CHARACTERISTICS OF SOIL STABILIZERS AND THEIR EFFECT ON HEAVY METAL MOBILITY IN SOIL

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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

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Approved by,

Signature : ____________________________

Supervisor : Assoc. Prof. Dr. Guo Xin Xin

Date : 7th May 2023

Specially dedicated to
my beloved brother, grandmother, mother and father

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ABSTRACT

Heavy metals are highly toxic and can lead to human and environmental health issues which poses a global threat and most heavy metal contamination happens through soil contamination. Many soil remediation methods have been proposed and researched to treat heavy metal contaminated soils and organic or biomass-based soil stabilizers are preferred to be applied as immobilizer as they are relatively cheaper compared to other soil stabilizers and can be nutritious to crops. However, studies on immobilization efficiency and mechanism of biomass-based materials needs more supporting evidence. In this research, plant extract (TM) and alkaline lignin (LGN) were used as a biomass-based soil immobilizer on a multiple element contaminated soil which consist of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). It was found that contaminated soil samples treated with LGN has higher reduction in mobility compared to TM. The mobility of Cd, Cu, Pb and Zn was reduced by 10.30%, 6.97%, 2.81% and 8.31% respectively through treatment by LGN while 8.05%, 4.93%, 1.28% and 4.03% respectively for treatment through TM. Functional groups like hydroxyl groups, amide groups and oxalic acid were found through the analysis of both soil stabilizers from FTIR and HPLC which affects the heavy metal mobility.

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LIST OF SYMBOLS / ABBREVATIONS

%	Percentage
pH	Acidity
cm	centimetre
˚C	Degree Celsius
g	Gram
µg	Microgram
µL	Microlitre
µm	Micrometre
mg/kg	Milligram per kilogram
mg/mL	Milligram per millilitre
mL	Millilitre
mL/min	Millilitre per minute
mm	Millimitre
M	Molar Concentration
mol/L	Mole per litre
N	Normality
ppm	Parts per million
psi	Pound per square inch
rpm	Revolutions per minute
v/v	Volume to volume
w/v	Weight to volume
w/w	Weight to weight
Al	Aluminium
As	Arsenic
Cd	Cadmium
Cd^{2+}	Cadmium (II) ion
Cu	Copper

Cu^{2+}	Copper (II) ion
Fe	Iron
Pb	Lead
Pb^{2+}	Lead (II) ion
Mn	Manganese
Zn	Zinc
Zn^{2+}	Zinc (II) ion
H_2SO_4	Sulphuric Acid
$Ca(OH)_2$	Calcium hydroxide
$CaCO_3$	Calcium carbonate
$CaMg(CO_3)_2$	Calcium magnesium carbonate
CaO	Calcium oxide
$CdCl_2 \cdot H_2O$	Cadmium chloride monohydrate
$CuSO_4$	Copper sulphate
D_2O	Deuterium Oxide
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric Acid
HF	Hydrogen fluoride
HNO_3	Citric Acid
KBr	Potassium bromide
NaH_2PO_4,	Sodium dihydrogen phosphate
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
$NH_2OH \cdot HCl$	Hydroxylamine hydrochloride
NH_4OAc	Ammonium acetate
OH	Hydroxide ion
P	Phosphorus
$Pb\ (NO_3)_2$	Lead nitrate
PO_4^{3-}	Phosphate ion
$ZnCl_2$	Zinc chloride
DI	Deionized
EDTA	Ethylenediaminetetraacetic acid
FAAS	Flame Atomic Absorption Spectrophotometry

H	Height
HM	Heavy metal
ICP-MS	Inductively coupled plasma mass spectrometry
L	Length
LGN	Alkaline lignin
MC	Moisture content
PTFE	Polytetrafluoroethylene
TM	TM Agriculture
TOM	Total organic matter
TSP	Trisodium Phosphate
W	Width
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Background

Soils is a complex substance as it consists variation in both physical and chemical composition(Baveye et al., 2016). They are considered primary medium for sustaining life as it supports a wide variety of ecosystem services and is the origin of food production, clean water, and air because of their wide range of abilities including filtering capacity; source of raw materials; habitat towards living organisms; and sequestration of carbon in the atmosphere which reduces the effect of global warming. Thus, it is distinctly acknowledged that soils have large contribution in maintaining our everyday life (Mandal and Sengupta, 2006). However, due to industrial revolution and anthropogenic activities, the soil has been highly contaminated with heavy metals over the last few decades and has been identified as a global threat as they are fragile, scarce, non-renewable and possesses significant effect between local and global environmental issues (Gasparatos, 2022). As heavy metals are not easily degraded and consist of long radioactivity reduction also known as half- life, the contamination of metals has expanded widely in soils and further to the food chain which poses significant concern to the human health due to their poisonous impacts (Zhang et al., 2009). Besides human impacts, excessive heavy metal deposition in soil may lead to soil erosion and environmental issues as mentioned they can exist for a long period of time, for example, lead persist in the soil for 150 to 5000 years depending on their source and the natural half-life for cadmium is 18 years (Azhar et al., 2022).

Heavy metal immobilization is one of the most sophisticated and effective remediations for treating heavy metal contamination in soil recently whereby heavy metals undergo biotransformation (Azhar et al., 2022). In this process of treating heavy metals, the reactions of heavy metals were reduced as their reactions were immobilized, this results in the reduction of bioavailability and mobility of heavy metals. With addition of reagents into the contaminated soil, heavy metals were able to be fixed as their mobility reduces and the fractions become less accessible and exchangeable which can be shown in an illustration in Figure 1.1 below(Derakhshan Nejad et al., 2018). Thus, preventing the migration of toxic metals to another environmental media. There are several mechanisms were undergone by heavy metals and the reagent added to immobilize them such as exchange reactions, complexation, surface adsorptions, chelation, and metal ion precipitation which alter the heavy metal properties from highly soluble to less soluble (Souza et al., 2020) . Besides common reagents like clay, phosphates, cement, zeolite and minerals, organic soil amendments were also widely applied to immobilize soil effectively as their two main advantage includes having relatively low cost compares to other soil amendments and their ability in facilitating revegetation of contaminated soils. (Lwin et al., 2018).

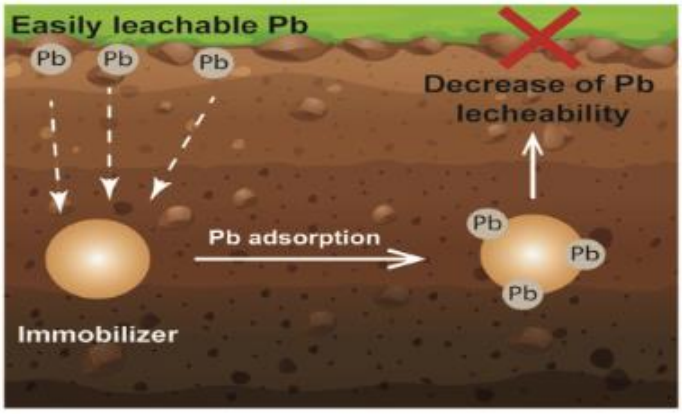


Figure 1.1: A schematic representation of an example on immobilization method (Souza et al., 2020)

1.2 Problem Statement

According to Lwin et al. (2018), considering the worldwide, there are over 10 million sites of soil contamination, and to add on to that, heavy metal and metalloids contamination has taken up more than 50% of it. Heavy metals can be lethal and can result in human and environmental health issues which poses a global threat. This makes the food chain to be very susceptible to the entrance of heavy metal through polluted soils, causing consequential food contamination as it exceeds the standard limits set by the World Health Organization (WHO) (Vareda et al., 2019). Other than that, heavy metal contamination not only affects the wellbeing of human and animals, but the key functions of the ecosystem may also be disrupted as well. Soils are one of the main media sources for the growth living things, however, if the contamination exceeds the allowable threshold, the soil is considered to lose its function which leads to adverse effects like the degrading quality of plant, yield, size and crucial impairment of the composition and microbial activities in the soil (Oliveira and Pampulha, 2006).

With the aforementioned, many soil remediation methods have been proposed and researched to treat contamination of soils with heavy metals. Many *in situ* immobilization remedial methods have been introduced to mitigate the adverse effects of heavy metal contamination in soil as they do not require expensive excavation process like ex-situ techniques (Souza et al., 2020). Organic or biomass-based soil stabilizers are preferred to be applied as immobilizer as they are relatively cheaper compared to other soil stabilizers such as thermal treatment, and they are able to benefit in terms of providing nutrients to the crops (Gong et al., 2018).

From previous research, it was proven that biomass-based soil stabilizers showed positive performance in the reduction of heavy metal mobility in soil (Lee et al., 2021). However, there are still many unknowns on the mechanisms that are related to immobilize heavy metals in soil through the soil stabilizers. Thus, it is important to understand the functional groups available in soil stabilizers that is responsible for heavy metal immobilization and relative changes in soil properties from the treatment of biomass-based soil stabilizers. Hence, through this knowledge, it opens up to further possibilities for biomass-based materials to act as heavy metal immobilizer which has benefits of cost efficiency and environmentally friendly traits.

This research focused on the application of plant extract (TM) and alkaline lignin (LGN) on heavy metal contamination of soil as biomass-based soil stabilizers. The effect of both soil stabilizers to the chemical and physical parameters to the soil were observed in this study. Screening of both soil stabilizers were done to characterise the functional groups and chemical compounds present that is responsible for immobilization of heavy metals.

1.3 Objective

This study focuses on researching the potentiality of TM and LGN as a heavy metal immobilizer in heavy metal contaminated soil in order to reduce the potential hazard of heavy metal exposure to plants, animals, and humans.

i. To observe the chemical and physical properties like pH, total organic matter (TOM) and moisture content of soil after applying TM and LGN.
ii. To compare the efficiency of TM and LGN in heavy metal immobilization.
iii. To identify the chemical components of TM and LGN through the functional groups present with analytical techniques.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals

Heavy metals are metallic elements that occurs naturally and can be found in the crust of the earth. It possesses high level of toxicity as it has a density of at least 5 times larger than water and consist of a high atomic weight. Natural heavy metal pollution comes from weathering phenomenon and volcanic eruption. However, due to industrialization heavy metals have become increasingly abundant to human exposure. Heavy metal in these days may come from different anthropogenic sources such as coal burning power plants, metal processing refineries, petroleum combustion, nuclear power plants and other material processing plants (Tchounwou et al., 2012).

Metal may come in many forms and the forms are highly related to the bioavailability to soil. According to Tao et al. (2017), there are various types of metal forms such as exchangeable, carbonate, reducible, organic and residual form. The author also mentioned that exchangeable form metals have the most adverse effects that are further elaborated in Section 2.2 as it is highly mobile. Heavy metal accumulation does not only associated with the total metal concentration in soil, but it is very dependable on the uptake mechanisms, soil physiochemical properties, chemical speciation of metals, texture of soil, nature and quantity of nutrients, climate and etc. According to Wuana and Okieimen (2011), nickle, mercury, lead, arsenic, chromium, zinc, cadmium, and copper are heavy metals that are most present in the contaminated sites. Heavy metals are a vague group of inorganic compounds that possess chemical hazards and rarely undergo chemical changes which are different from organic contaminants in soil which can be oxidized by microbial and chemical degradation. Therefore, they tend to linger for a very long time prior to their introduction to soil.

Metal mining and milling processes affects the heavy metal content in soil. This is due to the mining processes where tailing happens which indicates that heavy metals that are large and heavier will tend to sink to the bottom of the floatation cell. Subsequently, this causes the heavy metals to be directly discharge to natural depressions like onsite wetland causing the elevation of heavy metal contaminants (Devolder et al., 2003; Vareda et al., 2019). The combustion of fossil fuels are one of many types of source which leads heavy metals contamination of soil through air borne medium. Fossil fuel combustion are common factors that causes heavy metal combustion in surface soils as it produces a by-product of fly ash which are fine powdered particles. Coal consists of heavy metals like cadmium, chromium, arsenic, lead, mercury, cobalt, selenium, boron, berrylium, manganese, molybdenum and etc. Thus, without a precise pre-treatment of coal, the produced fly ash may consist high content of impurities which can combine with each other to form certain compounds of heavy metals with different properties (Shablimam et al., 2017; Mandal and Sengupta, 2006). Aside from that, applying excessive number of fertilizers and pesticides will contribute to heavy metal contamination as well (Lee et al., 2021). The fate of heavy metal has landed to food crops such as food grains, leafy vegetables, and corns (Rai et al., 2019). Besides food crops, heavy metals like mercury can be exposed from dental care such as dental amalgams, and be accumulated in fish (Tchounwou et al., 2012).

2.2 Effect of Heavy Metal Pollution

2.2.1 Effect of Heavy Metal Pollution on Soil

Many heavy metal contaminants happen in soil and as aforementioned, they persist in soil for long period. The main effect that can be influenced by heavy metal on soil is the soil microbial, enzymatic activity and the composition of nutrients in soil. It is shown from various studies that, low concentrations of heavy metal contamination in soil may cause to the release of carbon dioxide which increases the acidic properties and heavy metal pollution hinders soil microbial activity, therefore endangering the soil ecosystem function. As there is a very close proximation between soil enzymes and soil microbes, heavy metal contamination will cause the destruction of the active groups of enzymes causing a domino effect where the growth and reproduction of microorganisms are halted (Chu, 2018). Hence, resulting hindrance in plant growth. Further evidence shows a study that was conducted on paddy fields near a mining site which discharged high concentrations of heavy metals showed that rice seedlings would be killed if the water used for irrigation from a local well was not diluted (Hu et al., 2014).

2.2.2 Effect of Heavy Metal Pollution on Human

Humans are exposed to heavy metals through different pathways, including ingestion of contaminated food and drinking water and though air (Fu and Xi, 2020). Witkowska et al. (2021) mentioned that heavy metal ions may inhibit enzymatic pathways or be cofactors to activate enzymatic activities in human bodies. Exposures to heavy metals like mercury, arsenic, lead, aluminium, and iron have critical adverse effect on human body (Fu and Xi, 2020). Studies have also shown that exposure to cadmium may cause diseases like cancer and osteoporosis. Several case studies from the past during the 1950s to 1970s in Toyama, Japan also showed emergence of disease known as Itai-Itai Disease due to long term intake of cadmium contaminated rice (Hu et al., 2014). Once human is exposed to heavy metals accumulate in vital organs of the human body like the brain, heart, kidney, and liver. This deteriorates the normal biological functions of these organs. The heavy metals would then cause redox reactions in the biological systems resulting free radicals that will damage the proteins and DNA which indicates the mutagenic and carcinogenic properties (Fu and Xi, 2020).

2.3 Soil Stabilizer

Soil stabilisers also known as soil amendments are remedial methods for heavy metal pollution. They are substances that can amend the soil quality in terms of biological, chemical, and physical properties. Applying soil stabilizers to heavy metal contaminated soil diverts the fraction of unstable metal to stable and intransigent phase which are stabilized through actions such as precipitation, redox reactions, increasing metal complexation and sorption which reduces the soil metal bioavailability whereby the heavy metals have better separation from the soil (Lwin et al., 2018).

There are several methods to treat heavy metals in contaminated soils which includes *in-situ* and *ex-situ* remediation techniques. While *ex-situ* method requires the transporting of contaminated soil which includes excavation and removing of the soils that are contaminated from the site causing the diversion of focus towards *in-situ* treatment methods to be more preferred as it can be done directly on the contaminated site (Lee et al., 2021). The *in-situ* soil stabilizing methods introduces nutrients to the contaminated area, has low site disturbance, reduces risk of further contamination, applies to broad range of inorganic pollutants, has a relatively lower cost compared to other remedial treatment methods, has deemed to be quick and simple, and has gained high public acceptability (Tao et al., 2017a; Lwin et al., 2018).

2.3.1 Types of Soil Stabilizer and Their Stabilizing Mechanisms

Soil stabilization is unlike solidification which is the last resort for heavy metal remediation as it may halt future comprehensive restoration whereby it does not remove or destroy the heavy metals and mobility of heavy metals may relapse when the solid block is destructed by weathering; soil stabilization immobilizes soils without solidifying it and incorporates precipitation reagents and stabilizing chemicals into contaminated soils which will then undergo physiochemical interactions with the heavy metals to decrease their bioavailability and leaching potential.

There are many types of soil stabilizers for immobilization of heavy metals. They can be generally categorized into organic and inorganic soil stabilizers. Organic soil stabilizers are able to reduce the heavy metal availability in soil and allows the regeneration of vegetation. Whereas inorganic soil stabilizers form insoluble metal compounds which decreases the mobility through the soil profile and pool available for flora and fauna. Both applications possess possibilities of promoting plant growth and increases microbial activities of the soil (Branzini and Zubillaga, 2012). Many materials have been researched for its potential purpose in soil stabilization such as carbonates like lime; phosphates containing substances; alkaline agents like fly ash and calcium hydroxide; clay and iron containing materials and organic matter like chitosan, starch, xanthate, peat, compost, manure, activated carbon and biochar. Regardless, specific agents immobilize specific heavy metals through the particular mechanisms (Liu et al., 2018).

2.3.1.1 Biochar

Lwin et al. (2018) reported several types of soil stabilizers and their targeted heavy metals to be immobilized. Organic and inorganic soil stabilizers immobilize and reduces the toxicity of heavy metals by altering the original metal phase to a more geochemically stable phase. Immobilizing heavy metal involves a few mechanisms such as precipitation, adsorption/ ion exchange and complexation. From a few research, organic soil stabilizers like biochar uses sorption as mechanism for retaining of heavy metals due to the presence of highly porous structure which has exceptional amount of extractable humic and fulvic like substance. Besides, as biochar are highly aromatic substances with functional groups that are able to provide net negative charge on the surface of biochar that results in high cation exchange capacity in the soil. Other than adsorption, electrostatic interactions, precipitations, and complex formations mechanisms also come in together by reacting to the surface of biochar that consist abundant functional groups to immobilize heavy metals in the soil (Bashir et al., 2018; Lwin et al., 2018).

2.3.1.2 Manure and composted organic soil stabilizers

Manure and composted organic soil stabilizers are also highly researched these days as they can be procured from many different sources and is commonly used as many bioremediation techniques as possible on the immobilization of heavy metals in contaminated soil (Lwin et al., 2018). From research carried out by Lwin et al. (2022), besides improving the physical properties of soils, manure and compost are said to be able to improve soil chemical and biological properties, for instance like pH, TOM, cation exchange capacity and decreasing bulk density. Research proven that application on biosolid composts at As spiked contaminated soils were decreased through adsorption mechanism. Other than that, as pH of soil are crucial factors of metal solubility in soil, it is discovered that increasing pH in soil causes the mobility of heavy metal to decrease (Sintorini et al., 2021). Thus, addition of manures allows the pH of soil to increase as a result of the occurrence of definite adsorption of organic anions on hydrous Fe and Al surfaces which causes the release of hydroxyl ions. As for composting materials, the pH may increase or decrease in terms of the compost sources. However, the use of manure and compost must be carefully monitored as it may bring negative environmental impacts in the long run where pathogens may be produced from these organic amendments and dissolved salts may be discover to be at high level which may potentially commence in new unwanted sources of heavy metals (Lwin et al., 2018).

2.3.1.3 Sodium Alginate

Several studies proven that sodium alginate was also found to have the ability for removal of heavy metal in contaminated wastewaters and reduce the mobility of heavy metals in soil and sediments. Functional groups like carboxymethyl and hydroxyl groups that are present in sodium alginate improves the cation exchange capacity of soil. The functional groups alter the pH and TOM in the soil resulting in better condition for heavy metal adsorption (Lee et al., 2021). According to Gao et al. (2020), sodium alginate involves two adsorption mechanism that can be categorised into two which is physical adsorption and chemical adsorption. The physical adsorption consists of several mechanisms including chelation, electrostatic interaction, and ion exchange whereas chemical adsorption involves precipitation to be occurred at the surface or within the pores of the adsorbent.

2.3.1.4 Phosphate compounds

Research has also been undergone for di-ammonium phosphate, rock phosphate and single super phosphate which are phosphate sources for heavy metal stabilization. Metal phosphate will be produced as the phosphate sources are added on to the contaminated soils. Particularly for rock phosphate, reduction can be seen for metal mobility with the increase of soil pH since it is an alkaline material. Consequently, the sorption of metal on its surface will be enhanced as the metal solubility or bioavailability in the soil has been reduced. Aside from that, metal immobilization by phosphate is also intensified through the mechanism of adsorption and substitution by phosphate compounds, phosphate anion-induced metal adsorption, and precipitation of metal(loid) phosphate. Phosphate heavy metal immobilization technique has been highly used for the immobilization of lead and zinc through ligand exchange as well as ionic exchange that results in precipitation of pyromorphite type minerals which have poor solubility and bioavailability (Bahsir et al., 2018; Lwin et al., 2018).

2.3.1.5 Liming Materials

Lime is known to treat soil acidity and are highly utilized for managing the reduction of heavy metal toxicity in soils. Common liming materials include $CaCO_3$, CaO, $Ca(OH)_2$, and $CaMg(CO_3)_2$ which contains different acid neutralizing capacity. Lime acts as a precipitation agent for metal in soils as the addition of lime give rise to reactions of metal hydrolysis and precipitations bound with carbonates. Moreover, in some cases, the increase in pH> 7 from the addition of lime causes the decrease if metal availability in soil. However, this situation is an exception for molybdenum and arsenic species of metal as the availability of these metals increases with high pH (Lwin et al., 2018).

2.3.1.6 Gypsum ($CaSO_4$)

Gypsum can be both naturally mined and industrially made by man. Both sources were widely applied as amendments to heavy metal contamination in soils. It was discovered that the gypsum powder added to arsenic (As) contaminated soil has reduced the concentration of dissolved As in soil to less than 0.01 mg dm^{-3}. Unlike liming materials, gypsum does not increase pH of soil however arsenate ions were adsorbed onto soils particles while calcium ions was absorbed and neutralises the soils to more negatively charged environment, thus, increasing the arsenate binding sites.

Furthermore, dissolved organic matter concentration can be reduced by the application of gypsum in soil which reduces the phytoavailability of heavy metals in soil. It is explained as high dissolve organic carbon in the soil may cause dissolved organo-metallic complexes to form which results in the increase of heavy metal phytoavailability. Other than As, with application of as little as 3% of gypsum has also succeeded in reducing the Cd and Pb concentrations in root as there is a decrease in soil solution dissolved organic carbon (Lwin et al., 2018).

Table 2.3.1: Summary of organic and inorganic soil stabilizers and their stabilizing mechanisms (Bashir et al., 2018; Sintorini et al., 2021; Lwin et al., 2018; Gao et al., 2020; Lee et al., 2021).

Type of Soil Stabilizer	Soil Stabilizer	Mechanisms	Targeting Metals	Authors
Organic	Biochar	Adsorption/ Electrostatic interactions/ Precipitation/ Complex formation mechanisms	Cr/ Cd/ Pb/ As	(Bashir et al., 2018; Lwin et al., 2018)
	Manure and Composted Materials	Adsorption/ Complex formation mechanisms	Cd/ Cu/ Zn/ Pb/ Cr/ Ni	(Lwin et al., 2018; LWIN et al., 2022; Sintorini et al., 2021)
	Sodium Alginate	Adsorption	-	(Gao et al., 2020; Lee et al., 2021)
Inorganic	Phosphate Compounds	Adsorption/ Precipitation	Pb/ Cu/ Zn/ Cd	(Bashir et al., 2018; Lwin et al., 2018)
	Lime	Complex formation mechanisms/ Precipitation	Cd/ Pb/ Zn/ Cu	(Lwin et al., 2018)
	Gypsum	Adsorption/ Complex formation mechanism	Cu/ Pb/ Cr/ Cd/ As	(Lwin et al., 2018)

2.4 Biomass Based Materials as Remediation of Heavy Metal Contaminant

2.4.1 Seaweed

Seaweed has proven to be a promising bio sorbent of heavy metals as there are abundance of active functional groups present on the surface of the cell wall such as polysaccharides, protein, amino, hydroxyl, carboxyl, and sulphate which acts as a binding site for metals. Different seaweed consists of cell wall composition that varies which affects the biosorption capacity that is affected by the present of functional groups on the surface of the cell, the coordination number of sorbed ion, binding groups ability and affinity (Ahmed et al., 2021). In a study conducted by Musa et al. (2012) shows that the effectiveness of seaweed biosorption capacity is higher when high initial heavy metal concentration is present as it can overcome the mass transfer resistance. Musa et al. (2012) also stated that metal biosorption onto seaweed indicates alginates were present in the walls of the cells.

Seaweed's properties were discovered to have potential for remediation of heavy metal contamination since a few decades ago. Bakkaloglu et al. (1998) experimented various types of biomasses including bacteria, fungi, yeast, activated sludge and seaweed on the removal of heavy metal on industrial wastewaters. The results from Bakkaloglu et al. (1998) showed that marine algae species (*A.nodosum* and *F.vesiculosus*) exhibits excellent efficiency in removing zinc, copper and nickel ion through biosorption with amendments of pH and pre-treatments of oven drying, grinding procedures were applied.

In another study being experimented by Lee et al. (2021) exhibits that there is a significant decrease in unstable fractions of heavy metal through the utilization of sequential extraction procedure with the addition of liquid plant extract which contains high content of seaweed in contaminated soils. The study also shows that increasing the concentration of plant extract provides higher efficiency of heavy metal immobilization.

2.4.2 Alfafa meal

The research on Alfafa meal being used for heavy metal immobilization through phytoremediation has been existing for quite some time however with lacking information. A study experimented by Gardea-Torresdey et al. (1996) on the biosorption of heavy metals shows that African alfafa has higher tolerance of accumulating higher concentrations of heavy metals compared to other plants. The author discovered that pH affects the binding capacity of African alfafa shoots, observation shows that the metal bound on African alfafa shoots increases when pH increases to pH 6. Metals including Zn^{2+}, Pb^{2+}, Cr^{2+}, and Cd^{2+} except Cr^{6+} was not attracted to be bounded by biomass as it is present in an overall charge of -2 indicating no electrostatic interaction between two negative ions. Gardea-Torresdey et al. (1996) also stated that, the repulsion of electrostatic interaction could be true if the binding sites of the African alfafa biomass were carboxylate groups. Therefore, from his study, he found that African alfafa are more prone to remediate heavy metals of positively charged ions.

2.4.3 Lignin

Lignin is a great potential heavy metal immobilizer as it is one of the most abundant natural aromatic polymers can be obtained from the cell walls of vascular plants (Naseer et al., 2019). Many studies had shown the effectiveness of lignin immobilizing heavy metals due to its alkaline pH, functional groups that are active like methoxy, carbonyl, ether, phenolic, hydroxyl, alcoholic hydroxyl, aryl groups and benzene rings, to competitively bond with metal ions to form lignin metal complexes (He et al. 2021; Liu et al., 2022). However, there is another mechanism that was discovered by He et al. (2021) as there is a possibility of precipitation immobilization mechanism through PO_4^{3-} anion availability in the soil that is able to increase negative surface charges of soils. Considering that the available phosphorus content in lignin amended treatments results in increase in soil pH which increases the negative charges and anion repulsion of colloidal surface, therefore decreasing the phosphorus sorption onto acidic soils (He et al., 2022). The higher the P content available in the soils would enhance the facilitation of precipitation of heavy metal with P which then immobilizes it (He et al., 2021).

2.5 Screening Methods of Biomass Based Materials

Biomass analysis or screening is a process to narrow complex substances such as plant cell walls that consist of heterogenous networks that are tedious to analyse into more precise data by high throughput analysis (Decker et al., 2018).

Screening analysis is important in identifying the effectiveness of mechanism. Through changes in the vibrational frequencies and absorbance intensities of chemical groups in the plant extract indicates that the functional groups were bonded with heavy metals (Musa et al., 2012). The purpose of this review is to determine which screening method are most compatible in determining the functional groups present in the biomass.

2.5.1 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR screening method allows the discovery of functional groups present in a biological sample like microalgal biomass, intra- and extracellular metabolites (Ferro et al., 2019). It is able to identify the functional groups in the algal biomass as well as the chemical bonding and comparison with known physiological fingerprints (Sudhakar and Premalatha, 2015).

Yang et al. (2021) conducted FTIR analysis seaweed biomass that was pre-treated through freeze drying with the use of MAGNA 550 Nicolet (Madison, USA) which is coupled with a mercury cadmium telluride detector which is a photon detector. The samples were scanned under the spectra with 30 repetitions in the transmission mode with a typical resolution of 4 cm^{-1} and wavenumber range of 4000 cm^{-1} to 400 cm^{-1}. The air was scanned before measuring the samples to obtain the background spectrum. Table 2.5.1.1 below shows the functional groups that were detected from the analysis.

Table 2.5.1.1: Functional groups detected in marine seaweeds through FTIR spectroscopy analysis (Yang et al., 2021).

Peak Values (cm^{-1})	**Functional Groups**	**Associated Biocompounds**
3550 - 3200	O-H, N-H	Polysaccharides, amino acids
2930	C-H	Aliphatic Group
2140 - 2100	C≡C	Alkynes
1657 - 1648	C=O	Proteins, Glycoproteins and Alginate
1257–1254	S=O	Sulfate Group
1030 - 1058	C–O	Polysaccharides
840 - 790	C=C	-

Sudhakar and Premalatha (2015) carried out analysis on algae samples by using FTIR spectroscopy using the same apparatus, however, with different frequency wavelengths ranging from 450 to 4000 cm^{-1}. Furthermore, the algae samples were grounded with 100 mg of potassium bromide (KBr) powder that is of spectroscopic grade in an agate mortar. 10-12 tonnes of pressure were applied on to the mixture for 5 minutes to obtain transparent pellets of 1mm. The results were shown in Figure 2.5.1.2. The results from Figure 2.5.1.2 were summarized in Table 2.5.1.2 below.

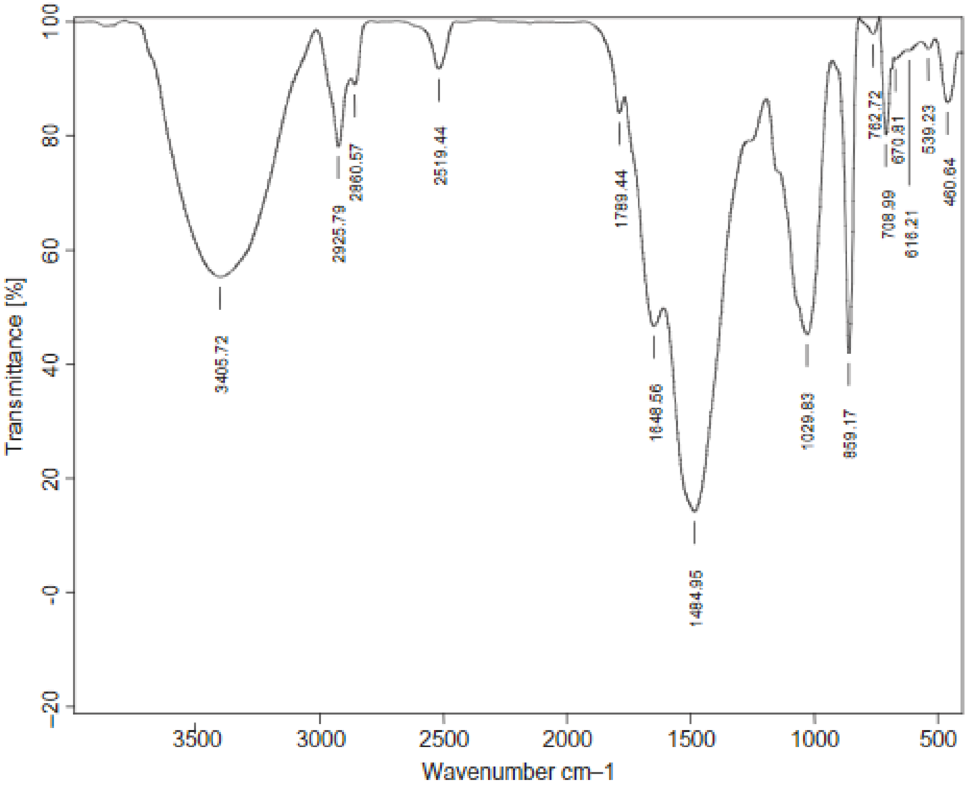


Figure 2.5.1.2: FT-IR spectra of *Scenedesmus* sp. (Sudhakar and Premalatha, 2015)

Table 2.5.1.2: Functional groups detected in *Scenedesmus* Algal Species through FTIR spectroscopy analysis (Sudhakar and Premalatha, 2015)

Peak Values (cm^{-1})	Functional Groups	Associated Biocompunds
3405	O-H	Polysaccharides
2925	Asymmetric C-H	Aliphatic groups
2519	Symmetric C-H	Aliphatic groups
1789,1648,1484	Esters	-
1700-1800	Carbonyl	-

The increment of mid and long chains of hydrocarbon was supported by the presence of aliphatic groups. Furthermore, the carbonyl stretch also shows large concentration of fatty acids. The overall results shows that the dried algal samples consist of surface bound alcoholic groups, carboxyl groups, and lipid groups (Sudhakar and Premalatha, 2015).

In another study carried out by Sasikala et al. (2016) on seaweed fertilizers, the three-seaweed liquid extract were being lyophilized before undergoing FTIR analysis of the biochemical functional groups in the samples. The three samples were of different preparation methods which is low temperature extract, cold water extract and boiling water extract. The analysis shows that there is presence of O-H group at the band waves of 3441 cm^{-1}, C-H stretch at 3031 to 2849 cm^{-1}, allene (1929 cm^{-1}) and C=O, acetate at 1732 cm^{-1}. Whereby O-H and C=O indicates the existence of alcohols. Other than that, C-O-S bending and S-O stretching of sulphate indicates that there are sulphate ester groups at peaks of 850 and 1256 cm^{-1}. Several groups were determined as well such as amide with the peak of 3654.12 cm^{-1}, alkynes and alkanes at 3307.55 cm^{-1} and 2918.44 cm^{-1} respectively, carboxylic acids at 2849.92 cm^{-1}, alkenes at 1643.73 cm^{-1}, aromatics, aliphatic amines and alkyl halide compounds at 1454.46, 1054.13 and 510.34 cm^{-1} respectively. The presence of primary amines was also detected through the stretching of N-H which has vibration at 3394 cm^{-1} and bands of N-H at 1620 and 1581cm^{-1}. At 1110 and 1024 cm^{-1} peaking relates to polysaccharides and a mild peak of 1451 cm^{-1} shows methyl groups of protein were present and can be derived from the asymmetric bending of CH_3.

A study utilizes FTIR analysis on aqueous leaf extract of *C.phlomidis* was done by Naveen and Kavitha (2021) to observe the biomolecular components available in the plant extract. Centrifugation is done at 3000 rpm for 10 minutes for plant extraction and sieved through Whatman No.1 filter paper under high pressure vacuum pump FTIR analysis. Ratio of 1:10 was used to dilute the sample. The extract was analysed using Perkin Elmer Spectrophotometer with a wavelength range between 300 to 1100 nm.

The results shows that there are eight major peaks between 800 and 3400 cm^{-1} which will be evaluated in the Table 2.5.1.3 below.

Table 2.5.1.3: Adsorption peaks and functional groups analysed by FTIR analysis (Naveen and Kavitha, 2021)

Adsorption peak	Functional groups	Associated compounds
3406.29	O-H	Alcohols and phenolics
2931.8	O-H	Carboxylic acids
2370.51	N-H	Amines and amide
1641.42	-C=C	Alkenes
1307.74	C-C (in rings)	Aromatics
1056.99	C-N	Aliphatic amines
802.39	C-Cl	Alkyl halides

2.5.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR is a quantitative technique whereby they can produce biological signatures or can be fingerprints of natural products that are of complex mixtures with unknown compositions. In one of the studies carried out to determine the organic composition of seaweed extracts, liquid extracts were being put on centrifuge at 15,000 g (relative centrifugal force) for 30 minutes to separate water soluble constituents for analysis. The clarified solution was then freeze dried prior to conducting the analysis. The solutions were then prepared by transferring freeze drying dried extract into volumetric flask of 3 mL with the weight around 99.00 mg. 1.50 mL D_2O with 99.9% purity containing reference 1.00 mg/mL TSP was added into the solution. Consequently, the flask was sealed with a stopper and was shaken vigorously in manual for approximately 2 to 3 minutes. The solutions were then made into volume of 3.00 mL with neat D_2O and a magnetic stirrer was put into the samples for vigorous stirring about 5 minutes. pH adjustments can be eliminated if the diluted solutions are in the pH range between pH 8.3 and pH 8.7. Otherwise, the sample will be adjusted to pH 8.5 with 1 M NaOH in D_2O before the solution was added to 3.00 mL. The frequency was set at 500.13 MHz at 20˚C or 25˚C for the 1H NMR experiment using Bruker DRX-500 Spectrometer (Craigie et al., 2008). The addition of D_2O is purposed to stabilize the magnetic field strength to prevent 1H containing solvents to create unnecessary inflation of NMR spectrum as high throughput measurements over several hours using auto sampling devices requires constant measuring conditions (Haslauer et al., 2019).

2.5.3 High Performance Liquid Chromatography (HPLC)

HPLC technique of isolating natural products is widely applied as it is highly versatile and robust. It is a chromatography technique that provides separation of a mixture of compounds and uses phytochemical and analytical chemistry to identify, quantify and purify the components individually in a mixture. A detector must be selected to begin with identification of any compound. Consequently, the detector is set to optimal detection setting and parameters of a separation assay should be developed which allows observation of a clean peak of known sample from the chromatography. A reasonable retention time is necessary for the identifying peak and must be well separated from the extraneous peaks at the detection levels where assay will be performed (Boligon and Athayde, 2014). UV-Vis diode array detector (DAD) and Radial Immunodiffusion (RID) are commonly incorporated into the analysis as the detector due to their high sensitivity and precise sample clean-up is necessary before injection (Misra et al., 2015).

Research done by Rajeshkumar and Jeyaprakash (2015) applied HPLC technique on *Acanthophora Specifera (Red Seaweed)* that was obtained from Gulf of Mannar, Tamilnadu, India to analyse the presence of flavonoids. The extract of the sample was prepared through dissolving 10 g of sample into 30 mL of methanol. Consequently, it was being swirled in an ultrasonic bath for 30 minutes. The extract was then filtered through Whatman 0.5 µm filter paper to filter out fibres and undissolved textures. Preconcentration of the extract was done by steaming it with nitrogen until it reaches to about 0.5 mL and another 0.5 mL of mobile phase solvent was added into it. Subsequently, the 20 µL of aqueous filtrate was injected to the HPLC column. Shimadzu Class-VPV6.14SP2, Japan that has an autosampler with 20 µL fixed loop was coupled with an UV-Visible detector was used as the HPLC instrument for this research. Due to the use of solvent A and solvent B which are water-acetic acid (25:1 v/v) and methanol respectively, the solvent gradients are formed due to elution. A dual pumping system was utilized to proportionate the solvents of solvent A to solvent B, 50% increase is designated for solvent B in 4 minutes and then increment to 80% in 10 minutes with a 1.0 mL/min flow rate. The samples were run for 25 minutes, and 280 nm wavelength was to detect by the UV detector (Lamp-D2). The results shows that there are several bioactive compounds were present in *Acanthophora Specifera* which is shown in Figure 2.5.3.1 and Table 2.5.3.1.

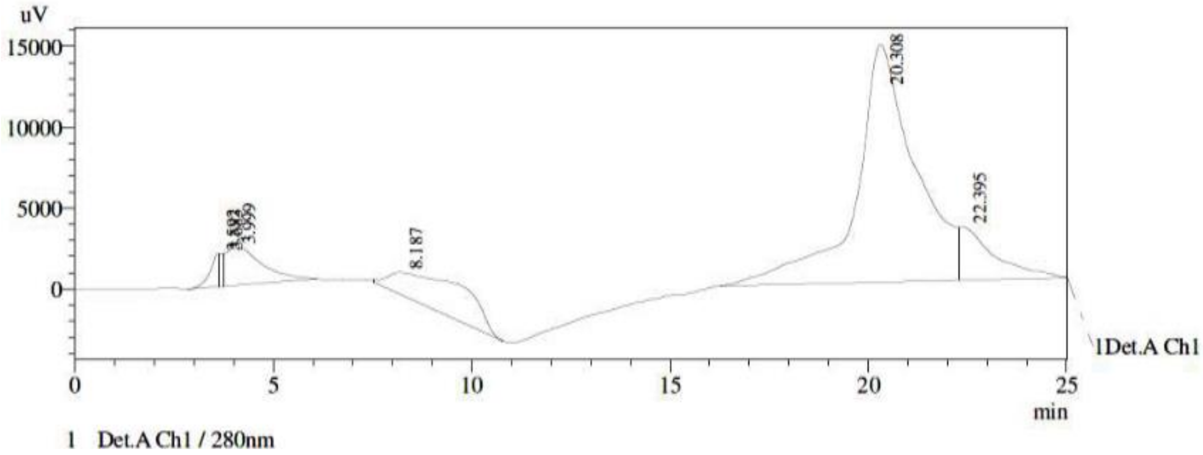


Figure 2.5.3.1: HPLC profile of Flavonoids (Rajeshkumar and Jeyaprakash, 2015)

Table 2.5.3.1: HPLC profile of Flavonoids (Rajeshkumar and Jeyaprakash, 2015)

Peak	Retention Time (minutes)	Compounds identified by literature
1	3.592	Quercetin
2	3.683	Catechol
3	3.999	-
4	8.187	Vitexin-rahmnose
5	20.308	Chlorogenic acid
6	22.395	Caffeic acid

2.6 Testing of Heavy Metals in Soil

2.6.1 Tessier Sequential Extraction

Sequential extraction is an analytical procedure to evaluate metal mobility in soils and sediments which was originally proposed by Tessier et al. (1979). The procedure allows partitioning of the metals into five fractions which includes, (i) exchangeable, (ii) bound to carbonate, (iii) bound to Fe-Mn oxides, (iv) bound to organic matter and finally (v) residual. Specific fractions are able to be extracted through the application of several reagents under a same soil.

Exchangeable phase is the first to be removed through the sequential extraction procedure where they are likely to undergo changes in sorption-desorption processes with the application of an electrolyte at a pH of 7.0. Subsequently, carbonate phase will be where trace metals that were associated with carbonate sediments are fractioned out through changes of pH to 5.0 and the application of sodium acetate and hydrogen acetate. The reducible phase (bound to Fe-Mn oxides) is usually present as nodules, concretions, cement between particles or just coating on particles and they are thermodynamically unstable under anoxic conditions where the extractions were done by reducing agents at pH 2.0 to 4.0 through hydroxylamine hydrochloride-acetic acid and sodium dithionite-citrate. The next is to extract the oxidizable phase (organic phase) through the adoption of hot hydrogen peroxide that is in a nitric acid medium of pH 2.0 coupled with metal bound to organic matter sulphides for the destruction of organic matter through oxidation. Lastly, the residual phase that is to dissolve the metals consist in crystal lattices of minerals that requires the use of digestion with a mixture of strong acids such as nitric acid or hydrofluoric acid (Ukiwe et al., 2011; Tessier et al., 1979).

A case study is done by Kumkrong et al. (2021) whereby Tessier's sequential extraction method was done to analyse 17 elements on three marine sediments. The author follows the steps of Tessier method where there were four extraction steps which is exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, bound to organic matter and sulphide materials, and lastly residuals, respectively. Before the extraction procedures, 1 g of soil samples were prepared in an extraction tube. The extraction solutions used were listed in the Table 2.6.1 below.

Table 2.6.1: Reagents used for Tessier's sequential extraction method.

Fraction	**Amount**	**Reagent**	**Remark**
Exchangeable	8 mL	1 mol/L NaOAc	Adjust to pH 8.2 using AcOH
Carbonates	8 mL	1 mol/L NaOAc	Adjusted to pH 5 using AcOH
Fe-Mn Oxides	20 mL	0.04 mol/L $NH_2OH·HCl$ in 25% (v/v) AcOH	-
Organic matter and sulphide materials	3 mL	0.02 mol/L HNO_3	Adjusted to pH 2
	5 mL, 3mL	30% (w/v) H_2O_2	Adjusted to pH 2
	5 mL	3.2 mol/L NH_4OAc in 20% (v/v) HNO_3	-

Each step was followed by centrifuging step to separate the exchangeable fraction and residue for the next step, the residue rinsed and centrifuged again to discard the supernatant. For exchangeable and carbonate phase, the steps were followed by shaking of the mixture at 30 rpm for 1 hour and 5 hours, respectively. As for Fe-Mn oxides, the mixture was agitated in a hot block continuously at 96˚C±3˚C for 6 hours. Consequently, to extract organic matter and sulphide materials, the first mixture was occasionally agitated for 2 hours with 85˚C±2˚C for 3 hours, and after cooling, the second mixture of added 3 mL of 30% (w/v) H_2O_2 was heated in a hot block for 3 hours with the previous temperature setting. Subsequently, another 5 mL of NH_4OAc in 20% (v/v) HNO_3 was added and followed by adding 15 mL of H_2O after the previous sub step was cooled, and finally the mixture was agitated continuously at 30 rpm for 30 minutes. The final result consists of fractions bound to organic matter and residue after centrifuging (Kumkrong et al., 2021). A final digestion of the residual was done by adding a strong acid that is 20 mL of aqua regia and heated at 70˚C for 30 minutes. The solution was then filtered with a 0.45µm syringe filter and subsequently diluted to 50 mL with distilled water (Kumkrong et al., 2021; Lee et al., 2021).

2.6.2 Modified Sequential Extraction for Arsenic

As arsenic (As) can be distributed into more fractions in the residual fraction that is stated by Tessier et al. (1979), a modified sequential extraction method is proposed to further break down the residual fraction of As into be bounded in silicates, pyrite, orpiment compounds as they do not contribute to the final residual fractions and only yield to approximately 40-50% (Javed et al., 2013a).

The fractions of arsenic can be categorized into several category as As can be sorbed onto material surfaces of compounds like oxides of iron, aluminium, and manganese (Mn). Other than that, arsenic can also be coprecipitated with iron oxyhydroxides which is a mechanism that strongly immobilizes arsenic in sediments. However, arsenic that are associated with hydrous manganese oxides are less stable compared to iron oxyhydroxides. Moreover, it is also discovered that As will be adsorbed on the edge of clay minerals as well as calcite surfaces. Additionally, arsenic bearing silicate materials and sulphide ores are considered more immobile forms of arsenic which occurs naturally in nature; although the sulphides and silicate materials tend to liberate arsenic to the environment through weathering which is likely to result in the formation of metastable new secondary minerals through thermodynamic processes.

Thus, a modified sequential extraction technique for As was introduced by Javed et al. (2013b), there are 10 fractionations of As that needs to be extracted. The fractions of As, reagents to be used and possible extraction mechanisms were listed in Table 2.5.2 below. Each extraction steps were applied with a minimum of 1:100 sediment to extractant ratio to ensure the solid phase was completely reacted with the extractant. However, a minimum 1:25 sediment to extractant ratio was allowed. Supernatant was obtained through centrifugation is done for 40 minutes with 6000 rpm on the sediment extractant suspensions that were shaken for a specified duration beforehand. The supernatant was filtered through a 0.45µm PTFE filters and the sediments were washed through for 30 minutes with nanopure water after each extraction was completed. Solution of preceding fraction immerses the washing solution after centrifugation. Subsequently, acidification was done on the poured extractants with 1% HNO_3 to maintain mobile conditions for ICP-MS analysis.

For the first step in extracting F1, 0.4 g of sediment was added into a 50 mL centrifuge tube followed by 40 mL of nanopure water and agitated vigorously for 30 minutes and centrifuged for separation of supernatant. For F2, 1 M NaOAc was added and shaken for 2 hours. Subsequently, to extract F3 the residue from F2 was agitated with 1 M NaH_2PO_4 for 16 hours and the process repeats for a second extraction for 24 hours. For F4, 5 hours is needed to recover residue from F3 and 1 M NaOAc was applied for the extraction. Tamm's reagent is used for F5 extraction whereby 0.2 M ammonium oxalate and oxalic acid in the dark where the tubes have to be wrapped in aluminium foil for 2 hours were prepared. Furthermore, to extract F6, the suspension was shaken with reagent mentioned for 2 hours and another repetition was done to ensure crystalline oxyhydroxides were completely being extracted. Extraction of F7 requires 10 M of HF was applied for 24 hours. Intermediately, at the 16th hour of the 24 hours, 5 g of boric acid was added to react HF with boron which produces fluoroboric acid that is equally strong as of HNO_3 to maintain soluble As form. To extract F8, 16 M of HNO_3 was added to extraction for 2 hours and the with another repetition. The extraction of F9 uses 30% H_2O_2 together with 1M NH_4OAc in the ratio of 1:2 for 16 hours. Lastly, the remaining sediment sample was extracted with concentrated HNO_3 with assistance with microwave digestion unit to determine F10 (Javed et al., 2013b).

Table 2.6.2: Modified sequential extraction procedure for arsenic fractionation (Javed et al.,2013b)

Fraction	Target phase	Reagents	Remarks	Possible mechanisms
F1	Soluble As	Deionized water	-	Dissolution with water
F2	Loosely absorbed As	1 M NaOAc	Adjusted to pH 8.2	Anion exchange of acetate for As
F3	Strongly absorbed As	1 M NaH_2PO_4,	Adjusted to pH 5	Anion exchange of PO_4^{3-} for AsO_4^{3-} and AsO_3^{3-}
F4	Carbonate bound As	1 M NaOAc	Adjusted to pH 5	Dissolution of carbonate minerals to liberate As
F5	As co-precipitated with amorphous Fe, Al and Mn oxyhydroxide	Tamm’s reagent (ammoniumoxalate /oxalicacid)	Adjusted to pH 3	Ligand promoted complexation and dissolution of Fe, Al and Mn oxyhydroxide
F6	As co-precipitated with crystalline Fe, Al and Mn oxyhydroxide	Ti-citrate-EDTA-bicarbonate	Adjusted to pH 7	Ti induced reduction of Fe^{3+} to Fe^{2+}
F7	As associated with As oxides and silicate clays	10 M HF	-	Dissolution of silicate clays and As oxides

F8	As coprecipitated with pyrite and amorphous orpiment	16 M HNO_3	-	Oxidation of pyrite and amorphous sulfides
F9	Organic matter and secondary sulphides	30% H_2O_2 + 1M NH_4OAc (1:2)	Adjusted to pH 2	Oxidation of organic matter and secondary sulfides
F10	Residual As	Concentrated HNO_3	-	Microwaved assisted digestion

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

Discussion on the setup and methodology which includes the materials and apparatus necessary, flow chart of experimental procedures, preliminary analysis of TM and LGN with the use of different screening methods and analysis of soil will be done in this chapter.

The flow chart of the experimental procedure was followed as shown in Figure 3.1.1.1. The experiment begins with soil sample preparation where heavy metal was added into soil sample for artificial contamination with 1 month incubation. While soil samples were under incubation, soil stabilizer was prepared for characterization by screening of TM and LGN through FTIR and HPLC with pre-treatment being done beforehand. After the first incubation of contamination with heavy metals, the pH, TOM, and moisture content were being determined with various experimental procedures. Consequently, sequential extraction procedures were being carried out on the contaminated soil samples to obtain the heavy metal amounts before treatment. Carrying on, the next incubation was being carried out to treat the contaminated soil samples with TM and LGN with the incubation period of 1 month. After the final incubation was done, soil properties were once again being determined and another round of sequential extraction was being carried out to observe the efficiency of TM and LGN in heavy metal immobilization.

3.1.1 Flow Chart of Experimental Procedure

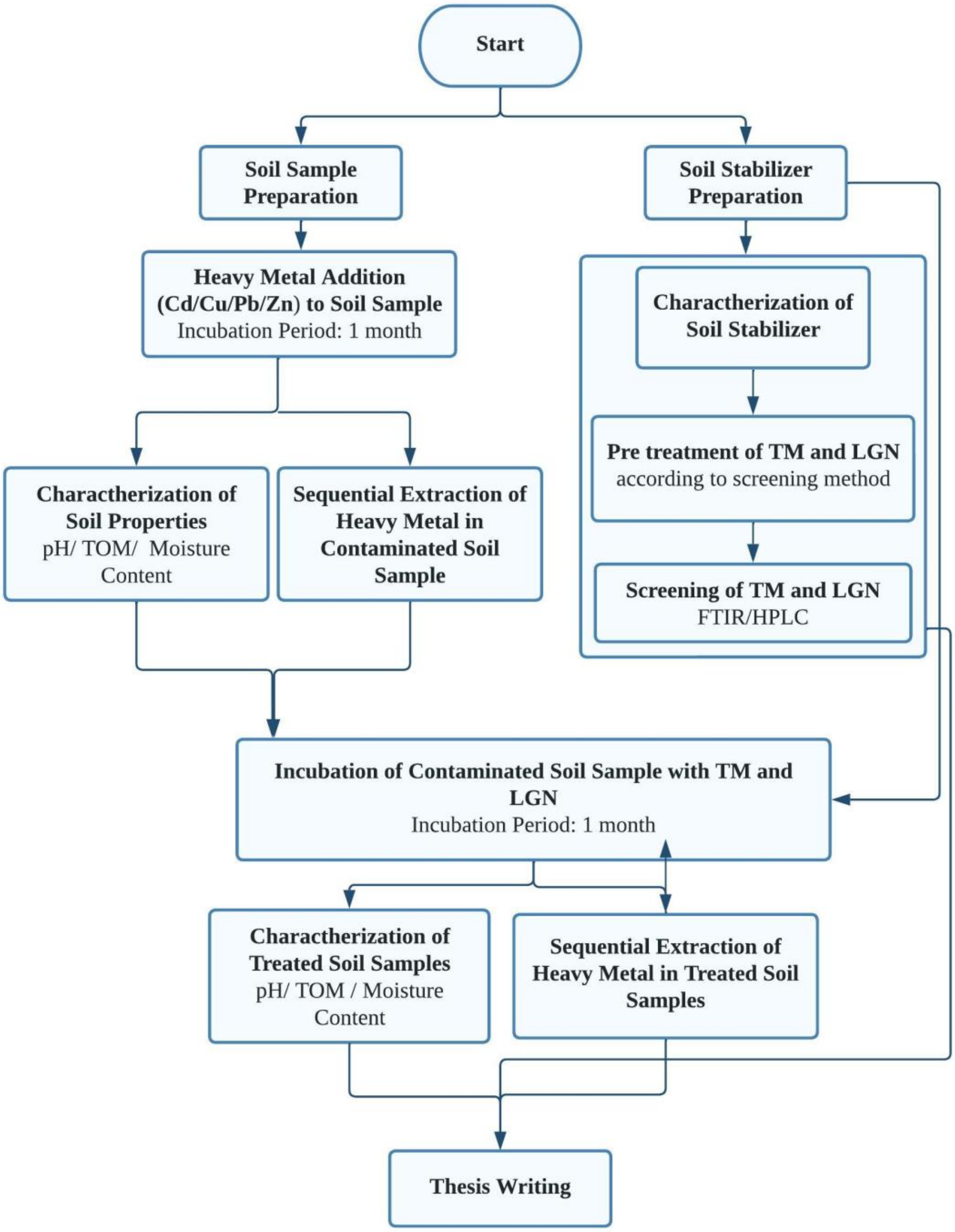


Figure 3.1.1.1: Flow Chart of the experimental procedure

3.2 Preparation of Materials and Apparatus

3.2.1 Soil Stabilizers

The plant extract, TM used in this study is a product of Best Environmental Technologies (M) Sdn Bhd. and it is available to be bought commercially. The main ingredients of TM include organic materials such as kelp, alfafa meal, barley grain, calcium carbonate, liquid fish, barley straw, molasses, wheat straw and sulphur. The compositions were illustrated in Figure 3.2.1 below.

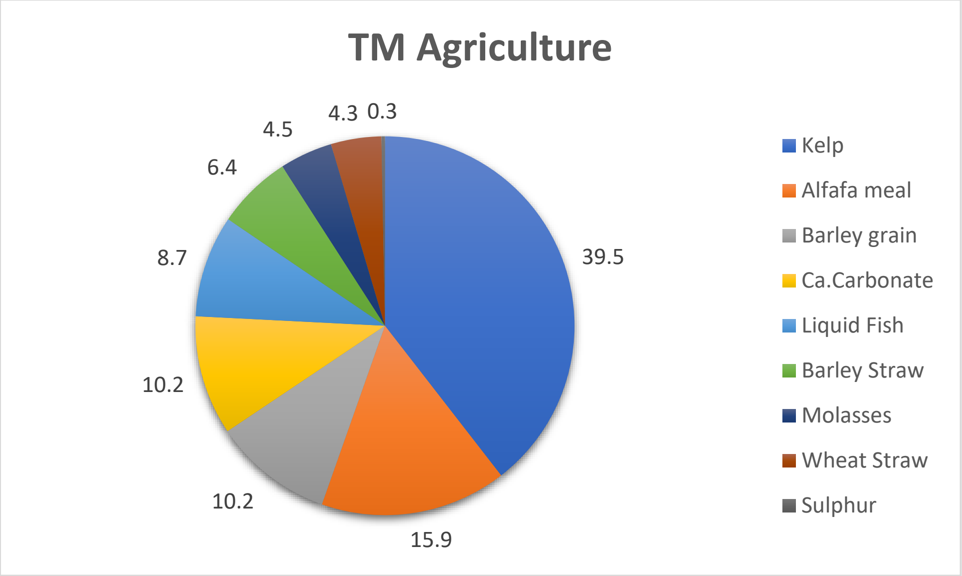


Figure 3.2.1.1: Compositions of plant extract from TM Agriculture.

The lignin (LGN) used in this study was alkaline lignin with CAS Number 8068-05-1 bought from Sigma-Aldrich in powder form which consist of less than 3.6% of sulphur content and is having an approximate pH around pH 10 to pH 11. The colour of the powder is considered brownish. The structure of the product was shown below at Figure 3.2.1.2

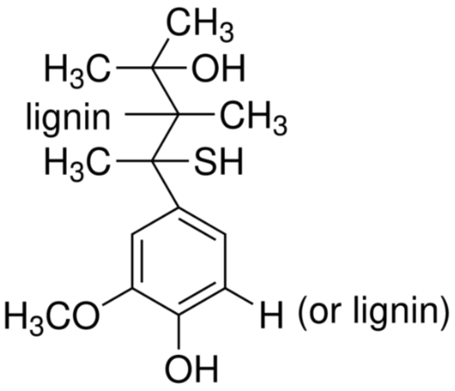


Figure 3.2.1.2: Structure of alkaline lignin (Sigma Aldrich, n.d.).

3.2.2 Soil Sample

Contamination of HM on commercially bought soil was done by preparing 6 kg of black soil samples bought from Lotus, Kampar into containers each with L×W×H of 30×20×25cm. The heavy metal solution was added into the soil and incubated for 1 month in room temperature before beginning the experiment and soil moisture was maintained by periodically spraying deionised water (Muhammad et al., 2009).

Heavy metal solutions were obtained by dissolving powder forms of the heavy metal such as $CdCl_2 \cdot H_2O$, $CuSO_4$, $Pb(NO_3)_2$ and $ZnCl_2$ to deionised water. Table 3.2.2 shows the number of heavy metals required to contaminate soil sample to its targeted contamination amount. After 1 month of incubation, 2% w/w of TM and LGN were added into duplicates of 100 g of contaminated soil samples for treatment of the contaminated soil samples as 2% w/w were able to provide significant results (Lee et al., 2021).

Table 3.2.2: Heavy metal contamination details

Heavy Metal	Heavy Metal (Powder Form)	Targeted amount to contaminate soil sample (mg/kg)	Amount of HM powder to be dissolved in DI water (mg)
Cd	$CdCl_2 \cdot H_2O$	8.33	89.50
Cu	$CuSO_4$	16.67	251.17
Pb	$Pb(NO_3)_2$	41.67	399.63
Zn	$ZnCl_2$	62.50	781.66

3.3 Characterization of Soil Sample

3.3.1 pH

pH meter

Calibration of electrode system on the pH meter was done before conducting pH testing. The calibration was done at pH 4, pH 7 and pH 10 using a buffer solution. Centrifugation was done on 20 g of soil sample on a 50 mL centrifuged tube with addition of 20 mL of deionized water. The suspension was continuously stirred for 5 minutes by using a shaker from Vortex-T Genie 2). The mixture was then centrifuged for 15 minutes with 4000 rpm to separate the suspension. Then, the supernatant was pipetted for pH measurement using the Hanna HI 2211 pH meter (Pansu and Gautheyrou, 2006). Care was taken to immerse the glass probe deep into the supernatant for sufficient electrical contact.

3.3.2 Total Organic Matter (TOM)

Loss of Ignition (LOI) Method

Empty crucibles were cleaned and dried prior to the experiment. The weight of the empty crucible was obtained using the weighing machine (W1). Consequently, 10 g of air-dried soil samples were placed in the crucible and was kept in the drying oven at 105 ˚C for more than 8 hours. After the sample was cooled down in the desiccator, the weight of the sample was obtained using the weighing machine (W2). The next step is to put the samples into the muffle furnace at 360 ˚C for 3 hours. The furnace was let cool until 100 ˚C until the samples could be taken out. After being cooled down in the desiccator, the weight of the sample was taken (W3) (Pansu and Gautheyrou, 2006). The TOM was obtained through the following formula:

$$OM\% = \left(\frac{W2 - W3}{W2 - W1}\right) \times 100\%$$

Whereby:

W1 is the weight of the empty crucible.

W2 is the weight of crucible and sample after oven drying at 105˚C.

W3 is the weight of crucible and sample after muffle furnace at 360 ˚C.

3.3.3 Moisture Content

Loss on Drying Method

Moisture content was determined through the loss on drying method. 10 g of soil sample was added into the crucible and weighed. The mass of empty crucible was weighed and recorded as W0. The mass of crucible and soil sample was obtained (W1). The soil samples were put into the oven at 105 ˚C for 8 hours. After 8 hours, the soil samples were taken out and put on a desiccator to be cooled down. After cooling down, the dried soil samples were weighed again (W2) (Pansu and Gautheyrou, 2006). The following formula calculates the moisture content of the soil samples:

$$Moisture\ Content\ (\%) = \frac{W1 - W2}{W1 - W0} \times 100\%$$

Whereby:

W0 is the weight of empty crucible.

W1 is the weight of crucible and soil sample before drying.

W2 is the weight of crucible and soil sample after drying.

3.4 Testing of Heavy Metals in Soil Sample

3.4.1 Sequential Extraction of Heavy Metals in Soil

Extraction was done in sequence of liberating exchangeable metals, carbonate phase, reducible phase, organic matter, and residual phase, respectively. The following extraction procedure were carried out in 50 mL polypropylene centrifuge tubes to reduce the loses of solid material. Tessier's method of sequential extraction procedures was followed in this experiment as reviewed in Chapter 2 at Section 2.6.1.

After each step of extraction was done, the solution is centrifuged at 4000 rpm for 30 minutes to separate the residue and supernatant. First four fractions of supernatant extracted which contains metal content was analysed through flame atomic absorption spectrophotometry (FAAS) whereas the residues were washed with 8 mL of deionized water to prevent solid materials being solubilized excessively (Tessier et al., 1979).

3.5 Screening Methods of Soil Stabilizer

3.5.1 Fourier Transform Infrared Spectrometry (FTIR)

FTIR-KBr method was performed on the screening of both soil stabilizer, TM and LGN.

i) Pre-treatment of Soil Stabilizer

TM plant extract was required to be air dried to solidify the original liquid form. Alkali lignin was bought commercially from Sigma Aldrich and did not undergo pre-treatment as it is already in a solid form.

ii) Pre-Cleaning of FTIR Accessories Kit

Pre-cleaning of FTIR accessories including sample holder, evacuable die set and grinder were done by using ethanol through gentle wipes.

iii) KBr pellet preparation

KBr pellet was dried in the oven before usage. After that, the sample and KBr were mixed in the ratio of 1:10. The mixture were homogenized and grinded into a fine powder. The powder mixture was then transferred to the evacuable die set. Pressure was applied to the sample with 4000 psi and care was taken to not over pressurize it. The KBr pellet was obtained after the pressure was released.

iv) Analysis of sample

The range was set at 400 - 4000 cm^{-1} before running the FTIR software. The peaks were identified, and values were reported in *Chapter 4* (Sudhakar and Premalatha, 2015).

3.5.2 High Performance Liquid Chromatography (HPLC)

Preparation of mobile phase was done by filtering 1 litre of 0.01 M with 0.22 µm filter to prevent column and capillary clogging (Gordobil et al., 2014).

i) Pre-treatment of soil stabilizer

1 mL of soil stabilizer was extracted with 0.5mL of 0.5M HCL and dilution was done to 50 mL with addition of deionized water. The sample was then put under ultrasonic

water bath for 1 hour. Subsequently, filtration of sample was done with 0.22 µm filter. The filtered sample was then injected into the Agilent Hi-Plex H, 300× 7.7 mm LC Column with injection volume of 20 µL under temperature of 65˚C.

ii) HPLC Analysis

Agilent 1100 HPLC Equipment was coupled with an UV-Visible detector was used as the HPLC instrument for this research. The mobile phase used was 0.01 M H_2SO_4 and the flow rate was controlled at approximately 0.60 mL/min. The data were recorded and analysed in *Chapter 4.*

3.6 Mobility Factor

Heavy metal mobility depends on the amount of mobile or unstable phase (exchangeable, carbonate phase) obtained in soil over the number of stationary or stable phases (FeMn oxides, organic, residual phase) of heavy metals in soil as the unstable phases poses a larger threat in harming the environment (Asmoay et al., 2019). The mobility factor is calculated through the formula shown below by Kabala and Singh (2001):

$$Mobility\ Factor\ (MF) = \frac{F1 + F2}{F1 + F2 + F3 + F4 + F5} \times 100\%$$

Whereby:
F1 is exchangeable fractions (unstable).
F2 is carbonate fractions (unstable).
F3 is FeMn oxide fractions (stable).
F4 is organic fractions (stable).
F5 is residual fractions (stable)

The mobility factor of each heavy metal was recorded and discussed in *Chapter 4*.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of Biomass-based Soil Stabilizer on Soil Properties

The following sub-sections will be discussing on the effect of biomass- based soil stabilizer on the changes in soil properties. The outcome of each parameter was shown in average with duplicated data where the raw data can be found in APPENDIX A, B and C.

4.1.1 pH

The pH of soils before contamination and treatment is 7.59 which is at the neutral site. After contamination with heavy metal solutions, the pH decreased in a considerably low manner to 6.03. According to Kicińska et al. (2022), the decrease of pH after contamination of heavy metals is caused by the dissolution of minerals and hydrous oxides of Fe, Mn and Al where heavy metal ions tend to be absorbed. Similar observations were also shown in the research from Schubert et al. (2019) whereby the pH decreases similarly as well after addition of heavy metal ions as it is mostly due to divalent ions like Cu^{2+} and Pb^{2+} which caused a decrease of pH from 7.40 to approximately pH 6.47 and 6.40 respectively. Meanwhile Cd^{2+} and Zn^{2+} provides less significant change in pH from the study conducted.

The treatment of contaminated soils with both soil stabilizers has caused increase in pH of the soils. Although the significance can be seen more for LGN treated soils compared to TM. Addition of LGN has increased the soil pH from 6.03 to 8.84 which caused the soil to have alkaline conditions since the method of extraction for the commercially bought LGN was done in alkaline conditions, the change of pH for soil is expected. Research shows similar results when adding LGN with increase of soil pH by around 2 units which is due to present of hydroxyl ions being increased the pore water of soils (He et al., 2022). For the case of TM, the pH increased from pH 6.03 to 7.00 which brings the soil conditions back to neutral.

Table 4.1.1: Results for pH before and after treatment.

Soil Samples		**pH**
Before Treatment	Before Contamination	7.59±0.02
	After Contamination	6.03±0.15
After treatment	TM	7.00±0.03
	LGN	8.84±0.14

4.1.2 Total Organic Matter (TOM)

The results of TOM before treatment and after treatment with soil stabilizers had disparity between TM and LGN. The TOM before treatment was 3.42% and after being treated with TM, the TOM increase insignificantly to 3.82% while for treatment with LGN, the TOM had a significant increase to 5.24%.

Table 4.1.2: Results for total organic matter before and after treatment

Soil Samples		**TOM (%)**
Before Treatment		3.424±0.10
After Treatment	TM	3.818±0.03
	LGN	5.241±0.03

The significant increase of soil OM for LGN treated soils is due to the organic nature of LGN properties. Studies conducted by Mohamed et al. (2010) is able to support this finding as similar results were obtained through his research on the application of organic amendments such as rice straws, green manure and pig manure are able to significantly increase the TOM of the soil. The organic matter content was considered to be slightly lower due to the nature of physiochemical of black soils which was used in this study, the statement is supported by Huat et al. (2012) on the content of organic matter of black soil which generally ranges from 0.5 to 5.0%.

4.1.3 Moisture Content

Table 4.2.4 below shows the changes of moisture content after treatment with different soil stabilizers. The treatment for moisture contents for TM treatment were not as remarkable compared to treatment with LGN. The moisture content before treatment was 1.420% and the after treatment with TM, the moisture content increased to 1.528%. Meanwhile, the moisture content increased significantly to 2.385% for LGN treatment on the soil samples.

Table 4.1.3: Results for moisture content before and after treatment

Soil Samples		**Moisture content (%)**
Before Treatment		1.420±0.06
After Treatment	TM	1.528±0.01
	LGN	2.385±0.10

The moisture content in soil is one of the most critical factors in the heavy metal mobility in soils. Through an experiment conducted by Dang et al. (2021) shows that the immobilization of exchangeable heavy metals can be increased with increasing soil moisture content during incubation period as immobilization with increasing moisture content results in more formation of crystallized FeMn oxides forms of heavy metal. Additionally, the increase in moisture content indicates that there are more negative charges on the surface of the adsorbent, triggering boundations with the adsorbents and increasing the transformation rate (Zheng and Zhang, 2011).

Having said that the soil moisture content did increase after treatment with both soil stabilizers, but the soil moisture content still tends to be lower in this research due to the shallow depth of soil samples. The shallow depth increases the evaporation rate causing the high moisture loss resulting in the inability to store water (Osman, 2018).

4.2 Effect of Biomass-based Soil Stabilizer on Heavy Metal Concentration

The following sub-sections will be discussing on the effect of biomass- based soil stabilizer on the changes in heavy metal concentration through fraction of exchangeable, carbonate, FeMn oxides, organic and residual. The result of each fraction was shown in average with duplicated data where the raw data can be found APPENDIX D.

4.2.1 Cd

From Figure 4.2.1, it is shown that the exchangeable fractions of Cd had decreased for both TM and LGN treatments, although, the result is more significant for stabilization with LGN compared to TM with approximately 8% decrease of exchangeable fractions from 19.61% to 8.37% while treatment of TM only decreased approximately 1% from 19.61% to 18.43%. As for carbonate fractions of Cd, there significance was seen for soil stabilized with TM whereby there is an approximate 7% decrease in carbonate fractions in the soil samples which is from 58.32% to 51.45%. Meanwhile, soil samples treated with LGN consist of a slight increase of 1% of carbonate fractions from 58.32% to 59.26%. Increase can be seen in FeMn oxides, organic and residual fractions of Cd for both LGN and TM treatments. Whereby for FeMn oxides, TM increased the fractions from 20.34% to 24.61% and treatment with LGN increased the FeMn oxides fraction to 27.15%. As for organic phase, both treatments increased the organic phase by approximately 2%, the soil sample treated with TM has an increase in organic phase from 1.14% to 3.62% while soil sample treated with LGN increased it to 3.04%. Similar results go for the residual phase where the percentage of residual fractions of Cd increased from 0.58% to 1.89% for treatment with TM while treatment using LGN increased it to 2.17%. This indicates that the mobility of Cd can be decreased as the Cd fractions were in more stable forms.

As analysed it is observed that most of the exchangeable fractions have diverted to the more stable parts like FeMn oxides, organic and residual fractions. This is due to the fact the addition of LGN increases the soil pH which resulted negative charges on soil colloidal surface to increase which is caused by the formation of hydroxides which leads to possible mechanisms of immobilization of Cd including precipitation and metal complexation (He et al., 2022). Although, the immobilization of Cd as observed from Figure 4.3.1 had a significant decrease in exchangeable fractions after treatment with both soil stabilizers, however, the overall unstable fraction of Cd still occupies the majority of the fractions. It is suggested by Singh and Kalamdhad (2011) that the large occupation of unstable forms of Cd can be explained by the highly toxic nature of Cd which consist of greater mobility and has lower affinity for soil colloids.

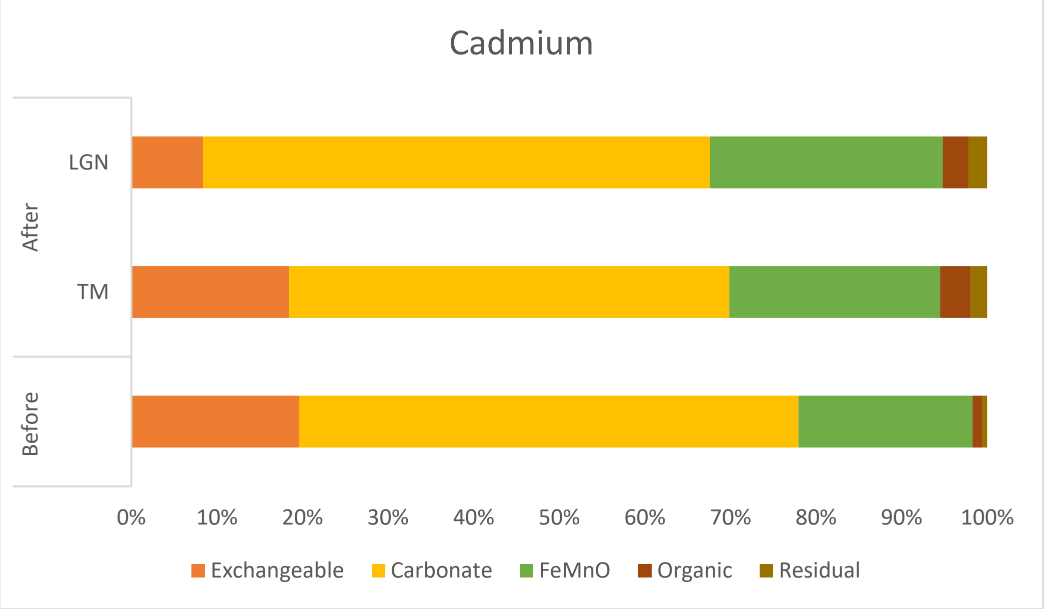


Figure 4.2.1: Distribution of Cd in soil samples before and after treatment by different soil stabilizer.

4.2.2 Cu

As shown in Figure 4.2.2, exchangeable fractions increased slightly for TM by about 0.2% from 1.14% to 1.36% while treatment with LGN has a larger increase of exchangeable fractions of Cu compared to TM which is from 1.14% to 2.55%. On the other hand, the carbonate fractions can be seen to decrease significantly for both treated soil samples. The percentage of carbonate fractions for soil samples treated with TM decreased it from 16.94% to 11.80% and carbonate fractions of soil samples treated with LGN decreased from 16.94% to 8.57%. Although, the FeMn Oxides fractions decreased at a similar amount for soil sample treatment with TM and LGN but there is a high increment for the rest of the stable forms like organic and residual phase. TM treatment decreased the fractions of FeMn oxides from 42.42% to 34.78% while LGN treatment decreased it to 35.43%. As for organic phase of Cu, TM increased the organic fractions of Cu from 32.84% to 43.14% and for LGN, the organic fractions increased to 44.09%. Lastly for the residual phase, TM increased the residual fractions of Cu by approximately 2% and 3% for LGN, from 6.65% to 8.92% and from 6.65% to 9.37% respectively.

The increase in organic fractions of Cu may be related to the dissolved organic carbon in the soil. As mentioned by Liang et al. (2017), Cu has a high preference of affinity to dissolved organic matter which may be the results of the reduction of Cu mobility in the soil samples. The presence of dissolved organic matter is highly in relation with the presence of functional groups in both soil stabilizers which favours the formation of strong metal binding. Studies showed that Cu has high affinity to bind with phenolic and carbonyl groups forms a highly stable ring structure with high stability constant which increases the organic forms of Cu under complex formations and possible mechanisms of chelation (Manceau and Matynia, 2010; Araújo et al., 2019).

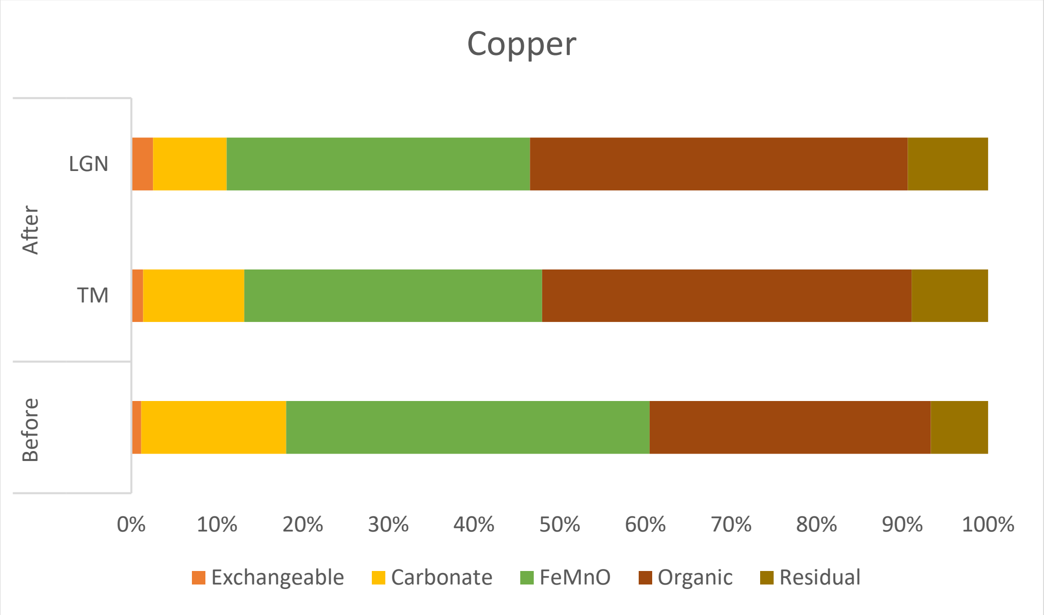


Figure 4.2.2: Distribution of Cu in soil samples before and after treatment by different soil stabilizer.

4.2.3 Pb

Pb had an increase in exchangeable fractions of Pb after treatment with both TM and LGN which can be seen in Figure 4.2.3. TM increased the exchangeable fraction of Pb from 0.78% to 5.77% while LGN increased it to 7.24%. As for the carbonate fractions, it can be seen that the fractions of Pb had both been decreasing after treatments. TM decreased it from 38.51% to 32.24% and LGN decreased it to 29.24%. Comparably fractions of FeMn oxides decreased as well for both treatments whereby treatment of TM decreased the percentage of fractions of FeMn oxides from 43.88% to 39.82% while treatment of LGN decreased it to 39.47%. The organic phase of Pb reduces notably too after using both treatments. From 11.55% to 10.39% for TM treated soil samples, and from 11.55% to 9.86% for treatment with LGN. The contrast goes for residual phase whereby it can be seen that there is an increase in the stable forms of lead. A large number of stable forms resulted in the residual phase whereby the amount increased from 5.27% to 11.78% for treatment of TM while LGN increased the percentage to 14.20% which is remarkably high immobilization in the residual phase.

According to research done by Zhang et al. (2004), exchangeable fractions of Pb increased as well from 2.86% to 3.64% after being treated with LGN in his soil sample which has a similar result in Figure 4.3.3. Although there was no specific explanation found in the research, however, there is a high possibility that the increase in exchangeable Pb has close proximity with the binding of hydroxyl ions in soil stabilizers. This statement is supported by Sasan Narkesabad et al. (2023) whereby the author stated that smaller ionic radius of molecules has higher affinity to bind on binding sites of the soil stabilizers. With the above statement, it can be deemed that Pb had high exchangeable fractions as most of the binding sites of soil stabilizers had been occupied by other heavy metals like Cd, Cu and Zn as Pb consist of a large ionic radius amongst them. Subsequently, it is also stated that with higher ionic radius, it would result in a higher reactivity to aqueous medium which could be the potential explanation of increase in exchangeable fractions of Pb (Okieimen et al., 2005).

Although there is an increase in exchangeable fractions, it is observed that the there is some redistribution between the FeMn oxide fractions and the residual fractions. The redistribution is highly dependent on the mechanism of adsorption of

Pb on to the surfaces of alkaline lignin with the combined action of electrostatic attractions (He et al., 2021; Lwin et al., 2018; Bashir et al., 2018).

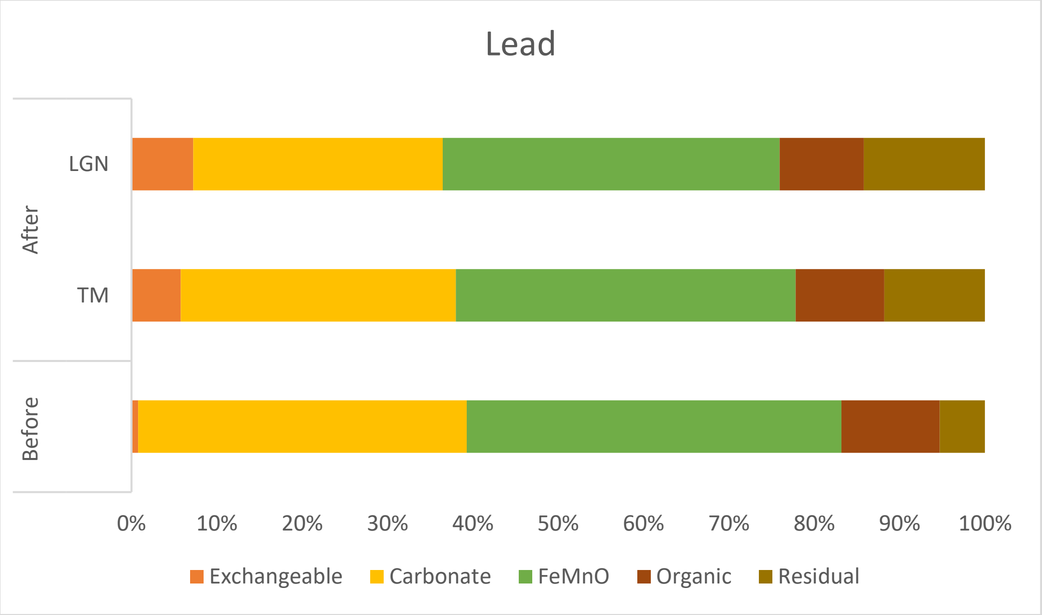


Figure 4.2.3: Distribution of Pb in soil samples before and after treatment by different soil stabilizer.

4.2.4 Zn

As shown in Figure 4.2.4, it can be seen that increase of exchangeable fractions of Zn is obvious in soil samples treated with TM, whereby the percentage increased from 0.09% to 1.06% and percentage increased from 0.09% to 0.28% for soil samples treated with LGN. Consequently, for carbonate fractions of Zn, both treatments manage to decrease it from 44.03% to 39.05% for TM and to 35.53% for LGN treatments. For Zn fractions of FeMn oxides, both treatments increased the percentage from 52.81% to 55.66% and to 60.52% for TM and LGN treatments respectively. However, there is a decrease in stable forms of Zn in the organic phase, whereby the percentage decreased from 2.42% to 2.06% for TM and to 1.99% for LGN. On the other hand, TM had more significant result in increasing the percentage of fractions of residual Zn from 0.63% to 2.18% while LGN treatment only increased it to 1.67%.

From Figure 4.2.4, it can be seen that the unstable forms, especially the carbonate forms of Zn were mostly diverted to FeMn oxide forms after treatment with soil stabilizers. According to Wu et al. (2018), the immobilization of Zn highly depends on the mechanism of precipitation from the complexation of functional groups present in the surface of the soil stabilizers. Even before treatment, majority of Zn is colluded with FeMn oxide fractions due to their nature of strong association with oxides (Xian and Shokohifard, 1989). Another factor which causes the amounts of Zn in Fe Mn oxides fractions to be higher is due to the increase in pH levels which is consistent with the result from Section 4.2.1 where the increment of pH after treatment where Zn was precipitated and being retained as oxides and hydroxides (Iyengar et al., 1981).

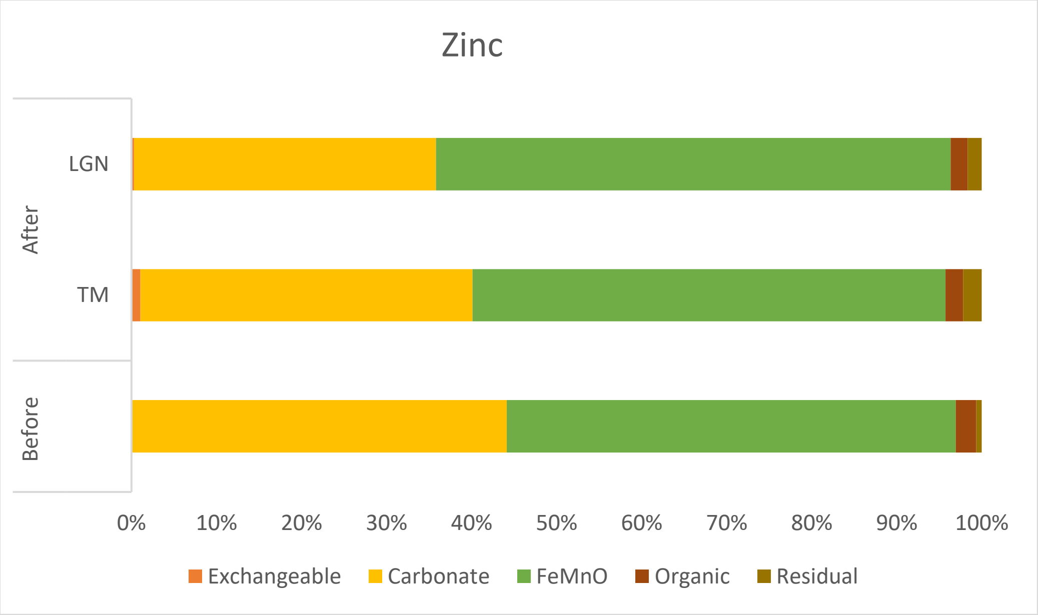


Figure 4.2.4: Distribution of Zn in soil samples before and after treatment by different soil stabilizer.

4.3 Characterization of Biomass-based Soil Stabilizer

The following sub-sections will be discussing on the results of characterization of both biomass-based soil stabilizers. Literatures were studied and compared with the result obtained from the analysis.

4.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The Figure 4.3.1 shows the FTIR results obtained through KBr method whereby the wavenumber spectra range from 400 cm $^{-1}$ to 4000 cm $^{-1}$. In the region of functional groups, it can be seen that both soil stabilizers show close to similar trends of peaks. Results shows that both TM and LGN consist of a strong broad peak that is ranging from 3200 cm $^{-1}$ to 3600 cm $^{-1}$ which designates the existence of hydroxyl group (Nandiyanto et al., 2019). Another peak observed for both soil stabilizer is at the wavenumber ranging around 1635 cm $^{-1}$ which shows the presence of hydrophilic aromatic C=C or amide C=O stretching which is also considered as hydrophilic functional groups (Pärnpuu et al., 2022).

While in the fingerprint region, it can be observed that LGN consist of more peaks were than TM, however there were no specific peaks. For LGN, fingerprint peaks that can be specify locates around at approximately 1514 cm $^{-1}$ which may indicate the present of C=C aromatic skeletal stretching or phenolic hydroxyl groups and at peak 1267 cm $^{-1}$ indicates the present of stretching of C-O in lignin and linkage of C-O in guaiacyl units (Chen et al., 2010). As for TM, there were no specific peaks that can be identified on the fingerprint region.

Similar results of FTIR analysis were obtained from lignin extraction of barley straw which shows remarkable result in adsorption capacity of heavy metal in water as the peaks mentioned in Table 4.3.1 was present in the research. According to Mohammadabadi and Javanbakht (2020), the guaiacyl units and OH groups present in the soil stabilizers are responsible for creating strong bonds with metal cations.

Table 4.3.1: FTIR peaks identified from literature review.

Wavenumber (cm $^{-1}$)		Assignment	Reference
Exp.	Lit.		
3446	3200 - 3600	O-H group	(Nandiyanto et al., 2019)
1635	1630 - 1635	C=C or amide C=O stretching	(Pärnpuu et al., 2022)
1514	1510	C=C aromatic skeletal stretching or phenolic hydroxyl groups	(Chen et al., 2010)
1267	1260-1270	Stretching of C-O in lignin and linkage of C-O in guaiacyl units	(Gordobil et al., 2014; Chen et al., 2010)

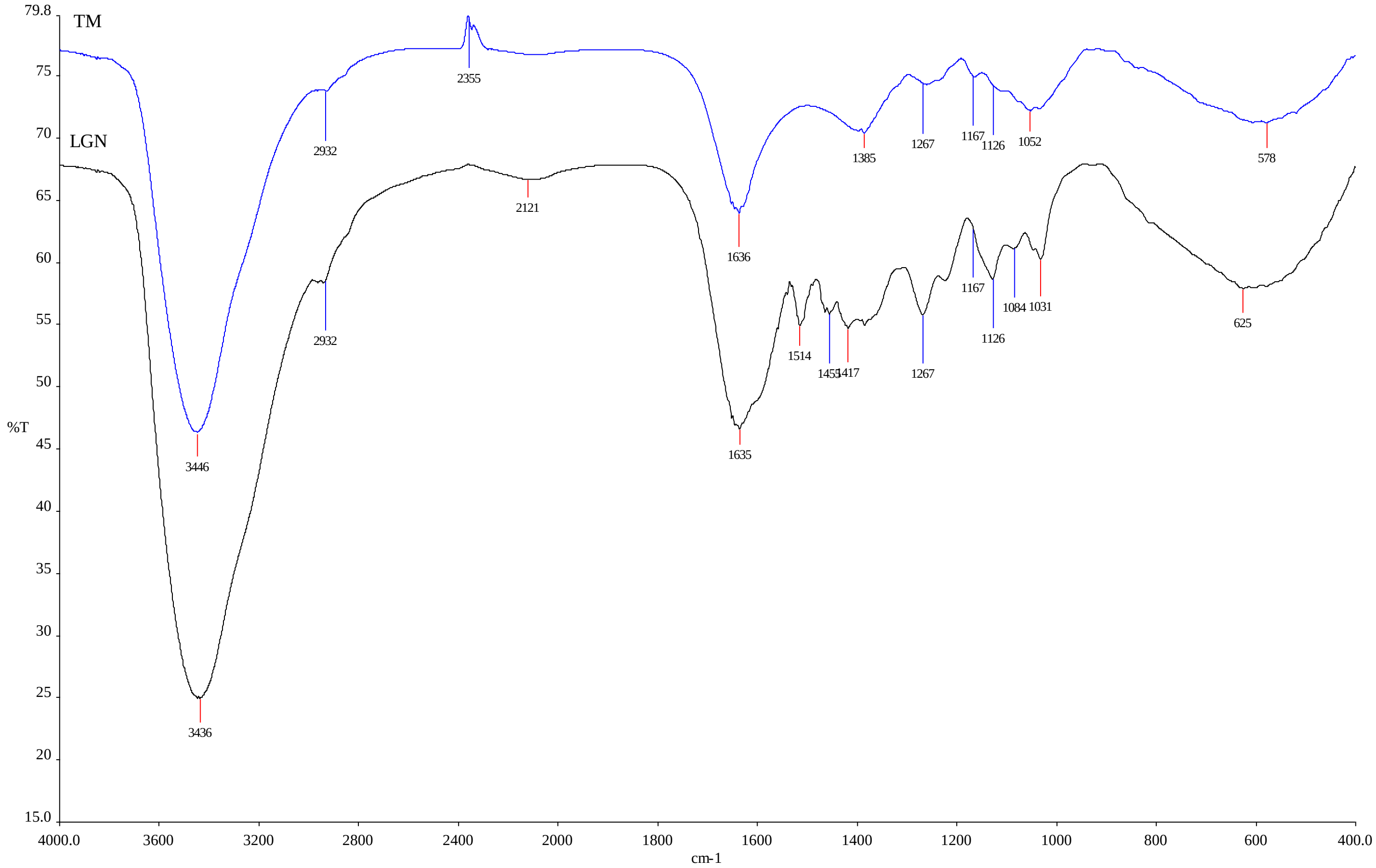


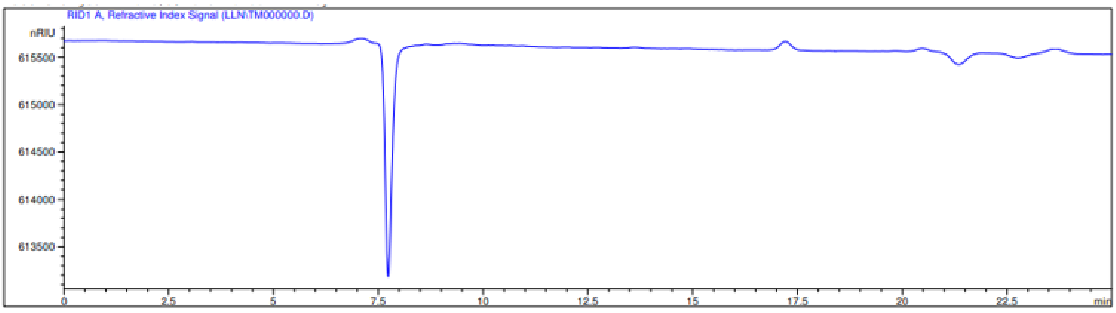
Figure 4.3.1: FTIR spectrum of soil stabilizers (TM and LGN) obtained through FTIR.

4.3.2 High Performance Liquid Chromatography (HPLC)

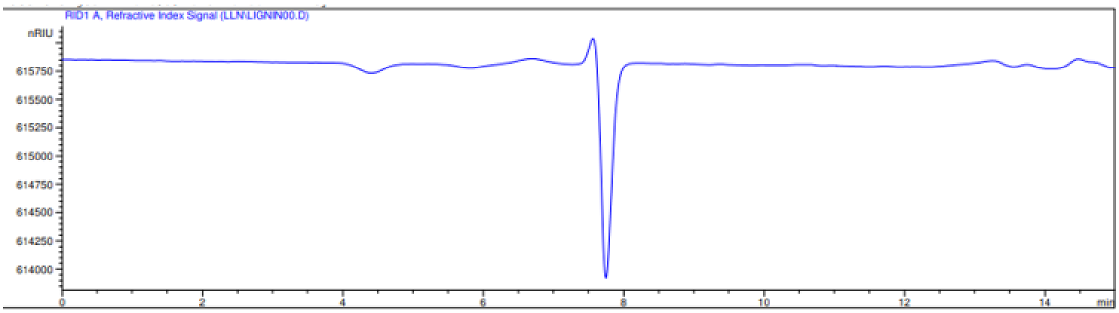
Figure 4.3.2 below shows the result of peaks obtained at respective retention time obtained from HPLC analysis. The test ran for 30 minutes for TM while LGN consist of 15 minutes run of the analysis.

As seen from Figure 4.3.2 below, the similar peaks of retention time observed for both soil stabilizers were approximately at 7.5 minutes which indicates the presence of oxalic acids (Din et al., 2020). The presence of oxalic acid is extremely crucial to the formation of soluble metals. According to Fomina et al. (2005), oxalic acids are able to transform metal minerals into metal oxalates which enhances the leaching efficiency which may explain the increase of exchangeable fractions in several HM. Thus, the ability of oxalic acid to solubilize HM is due to its high acidity constant. Aside from that, there were no significant peaks that were identified from the analysis for both LGN and TM.

Study conducted by Gordobil et al., (2014) on different types of acid insoluble lignin detected the presence of sugars using ROA Organic Acid (00H-0138-K0) column, nevertheless, the content was low which indicates the high purity of lignin. As for the case of LGN, the absent of peaks may be a result of using a different HPLC column used during the analysis which may not be compatible for the analysis (Szczerbowski et al., 2014). Another possible reason to the absent of peaks in the HPLC results is because of the nature of LGN in acid. As LGN is acid insoluble, the properties of LGN had precipitated as shown in APPENDIX E below whereby the precipitation occurs after being hydrolysed with 0.5M HCL. The phenomenon indicates that molecules of LGN had been separated from the liquid that was being tested in HPLC analysis. The results were consistent with research done by Zhang et al., 2015) where the author tried to analyse LGN on the content of sugar which precipitation occurs after the introduction of HCL solution.



(i) TM



(ii) LGN

Figure 4.3.2: Retention time of soil stabilizers (TM and LGN) obtained through HPLC.

4.4 Mobility Factor

As shown in Table 4.4.1 below shows the mobility factor of each HM after being treated by soil stabilizers to describe the biological availability and mobility of HM in soil. The mobility factor of each heavy metal had certainly decreased which indicates the decreased in mobility of HM as the unstable forms of HM decreased overall.

The most significant reduction can be seen for all soil samples treated with LGN whereby the percentage decrease for mobility factor of Cd falls at 10.30% while soil samples treated with TM decreased 8.03%. As for Cu, LGN managed to reduce the mobility factor by 6.97% while treatment with TM reduced Cu mobility by 4.93%. Consequently, Pb has the least significant reduction of mobility for both treatments with 2.81% and 1.28% for LGN and TM respectively. The reduction of mobility for Zn from LGN treated soil samples were 8.31% and 4.03% for TM treated soil samples.

Thus, it can be seen that LGN has higher efficiency in immobilizing HM compared to TM as the results obtained showed the lower mobility factor for LGN treated soil samples. The efficiency of LGN in immobilizing HM is highly related to increment of pH levels in soil which allows the phase transformations between the fractions (Husson, 2013). Even though both TM and LGN are biomass-based materials which possesses similar functional groups as shown in the FTIR analysis, however, the better efficiency of LGN in immobilization may be attributed to the difference of chain architecture of their structure and the distinct affinities of oxygen-containing functional groups on both LGN and TM for the different types of heavy metal (Tao et al., 2017b).

Table 4.4.1: Mobility factor of each soil stabilizer with their respective targeted HM.

Type of HM	Treatment		MF(%)	Percentage Decrease (%)
Cd	**Before treatment**		77.93	
	After treatment	**TM**	69.88	8.05
		LGN	67.63	10.30
Cu	**Before treatment**		18.09	
	After treatment	**TM**	13.16	4.93
		LGN	11.12	6.97
Pb	**Before treatment**		39.29	
	After treatment	**TM**	38.01	1.28
		LGN	36.48	2.81
Zn	**Before treatment**		44.13	
	After treatment	**TM**	40.10	4.03
		LGN	35.82	8.31

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This paper has shown the characterization of biomass-based soil stabilizers and their effect in heavy metal immobilization. Ultimately, the objectives stated in Chapter 1.3 was achieved through this study. It is found that both soil stabilizers were able to increase the soil properties in terms of pH, TOM, and moisture content. More significant increase was seen for LGN compared to TM which contributes to a higher percent reduction of mobility factor for all heavy metals treated with LGN. Although both soil stabilizers were able to decrease the mobility of HM in the soil but significance was seen for immobilization of Cd which showed the suitability of biomass-based soil stabilizers on the immobilization of Cd. The mobility factor decreased significantly for Cd from 10.30% and 8.03% for LGN and TM treatment respectively. Meanwhile the least efficient immobilization goes to Pb with an increase of exchangeable phase indicating the unsuitability of biomass-based materials to treat Pb contaminated soils with mobility reduction of 2.81% and 1.28% only for treatment LGN and TM respectively. Through the understanding of fraction increment after treatment from both soil stabilizers, the immobilization mechanism can be identified. Most of the mechanism of the biomass-based soil stabilizers involves mainly in adsorption, electrostatic attractions, and precipitation for heavy metals like Cd, Pb and Zn while immobilization of Cu depends more on chelation mechanism which is consistent with the explanation of increase in organic fractions. Characterization of both soil stabilizers were done to understand the functional groups present through

FTIR analysis; it can be identified that there were more functional groups that are able to immobilize HM for LGN compared to TM. However, in the HPLC analysis, although both of the soil stabilizers contain oxalic acids, the peak for LGN is higher compared to TM which indicates the higher unstable form of certain heavy metals like Pb to increase causing it to be more mobile. To sum up, LGN is considered a better biomass-based soil immobilizer compared to TM as there is more significant benefits of LGN in terms of the present of functional groups, changes in soil properties, and immobilization efficiency.

5.2 Recommendation and Improvements

Several recommendations and improvement were suggested in order to have more comprehensive and advance research of this study in the future. First of all, improvement has to be made for the studies regarding the soil properties. More repetitions of samples should be done to obtain a more consistent result which is able to eliminate the uncertainties which is not within the researcher's control as much as possible. Another recommendation is to conduct studies on more parameters of the soil properties such as nitrogen and phosphorus content to have a clearer understanding on how it will contribute to the immobilization efficiency.

As for the part on screening, it is recommended to use different chemicals for the hydrolysis of soil stabilizers in order to have a more consistent result and obvious peaks in the HPLC analysis. Another recommendation is on the remediation for Pb contaminated soils as both biomass-based soil stabilizers increase the exchangeable fraction of Pb which is in contrary with the goal in immobilization. Thus, it is recommended to conduct immobilization of Pb with different types of soil stabilizers for better immobilization efficiency of Pb. Lastly, individual artificial contamination of heavy metals should be done in the future to be able to have more homogenous contamination and may result in more precise prospects of affinity of heavy metals towards the soil stabilizers.

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APPENDICES

APPENDIX A: Raw data for soil pH.

Soil Samples		pH 1	pH 2	Average pH	Standard Error (%)
Before Treatment	Before Contamination	7.57	7.61	7.59	0.02
	After Contamination	6.18	5.88	6.03	0.15
After Treatment	TM	6.97	7.03	7.00	0.03
	LGN	8.7	8.98	8.84	0.14

APPENDIX B: Raw data for soil total organic matter (TOM).

Soil Samples		TEST 1				TEST 2				Average TOM (%)	Standard Error (%)
		W1	W2	W3	TOM 1	W1	W2	W3	TOM 2		
Before Treatment		39.02	48.54	48.221	3.321	39.525	48.66	48.33	3.527	3.424	0.10
After Treatment	TM	39.55	49.38	49.001	3.846	39.779	49.15	48.79	3.790	3.818	0.03
	LGN	40.14	49.72	49.214	5.269	40.131	49.55	49.06	5.213	5.241	0.03

APPENDIX C: Raw data for soil moisture content.

Soil Samples		TEST 1				TEST 2				Average MC (%)	Standard Error (%)
		W0	W1	W2	MC 1 (%)	W0	W1	W2	MC 2 (%)		
Before Treatment	After Contamination	39.023	50.02	49.86	1.482	39.028	50.003	49.85	1.358	1.420	0.06
After Treatment	TM	39.459	49.48	49.33	1.517	39.503	49.578	49.42	1.538	1.528	0.01
	LGN	40.059	49.14	48.93	2.290	40.161	49.232	49.01	2.480	2.385	0.10

APPENDIX D: Raw data from sequential extraction.

Cd (Before Treatment)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.295	0.291	0.293	8	1	10	1	23.44	19.62	0.002
Carbonate	0.873	0.869	0.871	8	1	10	1	69.68	58.32	0.002
FeMnO	0.238	0.248	0.243	20	1	5	1	24.30	20.34	0.005
Organic	0.018	0.016	0.017	16	1	5	1	1.36	1.14	0.001
Residual	0.007	0.007	0.007	20	1	5	1	0.70	0.59	0.000
Total amount								119.48	100.00	

Cd (TM)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)

Exchangeable	0.241	0.258	0.250	8	1	10	1	19.96	18.43	0.009
Carbonate	0.720	0.673	0.697	8	1	10	1	55.72	51.45	0.024
FeMnO	0.257	0.276	0.267	20	1	5	1	26.65	24.61	0.010
Organic	0.049	0.049	0.049	16	1	5	1	3.92	3.62	0.000
Residual	0.022	0.019	0.021	20	1	5	1	2.05	1.89	0.002
Total amount								108.30	100.00	

Cd (LGN)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.095	0.103	0.099	8	1	10	1	7.92	8.37	0.004
Carbonate	0.715	0.687	0.701	8	1	10	1	56.08	59.26	0.014
FeMnO	0.268	0.246	0.257	20	1	5	1	25.70	27.16	0.011
Organic	0.036	0.036	0.036	16	1	5	1	2.88	3.04	0.000
Residual	0.016	0.025	0.021	20	1	5	1	2.05	2.17	0.005
Total amount								94.63	100.00	

Cu (Before Treatment)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.02	0.018	0.019	8	1	10	1	1.52	1.15	0.001
Carbonate	0.278	0.282	0.280	8	1	10	1	22.40	16.94	0.002
FeMnO	0.482	0.64	0.561	20	1	5	1	56.10	42.42	0.079
Organic	0.565	0.521	0.543	16	1	5	1	43.44	32.84	0.022
Residual	0.107	0.069	0.088	20	1	5	1	8.80	6.65	0.019
Total amount								132.26	100.00	

Cu (TM)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.023	0.025	0.024	8	1	10	1	1.92	1.36	0.001
Carbonate	0.207	0.21	0.209	8	1	10	1	16.68	11.80	0.002
FeMnO	0.475	0.508	0.492	20	1	5	1	49.15	34.78	0.017
Organic	0.735	0.789	0.762	16	1	5	1	60.96	43.14	0.027

Residual	0.129	0.123	0.126	20	1	5	1	12.60	8.92	0.003
Total amount								141.31	100.00	

Cu (LGN)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.039	0.044	0.042	8	1	10	1	3.32	2.55	0.003
Carbonate	0.147	0.132	0.140	8	1	10	1	11.16	8.57	0.007
FeMnO	0.461	0.462	0.462	20	1	5	1	46.15	35.43	0.001
Organic	0.74	0.696	0.718	16	1	5	1	57.44	44.09	0.022
Residual	0.079	0.165	0.122	20	1	5	1	12.20	9.37	0.043
Total amount								130.27	100.00	

Pb (Before Treatment)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.033	0.009	0.021	8	1	10	1	1.68	0.78	0.012
Carbonate	1.039	1.041	1.040	8	1	10	1	83.20	38.51	0.001

FeMnO	1.005	0.891	0.948	20	1	5	1	94.80	43.88	0.057
Organic	0.352	0.272	0.312	16	1	5	1	24.96	11.55	0.040
Residual	0.123	0.105	0.114	20	1	5	1	11.40	5.28	0.009
Total amount								216.04	100.00	

Pb (TM)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.208	0.227	0.218	8	1	10	1	17.40	5.77	0.010
Carbonate	1.273	1.159	1.216	8	1	10	1	97.28	32.24	0.057
FeMnO	1.111	1.292	1.202	20	1	5	1	120.15	39.82	0.091
Organic	0.32	0.464	0.392	16	1	5	1	31.36	10.39	0.072
Residual	0.357	0.354	0.356	20	1	5	1	35.55	11.78	0.002
Total amount								301.74	100.00	

Pb (LGN)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)

Exchangeable	0.279	0.255	0.267	8	1	10	1	21.36	7.24	0.012
Carbonate	1.142	1.015	1.079	8	1	10	1	86.28	29.24	0.064
FeMnO	1.131	1.198	1.165	20	1	5	1	116.45	39.47	0.034
Organic	0.354	0.373	0.364	16	1	5	1	29.08	9.86	0.010
Residual	0.369	0.469	0.419	20	1	5	1	41.90	14.20	0.050
Total amount								295.07	100.00	

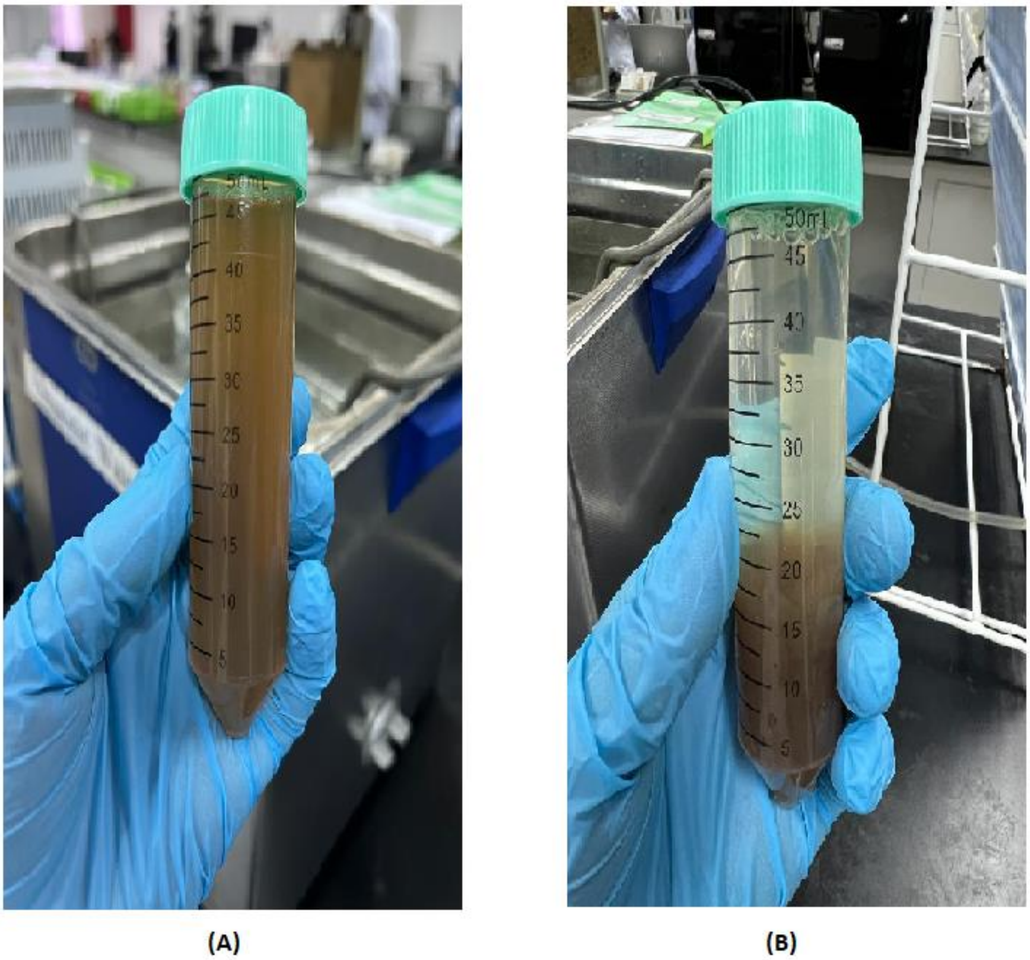
Zn (Before Treatment)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.001	0.006	0.003	8	1	10	1	0.27	0.10	0.002
Carbonate	0.823	0.684	0.753	8	1	10	2	120.54	44.04	0.069
FeMnO	0.795	0.651	0.723	20	1	5	2	144.58	52.82	0.072
Organic	0.081	0.085	0.083	16	1	5	1	6.64	2.43	0.002
Residual	0.025	0.009	0.017	20	1	5	1	1.68	0.61	0.008
Total amount								273.72	100.00	

Zn (TM)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.039	0.047	0.043	8	1	10	1	3.45	1.06	0.004
Carbonate	0.800	0.794	0.797	8	1	10	2	127.52	39.05	0.003
FeMnO	0.850	0.968	0.909	20	1	5	2	181.78	55.66	0.059
Organic	0.077	0.092	0.084	16	1	5	1	6.74	2.06	0.008
Residual	0.070	0.073	0.071	20	1	5	1	7.11	2.18	0.002
Total amount								326.60	100.00	

Zn (LGN)	Duplicate 1 (mg/L)	Duplicate 2 (mg/L)	Average (mg/L)	Vol. Acid (mL)	Soil Sample Amount (g)	Dilution Factor	New Dilution Factor	ppm (mg/kg)	Percentage (%)	Standard Error (%)
Exchangeable	0.0089	0.0128	0.011	8	1	10	1	0.87	0.28	0.002
Carbonate	0.6486	0.7282	0.688	8	1	10	2	110.14	35.54	0.040
FeMnO	0.9513	0.9245	0.938	20	1	5	2	187.58	60.52	0.013
Organic	0.0773	0.0771	0.077	16	1	5	1	6.18	1.99	0.000

Residual	0.0296	0.0738	0.052	20	1	5	1	5.17	1.67	0.022
Total amount								309.94	100.00	

APPENDIX E: Changes of LGN after being hydrolysed.



(A) (B)

**(A) before hydrolysis of LGN (B) after hydrolysis of LGN.*