REDUCING HEAVY METALS MOBILITY IN SOIL BY NATURAL PLANT EXTRACT

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UNIVERSITI TUNKU ABDUL RAHMAN

REDUCING HEAVY METALS MOBILITY IN SOIL BY NATURAL PLANT EXTRACT

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A project report submitted in partial fulfilment of the

requirements for the award of the degree of

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **“REDUCING HEAVY METALS MOBILITY IN SOIL BY NATURAL PLANT EXTRACT”** was prepared by **NG TECK BIN** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honors) Environmental Engineering at Universiti Tunku Abdul Rahman.

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Signature :

Supervisor : Dr. Guo XinXin

Date : 22 April 2022

Specially dedicated to

my beloved brother, mother, and father.

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REDUCING HEAVY METALS MOBILITY IN SOIL BY NATURAL PLANT EXTRACT

ABSTRACT

In this study, the incubation experiment was conducted to assess the effect of plant extract (TM) application on the immobilization of Pb, Zn, Cr, Cu and Cd in soil. Soils was amended with 1 %, 2 %, 5 %, and 10 % of TM and incubated for 30 days. As a conventional organic stabilizer, rice husk derived biochar (RHB) had been applied in this experiment for comparison purpose. The fractions of each heavy metals (HMs) in soil were evaluated by Tessier’s sequential extraction procedure. The finding suggested that RHB had significantly increased the soil pH, SOM, and TOC while TM performed a negligible effect on soil pH and SOM but remarkably improved the soil TOC. Both stabilizers were found containing abundant of O-containing and N-containing functional groups by using ATR-FTIR. TM had successfully decreased the mobility of Cd and Zn by 11.28 % to 17.53 % and 4.77 % to 7.92 % with the increment of dosage. However, an increasing trend of Cu and Pb mobility in soil was observed after the application of TM. The mobility of Cu and Pb increased by 5.66 % to 8.20 % and 7.74 % to 10.84 % respectively. There was also found a fluctuated effect on Cr mobility with the increase of TM dosage and the difference was ranging from -0.88 % to 1.16 % when compared to control sample. It might suggest that TM was more suitable for Cd and Zn contaminated soil. In comparison, the performance of RHB was found more stable than TM, in which had decreased the mobility of Cd, Zn, Cr, Cu and Pb by ranging from 1.86 to 9.53 %, 8.77 to 11.96 %, 5.46 to 6.87 %, 2.94 to 13.97 %, and 1.16 to 7.48% respectively.

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%	Percentage
°C	Degree Celsius
µg/g	Microgram per gram
µm	Micrometre
cm	Centimetre
g	Gram
g/ cm^3	Gram per centimetre cube
kJ/mol	Kilojoule per mole
m	Meter
M	Molar concentration
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
mL	Millilitre
mm	Millimetres
mmol/kg	Millimole per kilogram
MYR/year	Malaysia ringgit per year
N	Normality
pH	Acidity
ppm	Parts per million

rpm	Revolutions per minute
t/year	Tonne per year
v/v	Volume to volume
w/w	Weight to weight
Al	Aluminium
As	Arsenic
As_2O_3	Arsenic trioxide
C	Carbon
$CaCO_3$	Calcium carbonate
$Cd\ (NO_3)_2{\cdot}4H_2O$	Cadmium nitrate tetrahydrate
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
$CuSO_4$	Copper sulfate
$FeCr_2O_4$	Ferrochromite
$FeSO_4$	Iron (II) sulfuric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HCl	Hydrogen peroxide
Hg	Mercury
$Hg_2{}^{2+}$	Mercurous
Hg^{2+}	Mercuric
HOAc	Acetate acid
$K_2Cr_2O_7$	Potassium dichromate
K_2CrO_4	Potassium chromate
$MgCl_2$	Magnesium chloride

Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NaOAc	Sodium acetate
$NH_2OH \cdot HCl$	Hydroxylamine hydrochloride
NH_4OAc	Ammonium acetate
Ni	Nickel
O_2^-	Oxygen ion
OH^-	Hydroxide ion
P	Phosphorus
Pb	Lead
$PbCl_2$	Lead (II) chloride
$PbCO_3$	Lead (II) carbonate
PbS	Lead (II) sulfide
$PbSO_4$	Lead (II) sulfate
S	Sulphur
Sb	Antimony
Si	Silicon
Zn	Zinc
Zn^{2+}	Zinc (II) ion
$ZnCl_2$	Zinc (II) chloride

AB-DTRA	Ammonium bicarbonate-diethylenetriaminepentaacetic acid
BDAT	Best demonstrated available technology
CA	Citric acid
CAMOB	Citric acid modified Moringaoleifera bark
CAMOL	Citric acid modified Moringaoleifera leaf
CMC	Chicken manure compost

DI	Deionized
EDDS	Ethylenediamine disuccinic acid
EDTA	Ethylenediaminetetraacetic acid
Eh	Redox potential
EPA	Environmental protection agency
ER	Electrokinetic Remediation
F1	Exchangeable fraction
F2	Carbonate fraction
F3	Fe-Mn Oxides fraction
F4	Organic fraction
F5	Residual fraction
FE	Fagopyrum esculeritum extract
FF	Fordiophyton faberi extract
GDP	Gross domestic product
GPRB	grape-pruning-residue biochar
HMs	Heavy metals
MSE	Moringa olerifera seed extract
NAPL	Non-aqueous phase liquid
NTA	Nitrilotriacetic acid
PE	Plant extract
RHB	Rice husk derived biochar
RSB	Rice straw derived biochar
SOM	Soil organic matter
TM	TM Agricultural
TOC	Total organic carbon
USEAG	United states environmental action group
WEOC	Water-extractable organic carbon
ZVI	Zerovalent iron

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Soil is a valuable resource and foundation of agricultural sustainability as well as civilization of human. Population expansion, especially the rise of high-density urban populations, leads to global industrialization and creates significant stresses on our soil system, putting our environment’s sustainability in jeopardy. Soil pollution is mostly caused by the release of industrial, municipal, agricultural, and mining wastes that generated by anthropogenic activities (Masindi and Muedi, 2018). Despite the governments around the world had the responsibility to advocate their citizen to have a zero pollution in soil. However, this environment concerns had overshadowed by other concerns for economic growth, agricultural and industrial development for ever-increasing populations. As a result, this prioritizing in certain direction becomes the driving force behind the soil contamination.

Since the rising of the industrial revolution, the condition of heavy metals (HMs) contamination of the biosphere was increasingly serious and had become a major environmental concern throughout the world. Over the past decades, the worldwide released of HMs had up to 1,350,000 t/year for Zn, 939,000 t/year for Cu, 738,000 t/year for Pb and 22,000 t/year for Cd (Hakeem et al., 2014). It can say that metal productions had become one of the necessary of our living life in this model society. According to the data provided by "Our World in Data", the global metal production had considerably increase over the year as illustrated in Figure 1.1. However, the result of the rapid industrial development was the HMs contamination in environment.

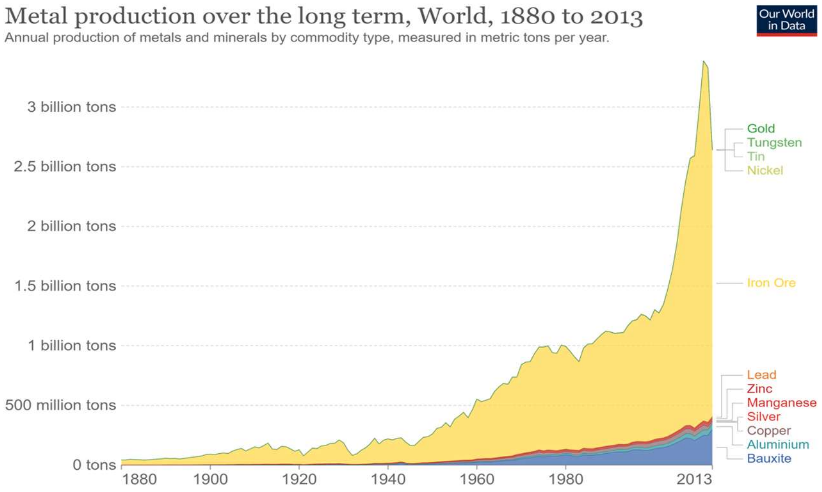


Figure 1.1: Metal production over the long term in the world from 1880 to 2013 (source: Our World in Data, 2013)

HMs contaminated in soil had increasingly attracted the global attention due to the rising cases of HMs-induced health issues had been reported during the past decades. According to the report of Khalid et al. (2017), there was over 50% of identified contaminated sites in the world were contaminated with HMs and metalloids. These HMs that found in soil commonly includes Pb, Cr, Hg, Cu, Zn, Cd, As, Co, Al, Ni, Mn and (Khalid et al., 2017; McGrath et al., 2001). Naturally, the HMs origin from the earth's crust and release into environment through the deterioration of parent material. Nowadays, the soil contamination with HMs is ubiquitous in the world especially in the developed countries such as Germany, USA, Sweden, Australia, and China due to rapidly expanding industrial areas, mining activities, large-scale application of fertilizers and pesticides (Gusiatin et al., 2020).

HMs contamination is difficult to be noticed because of its colorless and odorless characteristics. Unlike organic contaminants, HMs cannot be destroyed and hardly degraded into harmless forms through high temperature or the use of chemicals (Hakeem et al., 2014). Once they enter the soils, the total amount of HMs will persist for a long time. When the accumulation of HMs reached its toxic level in soil, it could cause a significant damage to not only the environment, but also plants, animals, and human. Generally, the presence of the toxic HMs in the soil will inhibit the biodegradation of organic contaminants, composition of microbial community and the enzyme activity in soil. HMs uptake by plants will result in yield depression, weak plant growth, chlorosis, and disorders in plant metabolism. Besides, human may expose to the risk and hazard through utilization of the HMs contaminated foods or absorption and inhalation of dust (Su, 2014).

Cleaning up the HMs contaminated sites is a sign of the time but is a tough task. To reduce the dangers of HMs in polluted soils, several techniques have been developed to remediate contaminated sites over the past three decades. The remediate techniques can be categorized into physical, chemical, and biological remediation. Those remediate techniques can be conducted in the way of in-situ or ex-situ or both. Physical remediation is using the physical technologies such as soil replacement and thermal treatment to distinguish the pollutants from the soil. The main physical remediation techniques include surface capping, landfilling and thermal desorption. Biological remediation is exploiting the inherent mechanisms of microorganisms and plants to uptake, destroy or immobilizing the contaminants (Ayangbenro and Babalola, 2017). Chemical remediation aimed to decrease the mobility, bioavailability and bioaccessibility of the pollutants by exploiting the chemical reagent, reaction, and principles. The typical chemical remediation techniques include soil flushing, soil washing, eletrokinetics, and stabilization (Li, 2019).

However, those ordinary methods employed to recover HM-contaminated soil was found ineffective because of high capital cost, limited land availability and potential of hazardous exposure of the remediation technique. Thus, the development of stabilization technique for soil remediation had accelerated due to its advantages of cost effective and environmentally friendly. (Palansooriya, et al., 2020). This method had been supported and investigated by numerous associations and research because of its potentialities (Shen et al., 2018; Yao et al., 2012; Gusiatin et al., 2020; Roote, 1997; Raffa et al., 2021; Rangga et al., 2019; Ohio, 2000). It had been admitted by Environmental Protection Agency (EPA) as the Best Demonstrated Available Technology (BDAT) for the HMs-contaminated soils remediation (Alaboudi et al., 2019). This technique is achieved by changed the soil texture and physicochemical properties thereby decrease the HMs mobility and bioavailability by using organic or inorganic stabilizers instead of directly removing the total amount of HMs in soil (Chirakkara et al., 2016). At recent, numerous soil stabilizers had been found and widely employed to the soil treatment. They can be either inorganic materials such as phosphate compounds, liming materials and industrial waste by-product or organic materials such as bio-solid compost, humic substances, and biochar (Wang et al., 2020). The biochar, as the conventional soil stabilizer, had been proved that it had an excellent performance in immobilizing HMs and enhancing soil properties through several research (Liang et al., 2017; Lu et al., 2017; Jiang et al., 2012; Hamzenejad and Sepehr, 2018; Rashid et al., 2022; Zhu et al., 2015; Derakhshan-Nejad and Jung, 2019). Nevertheless, most of the natural organic stabilizers were in solid forms but the research related to liquid-based soil stabilizers were limited.

Plant extract (PE) refers to a liquid residue that is formed through an extraction and separation process from the plants. The components of the PE are the same as their original plants (Keller, 2021). PE can be applied as natural pigments, natural sweeteners, Chinese medicine extracts, additives for cosmetics and so on. In addition, plant extract contains ample oxygen-containing groups, such as carboxyl, phenolic hydroxyl, and lactonic groups. Thus, it holds substantial potential as an efficient stabilizer of soil because its oxygen-containing group can easily chelate and immobilizing many metals.

In this study, PE was employed in HMs contaminated soil that collected from University Tunku Abdul Rahman, Kampar Campus, Block J in Malaysia. For comparison, a conventional organic stabilizer, rice husk derived biochar (RHB) was used with the same dose as the PE to treat the soil. The Tessier's method was applied for sequential extraction to all soil samples thereby assess the mobility of each type of HMs in the soil before and after the application of organic stabilizers.

1.2 Problem Statement

Soil is the one of the major sinks for the release of HMs through both natural and anthropogenic processes into the environment. Despite some of the metal such as Cu and Zn serve as essential element for human living, all the HMs has the toxic effect once the concentration over our acceptance threshold. Based on the report of United States Environmental Action Group (USEAG), there were over 10 million people were threatened by HMs contamination and mostly come from the developing countries such as Malaysia. In addition, 12% of national GDP and 16% of employment in Malaysia was coming from the agricultural production (Nations Ecyclopedia, n.d.). The health condition of soil directly influences the yield and quality of the crops thereby directly affect the economy of Malaysia. Several studies indicated that agricultural soil in Malaysia was being contaminated by HMs, including Fe, Pb, Cd, and Cu (Zarcina et al., 2004; Khairiah et al., 2009; Ismail et al., n.d.; Alrawiq et al., 2013). Although there are many technologies be innovated for remediating the HMs contaminated soil, the technologies are either non-cost-effective or low efficiency. Among those technologies, in-situ stabilization is known as the most user-friendly, cost-effective, and non-disruption method. Recent year, there are a lot of stabilizers have been found and their effect on the HMs mobility and bioavailability has been increasingly investigated in the laboratory and the contaminated site (Liang et al., 2017; Lu et al., 2017; Zhu et al., 2015; Nam et al., 2016; Rashid et al., 2022). However, the study about the changes in the physicochemical properties of contaminated soil with or without soil amendments is insufficient, especially the organic amendments. Further research is necessary for a better understanding of the mobilization and immobilization of HMs and the controlling processes, whereby find a better soil amendment for treating the HMs contaminated soil.

PE contains many functional groups involves carboxyl, phenolic hydroxyl and lactonic groups which can easily chelate and immobilize many metals. Therefore, it seems having a significant potential as an effective soil amendment. However, the study about the workability and efficiency of the plant extract for remediating HMs contaminated soil had not systematically investigated and recorded due to date. Thus, there are a huge research gate for this study and believe that result of this study is valuable for all researcher who try to find another alternative soil stabilizers with more cost-effective and efficiency.

1.3 Objectives of Research

This investigation focusses on the treatment of multi-HMs contaminated soil by using natural plant extract (PE) as an organic soil stabilizer. The mobility of HMs in soil is the main investigated parameter to determine the performance of PE. The aims of this study are listed as below:

1. To investigate the potentiality of PE become the soil stabilizer for HMs contaminated soil treatment through Tessier's sequential extraction.
2. To study the changes in the chemical properties of HMs contaminated soil after being amended with PE.
3. To evaluate the efficiency of PE with different dosages as the immobilizing agent to decrease the HMs mobility in soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Soil

Soil is defined as a natural body of inorganic matter that consists of mineral and organic matter derived from biomass, microorganisms, plants, and animals, which offer virous potential nutrients resources for living life. It serves as a shelter and habitat for countless living organisms, provides a physical support for root system and living medium for plant's growth. Soil also plays an essential role in the universal cycle of nutrient flow between the atmosphere, lithosphere, hydrosphere, and biosphere (Mirsal, 2004; Hakeem et al., 2014).

2.1.1 Soil pH

From a plant growth standpoint, soils pH is a vital chemical property that enormous affect the soil biogeochemical processes. It indicates the available concentration of hydrogen ion in soil solution. It had been known as the "master soil variable" because its effect on wide range of physicochemical, and biological properties and activities that affect plant's growth and biomass yield. Depending on soil pH, soils can be classified into neutral, acidic, and alkaline soil. Soil pH within 6.5 to 7.5 is consider neutral, over 7.5 is alkaline soil and less than 6.5 is deemed acidic soil. In general, soil with low pH will be toxic to microbes and flora and lead to decrease the inputs of organic matter to the soil and slowing decomposition and other microbial processes (Neina, 2019). For many decades, numerous researched revealed that soil pH is a main variable to control the solubility, mobility, and bioavailability of trace elements, as it controls the activity of metal hydroxide, carbonate, and phosphate solubility. For example, Cattlet et al. (2002) found that the activity of Zn^{2+} was limited in soil solution when the pH increased. They explained this tendency was due to organic matter adsorption and the development of franklinite. Besides, Bradl (2004) also observed that the Cd and Zn adsorption onto a sediment compound contents Al-, Fe- and Si-oxides decreased when the pH increase. Kabata-Pendias. (2000) had concluded that the solubility of most trace metal would decrease and leaded a lower concentration in soil solution with the increase of soil pH.

2.1.2 Soil Organic Matter (SOM)

SOM is the segment of the soil with different properties and structures that consists of plants, microbial substances, and animals in different phases of decomposition. Most of the agricultural soils had hold around 3% to 6% of SOM. With a stable SOM, it would bring abundant of benefits to that soil such as improved water holding capacity, improved the buffering capacity to resist pH changed and served as food resources for living organisms in soil (Baldock and Nelson, 2000). The main functional groups in SOM are electron-donating O- and N-containing groups, which undertake processes to create humic substances, a subtype of SOM made up of high-molecular-weight biopolymers that react strongly with organic and inorganic pollutants (Matocha, 2005). Numerous studies had observed that SOM played a very important role in trace metals solubility and mobility. For example, High-molecular-weight organic compounds have the potential bind and interact with trace metals from solution by the formation of stable metallic complexes thereby immobilize the trace metals (Schmitt et al., 2002; Karapanagiotis et al., 1991). Furthermore, the insoluble organic molecules were found that able to reduce the available Cu and Pb by the creation of insoluble complexes (Carrillo-González, 2006).

2.1.3 Total Organic Carbon (TOC)

TOC refers to a portion of organic compounds which only refers to its carbon component. The residues of animal and plant, root exudates, live and dead microbes, and soil biota, all are the source of TOC enter to the soil. It is the primary energy source for microorganisms in soil. The quality of soil is strongly correlated with TOC and the decrease of TOC will lead to negative impact the soil quality especially its physical resistance and resilience (Deluz et al., 2020). The organic carbon substances such as polysaccharides could help the binding of mineral particles and microaggregates. For example, Glomalin, one kind of organic matter which content 20% of organic carbon, can stimulate the combination of aggregates and enhanced the soil structure to improve the erosion resistance of soil. The organic acids such as oxalic acid, which are normally released from organic wastes and manures, avoid phosphorus fixation by clay minerals and improve its plant availability.

Therefore, increase TOC in soil can improve the biological diversity in the soil thereby enhance the biological control of plant diseases and pests (Rajan et al., 2010).

2.2 Heavy metals (HMs)

Over the past 20 years, the term "HM" had been widely applied in numerous journals and in regulations linked to chemical threats and the safe use of chemicals (Duffus, 2002). Because of the inconsistent used of the term "HMs" reflected inconsistency in the scientific literature, there was no specific definition of a HM (Duffus, 2002). Nowadays, the term HM is generally identified as a naturally existing element having a high density ($\rho > 4\pm1$ g/ cm^3) and high atomic weight ($w > 20$). It also had been usually used by referring to a group of metals and metalloids, and to their pollution that was often causing toxicity and environmental problems. There are about 45 metals that belong to HMs in the world, including Hg, As, Cr, Cu, Fe, Pb, Zn, Cd, and platinum-based elements. Among those HMs, Arsenic is a special condition because it is a non-metallic element, but its properties are similar to the HM and very toxic element, so it is classified as a HM (Bánfalvi, 2011).

2.2.1 Type of HMs

With the rapid growth of industrialization, HMs had become one of the important elements used in various productions such as electronic devices, paint, cosmetics, battery, fertilizer, pesticides and so on. However, due to improper disposal and storage of those HMs contaminated sludge and wastages, increasing studies found that the excessive concentration of HMs in many natural receptors such as air, river, lake and especially in soil. The most widespread HMs found in the contaminated soil are As, Cd, Zn, Hg and Cr, Pb, Cu (Masindi and Muedi, 2018). Table 2.1 summarizes the properties of the seven most common type of HMs and their application, arranging in descending order of world production.

Table 2.1: The properties, native form, and application of HMs

HM	Characteristic	Major Native Form	Application	World Production (t/year)	Reference
Cu	● **Density**: 8.96 gcm^{-3} ● **Atomic mass**: 63.55 ● **Heat of fusion**: 13.26 kJ/mol	Cuprite, Azurite, Malachite	Transport equipment, Industrial machinery, Electrical and electronics, Pesticides	20,000,000 (year: 2017)	(Raffa et al., 2021)
Cr	● **Density**: 11.4gcm^{-3} ● **Atomic mass**: 52 ● **Heat of fusion**: 21.0 kJ/mol	$FeCr_2O_4$, K_2CrO_4	Alloy, Tanning of leather, Chrome planting, Dyes and paints, Photography, Catalysts	15,000,000 (Year: 2017)	(Gregersen, 2020; Babula et al., 2009)
Zn	● **Density**: 7.14 gcm^{-3} ● **Atomic mass**: 65.38 ● **Heat of fusion**: 7.32 kJ/mol	Smithsonite, Heminorphite, Wurtzite	Rubber, Cosmetics, Batteries, Electrical equipment, Textiles, Inks	13,500,000 (Year: 2019)	(Raffa et al.,2021)

HM	● Characteristic	Major Native Form	Application	World Production (t/year)	Reference
Pb	● **Density**: 11.4gcm^{-3} ● **Atomic mass**: 207.2 ● **Heat of fusion**: 4.77kJ/mol	PbS, $PbSO_4$, $PbCO_3$	Paint Pigment, Battery, Gasoline additive, Building Construction	11,600,000 (Year: 2018)	(Wuana and Okieimen, 2011)
As	● **Density**: 5.72gcm^{-3} ● **Atomic mass**: 75 ● **Heat of fusion**: 24.44 kJ/mol	As_2O_3	Insecticides, Transistors, Laser material, Semiconductors	33,000 (Year: 2019)	(Jones, 2007)
Cd	● **Density**: 8.65gcm^{-3} ● **Atomic mass**: 112.4 ● **Heat of fusion**: 6.21 kJ/mol	Divalent Cd (II) ion	Pigment, Fertilizers, Detergent, Batteries, Machinery, Television phosphors	24,670 (Year: 2019)	(Campbell, 2006)
Hg	● **Density**: 13.6 gcm^{-3} ● **Atomic mass**: 200.6 ● **Heat of fusion**: 2.29 kJ/mol	Mercuric (Hg^{2+}), Mercurous ($Hg_2{}^{2+}$)	Thermometers, Blood pressure devices, Soap, Batteries, Dental preparations, Ultraviolet lamps	4000 (Year: 2019)	(Emsley et al., 2011)

2.2.2 The Sources of HMs

HMs origin as natural components of the earth's crust. They can be emitted into the environment from both anthropogenic and natural routes and subsequently end up in different environmental compartments including soil, water, and air. Several research have reported that the natural sources of HMs in the environment are generally less important compared with anthropogenic sources (Dixit et al, 2015; Xu et al., 2021; Zhang and Wang, 2020).

In nature, the key source of HMs is from their original parent materials. Total 95 % of the earth's crust is made up of ingenious rocks and the remain 5 % is made up of sedimentary rocks (Sarwar et al., 2017). Generally, basaltic ingenious rocks contain HMs such as cobalt, copper, cadmium, and nickel, while shales are rich in lead, copper, manganese, and zinc. The HMs contained in rocks could release to the soil as a result of natural processes including, volcano eruption, weathering, sea spray and hot spring.

For the anthropogenic sources, they could be classified into five main groups which were agricultural activities, metalliferous mining and smelting, industry processing, waste disposal and combustion of fossil and coal. Agricultural activities were the first major contributor to HMs contamination. A large number of fertilizers was regularly applied in farming systems to provide sufficient nutrients for crop growth. Those compounds included trace amounts of HMs as impurities that could lead to an increase in their content in the soil and indirectly cause soil pollution. Besides, pesticides are also large-scale used in horticulture and agriculture in the past contained substantial concentration of HMs. In recent years, there were approximately 10 % of the chemicals had been approved for application as fungicides and insecticides in UK (McLaughlin, 2000). On the other hand, mining and casting of metal ores were another principal source of HMs contamination. During mining, the tailings water which contained a high concentration of HMs were directly discharged into natural depressions caused the HM pollution to the onsite wetland and soil. Furthermore, an assortment of industries such as pharmaceutical facilities and tanning were also generating HMs during the production process (Sumner, 2000). The HMs would release to the environment because of the improper storage and disposal of the by-product and wastages. In the light of Zayed and Terry (2003), in India, a quantity that ranges from 2000 to 32000 tons of elementary Cd enters the environment annually due to the improper disposal and storage of contaminated sludge coming from the leatherworking industries. Last but not least, combustions of fossil fuels and coal are also the major anthropogenic sources of HMs. During the combustion, several HMs such as Cd, As, and Pb can volatilize and release into the air. Then, those released HMs subsequently end up to the soil and water body via wet and dry deposition (Tian et al., 2013).

2.3 Effect of HMs

Soil contaminated by heavy metals (HMs) contamination had received considerable attention of both public and government due to abundance, biomagnification, toxicity, and persistence of HMs prevailing in the environment and their subsequent accumulation in plants and crops which could lead to serious negative effects to the human health after consuming them.

2.3.1 Effects of HMs on Soil

Soil provides the primary resource for human beings living and serves as living channel for the growth of all kinds of floras. Soil is the most vital element of human production and the vector which can link the economic relationships together. Soil contains several microorganisms, bacteria, algae, and fungi actinomycetes, which can assist elements (C, N, P, S) cycle in nature. Those microbial substances are important for the decomposition of material elements and nutrient conversion (Chu, 2018). Almost all biochemical reactions are depended on the microbes in the soil.

However, many studies showed that the existence of HMs would affect those soil microbes, mostly included the effect of HMs on microorganic and enzyme activities in soil as well as the composition of soil microbial community (Abdu et al., 2017; Chu, 2018; Wang et al., 2007). The exist of HMs altered the soil pH, soil organic matter (SOM) and other chemical properties which could cause adverse influence on microbial activity in soil (Chu, 2018). With high concentrations of HMs pollution circumstances, the soil respiration would be inhibited, and the spatial active groups structure of the soil enzyme would be damaged. Wang et al. (2007) found that the microbial biomass of soil, enzyme activity, and community composition were decreasing significantly when near a copper smelter.

2.3.2 Effects of HMs on Plants

HMs can be uptake by plants through their root system. Some of these HMs such as Cu, Mo, Mn, Fe, Zn, and Ni are important ingredients needed for the normal plant’s metabolism and growth. However, these elements could effortlessly lead to poisoning if their concentrations were higher than permissible values (Garrido et al., 2005). Over the optimal concentration of HMs for the plant would cause chlorosis, inhibited the plant growth, decreased the plant yield, and even be attended by reducing the ability to nitrogen fixation in leguminous plants, decreasing the nutrient uptake and disordering the plant metabolism (Guala et al, 2010). According to Singh and Kalamdhad (2011), elevated Pb in soils even at very low concentrations might limit some essential plant processes, including water absorption, and mitosis photosynthesis with toxic symptoms of dark green leaves, short brown roots, yield depression and stunted foliage.

On the other hand, the reduction in several beneficial soil microorganisms and enzyme activities due to high metal concentration might cause a decrease in plant growth and subsequently results in plant death. Research showed that the 500 ppm of Cr (IV) in the soil would reduce germination up to 48 % in the bush bean Phaseolus vulgaris (Zeid, 2001).

2.3.3 Effects of HMs on Human

Vegetables and fruits are the main sources of energy for human living. However, the plant uptake of HMs from the soil at high concentrations might cause a serious health threat taking into consideration food-chain implications. One of the major food chain routes for human exposure is the application of those food crops polluted by the HMs. Some HMs such as Ca, Fe, Mg, and Zn had been reported to be bio-importance for human daily medicinal, while some other HMs such as As, Pb, Cd, had revealed to have not bio-importance for human living or even consumption could be toxic at minimal concentration (Duruibe et al, 2007). In 1972, a case of 5000-50000 fatalities occurred in Iraq, because of Hg contaminated wheat consumption (Takizawa, 2002).

HMs are non-biodegradable and cannot be broken down. Once HMs are swallowed or inhaled into our body, they will bio-accumulate in our system lead to biological and physiological complications (Briffa et al, 2020). HMs toxicities are caused by the formation of free radicals such as OH^-, O_2^- and H_2O_2, which causes DNA damage, lipid peroxidation and alteration of sulphydryl homeostasis (Eaton and Qian, 2002). The toxic effects of HMs in humans' body are highly relied on the types of metals and their concentration, emission rate and exposure duration. Table 2.2 had summarized the case study of harmful impacts of different HMs on humans' body.

Table 2.2 Summarized table of negative effect on human health

HMs	Negative Effect		Reference
	Acute Effect	Chronic Effect	
As	Diarrhea, death, vomiting, headaches	Skin lesions, bladder cancer, lung cancer, fetal loss, steatosis	Kapai et al., 2006
Cr	Hemorrhage, vomiting, diarrhea, blood loss in GI Tract	Skin irritation, kidney and liver damage, circulatory and nerve tissue damage, gastrointestinal carcinomas	Zhitkovich, 2011
Cd	Testicular injury, hepatic, pulmonary	Renal disfunction, lung damage, renal cancer bone defects	Dokmeci et al., 2009

Cu	Diarrhea, nausea, respiratory difficulties	Diabetes, Cardiovascular disease, Wilson's disease, cystic fibrosis	Uriu-Adams and Keen, 2005; Angelova et al., 2011
Hg	Loss of IQ, decrease in memory, impaired neurodevelopment	Brain damage, central nervous system damage, congenital malformation, abortion, development changes in young children	Roos and Dencker, 2012; Geier and Geier. 2007
Pb	Short attention span, vomiting, mild fatigue, distractibility	Affect the synthesis of haemoglobin, kidneys and liver damage, reproductive system damage, memory loss	Rogan and Ware, 2003
Zn	Diarrhea, respiratory disorder if inhale Zinc smoke, vomiting	Lethargy, local neuronal deficits, and prostate cancer	Plum et al., 2010

2.4 Global Scenario of Soil Pollution

In 38 European countries, there were more than 2.5 million sites potentially polluted, and 342,000 sites had been identified as contaminated sites caused by the wide fertilizer used and large volumes of municipal and industrial wastes (Bakshi et al., 2018). In European Union, Tòth et al. (2016) observed that the concentration of measured heavy metals (HMs) such as Cu, Cd, As, Cr, Hg, Ni, Pb, Zn, Co, and Sb in both of land area and agricultural soils were above the threshold limits that set by Finland government. France, Italy and Spain were indicated that the most polluted regions where the determined values of As overed the threshold values in the ranging of 10 % to 90 %. However, the concentration of Zn, Hg and Cu was not higher than 10 % of admissible value and most of regions were lower than the threshold limits about 2 %. Among the measured HMs, Ni was the most significant found in the soil, particularly in Greece and Italy, the concentration of Ni exceeded 90% of the guideline values.

In China, Yuan et al. (2011) reported that surface soil within 500m of the farmland around the soil mine area in Suzhou were polluted by Cr, Cd, Hg and As. The concentration of those elements had exceeded the national soil environmental quality standard (level 1). Besides, in light of the National Soil Pollution Investigation Bulletin 2014 of China, the results indicated that 16.1 % of arable soils had polluted by HMs with the concentrations exceeding the standard value of As, Cu, Cd, Pb, Hg, Cr, and Zn were 2.7 %, 2.1 %, 7.0 %, 0.9 %, 1.6 %, 1.1 %, and 1.5 % respectively (Li et al., 2014; Li et al., 2019).

In southeastern Missouri (USA), Ikem et al (2008) had defined that the background levels of HMs increased by 2-31%, majorly were caused by sewage sludge and the use of chemical fertilizers. In South Florida (USA), the concentration of Cu in agricultural soils had found greater than background levels (20 to 30 mg/kg) in soils about 50-75 times, which reached to 1500 mg/kg (Bakshi et al., 2018).

In Malaysia, Zarcinas et al. (2004) had found that the topsoil around Peninsular was contaminated by Cd, Co, Hg, Cr, Pb, Cu, Zn, and Ni due to the metal released from wastes, fertilizers, pesticides, and atmospheric sources. The concentration of Hg, Zn, Cd, Cu and Cr in the harvested crops was exceed the Maximum Permitted Concentration (MPC) that legislated in Malaysian Food Act (1983) and Food Regulation (1985).

Tavakoly Sany et al. (2013) also found that the concentration of Cd, As, and Pb at the coastal sediments of Port Klang was comparatively higher than background values.

Table 2.3: Regulatory standards of HMs in agricultural soils (mg/kg) (He et al., 2015; Chen et al.,2018; IDN et al., 2017; Chiroma et al., 2014; Maleki et al., 2014)

Country	As	Cd	Cr	Cu	Hg	Pb	Zn
Australia	20	3	50	100	1	300	200
Canada	20	3	250	150	0.8	200	500
China	20-40	0.3-0.6	150-300	50-200	0.3-1.0	80	200-300
Germany	50	5	500	200	5	1000	600
Japan	15	1.5	250(Cr VI)	N/A	15	150	N/A
Netherlands	76	13	180	190	36	530	720
New Zealand	17	3	290	$>10^4$	200	160	N/A
Norway	2	3	25	100	1	60	100
Tanzania	1	1	100	200	2	200	150
UK	43	1.8	N/A	N/A	26	N/A	N/A
USA	0.11	0.48	11	270	1	200	1100
WHO standard	20	3	100	100	1	100	300

All data were displayed in mg/kg

To control the release of the HMs to the soil, each country's government had set up the regulator standard of HMs and enforced with legislation to maintain the quality of the soils (Mohamed et al., 2009). The HMs concentration limits for the soils were different from country to country because there was no international legislations and regulations

(Raffa et al., 2021), as shown in Table 2.3. Unfortunately, not such of documents related to soil quality guidelines were available in Malaysia to protect the human, aquatic, and environmental health despite numerous existing HMs contamination (Rajoo et al., 2016). Most of research was compared to the standard permissible limit in soil that established by other country. For example, a study of HM-contaminated soil analysis in Perlis was conducted by Ripin et al. (2014). The result showed that the maximum Cu and Cd concentration were significantly exceed the limit value from Netherlands and China. Diami et al. (2016) also compared to guideline value that recommended by Canadian Council of Ministers of the Environment and Australian Department of Environment and Conservation. The result indicated that the concentration of Pb, Co, Cu in investigated site that closed to iron ore mining sites in Pahang.

The World Health Organization (WHO) had provided a standard for maximum HMs permissible limit in soil to raise the public concerns on this serious issue. Compared with other countries' standard, WHO standard seemed as an average data among other countries since the provided values was ranging within the highest and lowest values for each type of HMs as listed in Table 2.3. Therefore, WHO standard was considered a good reference for this study.

2.5 Physical Remediation Techniques

Physical remediation is a means which aim to separate the contaminants from the soil solid and reversal or stop the destruction to the soil by using physical technologies such as thermal treatment and soil replacement (Yao et al., 2012). Soil replacement is the process of mixing polluted soil with a substantial amount of unpolluted soil. The involving technologies were landfilling, surface capping, and encapsulation (Yao et al., 2012). The thermal treatment is achieved by heating the contaminated soil to make the contaminants volatile based on the contaminant’s volatility. Then, the volatile heavy metals (HMs) are collected and removed from the contaminated site (Li et al., 2010). This chapter will focus on discussing the surface capping, landfilling and thermal desorption methods which are the popular physical treatment for contaminated soil nowadays.

2.5.1 Surface Capping

Surface capping is known as an in-situ remediation technique that provide an impermeable barrier to surface water infiltration to polluted soil to prevent the further release of contaminants to the adjacent water body. This technique is not really removed or reduced the HMs from soil, but also efficiently eliminates risks associated with dermal contact and incidental ingestion of soil surface (Liu et al., 2018). The selection of an appropriate capping system may depend on characteristics, remedial objectives, and risk factors of the site. The design of the capping can be single-layer caps or multilayer systems as shown in Figure 2.1. Multilayered capping system may construct a hard cover, a layer of topsoil, and followed by revegetation. The hard cover and topsoil are used to divide ground surface to the underlying layers, whereas revegetation is used to strengthen the topsoil thereby reducing the risk of soil erosion and runoff. This technique is only applicable on a small area (<2000m^2) and it may poses the sliding risk (Ohio, 2000).

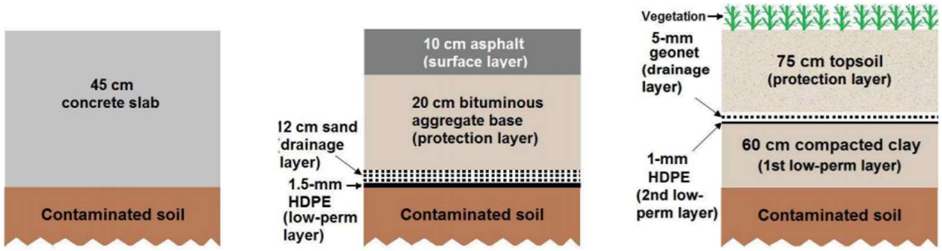


Figure 2.1: Examples of various surface capping systems (Ohio, 2000)

2.5.2 Landfilling

Landfilling is the easiest and traditional ex-situ soil remediation technique. This technique is achieved by removing the polluted soil from its original site to a secure landfill for disposal (Kuppusamy, 2016). The secure landfill is a carefully engineered site where to receive the dumped wastes. Generally, the secure landfill consists of three critical layers including, a top cover or cap, leachate collection system, and a bottom liner (Kuppusamy, 2016). In order to reduce rainfall penetration and minimize surface runoff from the landfill, capping or covering is designed to prevent leachate generation. Leachate collection system helps to collect leachate and to prevent leachate accumulate inside the landfill site. The collected leachate will be transported and treated by a waste treatment plant. The bottom liners are made used to avoid the accumulated waste from entering the groundwater and soil. Apart from secure landfill, there is an engineered and upgraded landfill known as the bioreactor landfill which serves as another option to treat polluted soil. The bioreactor landfill is designed for transformation and stabilization of toxins through microbial procedures within the first decade of bioreactor operation (Kuppusamy, 2016). However, the cost for operating a sanitary landfill is very high and the landfill operators are also will be charged license fees and required financial assurances. Landfill also may introduce hazards to humans due to the transportation and excavation activities.

A case study in Klang, Malaysia had indicated that the estimated cost for managing a landfilling was 1,705 million MYR/year for waste collection and transportation, 1,467 million MYR/year for leachate treatment, 593,928.57 MYR/year for land used and 8,382 million MYR/year for landfill tipping fees (Uding Rangga et al., 2019).

2.5.3 Thermal Desorption

Thermal desorption refers to a procedure of heating a medium under a monitored temperature to remove the volatile contaminants from the contaminated soil such as Hg and As. After that, those volatile contaminants can be collected using the vacuum negative pressure and further disposed (Li et al, 2010). This method can be conducted in either in-situ or ex-situ (Zhao, 2019). The major techniques used to heat the soil are steam-base heating, radio-frequency heating, electrical resistive heating, and conductive heating. Based on different temperature, this method can be classified into two categories which are high temperature desorption (320 to 560 °C) and low temperature thermal desorption (90 to 320 °C), depending on the contaminants boiling point (Dhaliwal et al., 2020). For instance, this method is commonly applied to treat the mercury-polluted soil because of its low boils point (357°C). Thermal desorption has the advantages of simple process, devices with mobility, produced minimal secondary contamination and required less energy compared with other processed (Chang and Yen, 2006; Yao et al., 2012). However, this method requires high capital cost, long desorption time and limited application in the soil remediation. According to the experiment result of Kunkel et al. (2006), the optimal temperature for mercury removal was 244 to 259 °C which its efficiency could reach 99.8 % within a very short period by using situ thermal desorption (ISTD).

2.6 Biological Remediation Techniques

Biological remediation is a method applied for the recovery of heavy metals (HMs) polluted soil in certain concentration of HMs. This method is removing or destroying or immobilizing the hazardous pollutants by exploiting biological mechanisms inherent in microorganisms and plants (Ayangbenro and Babalola, 2017). By using biological remediation, the HMs are not actually degraded by plants and microorganisms instead they accumulated and stabilized into less harmful volatile compounds (Raffa et al., 2021). Biological remediation is relative cost-effective and eco-friendly method for HMs removal when compared to the traditional physical and chemical techniques. A case study indicated that the total expenditure for treating one acre of lead-polluted soil by bioremediation could save 50 to 65% as compared with others conventional methods such as landfill (Blaylock, 1997). Biological remediation of HMs can be divided into two groups, which is microbial remediation and phytoremediation (Li et al., 2019). Sometimes this two remediation may combine to boost the performance and minimize the remediation time. For example, Mycorrhizal fungi had been found that it could affect the transformation of trace metals in the rhizophere which could help in phytostabiliztion (Mathur et al., 2017).

2.6.1 Microbial Remediation

Microbial remediation exploits the biological mechanisms of specific microorganisms to oxidize, reduce, immobilize, and metabolize the HMs. Some special microorganisms can also boost the decomposition of contaminants and enhance the characteristic of soil, such as fertility (Cui et al., 2017). The mechanisms perform in microbial remediation, including extracellular precipitation, complexation, intracellular accumulation, and oxidation-reduction reaction (Raffa et al., 2021). The primary microbial processes including biosorption and bioleaching (Zabochnicka-S´wiatek and Krzywonos, 2014).

Biosorption can be defined as a physicochemical process that can immobilize the HMs onto the microorganism's cellular structure. The HMs immobilization can be achieved via the extracellular binding created between the cell surface (anions) and metal ions (cations). The cell walls of microbial cells play a vital role in the HMs removal because of the existence of several active chemical functional groups with different charge and geometry for example sulfate, carboxyl, phosphate groups, and amino, which promote the binding mechanisms (Raffa et al., 2021). The mechanisms of biosorption are complex and they cover three important mechanisms includes ion exchange, physical adsorption, complexation, and (BABÁK et al., 2012; Ahalya et al., 2004). The efficiency is affected by numerous factors, including density of sorption centers, process conditions, types of metal ion precipitation, and immobilization agents. The common bioadsorbents used in soil treatments are bacteria, fungi, and algae. The bacteria cell wall has many functional groups that make HMs ions bond and accumulate on its polysaccharide slime layers (Jaafer et al., 2016; Cui et al., 2017). Fungi can adsorb the HMs via ion exchange and coordination in the glucuronic acid, chitin-chitosan, phosphate, and polysaccharides exist in their cells (Bano et al., 2018; Hassan et al., 2020; Iram et al., 2015). Algae can form peptides as a defense mechanism and its cell wall exist a lot of functional groups such as carboxyl, amino, sulfhydryl and sulfonate groups that can promotes the adsorption of metal ions (Raffa et al., 2021).

Bioleaching is achieved by using microorganisms such as Acidithiobacillus, Acetobacter, Arthrobactor, Pseudomonas and Acidophilum (Mulligan and Galvez-cloutier, 2003) to remove HMs from soil. In the bioleaching process, the mobility of the pollutants is reduced by utilizing the microorganisms' capacity to generate secretions such as organic acids with low molecular weight to dissolve HMs. This way promotes the easier solubilization of the HMs by the microorganisms' metabolism (Raffa et al., 2021).

Biosurfactant is an alternative bioleaching agent such as polysaccharides, lipopeptides, and lipids that are generated by microorganisms. Their high surface activity can allow the chelating metals formation and bounding of metal ions (Yang et al., 2018). A research of Deng et al. (2012) had reported that the indigenous Penicillium chrysogenum strain F1could remove 50%, 9%, 35%, and 40% of Cd, Pd, Cu, and Zn in one-step process respectively through bioleaching process.

Generally, microbial remediation is an economic and environmental-friendly method when compare with other physical and chemical remediation. However, this technique has some disadvantages such as some microbial organisms fail to bind harmful metals into stable and harmless metabolites, unstable efficiency, and time consuming (Raffa et al., 2021).

2.6.2 Phytoremediation

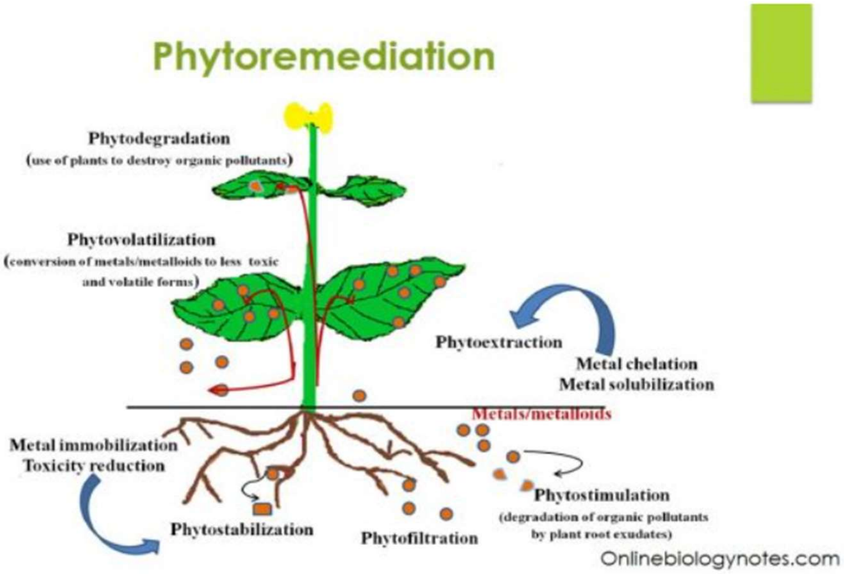


Figure 2.2: Schematic diagram of the phytoremediation mechanisms (Karki, 2020)

Phytoremediation refers a method that employs varieties of plants and associated soil microbes to eliminate, transfer, maintain, extract contaminants in the soils. The phytoremediation process target to isolate pollutants through the plant root to reduce the intake of those harmful substances or absorb into shoots and roots (Raffa et al., 2021). The species of plant used in this method should have the properties of fast-grow ability, heavy biomass, profuse root system, high metal-accumulation capability, and high tolerance ability (Li, 2019). Nowadays, there are many plants were found that have the high an ability of accumulate and tolerate the HMs, scientists call them "hyperaccumulators". According to Nwaichi and Dhankher (2016), hyperaccumlators were the plants that accumulate higher than 0.1% i.e., more than 1000mg/g of Cu, Cd, Cr, Pb, nickel cobalt or 1% (>10,000 mg/g) of Zn or Mg in the dry matter and their cropping can repeat until desired result is obtained. Depending on the plant types, contaminants, and elimination mechanisms, this method can be classified into phytoextraction, phytostabilization, phytovolatilization, phytofiltration, phytodegradation and rhizodegradation, as displayed in Figure 2.2. Among these mechanisms, rhizodegradation and phytodegradation are utilized to breakdown the organic contaminants. Therefore, the following subchapter will focus on phytostabilization, phytoextraction and phytovolatilization that are used for the elimination of inorganic matter from polluted soil (Yan et al., 2020).

Phytostabilization is one of the phytoremediation processes that used to immobilize HMs and reduce their bioavailability, at the same time avoiding their movement into the environment and minimizing the possibility of HMs penetrate the human food chain (Bolan et al., 2011). This process can be achieved via the reduction or precipitation, absorption, sequestration, adsorption of HMs within rhizosphere, root tissues, and root cell walls. To improve the efficiency, some recommended soil amendments include phosphates, organic matter, biosolids, and alkalizing agents can be added to the polluted soil during phytostabilzation treatment. With the soil amendment, the redox status and pH value of soil will be changed, thereby alter the metal speciation, and decrease the HM bioavailability and solubility. These soil amendments also help in increasing the essential nutrients as well as organic matter content of the soil. This can improve the physicochemical and biological characteristics of the soil and further advantage the colonization of plant and enhance water holding capacity (Yan et al., 2020). Besides, the microorganism habiting in the rhizophere such as mycorrhiza can aid phytostabilzation by improving the immobilization via adsorption of metals onto their cell walls, forming chelators and enhancing the precipitation procedures. They also can serve as a filtration barrier to prevent translocation of HMs ion from roots to shoots and improve plant root surface and depth to assist phtostabilization. The advantages of phytostabilization are that soil removal is not required, disposal of secondary pollutants or biomoass is unnecessary, the operation cost is less than other soil remedial technologies and ecosystem restoration is improved by the vegetation. However, the plants used for phytostabilization is required long-term maintenance or verification that the vegetation will be self-sustaining to prevent contaminants from future re-releasing and leaching into the ecosystem.

Phytoextraction is defined as a bio-absorption method of HMs from soil though the plant roots. The contaminants are then translocated and stored in their sprouts. The mechanisms of phytoextraction are complex, mainly involves a several procedures such as the HMs mobilization in rhizosphere, HMs uptake by plant roots, HM ions transportation from roots to aboveground parts of plant, sequestration, and compartmentation of HM ions in plant tissues (Yan et al., 2020). The two key factors that used to determine a potential plant species for phtoextration are the shoot metal concentration and shoot biomass. Hyperaccumulators, refers to the plant which have high ability of metal accumulation and high tolerance to contaminants, is the best selection for phytoextraction (Ali et al., 2013).

Besides, phytoextraction of HMs can also be classified into two ways, which known as natural and induced. Natural phytoextraction refers to the plants that applied for HMs removal under natural conditions without any addition of soil amendment. Conversely, induced phytoextraction is practiced by adding different chelating agents such as ethylenediaminetetraacetic acid (EDTA), ammonium sulfate, citric acid, and element sulfur to improve the HMs bioavailability in the soil for plants consumption (Ali et al., 2013).

Phytovolatilization is the different form of phytoextraction. In the phytovolatilization process, the pollutant is not predominantly concentrated in aboveground parts, but instead converted by the plant into volatile and less harmful status before emitting into the surrounding atmosphere (Chatterjee et al., 2013). According to Sakakibara et al. (2016), the maximum removal ratio of *P. vittata* could reach to 90 % of the total uptake of As from As-contaminated soils. In nature, the number of the plants which can conduct phytovolatilization is very less. To solve this problem, some plants are genetically modified such as transgenic tobacco plants that are modified to acquire the ability of mercury removal (Raffa et al., 2021). This technique provides the advantages of low-cost operation because it removes contaminants from the site without the requirement of disposal or harvest of polluted plants. However, this technique exists a unignorable issues which is the volatile elements released into the surrounding atmosphere can be hazardous form and cause secondary pollutants nearby the plants.

2.7 Chemical Remediation Techniques

Chemical remediation is a type of traditional technique that chemical reagent, reaction and principles are exploited to decrease the mobility, bioavailability and bioaccessibility of HMs and remove the HMs from the polluted soil (Liao and Li, 2011). The main chemical remediation technologies include soil washing, soil flushing, electrokinetics and stabilization (Li, 2019).

2.7.1 Soil flushing

Soil flushing is a technology applied in-situ to remove the pollutants from the soil by using an appropriate flushing solution to flood a zone of contaminated site. To achieve this process, an chemical solution is injected into polluted zone through vertical well. The contaminated zone may be within the vadose zone, saturated zone or both. After that, the solution will flow through the contaminated zone and the contaminants are then mobilized, gathered, and carried to the surface for disposal, or treated directly on-site and re-injection (Strbak, 2000). The aqueous solution mostly contains surfactants, cosolvents, or treated groundwater. Surfactants are the typical chemical compounds largely used in detergents and food products that change the properties of solution interfaces. Normally, surfactants consist of a strongly hydrophilic group and a strongly hydrophobic group, meaning that one end of the molecule is attracted to oil and the other to water. The addition of surfactant can improve the mobility and solubility of the contaminant through micellar solubilization (Strbak, 2000). Cosolvents are the chemicals that dissolve in both water and Non-Aqueous Phase Liquid (NAPL). The function of cosolvents is similar to surfactants, they can improve the solubility of many organic contaminants the cosolvent effect. The advantages of this technology are low operation cost and applicable to a wide range of contaminants. However, this technology also has some limitations such as long remediation time, uncertain performance and have the potential for spreading contaminants (Mulligan et al., 2001).

2.7.2 Soil washing

Soil washing is similar with soil flushing. It is an ex-situ method to eliminate contaminants from the soil by applying aqueous solutions. Soil washing can be conducted in physical separation and/or chemical extraction (Gusiatin et al., 2020). This technique also involves homogenization, the primary process of the separation of coarse particles by differences in density. During the physical separation, fine clay and slit particles are separated from the gravel and coarse soil particles. In this way, the pollutants are concentrated into a reduced volume of soil which can undergo further treatment such as incineration or bioremediation. In the chemically extraction, the selective pollutants are dissolved and follow with the chemical transformation or recover. The chemical reagents and additives added into washing solution may be varied according to the properties of the contamination to be treated. When the soil is polluted by various HMs with different characteristics, this technique should be conducted sequentially with different washing solutions. Soil washing is considered an economical method due to the diminishing volume of the contaminants that would require secondary treatment. Nevertheless, this technique will cause the deterioration of soil structure and soil composition such as nutrients will be removed during the treatment process (Gusiatin et al., 2020).

2.7.3 Electrokinetic Remediation (ER)

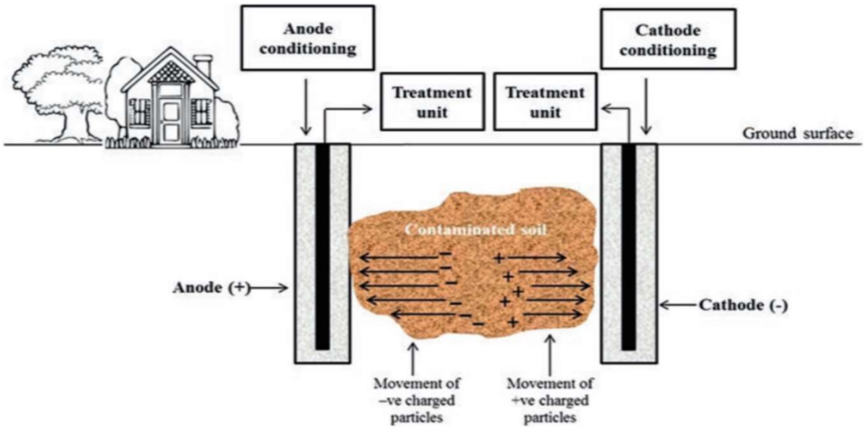


Figure 2.3: Schematic diagram for electrokinetics process (Raffa et al., 2021)

Electrokinetic Remediation (ER) is an in-situ innovative technique that used to promote the migration of HM to oppositely charged eletrodes (anode and cathode) under a direct-current electric field. The principle of ER depends on application of a low-intensity direct current at both sides of soil and further producing electric field gradient, as shown in Figure 2.3. Then, those metals will gather at the electrodes and subsequently be treated or removed with physical and/or chemical methods such as precipitation and sorption with ion-exchange resins. It includes the transportation of charged chemical particles through the substrate (electromigration), the motion of a liquid containing ions (electroosmosis), the mobility of charged species (electrophoresis) and the chemical reactions related to an electric current (electrolysis) (Raffa et al., 2021).

ER is appropriate for soil with low permeability and has the benefits of low operation cost, simple installation and operation as well as not need to remove or damage the nature ecosystem (Yao et al., 2012). However, the efficiency of ER is always low due to poor control over the pH value in soil. To enhance the efficiency of ER, some researchers are investigating the additive or chelating agents which can help to improve the mobility of HMs. Besides, Song et al. (2016) had investigated the effect of various chelating agents (ethylenediaminetetraacetic acid (EDTA), ethylenediamine disuccinic acid (EDDS), citric acid (CA)) and nitrilotriacetic acid (NTA) on the electrokinetic remediation of Cu, Pb, As, Cd, Cr, Ni, and Zn polluted dredged marine sediment. The result showed that the most suitable agent was the EDTA to improve removal of Pb (60.1%), Ni (52.8%), and Zn (34.9%), while EDDS showed the high efficiency in increasing the removal of Cu (52%) and CA was more applicable to improve Cd removal (40.2%). Furthermore, some researchers also combined the flushing and ER together either in series or integration to solve the inadequacy of flushing technology in fine soil. Li et al. (2016) had found that the Cr (VI) removal efficiency of Electrochemistry-flushing (E-flushing) technology was almost more than double of flushing technology. The result revealed that the efficiency of E-flushing had reached the maximum of removal efficiency determined by desorption kinetics. Ng et al. (2014) also reported that a two-stage electrokinetic washing process could improve the Pb removal efficiency by 4.98% ~

20.45% and 2.59 ~ 9.08% in comparison to a normal washing process and electrokinetic process.

2.7.4 Stabilization

Stabilization is a soil remediation process to reduce the mobility of pollutants by adding immobilizing agents to improve adsorption, complex binding, as well as precipitation. The aim of this technique is to decrease the mobility and solubility of the contaminants whereby minimizing their potential transport to plants, water, and other environment media instead of directly removing the contaminants form soils (Liu et al., 2018). Stabilization can be conducted in both in-situ and ex-situ ways. The typical used immobilizing agents can be classified into two groups which is natural organic materials and non-organic materials. The organic materials were included biochar, bio-solid compost, and humic substances, while the inorganic materials were included liming materials, phosphate compounds and industrial waste by-product (Wang et al., 2020). Each immobilizing agents had their own specific characteristics and dominant remediation mechanisms such as precipitation, complexation, ion-exchange, and adsorption.

2.8 Comparison Between Various Soil Remediation Techniques

By reviewing numerous of remediation methods, none of them were prefect, each treatments had their respective strengths and limitations. A summarized table of each remediation techniques in term of advantages and disadvantages were listed in Table 2.4 (Kuppusamy, 2016; Liu et al., 2018; Yao et al., 2012; Chang and Yen, 2006; Raffa et al., 2021; Li, 2019; Gusiatin et al., 2020). It is obviously to observe that the current ordinary remediation techniques are normally facing the problems of high cost, low efficiency, long treatment duration, loss of land and potential secondary pollution to the environment. Thus, further research on development of alternative methods with more cost-effective is needed to overcome the current limitations of those remediation techniques.

Table 2.4: Summary of advantages and disadvantages for each remediation techniques

Remediation technique	**Applicability**	**Advantages**	**Disadvantages**
Physical Remediation			
Surface capping	In-situ, high contamination sites	Easy to install, low in cost, high security	Limited to small areas and certain geographic locations, loss of land
Landfilling	Ex-situ, high contaminations sites	Immediate clean up, high security	High cost, requiring additional land for waste storage
Encapsulation	In-situ, high contamination sites	High security, fast install	Limited to small, shallow contamination areas, high cost, loss of land cropping function
Thermal Desorption	In-situ/ Ex-situ, volatile contaminants (e.g Hg, As)	High efficiency of the mercury removal, short period, long-	High capital cost, long desorption time, soil remediation in limited, potential gas emission

		term effectiveness	
Bioremediation			
Phytoremediation	In-situ, low to moderate contamination	Low-cost, easy to implement, suitable for large and low contamination areas	Limited to shallow contamination, time-consuming, low efficiency
Microbial Remediation	In-situ, low moderate contamination	Low-cost, eco-friendly	Unstable efficiency, time consuming, merely supplemental to remediation techniques
Chemical Remediation			
Soil Flushing	In-situ, moderate to high contamination	Contaminant removal, minimal soil disturbance, low cost	Potential groundwater pollution
Soil Washing	Ex-situ, moderate to high contamination	High efficiency, fast effects	Extreme soil disturbance
Electrokinetic	In-situ, moderate to high contamination	Contaminant removal, minimal soil disturbance, low energy	Time consuming, low efficiency, best for fine-textural soils with low permeability

		consumption, short duration	
Stabilization	In-situ, high contamination	Low-cost, easy to use, immediate effects	Temporary effectiveness, contaminants remaining in soil

2.9 Case Study on Organic Soil Stabilizers

In recent years, the development of organic soil stabilizer for soil remediation had become a hotspot due to its high cost-effectiveness, eco and user friendly and short treatment duration. Increasing research related to organic soil stabilizer had released and supported the applicability and versatility of the organic soil stabilizer to heavy metal (HMs) contaminated soil over few decades. Most of the studied organic soil stabilizers were derived from farmyard manure, poultry manure, straw, and husk of plant (Bolan et al., 2003a; Bolan et al., 2003b; Ok et al., 2011; Liu et al., 2009; Liang et al., 2017). The researchers intended to link our living wastes to the soil treatment to develop a win-win solution for both waste disposal and soil remediation. The following subchapters further discussed the finding related to organic soil stabilizers in detail.

2.9.1 Organic Soil Stabilizers

Organic wastes such as household waste, sewage sludge and manure compost could be utilized as a green soil stabilizer and in a way to slowly released nutrient sources as well as could be used to lower metal availability at the same time. The following Table 2.5 listed several case studies on organic stabilizers used in HMs contaminated soil treatment.

Table 2.5: Case study of organic soil stabilizer

Organic Stabilizers	**Soil Sample Source**	**Targeted Metals**	**Dosage**	**Incubation period**	**Observation**	**Reference**
Biosolid, farmyard manure, pig manure, poultry manure and spent mushroom	Mineral soil collected from Palmerston North, New Zealand	Cu	0, 2.5, 5 and 10 % w/w	4 weeks for incubation experiment, 12 weeks for plant growth (*Brassica juncea L*)	Exchangeable fraction decreased and increased the organic bound fraction; Increased dry matter yield because nutrients increased; Significantly reduced the phytotoxicity of Cu. The adsorption and complexation of Cu increased with the addition of manure compost.	Bolan et al., 2003a
Biosolid, farmyard manure, fish manure, horse manure, pig manure, poultry manure and spent mushroom	Mineral soil collected from North, New Zealand	Cr (IV)	0, 2.5, 5, 10 % w/w	4 weeks for incubation experiment, 12 weeks for plant growth (*Brassica juncea L*)	Enhance the reduction of Cr (IV) to Cr (III), thereby decreased bioavailability; Soil pH decreased with the addition of compost; Nutrients increased; Decreased the concentration of Cr in soluble fraction and increased the organic bound fraction in soil.	Bolan et al., 2003b

Organic Stabilizers	**Soil Sample Source**	**Targeted Metals**	**Dosage**	**Incubation period**	**Observation**	**Reference**
Humus compost, zerovalent iron (ZVI), lime, combination of these materials	Rice paddy soil close to an abandoned mine, Chung-buk, Korea	Cd	10 % (w/v)	1 month greenhouse experiment (*Orzya sativa L*)	(ZVI + humus) showed the highest efficiency on decreasing bioavailability; Exchangeable or adsorbed forms decreased, while the non-bioavailable fraction increased after the application of stabilizers	Ok et al., 2011
Maize straw derived- biochar & compost	Artificial contaminated soil collected from University of Agriculture Peshawar	Pb, Cd, Cr	0.5, 1, 2, 4 % (w/w)	60 days pot experiment (*Zea mays L.)*	Soil pH and organic matter increased significantly for both biochar and compost; reduced AB-DTPA extractable concentration of Pb, Cd, and Cr; decreased bioavailable Pb, Cd, and Cr to plant.	Irfan et al., 2021
Poultry manure compost	Topsoil from Huazhong Agricultural University, Hubei Province, China	Cd	0, 30, 60, 120 mg/kg soil	4 months pot experiment (*Triticum aestivum L.*)	Soil pH, OM and total P contents increased significantly; Decreased the exchangeable of Cd by 71.8 to 95.7 % and increased the organic fraction and inorganic precipitates Cd by 0.4 to 18.4 times; reduced phytotoxicity of Cd in wheat stems and seeds.	Liu et al., 2009

According to the review of the case study, the organic stabilizer had a good performance in decreasing the bioavailability and mobility of HMs in soil, while enhanced the soil properties. For example, the research conducted by Bolan et al. (2003a) to investigate the effect of organic manure on adsorption, complexation, and phytoavailability of Cu contaminated soil. Five different manure composts including, biosolid, farmyard manure, spent mushroom, pig manure and poultry manure, were used to treat the Cu contaminated soil collected from New Zealand at the doge of 0-100 % w/w. Incubation experiment for 1 month and plant growth experiment for 12 weeks were conducted in the author's study. The result showed that the exchangeable Cu fraction decreased with the addition of all manure composts but increased the Cu organic bound fraction. The yield of the plant increased remarkedly and the phytotoxicity of the plant decreased. The adsorption and complexation of Cu in manure amended soil increased. In the same year, Bolan et al. (2003b) also studies the effects of biosolid compost, farmyard manure, fish manure, horse manure, spent mushroom, pig manure and poultry manure on the reduction and phytoavailability of Cr in mineral soil collected from New Zeealand. Same experiment and dosage of organic compost with the author's pervious study. The author observed that the reduction of Cr (IV) to Cr (III) had a significant increase after the soil amended with those compost, thereby decreased the bioavailability of Cr in the soil. The added composts tended to acidify the soil and increased the nutrients for plant growth. Besides, the soluble or exchangeable fraction of Cr in soil decreased with the addition of composts and leaded to improve Cr bound to organic fraction.

Another study conducted by Ok et al. (2011) was focusing on the effect of combination of biosolid and inorganic stabilizers. The investigated site was located at rice paddy soil in Korea and the organic stabilizers used in experiment included compost, humus, ZVI and lime. Through the greenhouse experiment, the result indicated that the uptake rate for the plant was decrease followed the sequence: ZVI + humus (31 %) > lime (35 %) > ZVI + compost (39 %) > compost (54 %) > ZVI (58 %) > humus (86 %). The sequential extraction results also showed that the exchangeable fraction of Cd decreased and non-bioavailable fraction such as organic, carbonate and residual fraction increased after the application of soil stabilizers. Compost had the most efficiency to induce Cd immobilize and followed by ZVI and lime.

Besides, Irfan et al. (2021) was focusing on the application of Maize straw derived- biochar and compost on artificially contaminated soil with 20 mg/kg Pb 10 mg/kg Cd and 20 mg/kg Cr. The dosage of biochar and compost treatment was 0.5%, 1%, 2 %, and 4 % (w/w) applied separately to the contaminated soil. The soil was then planted with *Zea mays L.* and the crop was harvested after 60 days. The results showed that 4% of biochar had the most effective in reducing the Ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable Pb, Cd and Cr, which decreased by 79 %, 61 % and 78 % respectively. The highest reduction in shoot uptake for Pb, Cd and Cr was also observed in 4% biochar, which reduced by 71&, 63 % and 78 % respectively. The significant enhancement in soil properties such as soil pH and soil organic matter were observed after the application of both biochar and compost.

In addition, Liu et al. (2009) conducted an experiment to assess the effect of compost on Cd immobilization and biotoxicity of Cd in winter wheat (*Triticum aestivum L.*). The compost was derived from poultry and chaff and the soil was collected from the university farm located at Huazhong Agricultural University, Hubei Province, China. Pot experiment was carried out in greenhouse and the soil was treated with 0, 30, 60, 120 mg/kg of compost for 4 months. The result showed that the soil pH value increased by 0.5 to 1.2 units after addition of compost. The OM and total P content was found increased by 0.2 to 1.9 and 0.2 to 3.2 times respectively with the increase of compost dosage. The Cd concentration of wheat seeds and wheats seeds were decreased by 33 to 61.4% and 56.3 to 89.1% with the increase of compost dosage. The author also found that the application of compost resulted in decreased of exchangeable Cd by 71.8 to 95.7 and induced the Cd bounded to organic and inorganic precipitates Cd by 0.4 to 18.4 times.

2.9.2 Biochar

Biochar is a popular immobilizing agent obtained from organic materials under high-temperature anaerobic conditions. It has an excellent adsorption capacity due to the presence of many oxygen-containing functional groups, such as -C=O, -COOH, and -OH, and a large specific surface area, which can interact with HM in the soil, adsorbing them on the pore surfaces and potentially transforming them into carbonate, phosphate, and hydroxide precipitates (Wang et al, 2020; Guo et al., 2020). Table 2.6 had summarized the case study of biochar for soil remediation as the soil stabilizer.

Table 2.6: The summarized case study of soil treated with biochar

Biochar	Soil Sample Location	Targeted Metals	Dosage	Incubation Period	Observation	Reference
Compost (C) + Rice husk derived biochar	Wetland soil from Lujiao Port, Yueyang, Hunan	Cd, Cu, Zn	400g soil + 40g C; 2gB + 38gC; 4gB + 36gC; 8gB + 32gC; 16gB +2 4gC; 24gB +1 6gC; 40gB	1 week & 2 months	Increased soil pH but slightly decreased TOC and WEOC with the increase of biochar ratio; Exchangeable fraction of Cd and Zn decreased; the amendments containing compost would transform the Fe-Mn oxide and residual fractions to organic bindings.	Liang et al., 2017
Rice straw derived- biochar, bamboo biochar	Soil nearby Copper located at HangZhou, Zhe Jiang, China	Cd, Cu. Pb and Zn	0, 1, 5 % w/w	1 month	5% w/w biochar had the most effective to decrease bioavailable metals and immobilize Cd, Cu, Pb and Zn. Increased the HMs bound to organic matter fraction.	Lu et al., 2017

Biochar	**Soil Sample Location**	**Targeted Metals**	**Dosage**	**Incubation Period**	**Observation**	**Reference**
Rice straw	Subsoil of Ultisol derived from Quaternary red earth located at Liuzhou, Guangxi, China	Cu, Pb, Cd	0, 3, 5 % w/w	1 month	Significant increase soil CEC and pH; Markedly increase the reducible and oxidizable fraction for Cu and Pb, but sightly increase reducible and oxidizable fraction for Cd.	Jiang et al., 2012
Grape-pruning-residue biochar	Soil adjacent to lead and zinc mine located at Zanjan, Iran	Cd, Pb, Cu, Zn	0, 2, 5, 10 % w/w	1, 2, 4, 8 weeks	Significantly decrease the exchangeable and carbonate fraction, increase the organic, Fe-Mn and residual fraction. Reduction in mobility follow the sequence: Cu> Pb> Zn> Cd.	Hamzenejad and Sepehr, 2018
Rice husk biochar + compost (chicken manure)	Soil plant with Maize (*Zea mays* L.)	Cr, Pb, Ni, Cu, Zn	0, 2 % RHB; 2 % CMC; 1 % RHB + 1 % CMC	90 days	Both RHB, CMC and their combination decreased the mobility of HMs effectively; The highest residual fraction of Cr, Ni, Zn was found in 2 % RHB, 55.57 %, 70.07 % and 55.32 % respectively.	Rashid et al., 2022

Biochar	Soil Sample Location	Targeted Metals	Application Dosage	Incubation Period	Observation	Reference
Wine less derived biochar	Paddy soil nearby industry-concentrating area located in Chengdu plain.	Cr, Cu, Zn and Pb	0.5 % and 1 %	1 month	Soil pH increase with the increase of biochar dosage. The exchangeable decreased by 19.59, 10.19, 18.66, 17.84 % for Cr, Ni, Cu and Pb in 1 % biochar respectively; Significant decreased bioavailability.	Zhu et al., 2015
Raw rice husk, Rice husk biochar, raw maple leaves, maple leaves biochar	Agricultural soil in the vicinity of mining site located at Okcheon Country, South Korea	Cd, Cu, Pb, Zn	0.5, 1, 2 % w/w	2 months	Markedly increase the soil pH; the maximum immobility for Cd, Cu, Pb, and Zn was observed in the case of RHB and MLB (2 %), MLB (2 %), RHB (2 %) and RHB (2 %) treatments, by 61-62 %, 71 %, 76 % and 40 % respectively.	Derakhshan-Nejad and Jung, 2019

By reviewing virous case studies, it apparently found that biochar had an excellent performance in reduce the bioavailability and mobility of Cu, Pb, Zn, Cr and Cd as well as enhanced the soil pH and TOC. The biochar normally decreased the soluble or exchangeable fraction and increased the organic, Fe-Mn oxide and residual fraction of HMs in soil. For instance, Jiang et al. (2012) had investigated the effect of Rice Straw derived Biochar (RSB) to the mobility of Cu, Pb, and Cd in a simulated polluted Ultisol collected from Liuzhou, Guangxi Province. The 30 days incubation experiments were conducted with the RSB treatment at dose of 0, 30 and 50 % w/w. After 30 days, the samples were undergone Community Bureau of Reference (BCR) three-step sequential extraction for metal partitioning. The result indicated that the acid soluble fraction of Cu, Pb and Cd decreased with the increasing of RSB. The concentration of acid soluble fraction for Cu, Pb and Cd decreased from 3.56 mmol/kg to 2.16 mmol/kg, 4.05 mmol/kg to 2.71 mmol/kg, and 4.41 mmol/kg to 3.83 mmol.kg after amended by 3 % RSB. Similarly, the concentration of acid soluble fraction decreased from 3.56 mmol/kg to 1.89 mmol/kg, 4.05 mmol/kg to 1.97 mmol/kg and 4.41 mmol/kg to 4.00 mmol/kg for Cu, Pb and Cd respectively after amended by RSB. The CEC value increased significantly from 3.55 to 4.90 and 6.12 cmol/kg for 3 % and 5 % RSB, respectively. The soil pH increased from 5.38 to 6.72 and 7.46 for 3 % and 5 % RSB.

Besides, Hamzenejad and Sepehr (2017) found that grape-pruning-residue biochar (GPRB) effectively immobilize the Cd, Pb, Cu and Zn through the incubation experiment. The different rate of GPRB (0, 2, 5, 10 % w/w) were used to treat the contaminated soil collected from investigated the influence on Cd, Pb, Cu and Zn immobilization in a contaminated soil collected from a zin mining sites at Zanjan, Iran for 1,2, 4-, and 8-weeks incubation. The Tessier's sequential extraction was applied to determine the partitioning metal thereby evaluated the performance of GPRB. The result showed that the highest efficiency for HMs immobilization was observed at the 10 % GPRB at 8 weeks incubation. The mobility factor decreased by 47, 62, 70 and 49 % for Cd, Pb, Cu, and Zn respectively after 8-week incubation with 10 % GPRB.

Besides, Lu et al. (2017) also observed that the RSB and bamboo biochar significant decrease the acid extractable metals and increased the organic matter bound metals for Cd, Cu, Pb and Zn through the incubation experiments. The application rate of each biochar was controlled at 0, 1 and 5 % (w/w) and incubated in the HMs contaminated soil collected from China for one month. The result demonstrated that 5 % RSB had the most effective in mobilizing Cd, Cu, Pb and Zn, decreased by 11 %, 17 %, 34 % and 6 % respectively in acid extractable fraction, while increased by 37, 58, 68 and18 % respectively in organic matter fraction compared to control sample.

Apart from that, some research stated that the combination of biochar and compost cound lead to higher efficiency for HMs immobilization. For example, Liang et al. (2017) combined the Rice Husk derived Biochar (RHB) and compost (C) that made from the mixture of wood chips and pig slurry to treat the contaminated wetland soil collected from Dongting lake. The dosage for pot experiment represented as: 400 g soil without stabilizer, 400 g soil + 40 g C lonely, 400 g soil + 38 g C +2 g RHB, 400 g soil + 36 g C +4 g RHB, 400 g soil + 32 g C + 8 g RHB, 400 g soil + 24 C + 16 g RHB, 400 g + 16 g C+ 24 g RHB and 400 g soil + 40 g RHB lonely. After 2 months, all samples were conducted total organic carbon (TOC), pH, water-extractable organic carbon (WEOC), HMs availability and sequential extraction test. The result gave the slightly decrease trend for TOC and WEOC but increased soil pH significantly with the increase of amount of RHB in compost. For the result of sequential extraction, all the stabilizers decreased the exchangeable fraction and leaded to increase the organic bound fraction, Fe-Mn oxides fraction for Cd, and Zn.

In addition, the latest research conducted by Rashid et al. (2022) also stated that the combination of RHB and chicken manure compost (CMC) could effectively reduce the bioavailability of Cr, Ni, Zn, Cu and Pb in soil. The pot experiment planted by Maize (*Zea mays L.*) was conducted with the treatment of 0% (control sample), 2 %RHB, 2 %

CMC and 1 %RHB + 1 % CMC for 90 days. The sequential method was used to investigate the HMs speciation in treated and untreated soils. The result revealed that the highest residual fraction was obtained in 2 % RHB for Cr (55.57 %), Ni (70.07 %) and Zn (55.32 %), while Cu (30.64 %) and Pb (65.62 %) was found in RHB and CMC combination. The maximum reduction maize plants uptake in roots was observed for Zn (69 %), Ni (95 %), Zn (72 %) and Pb (72 %) after treated with RHB and CMC combination.

2.9.3 Plant Extract (PE)

Through large research on organic stabilizer, it was known that most of the organic stabilizer was found in solid form but very less research related to the use of PE in immobilizing, removing, and reducing the availability of HMs in soil. As a liquid base organic compound, PE had the huge potential in immobilizing HMs in soil because it was found that had a large number of functional groups and ligands which could easily chelated, bonded and interacted with HMs. Some research, as listed in Table 2.7, had indirectly proved that PE had the potential be a soil stabilizer for soil remediation.

Table 2.7: The case study on virous plant extract in different treatment for HMs contamination

Plant Extract	Application	Targeted Metal	Function of PE	Observation	Reference
Citric acid modified *Moringaoleifera* leaf and bark extract (CAMOL and CAMOB)	Lake water treatment in Bangalore	Zn, Pb, Ni, Cd, Cr	Bio-sorbent	Successfully adsorbed Zn, Pb, Ni, Cd and Cr with high adsorption percentage. The efficiency of CAMOB was higher than CAMOL.	George et al., 2016
Moringa olerifera seed extract (MSE)	Well water, drain water, ground water and sewage water treatment	Pb, Cd, Ni, Fe, Cu, Zn and Mn	Coagulant to precipitate HMs	Obviously decreased the Pb, Cd, Ni, Fe, Cu, Zn, and Mn uptake of lettuce plants compared to untreated water for both WW, DW, GW, and SW	Merwad, 2018
Canavalia ensiformis crude extracts	Mine waste collected from Busan, Korea	As, Mn, Zn, Pb, Cu, Cr	Catalyst of $CaCO_3$ for HMs precipitation	The concentration of As, Mn, Zn, Pb, Cr, and Cu decreased by 31.7 %, 65.7 %, 52.3 %, 53.8 %, 55.2 % and 49.0 % respectively.	Nam et al., 2016

Plant Extract	**Application**	**Targeted Metal**	**Function of PE**	**Observation**	**Reference**
Fagopyrum esculeritum (FE) and *Fordiophyton faberi* (FF) extract	Multi-HM contaminated mine soil and farmland soil	Pb, Zn and Cd	Soil washing agents	Soil OC and nutrients increased except for total P and K; the highest removal efficiencies of FE were Pb (6.69 %), Zn (26.64 %) and Cd (42.81 %) for mine soil; Pb (3.59 %), Zn (14.49 %) and Cd (12.76 %); whereas treated by FF were Pb (3.08 %), Zn (23.29 %) and Cd (25.05 %) for mine soil; Pb (1.54 %), Zn (8.93 %) and Cd (9.94 %) for farmland soil; increased concentration of exchangeable fraction for Pb and Zn but decreased for Cd	Feng et al., 2020

For instant, George et al. (2016) investigated the potential of *Moringa* leaf and bark extract as bio-sorbent to treat the HMs polluted lake water. The Moringa leaf and bark was extracted by citric acid to form citric acid modified Moringaoleiferal leaves (CAMOL) and Moringaoleiferal bark (CAMOB). Both CAMOB and CAMOL were applied into the collected lake water from Bellandur Lake, Varthur Lake and Hebbal Lake in Bangalore for lake water treatment. The result showed that both plant extract had a high adsorption percentage for Zn, Pb, Ni, Cd, and Cr. The adsorption efficiency of CAMOB was found higher than CAMOL. The highest adsorption percentage of Zn, Pb, Ni, Cd, and Cr reached to 71.4 %, 77.5 %, 44 %, 60 % and 53.8 % respectively. The author explained that the high efficiency of HMs adsorption was come from additional functional groups like -COOH and -OH added onto the surface of leaf and bark when treated with citric acid and NaOH. The functional group could enhance the mechanical strength of the bio-sorbent due to cross connecting of the integrated acidic groups with the complex polysaccharide.

Besides, Nam et al. (2016) studied the ability of *Canavalia ensiformis* extract to catalyze the precipitation of $CaCO_3$ in columns packed with HMs contaminated mine waste collected from an abandoned mine site located at Busan, Korea. The column experiments were conducted under three condition: without treatment (as negative control), treated with *C.ensiformis* extract and treated with purified urease (as positive control). The result showed that the concentration of As, Zn, Mn, Pb, Cr, and Cu in leachate from the mine waste decreased by 31.7 %, 65.7 %, 52.3 %, 53.8 %, 55.2 %, and 49.0 % respectively after the application of *C.ensiformis* extract. The author concluded that the *C.ensiformis* extract had the potential to stabilize and immobilize HMs in polluted mine waste to prevent further dispersion to the surrounding environment.

Moreover, the PE was also found a high efficiency in water treatment as a coagulant. For example, Merwad (2018) applied the *Moringa oleifera* seed extract (MSE) to treat the different water sources included well water (WW), drain water (DW), sewage water (SW) and ground water (GW) and compared to the water with untreated with MSE.

The pot experiment also had been conducted on lettuce (*Lactuca sativa*) and watered with treated water from different sources. The result showed that significant decreased in Pb, Cd, Ni, Fe, Cu, Zn and Mn uptake of lettuce plants for all water sources compared to untreated water. The decreasing percentage represented 33, 34, 6, 11, 24, 12, and 25 % respectively for WW; 7, 37, 23, 12, 14, 19 and 15% respectively for DW; 38, 45, 33, 13, 32, 34, and 27 %, respectively, for SW; and 36, 34, 31, 22, 23, 39, and 23 % for GW.

Another case study was about the use of PE in soil washing technique. Feng et al. (2020) applied the *Fagopyrum esculeritum* (FE) and *Fordiophyton faberi* (FF) extract as the soil washing agents to treat the mine and farmland soil that contaminated by Pb, Zn and Cd were collected from Huanyuan, Sichuan. The resulted displayed that the soil OC and nutrients increased remarkedly but slightly effect on total P and K. The maximum removal efficiencies of FE were found Pb (6.69 %), Zn (26.64 %) and Cd (42.81 %) for mine soil; Pb (3.59 %), Zn (14.49 %) and Cd (12.76 %) for farmland soil. Whereas the removal efficiencies for FF were found Pb (3.08 %), Zn (23.29%) and Cd (25.05 %) for mine soil; Pb (1.54 %), Zn (8.93 %) and Cd (9.94 %) for farmland soil. After single washing, the concentration of extractable fraction of Pb and Zn increased by 29.04 and 136.43 mg/kg for FE, and 18.07 and 152.89 mg/kg for FF in mine soil respectively, and in farmland soil by 61.46mg/kg and 101.76 m/kg for FE and 60 mg/kg and 74.94 mg/kg for FF respectively. However, the concentration of exchangeable and carbonate fraction for Cd in mine soil decreased by 17.15 mg/kg with FE and 17.11 mg.kg with FF, whereas deceased by 6.92 mg/kg with FE and 7.4 mg/kg with FF in farmland soil, respectively.

2.10 Sequential Extraction

The mobility and toxicity of heavy metals (HMs) highly depend on their specific chemical forms and binding state. In fact, the alterations of environment and geochemical conditions, such as changes in pH, alters in redox conditions, or rises in ligands concentrations of organic matter, can affect the mobilization of HMs from solid to liquid phase (Gleyzes et al., 2002). Generally, the assessment of soil quality was achieved by determining the total available metal concentration in soil. Nevertheless, not all the determined total metals in soil were posing hazard to the environment. Therefore, another specific method to analyze the labile metal fraction was required in this study.

Sequential extraction (SE) is the common method that used for understanding the trace element distribution in the solid phase as fractionation. This method based on the use of a series selective reagents to successively dissolve the various mineralogical fractions in order to simulate the various potential modifications of environment conditions. Among SE procedures, the 5 step-extraction that proposed by Tessier was the most widely used procedures to determine the mobility of HMS (Kumkrong et al., 2021). According to Tessier method, the author had partitioned the elements into 5 geochemical fractions including, exchangeable, bound to carbonate, bound to Fe-Mn oxides, bound to organic matter and residual (Tessier et al., 1979).

For the exchangeable fraction, the weakly-sorb metal species, such as those metal retained on the soil surface with weak electrostatic interactions, were easily be extracted in this operation. The reagents used for exchangeable should be electrolytes in aqueous solution. Normally, 1 M $MgCl_2$ was used in this fraction, and it did not attack organic matter, silicates, and metal sulfides (Kumkrong et al., 2021). Additionally, the carbonates fraction was very sensitive to the changes of pH and metal released through the dissolution of a fraction of the soil at pH approach to 5 such as sodium acetate solution or buffered acetic acid. Next, the fraction bound to Fe-Mn oxides were excellent hunters of metals.

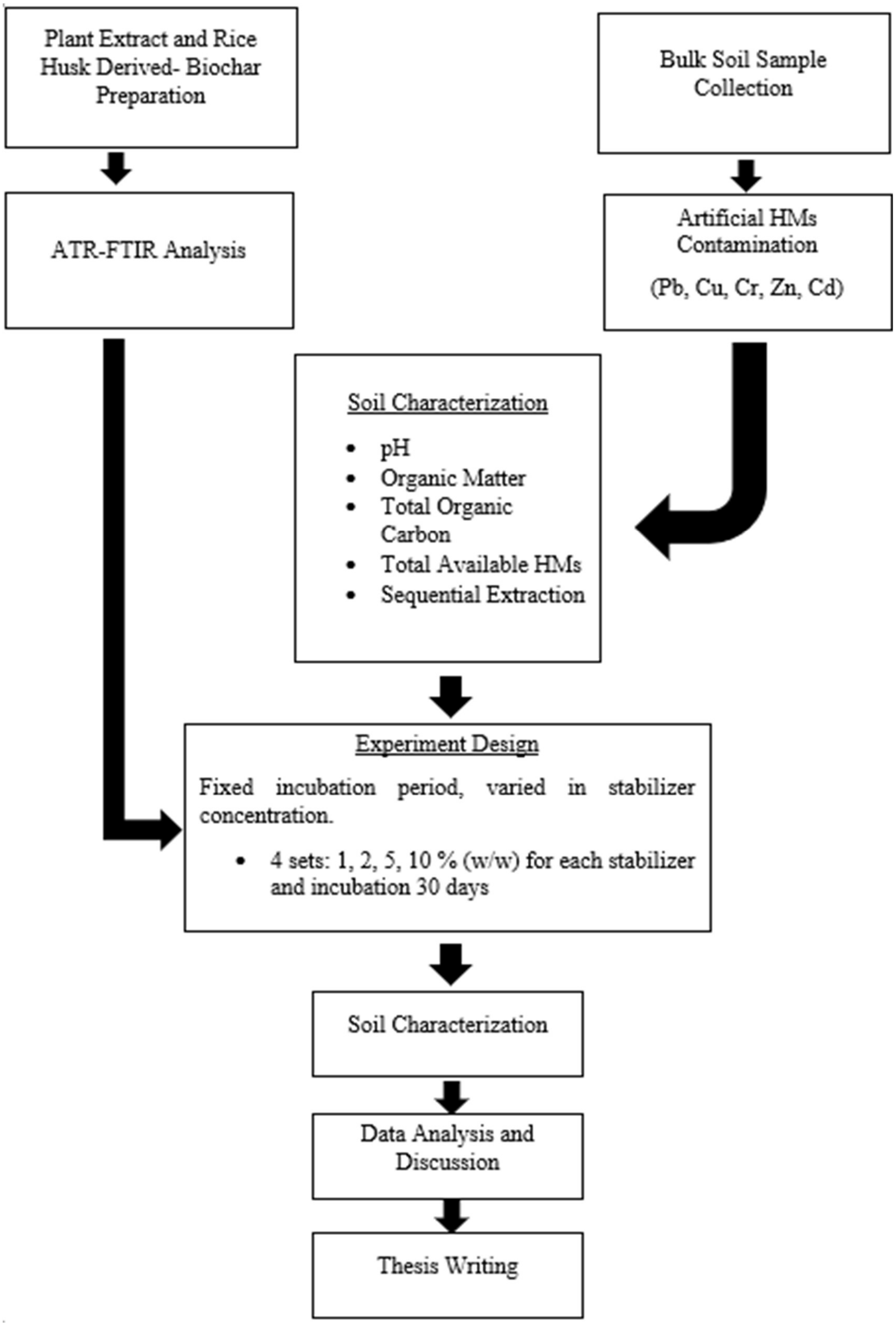
The dissolution of metal-oxides was succeeded by adjusting the pH and redox potential (Eh) of reagents. The extracted metals in this fraction were relative stable in environment compared to previous two fractions. Apart from that, the trace metal in soil might be interacted with organic matter including organisms, organic coverings on inorganic elements and biotic debris. Under oxidizing conditions, those organic matters prone to be destroyed and lead to the release of sorbed metals. Therefore, oxidizing reagents such as hydrogen peroxide were widely used in this organic matter fraction. The last fraction, residual, was the most stable fraction among all fractions in Tessier method. The bulk of this fraction was contributed by the primary and secondary minerals which containing metals in crystalline lattice form. Its extraction could be done by the digestion with strong acid, such as aqua regia, HF, and $HClO_4$ (Kumkrong et al., 2021; Filgueiras et al., 2002; Tessier et al., 1979). The stability of HM in each fraction was as followed the sequence: residual > organic > Fe-Mn oxides > carbonate > exchangeable.

CHAPTER 3

METHODOLOGY

3.1 Methodology Flowchart

Figure 3.1 illustrated the entire flowchart of this experiment. Initially, the bulk soil sample was collected from UTAR Kampar Campus, Block J. The collected soil sample was then artificial contaminated with HMs solution and incubated for 1 month before further used. There were two main soil stabilizers used in this experiment, which had the plant extract (TM Agricultural) and rice husk derived- biochar. For the soil characterization, pH, soil organic matter, total organic carbon and total available HMs were analyzed before and after adding soil stabilizer. The HMs mobility in soil was analyzed by using Tessier 5-steps sequential extraction method. In current study, the experiment was designed to determine the optimum dose of soil stabilizers for immobilize the HMs. The incubation period was fixed to 1 month but varied the concentration of soil stabilizer applied in contaminated soil (4 sets: 1, 2, 5, 10 % w/w). After 1 month, the treated soil was characterized again and compared with the original soil to determine the effect of those soil stabilizes in soil treatment.



Fixed incubation period, varied in stabilizer concentration.

- 4 sets: 1, 2, 5, 10 % (w/w) for each stabilizer and incubation 30 days

Figure 3.1: Methodology flowchart

3.2 Bulk Soil Sample Collection

The bulk soil sample were collected from the ground behind the Block J, where located at UTAR University Kampar Campus, Malaysia (coordination: **4°20'28.5"N 101°08'39.9"E**). Block J is a workshop for the engineering student conduct their experiment. Therefore, it was believed that the ground adjacent to the workshop were being contaminated because of some metal grinding or cutting activities that could cause metal runoff to the ground. In this study, total of 5 kg of soil was sampled from the surface up to 20 cm of depth by using hand auger. After sampling process, air drying process was conducted for the sampled soil for 24 hours followed by crushing process by using pestle and mortar. Then, the soil sample was sieved to smaller size particle by using 2 mm sieve to remove the unwanted impurities. Subsequently, the soil sample was kept and covered in plastic container prior to analysis.

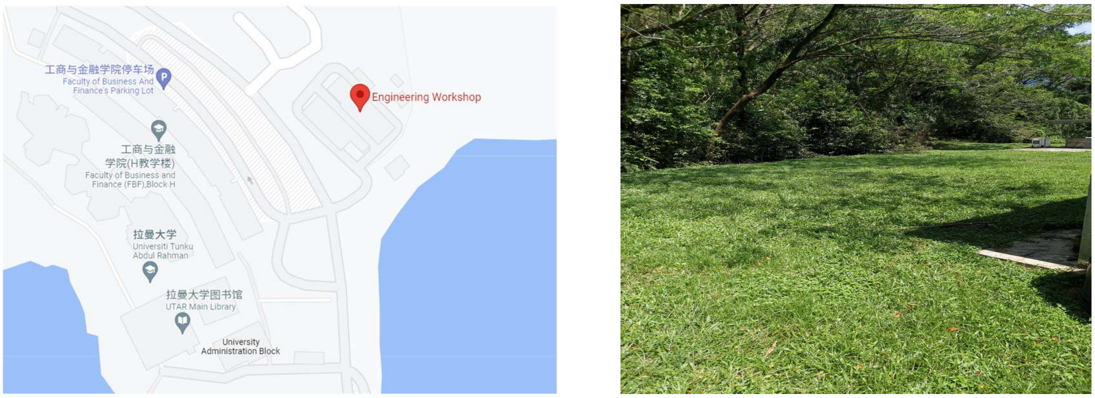


Figure 3.2: The location of the collected soil sample

3.3 Soil Amendment Preparation

In this study, two types of organic stabilizer would be applied to treat the HMs contaminated soil, namely plant extract and rice husk derived biochar. Both stabilizers were obtained from the market instead of self-prepared by author. Each pre-treatment steps were explained in the following sub-chapter.

3.3.1 Plant Extract

The plant extract applied in this experiment was donated by Best Environmental Technologies (M) Sdn. Bhd, namely TM Agricultural (TM). The TM solution consisted of various types of natural plant extract including Kelp (39.5 %), Alfalfa meal (15.9 %), Barley grain (10.2 %), Liquid fish (8.7 %), Barley straw (6.4 %), Molasses (4.5 %) and wheat straw (4.3 %) as shown in Figure 3.3. According to user manual, the TM solution was recommended to dilute into 600 to 1200 times before using. Thus, the concentrated TM was be diluted into 600 times in this experiment. To prepare the diluted TM solution, 1 mL of concentrated TM solution was transferred into a volumetric flask and topped up to 600 mL with DI water. Then, the volumetric flask was closed with cap and was inverted 10 times to thoroughly mixed the solution.

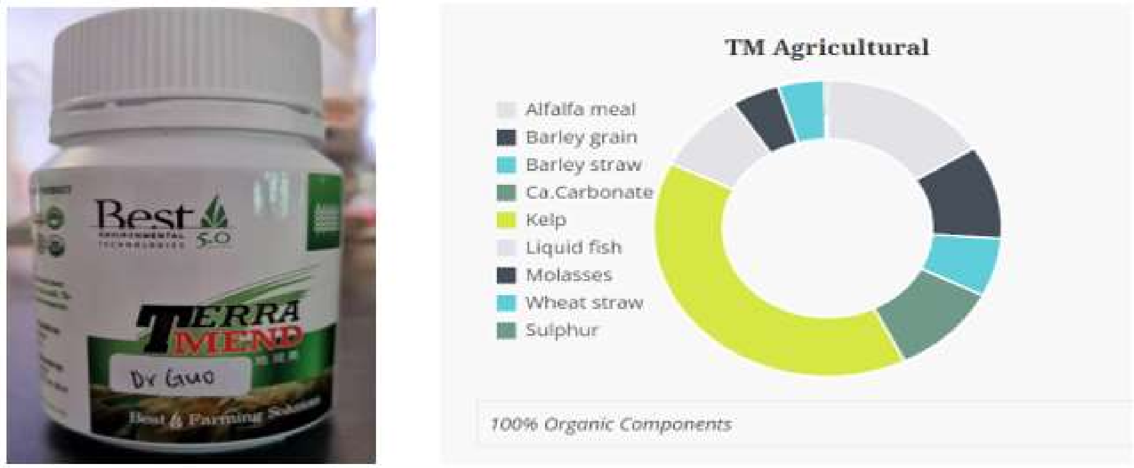


Figure 3.3: TM Agricultural and its components (Source: Best Environmental Technologies, n.d.)

3.3.2 Rice Husk Derived-Biochar (RHB)

As the conventional stabilizer, RHB was selected to compare the efficiency of the TM solution in HMs immobilizing. The RHB was purchased from the online store, called “Farid Agro Tech” in shopee. This RHB is a 100 % organic compound produced by the pyrolysis of natural rice husk under high temperature. The RHB was sieved by 2mm sieve and could be directly used without any treatment.

Figure 3.4: Rice husk-derived biochar

3.3.3 Stabilizer Characteristics

Attenuated Total Reflectance (ATR) Analysis

To identify the functional group of the soil stabilizer, both TM and RHB were analyzed by using ATR-FTIR spectrometer (Brand: PerkinElmer). Before analyzing, the diamond crystal and probe were gently cleaned with isopropanol in one-direction to prevent contamination. Then, the background date was setup for every sample before running the spectrum. After that, dropped an adequate sample on the diamond crystal and screwed down the probe with 60 to 80 force gauge to have a good surface contact. The samples were then analyzed by using Perkin Elmer Spectrum software. The obtained result was subsequently interpreted according to its wavenumber (cm^{-1}).

Figure 3.5: ATR-FTIR spectrometer

3.4 Artificial HMs Contamination (Cr, Cu, Cd, Pb and Zn)

Total of 5 kg of air-dried bulk soil sample was weighed and stored into a plastic container with the size of 30 cm in length, 20 cm in width and 25 cm in height. Then, 400 mg/ (kg soil) of $K_2Cr_2O_7$, 100 mg/(kg soil) of $Cd(NO_3)_2{\cdot}4H_2O$, 400 mg/(kg soil) of $CuSO_4$, 400 mg/(kg soil) of $PbCl_2$ and 400 mg/(kg soil) of $ZnCl_2$ were added into the soil and mixed thoroughly. After adding the metal solution, the contaminated soil was then incubated for 28 days at 40 % water content in room temperature before used. During the incubation, the soil sample was undergone a saturated and air-dried cycle for 5 times (6 days per time) (Muhammad et al., 2009).

Figure 3.6: The contaminated soil after incubation

3.5 Soil Sample Characteristics

The characteristics of all soil samples were analyzed before and after the incubation experiment. To ensure representative result, 5 points were selected for the soil sampling which included the four edges and the middle point of the container as displayed in Figure 3.7. Then, those sub-soil samples were transferred into a smaller plastic container and mixed them evenly. After that, a desired weight of the mixed soil sample from each small containers were then collected for the following analysis.

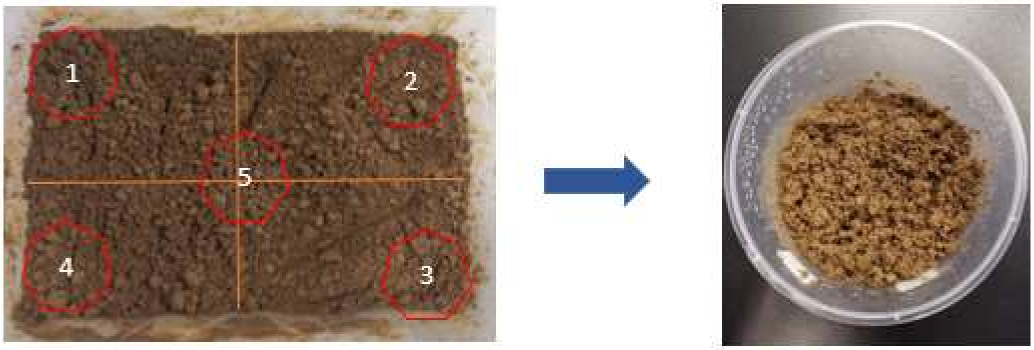


Figure 3.7: Soil sampling procedure

3.5.1 pH

pH meter

Before testing pH, calibration process of the electrode system was conducted with pH 4, pH 7 and pH 10 buffer solution. After that, 20 g of soil sample was weighed and transferred into a 50 mL centrifuge tube and 20 mL of deionized water (DI water) was added into it. Then, continuously stirred the suspension for approximately 5 minutes by using Votrex Shaker (Brand: Vortex-T Genie 2). After 5 stirring, centrifuged the mixture for 15 minutes with 4000 rpm to allow the suspension or clay settled down. After that, pipetted the supernatant for pH measurement by using pH meter (Brand: Hanna HI 2211). During measurement, the glass probe must be immersed enough deep into the supernatant to establish a well electrical contact (EPA 9045D).

Figure 3.8: pH meter and buffer solutions

3.5.2 Soil Organic Matters (SOM)

Loss-of-ignition (LOI) method

Weighed approximately 30 g of soil sample and placed into crucible. The soil sample was then dried in oven for continuously of 2 hours at 105 °C and subsequently transfer to the desiccator for cooling. After cooling, the soil sample was weighed and subsequently was combusted at 360 °C for 2 hours in a muffle furnace (Brand: Labtech). After combustion, the sample was placed into desiccator for cooling and weighed again after cooling. Then, the estimation of SOM percentage could be obtained by using the following equation (Salehi et al., 2011):

$$\mathbf{SOM_{LOI}} = \frac{(oven-dry\ soil\ weight)-soil\ weight\ after\ combusttion}{oven-dry\ soil\ weight}\ \mathbf{x\ 100}$$

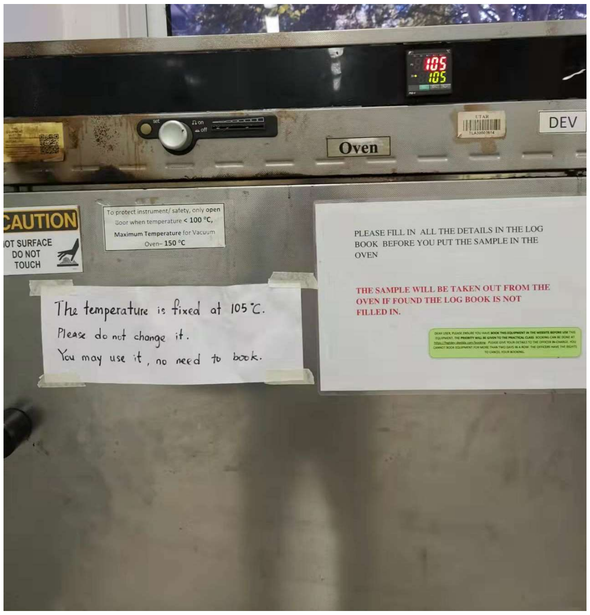


Figure 3.9: Oven

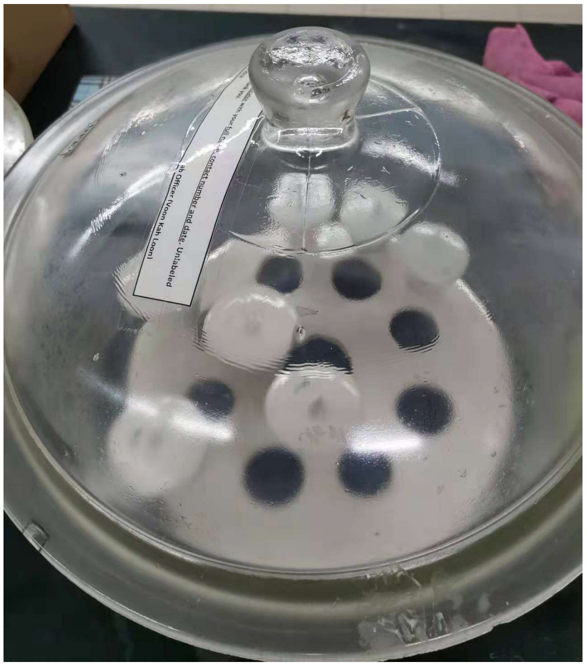


Figure 3.10: Desiccator

Figure 3.11: Muffle furnace

3.5.3 Total Organic Carbon (TOC)

Walkley-Black Titration Method

Approximately 1 g of dried soil sample was weighed and transferred into a wide mouth graduated Erlenmeyer flask of 250 mL. Then, 10 mL of 1N $K_2Cr_2O_7$ was pipetted into the flask and swirled slowly to ensure the soil was fully dissolved the soil in the solution. After that, 20 mL of concentrated H_2SO_4 (98 %) was carefully added into the flask and gently mixed under the fume hood. After mixing, the flask was heated to 135 ^{0}C for around 0.5 minutes by using hot plate under the fume hood. A 200 ^{0}C thermometer was inserted to the flask to confirm the heating temperature. Then, the flask was set aside for cooling about 30 minutes under the fume hood. After cooling, the solution was topped to 200 mL up with DI water and proceed to $FeSO_4$ titration. For the titration, 3 or 4 drops of Ferroin indicator were added into the flask and immediately titrated with prepared 0.4 N $FeSO_4$.

If the mixture solution approached to the end point, the color of the solution would change from a dark green to brownish green as illustrated in Figure 3.12. At this point, $FeSO_4$ was added drop by drop until the color steeply change to reddish grey. the titrant volume was recorded accurately to nearest 0.1 mL. In addition, two blank titrate samples without soil sample were conducted in the same way before testing the TOC of the soil sample to standardize the $FeSO_4$ solution. The titrant volume of the two blanks sample must within 0.2 mL. If not, the burette and Erlenmeyer flask were required to clean and repeated the test again (DSNR, n.d.).

Finally, the total organic carbon of soil was calculated by using the below equation:

$$\mathbf{2Cr_2O_7^{2-} + 3C + 16H^+ \rightarrow 4Cr^{3+} + 8H_2O + 3CO_2 \uparrow}$$

Follow the equation above, 1 mL of 1N $K_2Cr_2O_7$ solution is equal to 3mg of carbon. Where the volume and concentration of $K_2Cr_2O_7$ used to react with the organic carbon were known in the method. Therefore, the percentage of the TOC could be determined from the following formular:

$$\boldsymbol{TOC}\ (\%) = \frac{\mathbf{0.003}\boldsymbol{g}\ \times \boldsymbol{N}\ \times \mathbf{10}\boldsymbol{mL} \times \left(\mathbf{1} - \frac{\boldsymbol{F}}{\boldsymbol{F_0}}\right) \times \mathbf{100}}{\boldsymbol{W}}$$

$$= \frac{\mathbf{3}\ (\mathbf{1} - \frac{\boldsymbol{F}}{\boldsymbol{F_0}})}{\boldsymbol{W}}$$

Where:

N = The normality of Dichromate solution
F = Volume of Iron (II) sulfate solution (mL)
F_0 = Volume of blank sample (mL)
W = Oven-dried sample weight (g)

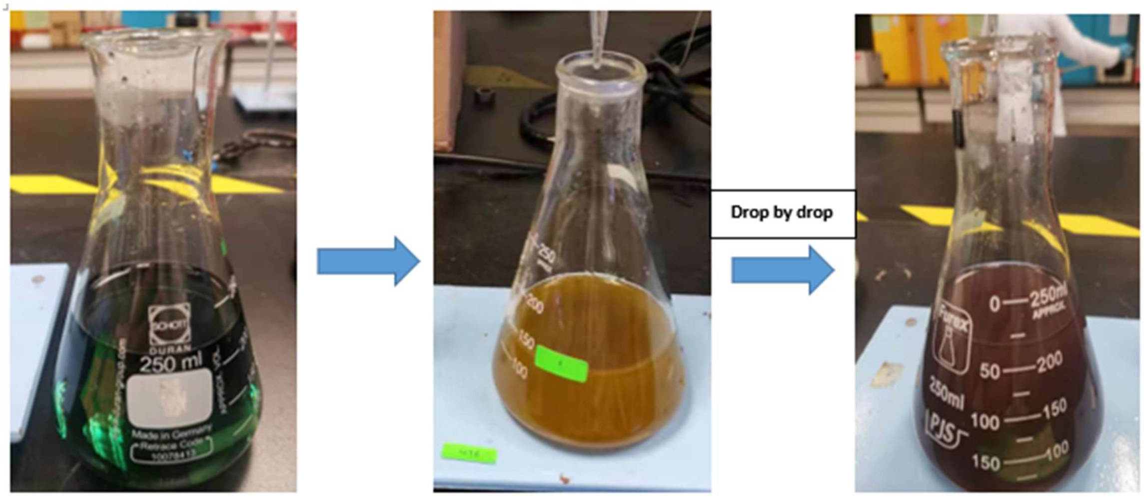


Figure 3.12: The color transformation of the mixture during titration process (Dark Green → brownish green → reddish grey)

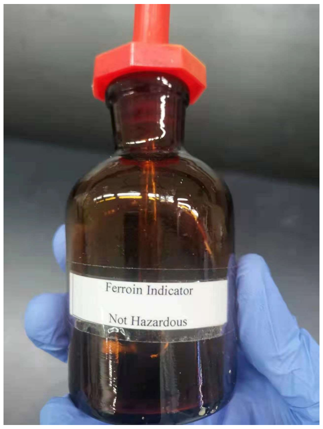


Figure 3.13: Ferroin indicator

3.5.4 Total Available HMs

Figure 3.14: Flame atomic absorption spectrophotometry (AAS)

Acid Digestion

The 1 g of dried-soil sample was weighed and placed into a beaker of 100 mL. After that, aqua regia was prepared by mixing of 37% of HCl with 65 % of HNO3 in 3:1 ratio. 20 mL of aqua regia was pipetted into the beaker and swirled gently to ensure the soil sample was wetted by the reagent. Then, the beaker was heated to 70 ^{0}C on a hot plate and for 30 minutes until the mixture become transparent under the fume hood. After heating, allowed the beaker for cooling and then filtered the resulting solution into a 50 mL volumetric flask through a 0.22 µm syringe filter after cooling. Then, the filtered solution was then dilute into 50 mL and readied to test the HMs concentration by using flame atomic absorption spectrophotometry (AAS) (Brand: Agilent Technology 280F AA) (Maurya et al., 2018).

3.5.5 HMs Mobility

Sequential Extraction (Pb. Zn. Cu, Cd, Cr)

The sequential extraction method was referring to the method as used as described by Tessier et al. (1979) and further modified by Cortinovis, (2016). This is a 5-steps extraction where different reagents were be used in different stage of extractions under certain condition as listed in Table 3.1. To reduce the losses of sample, each stage of extractions was performed in the centrifuge tubes made of polypropylene with volume of 50 mL to ensure minimal losses of sample except for the residual fraction. For the residual fraction, the sample needed transferred to a 50 mL beaker to ovoid the centrifuge tube be deteriorated by strong acid.

Table 3.1: Schematic overview of the Tessier's extraction procedure (Cortinovis, 2016; Tessier et al., 1979**)**

Fraction	Targeted Substance	Exacting Reagents	Shaking Time & Temperature
F1	Exchangeable	8 mL of $MgCl_2$ (1M)	1 hour, room temperature
F2	Carbonates	8 mL of NaOAc with pH 5 adjusted by HOAc)	5 hours, room temperature
F3	Fe + Mn Oxides	20 mL $NH_2OH{\cdot}HCl$ (0.04 M) in 25% (v/v) HOAc	Occasional shaking 6 hours, 96 ^{0}C
F4	Organic Complexes	3 mL HNO_3 (0.02M) + 5 mL H_2O_2 (30 %, pH 2 adjusted with HNO_3); 5 mL NH_4OAc (3.2M) in 20 % (v/v) HNO_3	Occasional shaking 2;3 hours in 85 ^{0}C; 30 minutes in room temperature
F5	Residual	8 mL of aqua regia (HCl + HNO_3, ratio: 1:3)	2 hours, 110 ^{0}C

3.5.5.1 Exchangeable (F1)

For the first fraction, approximately 1 g of dried soil sample was weighed using top loading balance and added into a centrifuge tube. Then, transferred 8 mL of $MgCl_2$ into the centrifuge tube and continuously shaking by using vortex shaker (Brand: Vortex-T Genie 2) for 1 hour at room temperature**.** After shaking, the sample was proceeded to centrifugation.

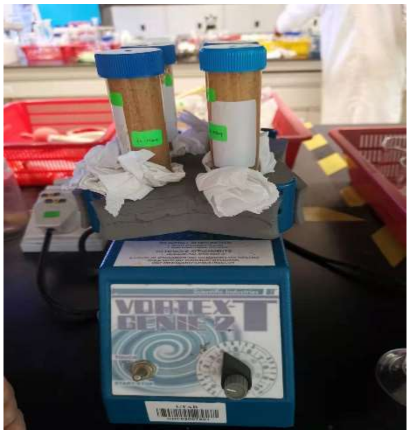


Figure 3.15: Vortex Shaker

3.5.5.2 Carbonate (F2)

For the carbonate fraction, pH adjustment of the NaOAc was conducted by using HOAc to obtain the pH value of 5. Then, 8 mL of the NaOAc was added into the centrifuge tube and mixed with the residual from the **F1**. After adding the reagent, continuously agitated the centrifuge tube for 5 hours under room temperature. Then, the resulting mixture was proceeded to centrifugation.

3.5.5.3 Fe-Mn oxide (F3)

After that, the residual from **F2** was then used in this fraction. 20 mL of 0.04 M $NH_2OH \cdot HCl$ that had prepared in 25 % (v/v) of acetic acid was accurately pipetted into the centrifuge tube. Heated the mixture to 96 ^{0}C for 6 hours with occasional agitated by using stirring water bath with lid (Brand: Major Science). After 6 hours, allowed the mixture for cooling under fume hood and was proceeded to centrifugation.

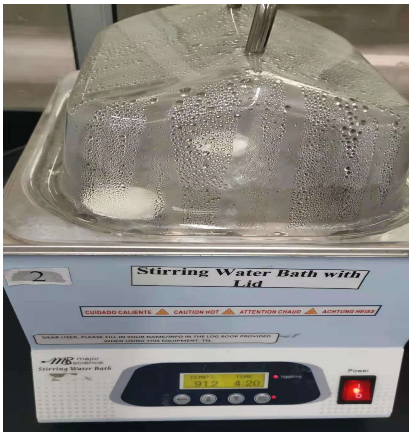


Figure 3.16: Stirring water bath with lid

3.5.5.4 Organic Matter (F4)

For organic fraction, 30 % of hydrogen peroxide was first adjusted to pH 2.0 by using nitric acid. Then, the residual from the **F3** was extracted by adding 3 mL of 65 % 0.02 M nitric acid and 5 mL of the adjusted hydrogen peroxide. After that, heated the mixture up to 85 ^{0}C for 2 hours with occasional agitated by using water bath with stirring function. After 2 hours, a second dose of 3 mL adjusted hydrogen peroxide was mixed into the mixture and heated continuously for another 3 hours with 85 ^{0}C with occasional stirring. Subsequently, placed the mixture to the fume hood for cooling. Then, 5 mL of 3.2 M NH_4OAc in 20 % (v/v) nitric acid was transferred into the solution and further diluted to 20 mL with DI water. After that, the solution was constantly agitated for 30 minutes by using vortex shaker and proceeded to centrifugation.

3.5.5.5 Residual (F5)

Transferred the residual of the **F4** from centrifuge tube to a 50 mL beaker. After that, 20 mL of aqua regia was transferred into the beaker. Then, placed the beaker on the hot plate and heated to 70 ^{0}C for 30 minutes under the fume hood. After heating, allowed the mixture cooled for 15 minutes under fume hood before proceeding to centrifugation.

3.5.5.6 Centrifugation

The separation of supernatant and residual for each successive extraction was done by using centrifuge (Brand: Hermle Labortechnik GmbH**)** at 4000 rpm for 30 minutes. Then, the supernatant was extracted into a 50 mL volumetric flask and dilution into 50 mL was done by using DI water for HMs concentration analysis. The remaining residual was subsequently rinsed with 8 mL of DI water to prevent the sample from excessive solubilization. The rinsed sample was centrifuged for another 30 minutes but the secondary supernatant was discarded in this time. Finally, the diluted sample could proceed to HMs concentration analysis by using atomic absorption spectrophotometry

(AAS). The samples were stored in fridge at 4 ^{0}C if the samples were not immediately analyzed.

Calculation:

The result of HM obtained from AAS can be converted into the concentration mass/mass of soil by using the following formula:

Y (mg/kg) = (X mg/L (AAS result) x V) x dilution factor/ W

Where,

Y = Actual concentration of HMs in soil (mg/kg)

X = AAS result expressed in mg/L

V = volume of sample in mL

W = weight of sample in gram.

Figure 3.17: Centrifuge

3.6 Incubation Experimental Design

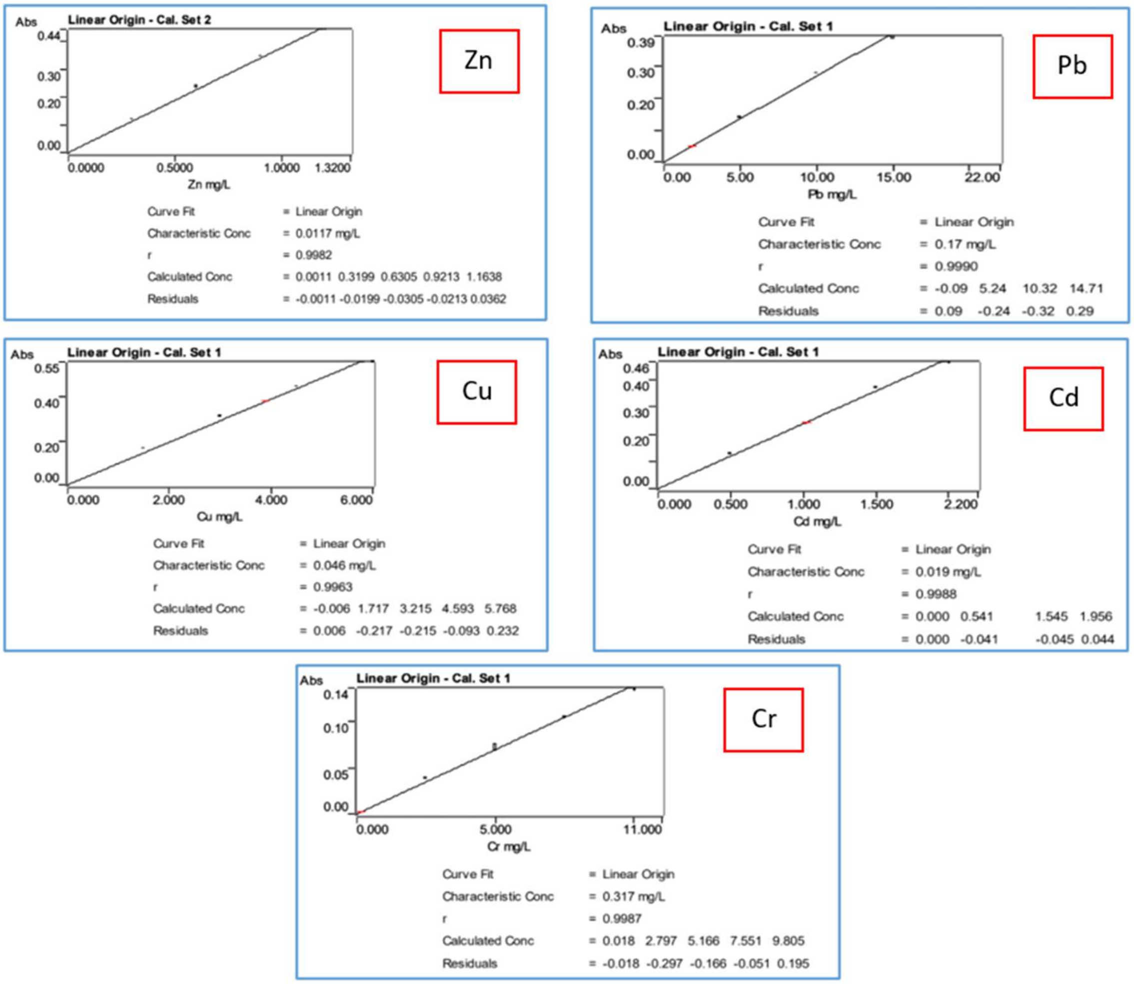
To determine the accurate optimum dose of plant extract, 100 g air-dried soil samples were be mixed with the diluted plant extract (TM) at the weight percentage 1, 2, 5, 10 % w/w respectively and then stored in a plastic container with covered by parafilm. For comparison, RBH also be mixed with 100 g air-dried soil samples at same weight percentage with the plant extract, which are 1, 2, 5, % respectively as well as stored in the labeled container. A control sample without soil amendment also be prepared and stored in a plastic container. All treated soil samples were stored at room temperature with approximately 60-70 % of water holding capacity for 1 month. The weight of all the soil samples were maintained by constant watering in every 3 days to maintain moisture at a constant level during the incubation period. After 30 days, all the soil samples were measured the physical and chemical characteristics again.

Figure 3.18: Treated soil samples that were incubated in plastic container with covered for 30 days

3.7 Quality Control

Before conducting the experiment, all required glassware were soaked with 1M HCl for overnight and washed with soap to remove any residue. After cleaning, rinsed the glassware with ultra-pure water and dried with drying cabinet at 70 ^{0}C. All the testing sample were measured as fast as possible. If immediate measure was not possible, the samples were kept in fridge at 4 ^{0}C and allowed the temperature rose to room temperature before analysis. Stored the samples in the glassware must be avoided. Each treatment had duplication and a control sample without stabilizer was prepared for reference. For the calibration curve of AAS, at least 5 non-zero calibration standards should be prepared to form the curve. The range of the calibration standards should within the test range of the AAS. The value of r should be as close to 1.00 as possible to make more accurate curve represented the detect response. Generally, the r values equal or greater than 0.995 are considered good calibration curve (Rigdon, 2016).

Figure 3.19: Acid washed glassware



Zn

Curve Fit = Linear Origin
Characteristic Conc = 0.0117 mg/L
r = 0.9982
Calculated Conc = 0.0011 0.3199 0.6305 0.9213 1.1638
Residuals = -0.0011 -0.0199 -0.0305 -0.0213 0.0362

Pb

Curve Fit = Linear Origin
Characteristic Conc = 0.17 mg/L
r = 0.9990
Calculated Conc = -0.09 5.24 10.32 14.71
Residuals = 0.09 -0.24 -0.32 0.29

Cu

Curve Fit = Linear Origin
Characteristic Conc = 0.046 mg/L
r = 0.9963
Calculated Conc = -0.006 1.717 3.215 4.593 5.768
Residuals = 0.006 -0.217 -0.215 -0.093 0.232

Cd

Curve Fit = Linear Origin
Characteristic Conc = 0.019 mg/L
r = 0.9988
Calculated Conc = 0.000 0.541 1.545 1.956
Residuals = 0.000 -0.041 -0.045 0.044

Cr

Curve Fit = Linear Origin
Characteristic Conc = 0.317 mg/L
r = 0.9987
Calculated Conc = 0.018 2.797 5.166 7.551 9.805
Residuals = -0.018 -0.297 -0.166 -0.051 0.195

Figure 3.20: The calibration curves of different HMs for AAS

3.8 Research Timetable

Table 3.2: Timetable for May Trimester 2021

Tasks	Date												
	7/6	**14/6**	**21/6**	**26/6**	**5/7**	**12/7**	**19/7**	**26/7**	**2/8**	**9/8**	**16/8**	**23/8**	**30/8**
Preliminary Title Analysis													
FYP Title Selection	■	■	■										
Introduction Meeting				■									
Case Studies Reviews					■	■	■	■	■	■			
Preliminary Report Writing													
Chapter 1: Introduction						■	■	■	■				
Chapter 2: Literature Review						■	■	■	■	■	■	■	■
Chapter 3: Methodology									■	■	■		
Presentation													
Presentation Slide Preparation												■	
Presentation Rehearsal													■
Presentation (VIVA 1)													■

Table 3.3: Timetable for October Trimester 2021

Tasks	Date									
	18/10	25/10	1/11	8/11	15/11	22/11	29/11	6/12	13/12	20/12
Experiment Materials Preparation										
Soil Sample Collection	■									
Plant Extract	■									
Biochar	■									
Chemical Preparation		■								
Artificial Soil Contamination										
Soil Characterization			■							
HMs Solution Addition & Incubation				■	■	■	■			
Incubation Experiment										
Soil & Stabilizer Characterization								■		
Soil Treatment & Incubation									■	■
Report Writing										
Chapter 1: Introduction (Revise)				■	■	■				
Chapter 2: Literature Review (Revise)	■	■	■	■	■	■	■	■	■	■
Chapter 3: Methodology	■	■	■							

Table 3.4: Timetable for January Trimester 2022

Tasks	Date																
	3/1	10/1	17/1	24/1	31/1	7/2	14/2	21/2	28/2	7/3	14/3	21/3	28/3	4/4	11/4	18/4	25/4
Incubation Experiment																	
Soil Incubation	■																
Soil Characterization		■	■	■	■	■	■	■	■								
Result Analysis			■	■	■	■	■	■	■	■							
Report Writing																	
Chapter 2: Literature Review	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		
Chapter 4: Results and Discussion											■	■	■	■	■	■	
Chapter 5: Conclusion & Recommendation															■	■	
Final Report Submission																■	
Presentation																	
Presentation Slide Preparation																■	
Presentation Rehearsal																	■
Presentation (VIVA 2)																	■

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Characteristics of Soil and Stabilizer Before Treatment

Before starting the incubation experiment, the concentration of HMs in the soil sample should be ensured high enough, otherwise, the obtained results might not be obvious and difficult to compare the performance of each stabilizer. Based on WHO standards for soil, all the concentration of investigated heavy metals (HMs) was within the maximum permissible level for the natural soil collected from the ground behind the UTAR, Kampar Campus, Block J, Malaysia. It was good to know that the investigated site was considered healthy. After adding HMs solutions ($K_2Cr_2O_7$, Cd $(NO_3)_2{\cdot}4H_2O$, $CuSO_4$, $PbCl_2$, $ZnCl_2$) into the soil samples, the concentration of total available HMs in the soil had reached 106.25 ± 9.88, 350.73 ± 3.84, 411.58 ± 30.32, 387.16 ± 4.01, 433.93 ± 22.38 mg/kg, for Cd, Cr, Cu, Zn and Pb respectively. The concentration of Cd, Zn, Cr, Pb and Cu in the artificially contaminated soil was exceeded 3 to 4 times the maximum permissible level. It was considered seriously contaminated by Cd, Cr, Cu, Zn and Pb.

Table 4.1: Comparison between Maximum Permissible Limit in WHO standards and soil before and after being contaminated (Chiroma et al., 2014)

Types of HMs	SBC (mg/kg)	SAC (mg/kg)	Maximum Permissible Level in Soil (mg/kg)	Remarks	
				SBC	SAC
Cd	0.125 ± 0.03	106.25 ± 9.88	3	Within	Exceed
Cr	8.63 ± 0.25	350.73 ± 3.84	100	Within	Exceed
Cu	3.32 ± 0.13	411.58 ± 30.32	100	Within	Exceed
Zn	30.46 ± 0.6	387.16 ± 4.01	300	Within	Exceed
Pb	18.28 ± 1.14	433.93 ± 22.38	100	Within	Exceed

*** The values are presented as means of duplication ± standard deviation for SBC and SAC.* ***SBC****: Soil before being contaminated;* ***SAC****: Soil after being contaminated.*

The pH values, SOM, TOC and total HMs contents of tested soil and soil stabilizers before remediation were summarized in Table 4.2. According to the table, the pH value of SAC was highly acidic (pH = 4.825 ± 0.08) after contaminated by HMs solution. The SOM and TOC content of the SAC were observed in the lower level, with only 0.89 ± 0.1 % of SOM and 0.52 ± 0.01 % of TOC in soil.

For the characteristics of the stabilizers, the result indicated that the RHB was an alkaline compound with a high pH value of 8.915 ± 0.06. In general, the alkaline nature of biochar was obtained from the separation of alkaline salts during pyrolysis of biomass under high temperatures (Ifran et al., 2021). RHB seemed that have a high potential to become buffer materials used for enhanced acidic soil. However, the pH of diluted TM was almost closed to DI water pH (7.705 ± 0.055), the value was found 7.80 ± 0.05 in this experiment. Originally, the concentrated TM was an alkaline liquid with pH 8.92 ± 0.06. The Low pH value found in TM might be due to the high dilution effect to TM (1:600).

Table 4.2: The characteristics of tested soil and utilized stabilizer

Property	Unit	SAC	Stabilizer	
			RHB	TM (1:600)
SOM	%	0.89 ± 0.1	-	-
TOC	%	0.52 ± 0.01	2.32 ± 0.15	0.635 ± 0.015
pH	-	4.825 ± 0.08	8.915 ± 0.06	7.80 ± 0.05

*** All values are presented as means of duplication ± standard deviation.* ***SAC****: Soil after being contaminated;* ***RHB****: rice husk derived biochar;* ***TM****: TM Agricultural;* ***SOM****: Soil Organic Matter;* ***TOC****: Total Organic Carbon*

4.2 The Nature of HMs in Soil

Figure 4.1 displayed the percentages of each fraction obtained via sequential extraction (SE) for Cd, Zn, Cu, Pb and Cr in the contaminated soil sample without treatment. Cd and Zn were the most unstable metal among the tested HMs, which had the highest and second-highest percentages in exchangeable fractions, respectively. The percentage in F1, F2, F3, F4 and F5 were found in 93.6 %, 3.54 %, 1.89 %, 0.16 % and 0.82 % for Cd respectively, whereas 80.54 %, 6.06 %, 8.28 %, 0.59 % and 4.54 % for Zn, respectively. Cr and Pb were likely bounded to Fe-Mn oxides fraction because the highest percentage of F3 was found via SE. The resulted showed that 2.32 % (F1), 10.84 % (F2), 76.93 % (F3), 6.5 % (F4) and 3.42 % (F5) were found in Cr and 8.88 % (F1), 19.98 % (F2), 67.68 % (F3), 1.34 % (F4) and 2.15 % (F5) were found in Pb. The SE result for Cu displayed that Cu had a higher percentage in the first three fractions but a very lesser percentage in the last two fractions. The partitioning of Cu showed as 8.86 %, 32.51 % and 47.14 %, 1.48 %, 0.46 % for F1, F2, F3, F4 and F5, respectively.

Among all chemical fractions of HMs, the exchangeable and carbonate fraction of HMs are typically used to estimate the true environmental risk. Exchangeable and carbonate fraction could easily become bioavailable under when increased in environmental salinity. Thus, assessing the influence of the specified amendments on the immobilization of Cd, Cr, Cu, Zn and Pb can be done based on changes in the exchangeable and carbonate fractions. The mobility factor could be calculated by using the following formular (Kabala and Singh, 2001):

$$\boldsymbol{MF = \frac{F1 + F2}{F1 + F2 + F3 + F4 + F5} \times 100\%}$$

Where,

MF: Mobility Factor

F1: exchangeable fraction (unstable)

F2: carbonate fraction (unstable)

F3: Fe-Mn oxide fraction (stable)

F4: Organic fraction (stable)

F5: Residual fraction (stable)

The presence of a high MF for HMs in soil had been interpreted as indicating a relatively high level of biological availability and lability (Kabala and Singh, 2001). Metal mobility can be divided into 4 groups depended on the MF including, low mobility (MF: 1 % to 10 %), medium mobility (MF: 10 % to 30 %), high mobility (MF: 30 % to 50 %) and extreme high mobility (MF over 50 %) (Rodríguez et al. 2009).

By comparing the mobility factor of each HMs, the Cd had the highest MF (97.2 %) which means it had a very high potential to cause the environmental risk followed by Zn, Cu, Pb and Cr. This finding implies a greater fraction of inefficient energy linkages (electrostatic), and hence a higher mobility of Cd in soil. (Hamzenejad and Sepehr, 2017). The Cr was the most stable among other metals, in which had the lowest MF (13.2 %), but still posed a medium risk to the environment. The low mobility nature of Cr could be caused by the reduction effect in natural environment. In fact, the toxic and mobile form Cr (VI) could be naturally converted to less mobile form Cr (III) with the assistant of organic matter under alkaline soil pH (Kumpiene et al., 2008). Overall, the mobility in soil of each HMs could arrange in descending order: Cd> Zn> Cu> Pb> Cr.

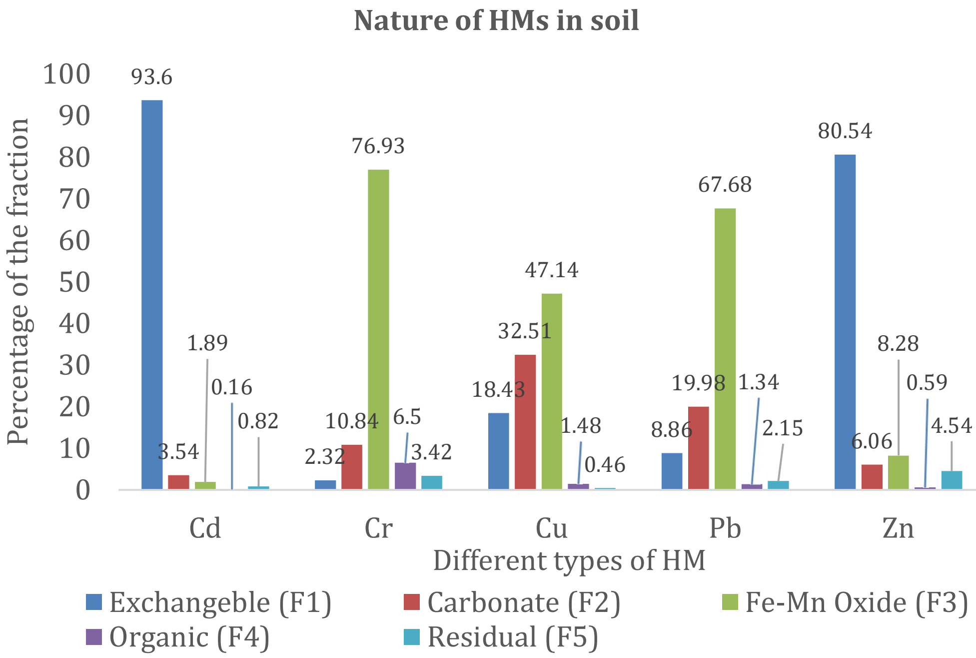


Figure 4.1: The distribution of different types of HMs in the untreated soil in each extraction fractions

4.3 Functional group of Stabilizers

Figure 4.2 was a stack plot of the ATR-FTIR spectrum for wavenumbers from 400 cm^{-1} to 4000 cm^{-1} for TM and RHB. For the result of TM, the intensity of the peaks at wavenumbers 3316 cm^{-1} (represent N-H stretches/ O-H stretches), 2122 cm^{-1} (represent -N=C=S stretches/ C triple bond), 1638 cm^{-1} (represent C=O stretches) and 632 cm^{-1} (represent Acetylenic C-H bends/ C-Cl stretches). Besides, the result reported that RHB had several functional groups on its surface including, the C-N stretches, C-O stretches, C-O-C stretches, N-H bends, and C-H bends as the intensity of the peaks at wavenumbers showed at 1037, 795, and 461 cm^{-1}. In comparison between TM and RHB in terms of functional groups, both had contained high amount of the oxygen-containing (O-containing) functional groups. The O-containing functional groups had been reported had the potential ability to adsorbed Zn, Pb, Cu and Cd through the interaction of metal electron to C=C (π-electron) bond and ion exchange, which resulted in the immobilization of HMs in soil (Park et al., 2016; Mahmound et al., 2018; Zhang et al., 2020). Besides, nitrogen-containing (N-containing) functional groups such as N-H and C-H as presented in both TM and RHB surface had also been recognized by several studies that could assist the adsorption of HMs on the stabilizer (Zhang et al., 2020; Yang et al., 2019).

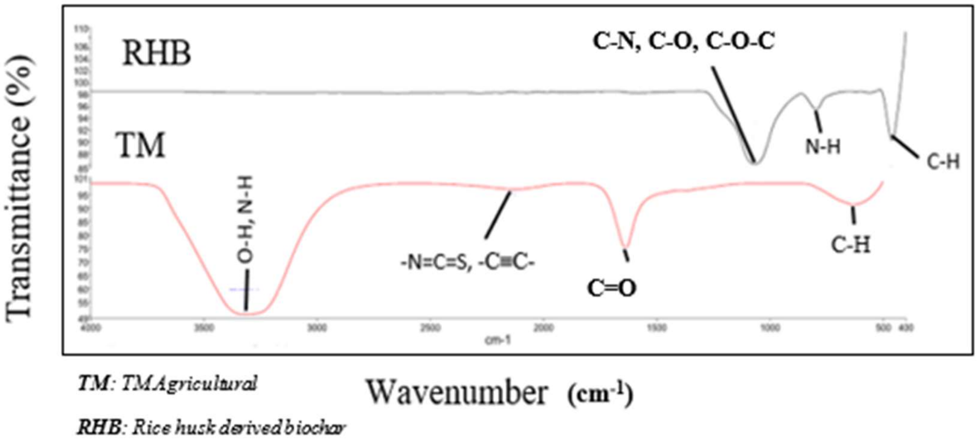


Figure 4.2: ATR-FTIR spectroscopy of tested stabilizers

4.4 Effects of Stabilizer on Soil Properties

According to the result as displayed in Table 4.3, the pH, SOM, and TOC all increased with the incorporation of both RHB and TM at different dosages compared to the untreated soil. RHB had a nature of alkaline at pH 8.915, which could significantly increase soil pH and promote metal precipitation (Hamzenejad and Sepehr, 2017). Soil pH was increased by 0.085, 0.255, 0.675 and 1.315 units with the application of RHB at 1 %, 2 %, 5 % and 10 % respectively. While the result reported that the TM had a minor effect on the soil pH compared to control sample. The pH value obtained as 4.98, 5.01, 5.035 and 5.04, for 1 %, 2 %, 5 % and 10 % of TM, respectively. The ascent in soil pH after the application of stabilizers could be justified by the binding effect of the functional groups such as hydroxyl and carboxyl groups in the stabilizers with H^+ in soil led to the increased the pH values, the pH buffering effect achieved. It had been found that TM and RHB could offer several functional groups as showed in Figure 4.2, promoting the complicated interaction of oxidation and sorption on the surface of TM and RHB with the formation of O-containing functional groups (Liang et al., Wiedner et al., 2015). When the RHB percentage was low, the interaction was weak due to the smaller number of functional groups, thus the soil pH increased slightly, vice versa. The slightly effect on soil pH that treated by TM was possibly caused by the extremely low concentration since it had been diluted to 600 times and caused the initial pH dropped to neutral.

Besides, the data showed that TM performed a marginally increasing in SOM content in relative with the control sample. The SOM percentage increased with a stable trend, from 1.610 % to 1.625 % and further increased to 1.655 % and 1.690 % for 1 %, 2 %, 5 % and 10 % of TM, respectively. However, as the compared material, RHB was observed to have a fluctuating effect on SOM content after one month of incubation. Still, the overall date displayed that the RHB had significantly increased the SOM content in the soil. Initially, the SOM increased from 1.625 % to 2.135 % after being amended with 1 % RHB compared to the control sample. Then, a slightly decreased trend was observed after the concentration of RHB increased, decreasing to 1.98 % and 1.930 % for 2 % and 5 % of RHB, respectively. Subsequently, the SOM content increased sharply when the treating concentration reached 10 % of RHB, increasing by 3.13 % compared untreated soil (1.625 %). Increased in SOM content in soil after the application of biochar had been reported in general and could be clarified by high SOM concentration in RHB (Naval et al., 2009; Gamage et al., 2016; Masuli et al., 2010; Zhang et al., 2017). Several studies had reported that the SOM could assist the formation of the complexation with metals in soils thereby affect the HMs mobility (Debela et al., 2012; Hashimoto et al., 2009). Same reason for TM, diluted TM to low concentration would directly lead to dilute the amount of SOM and caused the low performance on SOM enhancement.

Furthermore, the result showed that TM had a fluctuating trend on soil TOC effect where the TOC percentages were obtained between 0.59 % to 0.71 %. The slight fluctuation could cause by human error during experiment, but still showed a positive effect on soil TOC compared to the control sample. The performance of RHB on TOC enhancement was relatively stable compared to TM. The TOC content in soil was consistently increased from 0.45 % to 0.51 %, 0.53 %, 0.56 % and 0.60 % for the 1 %, 2 %, 5 % and 10 % of RHB treatment, respectively. The gradual incresed in soil TOC upon the application of these stabilizers could be essentially caused by the high TOC content in RHB and TM, especially in biochar. Sevral studies showed that rice hush derived biochar had relaitvely stable and insoluble carbon which could highly increased the TOC content in soil (Munda et al., 2018; Abrishamkesh et al., 2015; Liang et al., 2017). Besides, the result also showed that the performance of TM in TOC enhancement was found better than RHB desipte the TOC contain in TM was much lower than RHB. Nevertherless, the research related to TM increased the TOC content in soil was not recorded yet. It was believed that reason cause for increasing TOC in soil by treated TM was related to its high ability of non-labile carbon stabilization in TM (Best Environment Technologies, 2014). More finding about the efficiency of non-labile carbon stabilization in TM could be conducted in future experiment to support this statement. Another potentail factor would be the decomposition of organic matter in TM which could result in the formation of dissolved organic carbon (DOC) (Zhao et al., 2007), because the TM used in this study was prepared and collected since 3 years ago instead of the freash one.

Table 4.3: The soil properties after amended with stabilizers

Soil Stabilizer	pH	SOM (%)	TOC (%)
RHB (1%)	4.965 ± 0.075	2.135 ± 0.205	0.51 ± 0.02
RHB (2%)	5.135 ± 0.015	1.980 ± 0.010	0.53 ± 0.03
RHB (5%)	5.555 ± 0.015	1.930 ± 0.060	0.56 ± 0.01
RHB (10%)	6.195 ± 0.005	4.755 ± 1.085	0.60 ±0.08
TM (1%)	4.980 ± 0.040	1.610 ± 0.070	0.67 ± 0.08
TM (2%)	5.010 ± 0.030	1.625 ± 0.025	0.71 ± 0.04
TM (5%)	5.035 ± 0.025	1.655 ± 0.165	0.59 ± 0.01
TM (10%)	5.040 ± 0.050	1.690 ± 0.040	0.63 ± 0.03
Control	4.880 ± 0.010	1.625 ± 0.345	0.45 ± 0.01

*** All values are presented as means of duplication ± standard deviation.* ***RHB****: rice husk derived biochar;* ***TM****: TM Agricultural;* ***Control:*** *soil without stabilizer*

4.5 Effects of Stabilizer on Speciation Distribution of HMs

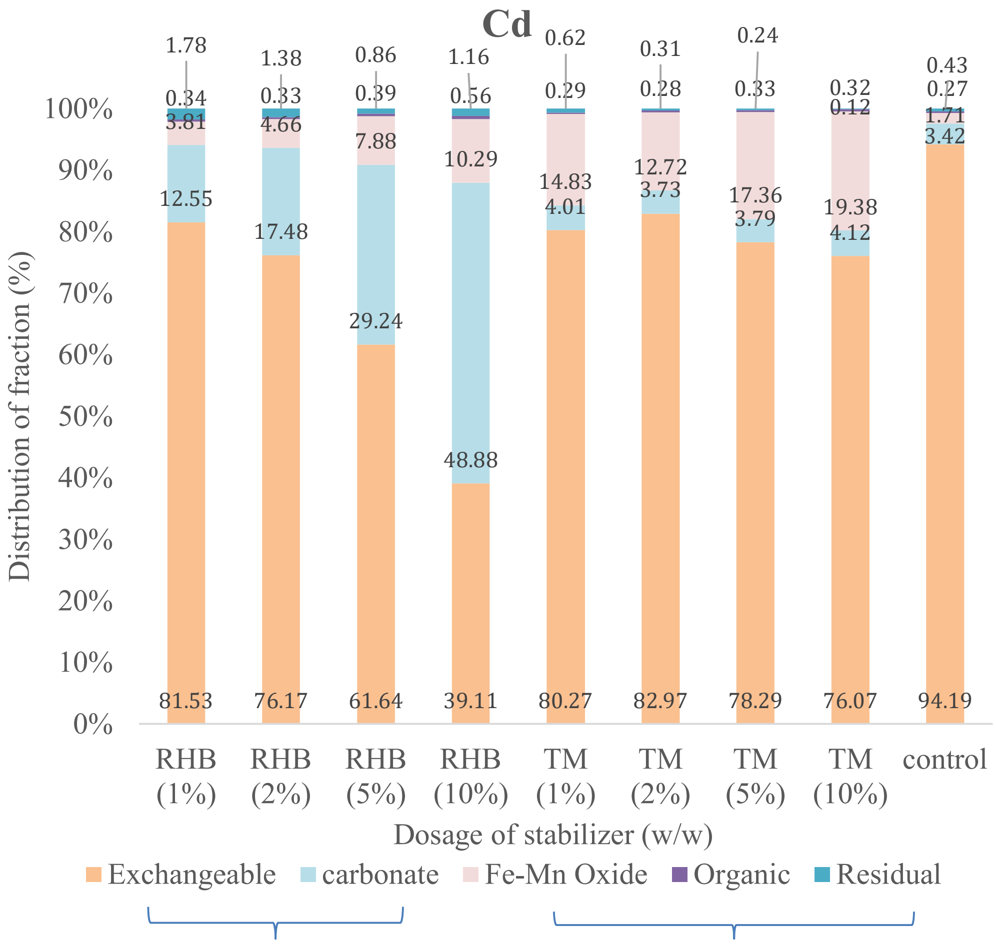
The speciation distribution characteristics of Cd, Cu, Cr, Zn and Pb were discussed detailly in the following sub-chapter. The result of each fraction was shown in average of duplication and the data had been converted into percentage.

4.5.1 Cadmium

A considerably decreasing trend was observed in exchangeable fraction of Cd as the RHB concentration slowly increased from 1 % to 2 %, 5 % and 10 %. 10 % of RHB was the maximum dose among other concentration, in which had decreased by 55.07 % of exchangeable fraction after 30 days incubation. The RHB was attributed to induce Cd bounded to carbonate fraction and Fe-Mn oxides fraction since a highly increasing concentration were observed in these two fractions. The carbonate fraction of Cd surged from 3.42 % (control) to 12.55 % (1 % RHB), 17.48 % (2 % RHB), 29.24 % (5 % RHB) and 48.88 % (10 % RHB), whereas the Fe-Mn oxides fraction of Cd increased from 1.71 % (control) to 3.81 % (1 % RHB), 4.86 % (2 % RHB), 7.88 % (5 % RHB) and 10.29 % (10 % RHB).

The soil treated with TM showed a slightly decreased trend in exchangeable fraction of Cd and significantly increased Fe-Mn oxides fraction compared to untreated soil. The percentage of exchangeable ranged from 82.92 % to 76.07 % after treated by different dose of TM. Similarly, the maximum dose was found in 10 % TM treatment which had decreased the percentage of exchangeable fraction by 18.12 %. It was worth to mentioning that the performance of TM in increasing Fe-Mn oxides fraction of Cd was much better than RHB. The organic fraction increased almost 4 to 6 times compared to control after the addition of TM. The Fe-Mn oxides fraction in 1 % TM, 2 % TM, 5 % TM and 10 % TM were obtained 14.83 %, 12.72 %, 17.36 % and 19.38 % respectively.

This finding had supported that RHB could cause a significant decreased in extractable fraction and increased the Cd bounded to carbonate and Fe-Mn oxides in soil followed the application rate. A similar metal-immobilizing impact caused by biochar was also discovered by Zhang et al. (2017), who noticed that the used of rice straw biochar considerably descended the exchangeable fraction of Cd and increased the Cd bounded to oxide and organic fraction. TM was observed slightly decreased exchangeable fraction and highly induced Cd bound to Fe-oxides fraction. This finding may imply that TM may cause the conversion of Cd from a soluble to a stable state, particularly for the formation of metal-oxide.



Unstable

More stable form

Figure 4.3: Distribution of Cd in soil samples that had been treated by different dose of TM and RHB

4.5.2 Chromium

In the case of Cr, it was tightly bounded in all soil samples due to over 80 % of Cr total concentration was found in Fe-Mn oxides, organic, and residual fraction. Overall, both stabilizers had slightly reduced the Fe-Mn oxide fraction percentage of Cr and leaded to increase the organic fraction and residual fraction around 2 to 3 times compared to control sample. Although there were observed the slightly fluctuated trend in all extracted fractions for both RHB and TM treatment, all data showed the downward trend as the increase of stabilizers in soil. TM had decreased the percentage of Fe-Mn oxide fraction of Cd by 9.33 % to 11.74 % and increased 3.63 % to 6.26 % in organic fraction as well as 4.44 % to 5.54 % in residual fraction. Similarly, the RHB was found slightly increased the last two stable fractions, in which had increased 6.33 % to 7.12 % in residual fraction and 6.05 % to 6.68 % in organic fraction. However, the RHB was found slightly increased in exchangeable and carbonate fraction with the increase of RHB concentration. The percentage of exchangeable fraction obtained at 2.53 %, 3.05 %, 3.48 % and 5.28 % for 1 % RHB, 2 % RHB, 5 % RHB and 10 % RHB respectively, whereas the percentage of carbonate for 1 % RHB, 2 % RHB, 5 % RHB and 10 % RHB were obtained at 2.53 %, 3.05 %, 6.48 % and 6.18 % respectively. The effects of TM on exchangeable and carbonate fraction were considered insignificant as compared to RHB. The percentage of exchangeable fraction was found slightly dropped with the application of TM, decreased from 4.35 % (control) to 3.36 % at 10 % TM treatment.

In a nutshell, the performance of both TM and RHB was not significant since only slightly change for oxide bound, organic bound and residual bound fraction were found in this study. This result could be explained by the naturally reduction of the most unstable form Cr (VI) to less mobile form Cr (III) during incubation period and led to the low mobility nature of Cr (Kumpiene et al., 2008). Thus, even if the Cr added to soil is in its mobile form, Cr (VI), when it comes into touch with the natural environment, it has a strong tendency to be changed to the trivalent oxide form which was relatively less mobile in soil (Bandara et al., 2020). Thus, less reactivity was created between stabilizers and hydrous chromium oxide in soil.

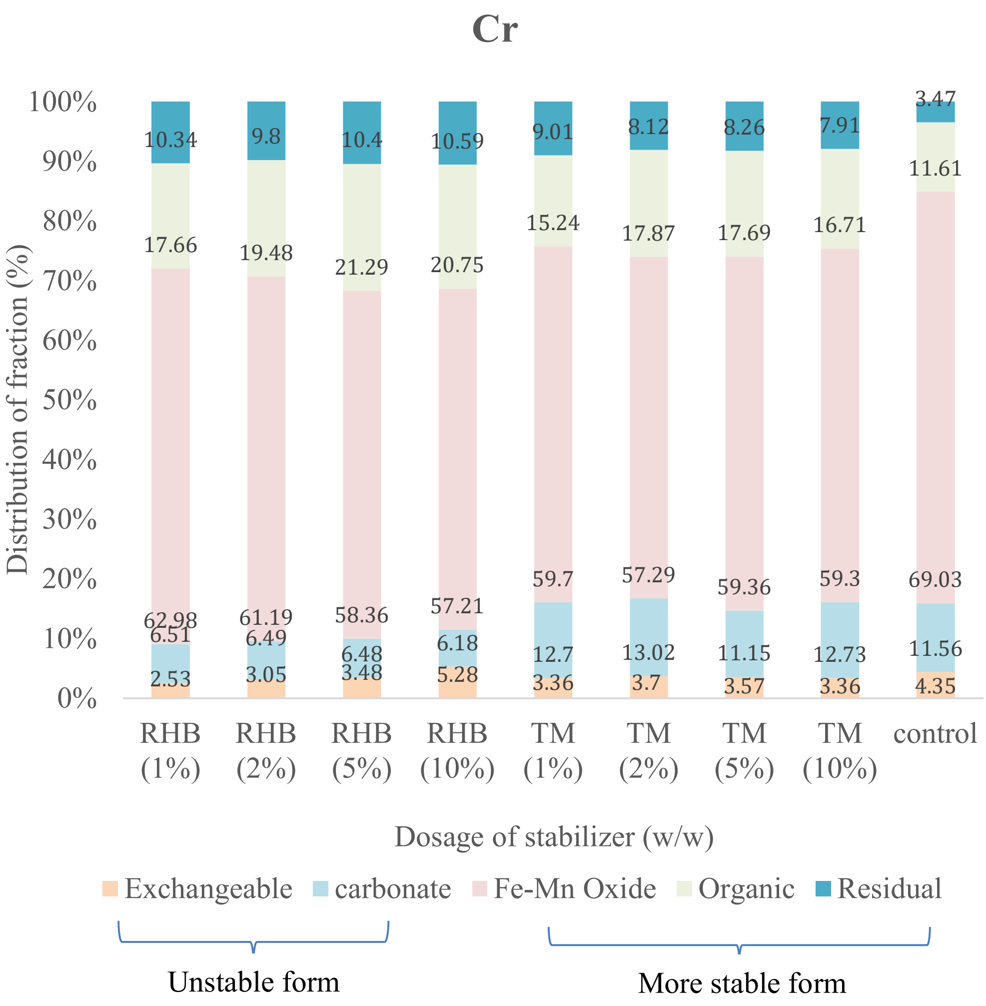


Figure 4.4: Distribution of Cr in soil samples that had been treated by different dose of TM and RHB

4.5.3 Copper

Two different stabilizers obtained an opposing trend for exchangeable fraction of Cu, where the RHB had decreased the percentage of exchangeable fraction from 19.85 %

(control) to 1.11 % (10 % RHB) as the concentration of RHB increased, but the TM had marginally increased the same fraction from 19.85 % (control) to the highest percentage as 22.95 % at 2 % TM, slightly fluctuation was found in this fraction for TM treatment. The carbonate fractions of Cu were obtained an increasing trend with slightly fluctuation for both stabilizers, The carbonate fractions were obtained 39.56%, 39.48 %, 37.84 % and 39.82 % for 1 %, 2 %, 5 % and 10 % of TM, while obtained 37.76 %, 41.34 %, 43.32 %, 39.03 % for 1 %, 2 %, 5 % and 10 % of RHB respectively. For the stable fractions, the effect of TM in those three fractions was not significant. TM tended to decrease the Fe-Mn oxide fraction while increased organic fraction and residual fraction. The Fe-Mn oxide fraction was slightly decreased by 9.02 % to 10.87 %, organic fraction increased by 1.08 % to 1.7 % and residual fraction increased by 0.79 % to 1.84 %. As the compare materials, the RHB successfully immobilize the Cu by increasing its Fe-Mn oxide, organic and residual. The maximum dose of RHB was found at 10% which had caused the Fe-Mn oxide fraction increased by 2.87 %, organic fraction increased by 7.33 % and residual fraction increased by 3.57 %.

In current finding, RHB significantly decreased the exchangeable fraction percentage of Cu and increased the stable fractions such as oxide bound, organic bound and residual bound portion. The obtained result was consistent with the research of Jiang et al. (2012) who observed a significant descending trend in exchangeable of Cu, ranging from 19.7 % to 100.00 % and increased in oxides fraction by 8.13 and 7.16 times as the increase of rice straw derived biochar. The oxidizable Cu increased was mainly caused by the formation of complexes with soil and multi-functional groups on the surface of RHB. The mobility of Cu was also sensitive to the soil pH and SOM. At slightly alkaline pH or not over pH 10, the mobility of Cu could be at the lowest within this range (Kumpiene et al., 2008). High organic soils could significantly bind with Cu to increase the percentage of organic bound fraction (Chirenje and Ma, 1999). In contrast, the TM was found increased the exchangeable and carbonate fraction percentage of Cu, prone to mobilize the Cu. However, not such research related to TM mobilize the Cu in soil was found up to date. This result was worth to take note since it could be an important information to future study.

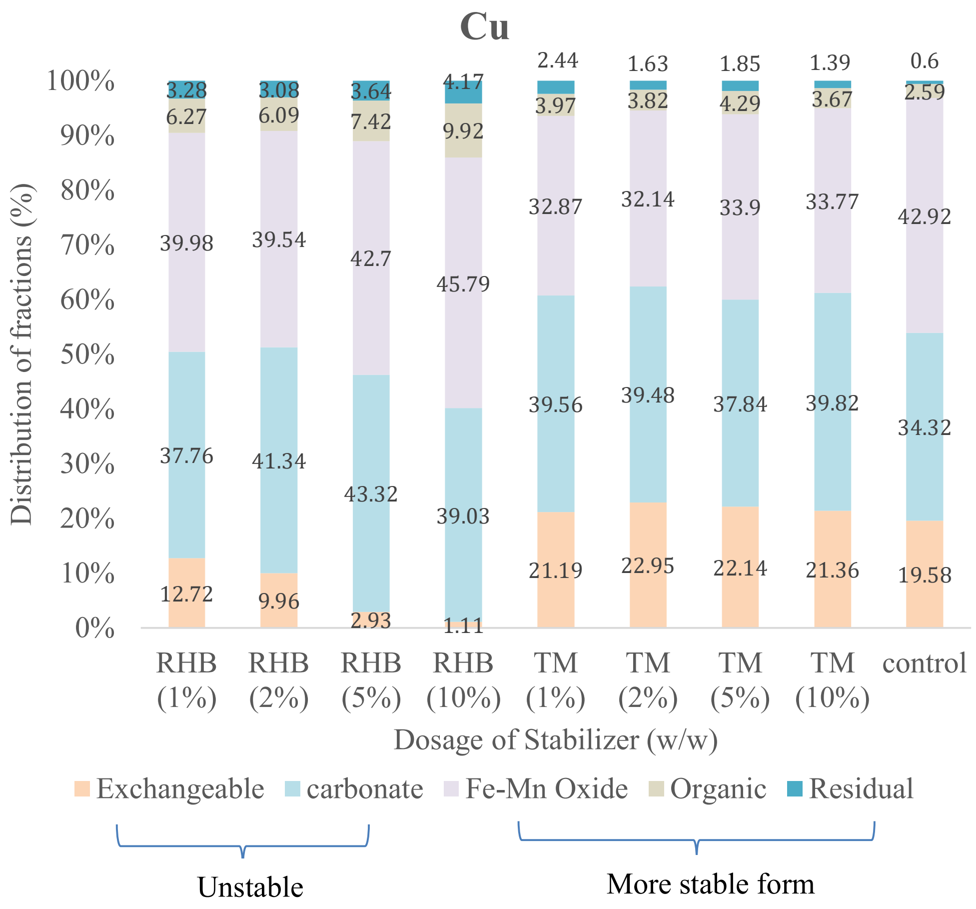


Figure 4.5: Distribution of Cu in soil samples that had been treated by different dose of TM and RHB

4.5.4 Zinc

According to Figure 4.6, RHB treatment realized a significant reduction in exchangeable fraction and increase in carbonate fraction found in RHB treatment. The exchangeable fraction decreased sharply from 79.98 % to 38.77 %, 57.13 %, 36.54 % and 19.36 % for 1 %, 2 %, 5 % and 10 % RHB treatment. RHB was attributed to induced Zn bounded to carbonate since the carbonate fraction had suddenly surged with the increase of RHB dose. The carbonate fraction had increased about 2 to 8 times when compared to control sample. However, there was found a fluctuated trend in increasing Zn bounded to oxides, organic and residual with the level of RHB treatment. The maximum concentration (11.39 %) of Zn bounded to Fe-Mn oxides was observed at 10 % RHB, while the minimum concentration (7.56 %) was found at 2 % RHB; the greatest concentration (1.77 %) of Zn bounded to organic was showed at 5 % RHB, while the least concentration (1.04 %) was showed at 2 % RHB; the highest percentage of residual was found at 1 % RHB, while the lowest was found at 5 % RHB.

As compared to RHB, the performance of TM seemed not significant in decreasing exchangeable and carbonate fraction but had a slightly increased in residual fraction with a decreasing trend as the increase of applied concentration. There was an irregular trend of reducing Zn bound to exchangeable and carbonate fraction with the concentration of TM application. The TM at 10 % showed that highest Zn concentration bounded to exchangeable (72.83 %), while TM at 1 % showed the lowest one (71.01 %). Besides, TM at 5 % displayed the highest concentration bounded to carbonate (6.89 %), while TM at 1 % displayed the lowest concentration (6.77 %). Besides, the Fe-Mn oxide and organic bound fractions were observed in a minor fluctuated trend with the treatment concentration of TM. The percentage of Fe-Mn oxide fraction varied from 8.44 % to 10 % and the concentration of organic bound varied from 1.04 % to 1.85 % after amended with TM. Other than that, the Zn bounded to residual was observed in a decreasing trend with the increase of TM level. The concentration of residual fraction was obtained as 11.87 %, 10.75 %, 10.3 % and 9.5 % for 1 %, 2 %, 5 % and 10 % TM treatment.

To conclude, the percentage of exchangeable fraction for Zn had been significantly decreased and the percentage of carbonate bound, Fe-Mn oxides bound, and residual bound fractions considerably increased with the increase of RHB application rate. A similar trend was found in the report of Hamzenejad and Sepehr (2017) who noticed that the exchangeable bound fraction percentage of Zn decreased from 9 % to 5 % and oxides bound fraction percentage of Zn increased by 22 % with the 10 % of biochar treatment. This result could cause by the increase of pH in soil after biochar application, which could lead metal sorption on the oxides. Generally, the amorphous iron-oxides have a strong attraction for Zn. Thus, Zn was transferred from the exchangeable fraction to Fe-Mn oxides fraction at higher pH levels in biochar-amended soil (Gusiatin and Kulikowska, 2015). For the TM, this finding suggested that the TM had a slight effect on Zn retribution from exchangeable fraction and residual fraction which had confirmed by the SE result of Zn via this experiment.

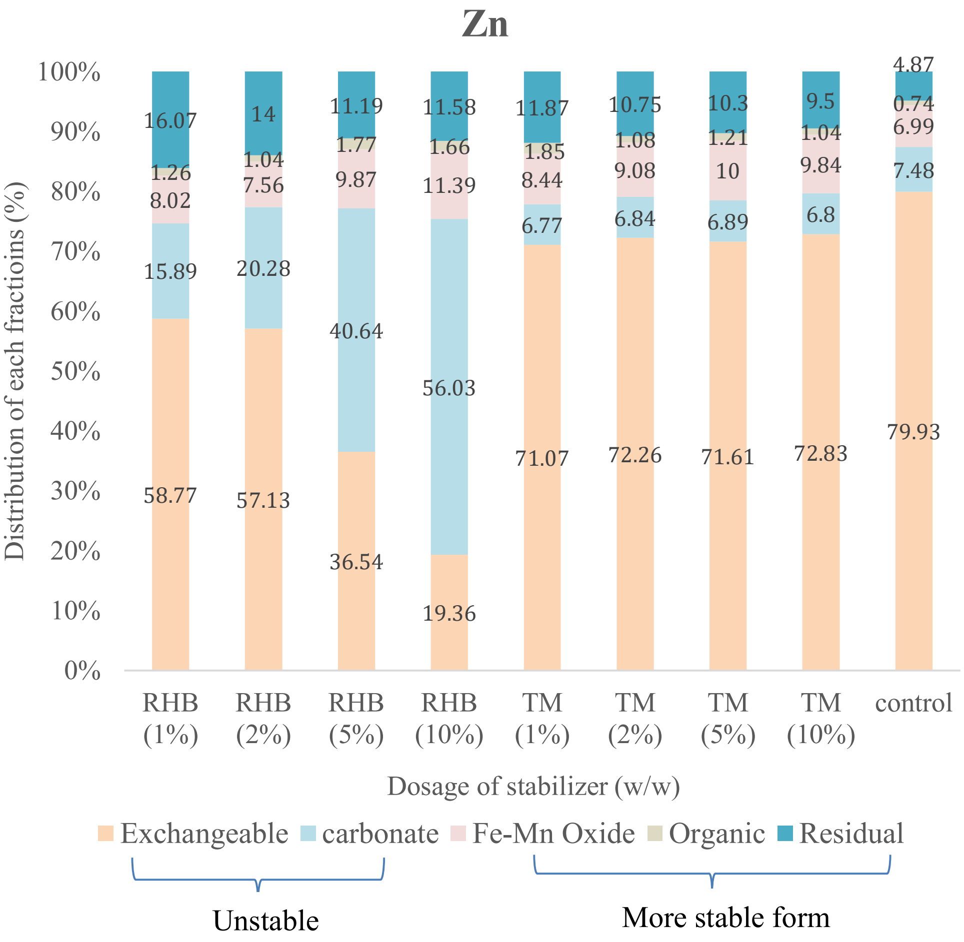


Figure 4.6: Distribution of Zn in soil samples that had been treated by different dose of TM and RHB

4.5.5 Lead

From the result of sequential extraction for Pb, there was a slight increase in exchangeable and Fe-Mn oxides fraction with an irregular trend. The highest percentage of exchangeable and carbonate fraction were observed at 2 % TM and 1 % TM respectively, while the lowest percentage for both exchangeable and carbonate was found at 10 % TM. The increase of exchangeable and carbonate fraction was contributed from Fe-Mn oxide fraction due to a significant decrease was found in this fraction compared to control sample. Conversely, the application of RHB showed a slight decreasing trend in exchangeable fraction and increasing trend in carbonate fraction when the increase of RHB treatment dose. The percentage of exchangeable fraction decreased from 5.7 % to 3.45 %, while the carbonate fraction increased from 16.4 % to 24.90 % as the 10 % RHB applied to the soil. Similarly, RHB also decreased the Fe-Mn fraction percentage of the soil with fluctuated trend with the treatment level. The maximum dose for immobilizing Pb was observed at 10 % RHB and 10 % TM, in which had the highest percentage in metal oxides, organic bound and residual fraction and the lowest percentage in exchangeable and carbonate fraction.

This finding supported that the application of biochar had significantly decrease the concentration of exchangeable Pb and increased the concentration of more stable fractions such as metal-oxides and residual fraction. This is an agreement with the result found by Jiang et al. (2012) who observed that the exchangeable Pb decreased by 18.8 to 77 % and the oxidizable Pb increased by 1.18 to 1.94 times as the rice straw derived biochar dosage increased. The decreasing in exchangeable Pb could be resulted by the increase of soil pH, TOC, and oxygen-containing groups (Bandara et al., 2020). RHB increased the soil pH could enhance Pb precipitation; TOC could increase the soluble DOC-Pb complexes adsorption on the biochar surface; oxygen-containing functional groups could help to enhance the surface complexation of Pb (Bandara et al., 2020). In contrast, TM was found had a negative effect in Pb immobilization compared to control sample. While, if no compared with the control sample, TM was observed slightly decreased the exchangeable fraction and increased the residual fraction of the Pb followed with the increase of TM application.

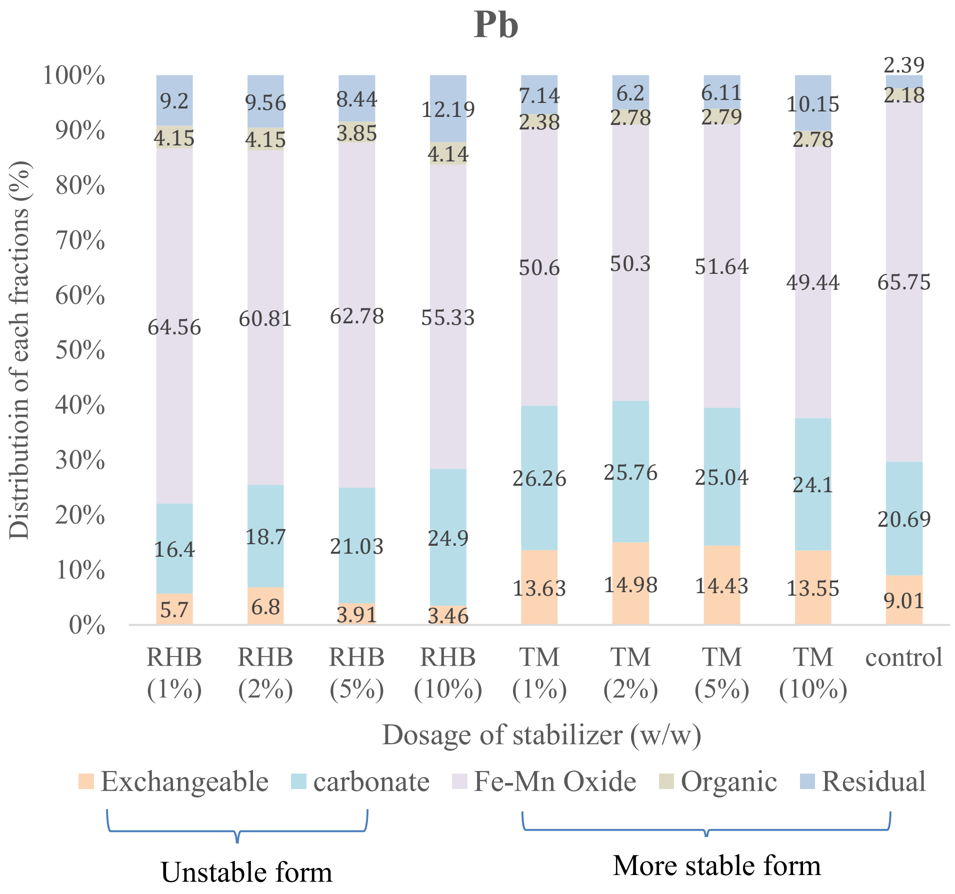


Figure 4.7: Distribution of Pb in soil samples that had been treated by different dose of TM and RHB

4.6 Mobility Factor (MF) comparison

By comparing the MF, the results indicated that RHB had a better performance than TM in immobilizing Pb, Cu, Cr and Zn but not for Cd. Both TM and RHB decreased Cd mobility with the increase of treatment ratio, although an outlier was observed at the soil sample treated with 2 %TM. The reduction percentage of MF for Cd after treated by TM and RHB was ranging from 11.28 % to 17.53 % and 1.86 % to 9.53 %

respectively. A higher performance was found in TM for immobilize Cd compared the finding of Lee et al. (2021), who had observed that the application of TM in contaminated soil decreased the unstable Cd with 0.1 % to 1.1 % at the minimum dose of 1% and maximum dose of 10 % TM compared.

Besides, the result also indicated that the TM increased the mobility of Pb by 7.74 % to 10.84 % with the decrease of TM treatment ratio, while the RHB decreased the immobility efficiency of Pb by 6.32 % with the increase of treatment ratio. This result was inconsistent with the research of Derakhshan-Nejad and Jung (2019) who observed that the mobility efficiency for Pb increased with the rise of RHB treatment. The RHB and TM were suspected to be contaminated by Pb during the incubation experiment or could be contaminated during its producing processes. The total available HMs in each stabilizer was suggested being measured before applying to the contaminated soil.

Furthermore, Cu mobility was found decreased by 2.94 % to 13.97 % after amended by RHB in this experiment. This result was under expectation since high efficiency of biochar in immobilizing Cu had been reported in several studies (Jiang et al., 2012; Zhu et al., 2015; Lu et al, 2017; Hamzenejad and Sepehr, 2018). However, the performance of TM was out of expectation in which had increased the Cu mobility with the decreased of treatment ratio. The increment percentage was ranging from 5.66 % to 8.20 %. This result was suspected to be related to the increase of DOC in soil. TOC could include non-soluble OC and DOC. The presence of DOC could form a strong complex with Cu in soil solutions thereby translocate and mobilize the Cu in soil (Zhao et al., 2007). This hypothesis could be supported by the TOC as stated in Chapter 4.4, which had observed that the TOC content in soil was significantly increased after treated with TM. In addition, the total OC in TM solution could be decomposed over the time and led to increase the concentration of DOC. However, the statement could not be proved by only the TOC result. DOC test was recommended for TM in future study to support this statement.

Apart from that, both TM and RHB showed a noticeable decreasing trend of MF for Zn as the dosage increased, despite having an outlier at 1 % RHB. The reducing percentage in Zn MF was ranging from 4.77 % to 7.92 % and 8.77 % to 11.96 % for TM and RHB, respectively. The outlier detected at 1 % RHB could be caused by the unintended human error during the experiment period, extrinsic contaminant presented in soil before or after incubation experiment and the uneven mixed process for soil that contaminated by HMs solution (Lee et al., 2021; Zhang et al., 2017; Chen et al., 2019). Chen et al. (2019) had stated that the conventional soil spiling method was difficult to mix well the metal solutions with the soil and could pose a potential risk to obtain the soil with high heterogeneity.

On the other hand, RHB was found able to decrease the MF percentage of Cr by 5.46 % to 6.87 % with a decrementing trend as the treatment dosage increase. However, there was a fluctuated trend of MF for Cr was found in TM treatment. The MF of Cr increased from 0.88 % at 2 % TM and suddenly decreased by 1.16 % at 5 % TM. This data was not consistent with the finding of Lee et al. (2021) who observed that the percentage of unstable fraction of Cr decreased by 6.1 % to 7.9 % with the rise of TM treatment ratio. The fluctuation could be resulted by the same reasons as described above such as unwanted human error, incompletely mixed metal solutions with soil as well as extrinsic contaminant entered to soil sample during incubation period. Moreover, the redistribution of HMs in the fractions or incomplete extraction during the SE stages could also contribute to the fluctuated trend obtained for TM treatment in this study (Bacon et al., 2008). Soil sample drying and crushing had been reported that would lead to redistribution of the HMs to more soluble and extractable forms (Hall et al., 2006).

Overall, RHB treatment resulted in a considerable decrease in MF in order of Cu> Pb> Zn >Cd> Cr. A Similar trend was found in the finding of Hamzenejad and Sepehr (2017), who stated the grape-pruning-derived biochar remarkable decreased the MF of metal in order of Cu> Pb> Zn> Cd. For the TM treatment, the immobility efficiency of TM for each HMs was found in descending order: Cd> Zn> Cr> Cu> Pb, while the Cu and Pb mobility were found increased by the TM. The immobility efficiency was found not significant for TM as compared to RHB since the maximum immobility percentage was observed only 17.53% in Cd. The main reason might come from the extremely high dilution factor for TM which may cause low performance in soil properties as confirmed by pervious chapter 4.4. TM had been diluted by 600 times and added by percentage per soil weight (% w/w), which means 1 % w/w of TM was only 1/60000 contained in soil eventually. It was different with solid biochar which was fixed 1 % of soil mass. A higher concentration of TM was suggested to determine more significant result in future. However, TM still showed a positive result for Cd and Zn even the concentration was low. Thus, it is suggested that TM is more suitable for Cd and Zn contaminated soil.

4.7 Mechanisms of HMs immobilization by RHB and TM

Overall, the results had shown that the RHB decreased the mobility of the Cr, Cu, Cd, Zn and Pb with certain intensity compared to untreated soil according to the MF. Nowadays, the mechanisms of biochar for HMs immobilization had been explained in numerous studies (Lahori et al., 2017; Awad et al., 2021; Jiang et al, 2012; Bandara et al., 2020).

Based on present studies, the immobilization of HMs in acidic soil by RHB could be explained by two mechanisms which were the formation of precipitates and increased in the surface adsorption of HMs. As the soil pH rose, the hydrolysis of HMs would cations increase, resulting in the formation of precipitates of metal hydroxides. The RHB had an alkaline pH in nature. Therefore, the application of RHB could increase as confirmed by soil pH tested. Besides, increase in soil pH could led to improve the negative surface-charge and CEC of soil, which could lead to increase specific HM adsorption by variable charge soils because the soil surface had a higher adsorption attraction for metal hydroxide species than free HM ions (Jiang et al.,2012).

On the other hand, RHB owned abundant of oxygen- containing functional and nitrogen-containing groups such as C-O, C-O-C, S-O, C-N and N-H bend on its surface. These types of functional groups could aid in the formation of complexation with HMs and thus enhanced the specific adsorption of HMs by the soil (Uchimiya et al., 2011a; Uchimiya et al., 2011b; Zhang et al., 2017). The π-electrons present the aromatic graphite structure of RHB could help in Cr (IV) reduction to less harmful form Cr (III) and ease the formation of complexation with surface functional groups via donating protons (Bandara et al., 2019).

Besides, some adsorbed metals made their way into the soil colloid's stern layer, causing adsorption. That is due to only positive ions invading the stern layer of soil particle's double electric layer could result in a difference in zeta potential, and these adsorbed metal cations would cause the surface of soil charge less negative. Consequently, these cations were more difficult returned to bulk solution. And made HMs interact more strongly with the soil (Jiang et al., 2012; Yu, 1997). Furthermore, the increase in SOM due to RHB treatment as described previously might allow more trace metals to create very stable organic complexes that are not easily dissolved in water (Zhang et al., 2017).

For TM, the overall results showed that TM had a better performance on immobilizing Cd and Zn but worse result for Cu, Cr and Pb as compared to RHB. The mechanisms of TM were hypothesized similar with the RHB mechanisms since it had showed a positive result on soil properties although the effects were not significant. Based on current study, the mechanisms of TM could also be explained by its large amount of O-containing and N-containing groups which could easily chelated the HMs and increased the soil adsorption. However, the current information was not enough to confirm the mechanisms of TM on immobilizing HMs. In addition, the existing research relating to the mechanisms of plant extract on immobilizing HM in soil was limited. Thus, more afford should be focused on the effect of TM to the soil properties for a better understanding on the mechanisms of TM.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this research, plant extract (TM) and rice husk-derived biochar (RHB) were selected as an organic soil stabilizer to investigate their effects on decreasing HMs mobility in soil. TM and RHB were used in artificially contaminated soil at the different dosages of 1 %, 2 %, 5 % and 10 % w/w, intended to determine the optimum dosage of these soil stabilizers. Through this incubation experiment, all the objectives as stated in **Chapter 1.3** had been achieved. The result suggested RHB had significantly improved the soil pH, TOC, and SOM with the increase in dosage. However, TM showed a negligible effect on soil pH and SOM but significantly increased the soil TOC with the raise in dosage. The maximum dosage for soil properties enhancement was found at 10 % TM and 10 % RHB, which had increased 3.28 %, 4 % and 40 % of pH, SOM, and TOC respectively by using TM, while 26.94 %, 192.62 % and 33.33 % respectively for RHB. For the ATR-FTIR result, the amount of O-containing functional groups and N-containing functional groups presented in TM was more than RHB. Besides, the result also suggested that both TM and RHB noticeably reduced the unstable fraction percentage of Cd and Zn with the increase of dosage. The reduction percentage of MF for Cd and Zn was ranging from 11.28 % to 17.53 % and

4.77 % to 7.92 % respectively after soil amended with TM. In comparison, the reduction percentage of MF for Cd and Zn was ranging 1.86 % to 9.53 % and 8.77 % to 11.96 % by using RHB. Furthermore, TM increased the bioavailable Cu percentage by 5.66 % to 8.20 % while RHB decreased the bioavailable Cu percentage by 2.94 % to 13.97 %. On the other hand, TM increased the mobility of Pb by 7.74 % to 10.84 % with a decreasing trend as the dosage of TM increased. For RHB, the mobility of Pb decreased by 1.16 % to 7.48 % with the increasing trend as treatment dosage increased. Moreover, the RHB was found able to decrease the mobility of Cr by 5.46 % to 6.87 % while the TM yielded a fluctuated result on affecting the mobility of Cr with the ranging from -0.88 % (increased mobility) to 1.16 %. By comparing the immobility efficiency, RHB showed a better performance in immobilizing Pb, Cu, Cr, and Zn but TM was observed to have higher effectiveness in Cd stabilization. The immobility efficiency for each HMs could be arranged in descending order: Cd> Zn> Cr> Cu> Pb for TM and Cu> Pb> Zn >Cd> Cr for RHB. The optimum dosage of both TM and RHB was found at 10 % w/w since most of the best results in decreasing unstable fractions had been observed in treated soil when compared with other soil samples treated with a lower dosage. To conclude, TM might be more suitable for Cd and Zn contaminated soil in low concentration.

5.2 Recommendation

There are several recommendations that need to be updated in future study. Firstly, more replication samples should be conducted in the future experiment to obtain more consistent results since there would be many uncertainties occurring in soil circumstances that might not be able to control by the researcher. A higher concentration of TM was also suggested to apply in future study to find the relationship between the stabilizer concentration and immobility efficiency. Besides, the stabilizer should be analyzed for its total HMs concentration before applying it to the experiment to ensure that the stabilizer did not contaminate by HMs which would have the potential to affect the accuracy of the result. The pot experiment was recommended to determine the bioavailability of HMs after being treated by TM. More parameters that might influence the immobility of the soil stabilizer such as dissolved organic carbon, cation exchange capacity, and so on should be considered as one of the testing parameters in the experiment to have a better understanding of the effect of TM as a soil stabilizer. Last but not least, the other HMs such as mercury and arsenic which might pose the risk to the environment should also be included and focused on in the future study.

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APPENDICES

APPENDIX A: Raw data of experiment

(Cd)	F1	F2	F3	F4	F5	**MF (%)**
Soil before treatment	146.06	5.51	3.69	0.28	1.56	96.5
RHB (1%)	65.32	6.92	2.54	0.23	0.62	95.52
RHB (2%)	50.85	8.9	2.99	0.2	0.47	94.23
RHB (5%)	46.39	18.28	5.64	0.25	0.4	91.14
RHB (10%)	23.63	28.7	7.53	0.42	0.46	86.15
TM (1%)	102.34	5.11	24.57	0.47	0.45	80.83
TM (2%)	100.01	4.45	19.77	0.43	0.24	83.63
TM (5%)	91.94	4.43	25.83	0.48	0.17	78.45
TM (10%)	82.97	4.49	26.61	0.44	0.08	76.32
control	153.52	5.59	3.47	0.56	0.85	97.6

(Pb)	F1	F2	F3	F4	F5	**MF (%)**
Soil before treatment	86.61	195.19	831.52	15.97	25.55	24.4
RHB (1%)	34.65	100.66	491.66	31.34	34.79	19.52
RHB (2%)	34.77	96.25	394.3	27.01	31.15	22.45
RHB (5%)	21.25	114.53	421	26	28.84	22.2
RHB (10%)	16.92	122.16	336.02	25.08	37.23	25.88
TM (1%)	89.94	173.18	418.34	19.64	29.46	36.02
TM (2%)	87.03	151.21	371.1	20.24	22.8	36.52
TM (5%)	81.87	141.31	370.74	19.97	21.68	35.11
TM (10%)	73.32	130.15	335.88	19.05	35.15	34.28
control	80.49	185.31	735.96	24.13	26.53	25.26

(Cr)	F1	F2	F3	F4	F5	**MF (%)**
Soil before treatment	18.19	84.71	753.45	61.98	32.65	10.8
RHB (1%)	11.36	29.36	306.95	98.94	28.9	8.56
RHB (2%)	10.78	22.98	287.55	86.84	21.79	7.85
RHB (5%)	12.79	24	290.78	97.87	23.95	8.19
RHB (10%)	17.42	20.38	269.19	85.71	21.89	3.12
TM (1%)	13.83	52.11	306.95	78.32	23.15	13.9
TM (2%)	14.8	51.63	287.55	88.86	20.23	14.35
TM (5%)	13.71	43.2	290.78	86.18	20.08	12.54
TM (10%)	12.21	46.23	269.19	75.8	17.94	13.87
control	30.33	83.58	633.26	99.24	30.63	12.99

(Cu)	F1	F2	F3	F4	F5	**MF (%)**
Soil before treatment	128.61	226.93	411.13	12.53	3.91	45.4
RHB (1%)	62.85	187.06	247.86	38.59	10.06	45.74
RHB (2%)	40.44	168.79	201.98	31.3	7.9	46.45
RHB (5%)	12.4	185.97	226.42	39.36	10.02	41.84
RHB (10%)	4.41	155.1	227.19	48.97	10.29	35.77
TM (1%)	108.93	203.03	211.34	25.45	7.72	56.06
TM (2%)	106.23	183.46	186.98	22.16	4.72	57.06
TM (5%)	100.35	170.27	192.67	24.2	5.19	54.93
TM (10%)	90.58	168.71	179.02	19.44	3.7	56.19
control	139.33	243.12	375.85	23.49	5.32	48.59

(Zn)	F1	F2	F3	F4	F5	**MF (%)**
Soil before treatment	326.64	24.57	17.26	2.92	22.38	89.19
RHB (1%)	164.7	41.95	31.3	4.22	25.97	77.07
RHB (2%)	161.22	53.24	27.27	3.45	22.67	80.07
RHB (5%)	108.06	113.3	40.1	5.57	19.99	77.12
RHB (10%)	56.91	154.44	42.92	5.52	19.96	75.55
TM (1%)	272.79	26.04	17.69	8.61	28.12	84.59
TM (2%)	269.07	25.47	40.95	5	24.9	80.61
TM (5%)	247.79	23.87	41.99	5.17	22.12	79.68
TM (10%)	230.83	21.54	43.36	4.14	18.8	79.19
control	328.37	31.76	39.58	4.1	25.53	83.88