other chemicals on their nails waited for three months and then one extra month for nails to grow before sample collection, i.e. four months.

Inclusive Criteria for farmers of hair samples

- No dermatological disease affecting the hair.
- No dyed or bleached hair. Hair was free of creams, oil and gels before sample collection.
- Participants would be informed four weeks in advance about the study, instructed about the dates for hair sample collection, and advised that they should not get a haircut during this time. If the participant has had a haircut, perm or colouring during the last four weeks, sample collection would be postponed for four weeks as hair grows at about 0.4 mm/day or an average of about 1 cm/month.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

Participants will receive an incentive of Twenty Ringgit Malaysian (RM20) as a token of appreciation upon completing all data collection methods. Upon request, you will receive the output of the trace element detected in your hair and nail sample. Besides, your participation will increase researchers' understanding of the trace element contamination following synthetic pesticides and fertilizers usage among the farming community. Participants would be one of the subject matter that contributes to the data in building healthy and sustainable agricultural practices in Malaysia.

(b) TO THE INVESTIGATOR?

Researchers were able to generate new insights into the trace elements contamination from the synthetic pesticide and fertilizers used during farming activities. The information obtained in this study could support the prevention and control of pesticide-related health risks and create a healthy and sustainable agricultural practice in Malaysia.

6. WHAT ARE THE POSSIBLE RISKS?

Minimal nail injury might occur during nail (finger and toenail) clipping while the nail is torn away from the skin. Researcher with first aid knowledge will monitor participants while cutting their nails; if any injury happens, an immediate first aid procedure will be performed to reduce the pain.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

Information & identity of the subjects will remain confidential & will be used for research purposes only. Besides, all collected biological sample would be disposed immediately at the end of the study by follow strictly the clinical waste management system in Malaysia.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

For further information related to the research, you may contact the principal investigator,

Dr Vivien How
Department of Environmental and Occupational Health
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
Email: vivien@upm.edu.my
Contact: 016-6193697

If you have any questions about your rights as a participant in this study, please contact: The Secretariat, JKEUPM, at email address **jkeupm@upm.edu.my**

*Please initial here if you have read and understood the contents of this page*______

9. CONSENT

I ………………………………….. Identity Card No. …………………………… address………………………………………………………………………………………………
……... ………………………………………………….hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ……..………………………… (Respondent)

Date :……………………………………..

Signature ……..…………………………… (Witness)

Name :……………………………………..…..

I/C No. :…………………………………..…..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ……..…………………………

Signature ……..………………………… (Researcher)

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UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN: Mineral Mikro dalam Kuku dan Rambut Petani yang Terdedah kepada Racun Perosak Sintetik dan Baja Kimia

2. PENGENALAN

Mineral Mikro adalah komponen penting dalam struktur biologi manusia. Mereka terlibat dalam kebanyakan proses biokimia dalam tubuh badan manusia. Perkembangan pengeluaran di dalam bidang pertanian telah menyebabkan penggunaan racun perosak dan baja kimia secara berleluasa. Sisa bahan kimia ini akan berada di dalma kitaran atmosfera, air, tanah dan biosfera. Di dalam situasi yang paling teruk, sisa bahan kimia ini akan berada di permukaan air dan di dalam kitaran air bawah tanah, di mana risiko pendedahan bahan kimia ini kepada petani adalah cepat berdasarkan cara kerja mereka di ladang. Kajian terdahulu telah menunjukkan bahwa pendedahan berpanjangan kepada racun perosak dan baja kimia melalui melalui pekerjaan dan alam sekitar dapat menurunkan tahap mineral mikro dalam tubuh manusia kerana bahan kimia ini mampu memberikan kesan antagonis terhadap manusia. Kekurangan unsur-unsur mineral mikro ini boleh mengganggu beberapa proses metabolisme di dalam badan dan akhirnya membawa kepada pelbagai masalah kesihatan.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Kajian ini melibatkan persampelan rawak mudah berdasarkan senarai kerangka persampelan. Mereka yang memenuhi kriteria inklusif perlu melibatkan diri dalam tiga (3) bahagian pengumpulan data seperti di bawah,

Stesen 1: Soal Selidik Bersemuka menggunakan borang soal selidik yang akan dijawab oleh petani. Maklumat yang akan ditanya semasa soal selidik secara bersemuka dijalankan adalah seperti berikut: (Anggaran tempoh: 5 -10 minit)

- Bahagian A : Maklumat Diri
- Bahagian B : Maklumat Gaya Hidup
- Bahagian C : Latar Belakang Pekerjaan
- Bahagian D : Maklumat Penggunaan Racun Perosak dan Baja Kimia
- Bahagian E : Maklumat Penggunaan Kelengkapan Pelindung Diri

Stesen 2: Penyelidik akan menyemak senarai racun perosak berbahaya yang digunakan oleh petani di tempat kerja. Peserta dikehendaki mengumpulkan semua botol racun perosak untuk penyimpanan rekod daripada pihak penyelidik. Semasa proses ini dijalankan penyelidik akan mengambil gambar setiap botol racun perosak yang dikumpulkan daripada petani. (Anggaran tempoh: 5 -10 minit)

Stesen 3: Dibahagian in, sampel rambut dan kuku (jari dan kuku kaki) peserta akan dikumpulkan dan setiap peserta akan dibekalkan dengan gunting keluli tahan karat dan pengetip kuku keluli tahan karat untuk tujuan kebersihan.

Sampel rambut sebanyak 20-30 helai akan dikumpulkan dari kawasan oksipital, dalam jarak 2 mm dari kulit kepala dan lebih kurang 3 cm panjang. Sampel rambut ini akan diambil menggunakan gunting keluli tahan karat. Bagi sampel kuku pula, sampel kuku akan di ambil daripada kedua-dua jari kaki dan tangan peserta. Anggaran sampel kuku yang diperlukan daripada peserta adalah sebanyak 5g. Sebelum sampel kuku diambil daripada peserta, penyelidik akan meminta kebenaran terlebih dahulu untuk memotong kuku peserta menggunakan gunting kuku keluli tahan karat yang bersih. (Anggaran tempoh: 5 -10 minit)

Jangkaan tempoh pengumpulan data secara keseluruhan ialah tiga puluh (30) minit. Semua kaedah pengumpulan data akan dijalankan di lapangan pada hari yang sama. Para peserta akan menerima RM20 sebagai tanda penghargaan kerana mengambil bahagian dalam kajian ini. Setiap peserta juga akan menerima satu set gunting rambut keluli tahan karat dan pengetip kuku keluli tahan karat sebagai hadiah dibawa pulang berikutan pengumpulan sampel rambut dan kuku. Penyertaan adalah secara sukarela, dan peserta boleh menarik diri pada bila-bila masa tanpa penalti atau kehilangan manfaat yang mereka berhak.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?
Seramai 100 orang petani akan diambil dalam kajian ini.Penyelidik akan menyemak dua (2) fasa kriteria inklusif dan eksklusif untuk mengesahkan penyertaan peserta di dalam kajian ini. Semasa fasa pertama pemilihan, kedua-dua petani sayur-sayuran dan nurseri organik dan konvensional perlu memenuhi keperluan berikut,

Kriteria inklusif untuk petani konvensional
- Lekaki dan perempuan, dalam umur 18 – 60 tahun
- Telah terlibat dalam pertanian konvensional di lokasi yang dikaji sekurang-kurangnya 12 bulan
- Hidup dalam komuniti pertanian sejak 12 bulan yang lalu
- Mengendalikan aktiviti pertanian konvensional, termasuk menggunakan racun perosak dan baja sintetik dalam aktiviti pertanian rutin mereka

Kriteria inklusif untuk petani organik
- Lekaki dan perempuan, dalam umur 18 – 60 tahun
- Telah terlibat dalam pertanian organik di lokasi yang dikaji sekurang-kurangnya 12 bulan
- Hidup dalam komuniti pertanian sejak 12 bulan yang lalu
- Mengendalikan aktiviti pertanian organik/semulajadi/agroekologi tidak menggunakan sebarang agrokimia racun perosak dan bahan baja selama 12 bulan yang lalu

Walau bagaimanapun, peserta yang termasuk dalam mana-mana kriteria di bawah akan dikecualikan daripada menyertai kajian ini,

Kriteria Eksklusif untuk komuniti petani
- Mengalamimasalah kesihatan, cth. anemia, masalah nefritik dan penyakit hati
- Mempunyai sejarah pendedahan kepada ubat terapeutik sitotoksik (cth. kemoterapi) dan radiasi

Semasa pemilihan fasa kedua, peserta yang telah memenuhi pemilihan fasa pertama akan menjalani saringan kali kedua untuk memastikan mereka memenuhi kriteria berikut untuk meneruskan penyertaan akhir di dalam kajian ini,

Kriteria Inklusif untuk petani sampel kuku
- Tiada penyakit dermatologi, trauma atau kecederaan yang menjejaskan kuku.
- Tiada pengilat kuku atau bahan kimia lain pada kuku selama sekurang-kurangnya tiga bulan sebelum tarikh pengumpulan sampel kuku.
- Para peserta akan dimaklumkan tentang kajian terlebih dahulu dan dinasihatkan supaya tidak memotong kuku mereka selama empat minggu sebelum pengambilan sampel. Peserta yang mempunyai pengilat kuku atau bahan kimia lain pada kuku mereka perlu menunggu selama tiga bulan dan kemudian satu bulan tambahan untuk kuku tumbuh sebelum pengumpulan sampel, iaitu pada bulan keempat.

Kriteria Inklusif untuk petani sampel rambut
- Tiada penyakit dermatologi yang menjejaskan rambut.

- Tiada rambut yang dicelup atau diluntur. Rambut bebas daripada krim, minyak dan gel sebelum pengumpulan sampel.
- Peserta akan dimaklumkan empat minggu lebih awal tentang kajian itu, dimaklumkan tentang tarikh pengambilan sampel rambut, dan dinasihatkan supaya mereka tidak menggunting rambut pada masa ini. Jika peserta telah menjalani potongan rambut, perm atau pewarnaan selama empat minggu yang lalu, pengumpulan sampel akan ditangguhkan selama empat minggu apabila rambut tumbuh kira-kira 0.4 mm/hari atau purata kira-kira 1 sm/bulan.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Peserta akan menerima insentif sebanyak Dua Puluh Ringgit Malaysia (RM20) sebagai tanda penghargaan setelah melengkapkan semua kaedah pengumpulan data. Atas permintaan, anda akan menerima output unsur surih yang dikesan dalam sampel rambut dan kuku anda. selain itu, Selain itu, penyertaan anda akan meningkatkan pemahaman penyelidik tentang pencemaran unsur surih berikutan penggunaan racun perosak dan baja sintetik dalam kalangan komuniti petani. Peserta akan menjadi salah satu subjek yang menyumbang kepada data dalam membina amalan pertanian yang sihat dan mampan di Malaysia.

b) KEPADA PENYELIDIK?

Penyelidik dapat menjana pandangan baharu tentang pencemaran unsur surih daripada racun perosak sintetik dan baja yang digunakan semasa aktiviti pertanian. Maklumat yang diperolehi dalam kajian ini boleh menyokong pencegahan dan kawalan risiko kesihatan berkaitan racun perosak dan mewujudkan amalan pertanian yang sihat dan mampan di Malaysia.

6. ADAKAH IA BERISIKO?

Kecederaan kuku minimum mungkin berlaku semasa proses pemotongan kuku (jari dan kaki). Penyelidik yang mempunyai pengetahuan pertolongan cemas akan memantau peserta semasa memotong kuku mereka; jika berlaku sebarang kecederaan, prosedur pertolongan cemas akan dilakukan segera untuk mengurangkan kesakitan.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Maklumat & identiti subjek akan kekal sulit dan akan digunakan untuk tujuan penyelidikan sahaja. Selain itu, semua sampel biologi yang dikumpul akan dilupuskan serta-merta pada akhir kajian dengan mematuhi sistem pengurusan sisa klinikal di Malaysia.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Untuk maklumat lanjut berkaitan penyelidikan, anda boleh menghubungi penyelidik utama

Dr Vivien How
Jabatan Kesihatan Persekitaran dan Pekerjaan
Fakulti Perubatan dan Sains Kesihatan
Universiti Putra Malaysia
E-mel: vivien@upm.edu.my
Hubungi: 016-6193697

Jika anda mempunyai sebarang pertanyaan berkaitan dengan hak-hak anda sebagai peserta dalam penyelidikan ini, sila hubungi: Sekretariat, JKEUPM, melalui email: **jkeupm@upm.edu.my**

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _______

9. PERSETUJUAN

Saya………………………………………… No Kad Pengenalan. …………………………… beralamat……………………………………………………………………………………… ……………………………………..dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan. Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya * berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imej/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan ……..………………………… (*Responden*)

Tarikh :……………………………………..

Tandatangan ……..…………………………. (*Saksi*)

Nama :……………………………………..

No. K/P: ……………………………………..

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh ……..…………………………

Tandatangan ……..…………………………. (*Penyelidik*)

ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS (JKEUPM) UNIVERSITI PUTRA MALAYSIA

Research title	:	**Trace Elements in the Nails and Hair of Farmers Exposed to Synthetic Pesticides and Chemicals Fertilizers.**
Study Site	:	**Cameron Highlands, Johor, Malacca, Selangor, Perak and Penang.**
JKEUPM Ref No.	:	**JKEUPM-2023-187**
Principal Investigator	:	**Dr. Vivien How**

Documents received and reviewed with reference to the above study:

1. Ethics Application Form, Version 1 dated 23/02/2023
2. Respondent's Information Sheet / Consent (English), Version 1 dated 23/02/2023
3. Respondent's Information Sheet / Consent (Malay), Version 1 dated 23/02/2023
4. Proposal (English), Version 1 dated 23/02/2023
5. Questionnaire / Interviews (English), Version 1 dated 23/02/2023
6. Questionnaire / Interviews (Malay), Version 1 dated 23/02/2023
7. Curriculum Vitae of:
   a. Dr. Vivien How

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.
Decision by JKEUPM:

[X] Approved

[X] **Permission MUST BE OBTAINED from the respective hospitals/ institutions before conducting the research**

[ ] Disapproved

Please note that the approval is **VALID UNTIL 11 APRIL 2024**

Researchers should comply with the following:

I. Complete a Study Final Report upon study completion (Form 3.2).

II. Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

III. Applicable for Clinical Trial Studies and Clinical interventional Studies only: Progress Report has to be submitted to JKEUPM at every 6 months from the date of approval (Form 3.1). Report occurrences of all Serious Adverse Events (SAEs), Suspected Unexpected Serious Adverse Reaction (SUSARs) and Protocol Deviation/ Violation at all JKEUPM approved sites to JKEUPM. All serious adverse events (SAEs) detected or being notified should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports.