PHYTOREMEDIATION OF SOIL CONTAMINATED WITH PLUMBUM AND CHROMIUM USING *LANTANA CAMARA* AND *PILEA CADIEREI* WITH ORGANIC WASTE AMENDMENTS

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Field of Study: **Waste and Environmental Management**

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ABSTRACT

Anthropogenic activities cause an estimated 10 billion tonnes of topsoil contamination from metals annually, this puts humans at risk because of metal trophic-level accumulation. This study was aimed at evaluating the efficiency of biowaste amendments, in phytoremediation of Pb and/or Cr contaminated soil, using 6% of empty fruit bunch (EFB) or spent mushroom compost (SMC) biowaste, with *Pilea cadierei* and *Lantana camara* plants on different Pb and/or Cr concentrations on (Mollisol) soil for 98 days, using a complete randomized experimental design. Based on the background and intervention guidelines from the Department of Environment (DOE) Malaysia on soil heavy metal standards. Results indicated Pb reductions between 52 to 88% and 45 to 82% in EFB and SMC amended soils under *Lantana camara*, and 44 to 83% and 42 to 79% for *Pilea cadierei*, respectively. Then 47 to 72% and 38 to 67% removal of Cr under *Pilea cadierei*, and also 53 to 75% and 46 to 72% for *Lantana camara* at similar order of biowaste above. Heavy metal resistant bacteria (HMRB) counts, pooled at 79.67 x 10^6 to 39.67 x 10^6 cfu/g, and 63.33 x 10^6 to 39.0 x 10^6 cfu/g were recorded at EFB and SMC metal-soils, respectively. Half-life's ($t_{1/2}$) of 7.24-2.26 days for (amended), 18.39-11.83 for (unamended), and 123.75-38.72 for (control) soils, were recorded. Bioaccumulation and translocation factors of *Pilea cadierei* and *Lantana camara* recorded < 1. Supposedly, a potential metallophytes. Freundlich isotherms, showed less Pb and Cr adsorption intensity (***n***) to soil between 2.44 to 2.51 when amended. Indicating opposite metal movement away from the bulk soil, via plant-root absorption, and ΔG also ranged -5.01 to -0.79 $KJmol^{-1}K^{-1}$ (exothermic), hence with intraparticle diffusion rate constant of 7.064 to 1.669 $mg/kg^{-0.5}$. The pH of 5.02-6.03 was optimum for phytoremediation. In conclusion, *Pilea cadierei* and *Lantana camara* was sustainable in enhanced remediation of Pb and/or Cr contaminated soil, when amended with these biowastes.

ABSTRAK

Aktiviti antropogenik menyebabkan kira-kira 10 tan billion pencemaran tanah daripada logam setiap tahun, yang memberikan risiko kepada manusia akibat daripada pengumpulan logam aras trofik. Kajian ini bertujuan untuk menilai keberkesanan pindaan sampah biologi, dalam fitoremediasi Pb dan/atau Cr tanah yang tercemar, dengan menggunakan 6% tandan buah yang kosong (EFB) atau kompos sisa cendawan (SMC) sampah biologi, dengan *Pilea cadierei* dan tumbuhan *Lantana camara* ke atas kepekatan Pb dan/atau Cr yang berbeza terhadap (Mollisol) tanah untuk 98 hari, menggunakan bentuk experiment rawak lengkap. Berdasarkan latar belakang dan garis panduan daripada Jabatan Alam Sekitar (JAS) Malaysia ke atas standard logam berat tanah. Keputusan menunjukkan pengurangan Pb antara 52 kepada 88% dan 45 kepada 82% untuk EFB dan tanah pinda SMC kepunyaan *Lantana camara*, dan 44 kepada 83% dan 42 kepada 79% untuk *Pilea cadierei* masing-masing. Kemudian 47 kepada 72% dan 38 kepada penyingkiran 67% Cr kepunyaan *Pilea cadierei*, dan juga 53 kepada 75% dan 46 kepada 72% untuk *Lantana camara* pada susunan sampah biologi di atas yang sama. Kiraan bakteria tahan logam berat (HMRB), jatuh dari 79.67×10^6 kepada 39.67×10^6 cfu/g, dan dari 63.33×10^6 kepada 39.0×10^6 cfu/g telah direkodkan untuk EFB dan tanah logam SMC masing-masing. Hayat separuh ($t_{1/2}$) 7.24-2.26 hari untuk (telah dipinda), 18.39-11.83 untuk (belum dipinda), dan 123.75-38.72 untuk (kawalan) tanah, telah direkodkan. Pengumpulan biologi dan faktor pemindahan *Pilea cadierei* dan *Lantana camara* direkodkan < 1. Sepatutnya, metallofit berpotensi. Freundlich isotherm, menunjukkan kurang Pb dan Cr keamatan penyerapan (***n***) terhadap tanah antara 2.44 kepada 2.51 apabila dipinda. Menunjukkan pergerakan logam yang bertentangan menjauhi tanah pukal, melalui penyerapan akar tumbuhan, dan ΔG juga berjulat -5.01 ke -0.79 $\mathrm{KJmol^{-1}K^{-1}}$ (exotermik), maka dengan kadar resapan intrapartikel yang tetap, iaitu 7.064 ke 1.669 $\mathrm{mg/kg^{-0.5}}$. pH 5.02-6.03 adalah optima untuk fitoremediasi. Kesimpulannya, *Pilea cadierei* dan *Lantana camara* adalah lestari dalam meningkatkan remediasi Pb dan/atau Cr tanah yang tercemar, apabila dipinda dengan sampah biologi ini.

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While, I alone, am responsible for this thesis, it is nonetheless at least a product of years of support and persistent prayers from my dad and mom, siblings, niece and/or nephews “The ALARIBE’S”. Therefore, I remain indebted and thankful to them always. Regrettably, may the souls of my lovely departed sisters (Oluchi Alaribe; who exited mid-way during this study and Ijeoma Alaribe) continue to rest in perfect peace, because they encouraged me so much on this goal, but untimely could not live and/or wait until this moment.

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LIST OF SYMBOLS AND ABBREVIATIONS

AET	Actual Evapotranspiration
AMD	Acid Mine Drainage
ASEAN	Association of South East Asian Nations
ATSDR	Agency for Toxic Substance and Disease Registry
BAF	Bioaccumulation Factor
BI	Blacksmith Institute
CEPA	Chinese Environmental Protection Administration
CM	Chicken Manure
DDT	Dichlorodiphenyltrichloroethane
DOE. My	Department of Environment Malaysia
EFB	Empty Fruit Bunch
ENS	Environmental News Service
FBA	Fluidized Bed Boiler
FRTR	Federal Remediation Technologies Roundtable
GC-MS	Gas Chromatography- Mass Spectroscopy
GWB	Green Waste Biochars
GWRTAC	Ground Water Remediation Technologies Analysis Centre
HMRB	Heavy Metal Resistant Bacteria
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
MSF	Médecins San Frontières
NISF	National Institute of Solid Fertilizer
NMR	Monitored Natural Recovery
PCBs	Polychlorinated biphenyls
REE	Rare Earth Element
SEM	Scanning Electron Micrograph
SMC	Spent Mushroom Compost
TL	Translocation Factor
USEPA	United States Environmental Protection Agency
USITC	United States International Trade Commission
WHO	World Health Organization
WQI	Water Quality Index
PICT	Pollution Induced Community Tolerance

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CHAPTER 1: INTRODUCTION

1.1 Background of study

Metals with atomic mass over 20 and specific gravity higher than 5gm/cm^3 are often classified as heavy metals, such as Cd, Cu, Ni, Zn, Pb and Cr etc., as depicted by Jankaite & Vasarevićius, (2007), Babula et al., (2008), Meera & Agamuthu, (2011). Sometimes when we talk about heavy metal contamination of soil, we may not see the effects with clarity as opposed to air and water pollution or contamination. This is because soil heavy metal contamination is physically less glaring, and consistent soil abuse goes un-noticed. For the function of this thesis, *soil contamination* is considered to mean the introduction of metals or metallo-organic compounds that can significantly impair the biodiversity equilibrium of a well-defined biogeochemical and ecological system.

Though metals are indispensable tools for building modern-day infrastructural capacities, hence their demands are steadily on the rise, particularly in the world's emerging countries. So as prosperity increases, so too does demand for consumer products like electronic goods and cars. That means to say "metal is all around us; you only need to look around to see hundreds of objects containing metals". Much as the importance might sound apparent, heavy metals still constitutes chemical hazards which is notorious for inducing toxicity, mutagenicity, carcinogenic effects to humans, animals, microorganism and plants based on their chemical reactivity, radioactivity and cumulative tendencies in biological systems (Fotakis & Timbrell, 2006; Boder et al., 2006; Doumett et al., 2008).

Heavy metal contamination of the soil is one of the most important problems throughout the world, and edaphic qualities are affected by past land use (Hou et al. 2007; Bai et al., 2010; Christian et al., 2008). Soil functions as a dynamic and active sink, receiving inputs and releasing outputs of both energy and matters from many chemical, physical and biological activities resulting from industrialization, urbanization, agriculture, poor management of liquid and solid wastes, may cause soil contamination (Teng et al., 2010; Miao et al., 2012). According to Rodríguez et al., (2009) and Guannan et al., (2013), mining activities and metal smelting have been pointed out as the major sources of hazardous heavy metal. As mining and smelting processes take place, waste water and dust containing heavy metals are discharged into the environment, conveyed to surface and ground waters, then leached into agricultural soils and living organisms. As important biogeochemical sink for contaminants, arable lands close to non-ferrous metal mines are often contaminated by heavy metals due to irrigation and flooding (El Khalil et al., 2008; Cheng et al., 2011). Natural pedogenic processes are supposedly impeded upon soil contamination.

In 2008, global production of metals reached 1.4 billion tonnes, which was a production rate seven times than that in 1950 (Prasad et al., 2010). Metal consumption was 77 kg per capita in 1950, which increased to 213 kg per capita at 2008, varying tremendously among countries (Götz & Erika, 2012). Globally, mining occupies a territory of approximately 37,000 km^2 which relatively equals to the area of Belgium or 0.2% of the world land surface (Dudka & Adriano, 1997). In addition, approximately 240,000 km^2 land spaces are influenced by metals released from waste dumps and open mines (Furrer et al., 2002). Collation for annual loss of farming lands predominantly through contamination, industrialization, urbanization and desertification ranged between 70 to 140,000 km^2. Also reported were 4.3 million km^2 of arable land estimated to have been abandoned during the last 40 years and 10 billion tonnes of topsoil are lost every year to contamination (Döös, 2002; Götz & Erika, 2012).

Rapid transition from an agricultural economy to an industrial economy in some developing countries like Malaysia makes it probable that heavy metal environmental pollution is a significant issue. However, there is no statistically significant data on potential soil contamination in Malaysia. Much work remains to identify potential contamination sites and suggest remediation measures (Heng Keng et al., 2009). According to Rajiv et al., (2009), there are about three million contaminated sites in the Asia-Pacific region. These include the abandoned mine sites along with closed landfills. The contaminated sites mostly contain heavy metals Cd, Pb, Hg, Zn etc., and chlorinated compounds like the Polychlorinated biphenyls (PCBs) and Dichlorodiphenyltrichloroethane (DDT).

1.2 Heavy metal risk impacts on soil ecosystem, environment and human health

Previous research has suggested that in most cases, there are more heavy metal loads in soils around mining vicinities and those irrigated with waste water e.g. landfill leachate, were significantly higher than those in the background soils (Wang et al., 2010). Though, many indexes have been explored for the assessment of soil heavy metal contamination, such as total concentration of heavy metal (Liu et al., 2011; Sheng et al., 2012; Kabala & Ram, 2001; Delgado et al., 2011). Therefore, the total content of heavy metals has been used to assess the risk of soil pollution by equating it with some background indexes like enrichment factor, geoaccumulation index, contamination factor and complex quality index (Bhuiyan et al., 2010). However, suggestion from El et al., (2010) and Pueyo et al., (2008) read that it is insufficient to only consider total concentration of heavy metals in soils for environmental risk assessments, because when heavy metals are discharged into the soil, they become transformed into different geochemical forms through a series of physical, chemical and biological process (adsorption, dissolution, complexation and uptake by biota, etc.). This may increase risk diversification and promote toxicity effects on soil microbes, microfaunas/floras and nutrient loss (Consuelo et al., 2015). In such instance, drop in soil biota metabolic processes and/or biomass or both are inevitable. Impacts like this, renders soils unfit for agricultural purposes as reported by Gimici et al., (2008) and Türker et al., (2013). Unlike organic substances, heavy metals are essentially nonbiodegrable and therefore may accumulate in the environment. This accumulation of heavy metals in soils and waters poses risks to the environment and human health (Khan et al., 2010). Following this, bioaccumulation may occur in living body tissues, through food chains and their concentrations increases as they pass from lower trophic levels to higher trophic levels; a phenomenon known as biomagnification (Ping et al., 2009; Sánchez-Chardi et al., 2009; Dabonne et al., 2010; Ali et al., 2013).

Incidences of heavy metals poisoning around the world have been well documented, especially among children. A typical case example; Galadima & Garba, (2012) reported the Zamfara "a state in north-west Nigeria" Pb poisoning in the year 2010, where the worst and most recent heavy metals lethal impact took place in Nigeria and killed over 500 children within seven months. This was because, some unprofessional miners within Zamfara brought back rocks containing gold ore into the villages from a small-scale mining operations (Word Health Organisation, 2011). However, these ores were said to also contain extremely high levels of Pb, which was crushed inside village compounds, discharging Pb polluted dust and soil sediments within the affected communities. In response to this menace, joint field studies were carried out by international organisations such as Médecins Sans Frontières (MSF) and Blacksmith Institute (BI) in collaboration with affected local governments in Zamfara state and the Federal authorities in Nigeria, to measure the blood-Pb concentrations in samples from 113 young children in the affected villages. The outcome revealed that 100% of the children had blood-Pb levels exceeding 10 µg/ dL which is the international standard for the maximum safe levels of

Pb in blood, 96% exceeded 45 µg/dL, and 84% exceeded 70 µg/dL (MSF, 2010; BI, 2010). The same study, revealed that an estimated 2,500 children within that whole area have life-threatening levels of Pb in their bloods (Galadima & Garba, 2012). Five additional villages, where similar kinds of ore processing activities were practised, were also identified in the process. Many areas sampled in the village including family homes and compounds, indicated that soil Pb concentration exceeded 100,000 parts per million (ppm), against the maximum 400 ppm considered acceptable for residential areas. The ingestion of contaminated soil and air inhalation has been the primary pathway of Pb exposure as their studies concluded for Zamfara metal epidemics.

Furthermore, numerous authors have reported the decline in semen quality, causing infertility in men to be associated with both Pb and Cd concentrations in blood (Eibensteiner et al., 2005; Telisman et al., 2000 and 2007). Other reports from Hernández-Ochoa et al., (2005) and Benoff et al., (2009), have shown an association between impaired sperm motility and Cd and/or Pb concentrations in sperm or seminal fluid. Therefore, studies suggest that the presence of heavy metals in the reproductive tract (of men attending infertility clinics) may be related to a moderate alteration of their seminal parameters (Mendiola et al., 2011).

1.3 Environmental Biotechnology for decontamination of heavy metal impacted soil

Environmental biotechnology in metal clean-up, implies the use of biological techniques to remediate contaminated environment (Whiting et al., 2004). Heavy metals are not subject to degradation processes and therefore may remain almost indefinite in the environment if no active remediation interventions are adopted.

Appropriate clean-up of contaminated sites are legal requirement in many countries. For instance, since the adoption of the "German Soil Protection Act (BBodSchG) in 1998, which states that soil which present a danger to humans and environment, must be remediated". The choice of remediation method depends on the type, mobility and concentration of the contaminant and the future land use. Maike & Filser, (2007) and Geller (2002), argued that biological remediation is used when the pollution level is moderate and non-biological methods are not economical.

Therefore, based on the European Thematic Strategy for Soil Protection framework adopted by the European Commission in September 2006 at COM (2006) 231, had advocated for a new viable and in *situ* technologies for soil protection and reclamation as priority objective (Petra et al., 2009). Use of eco-friendly and gentle soil remediation techniques to remove or reduce the concentration of pollutants in an environment using plants species and associated soil microbes are collectively referred to as phytoremediation (Odjegba & Fasidi, 2007; Lone et al., 2008; Kawahigashi, 2009; Greipsson, 2011;Vithanage et al., 2012; Ali et al., 2013). It is generally considered to be less invasive, more cost-effective in restoration of soil structure and functions compared to conventional civil engineering methods that are based on techniques like leaching of pollutants, solidification/stabilisation, size selection and pyrometallurgical processes, electro-kinetical treatments, chemical oxidation/reduction of pollutant and excavation (McGrath & Zhao, 2003). Studies from Di Baccio et al., (2011), have revealed that *Thlaspi* spp can accumulate more than 3% of their shoots weight with Zn, 0.5% Pb and 0.1% Cd, while *Arabidopsis halleri* plant have demonstrated to accumulate more than 1% of its aboveground biomass of Cd and Zn.

Diversity of bioremediation techniques has been developed to increase the clean-up rates of contaminated sites (Jingchin et al., 2009). Bioaugmentation (application of specifically selected bacteria on contaminated soil) has been well studied and demonstrated to be a needful method in bioremediation of contaminated soil (Sadin et al., 2013). Some studies have reported various bacterial species with the capacity of mineralizing heavy metals from industrial waste (Congeevaram et al. 2007; Őzdemir & Kılınç, 2012). Sadin et al., (2013), have also reported *Bacillus* spp, *Geobaccillus thermantarcticus, and Anoxybaccillus amylolyticus* on heavy metal removals from polluted substrates.

1.4 Problem statement

Environmental pollution by heavy metals has become an alarming problem in the world due to rapid industrialization and solid waste generation dynamics. Mobilization of heavy metals through extraction from ores and subsequent processing for different applications has led to the harsh bombardment of these elements into our environments (Fulekar et al., 2009; Conesa & Schulin, 2010; Wuana & Okieimen, 2011; Wolska & Bryjak, 2013). The dangers imposed are infinite, it includes but not limited to mining activities alone.

In the Campine region of Belgium and the Netherlands, 700 km^2 is said to be diffusely contaminated by atmospheric deposition of Cd, Zn and Pb (Meers et al., 2010). Reports from Xia, (2004), implicated China to have a total area of 2.88 x 10^6 ha of destroyed lands as a result of mining, and additional mean area of 46700 ha are contaminated annually. These spiflicate lands, lacks complete vegetation (**Plate 1.1**), due to serious heavy metal contamination and ultimately cause severe soil erosion and offsite metal pollution cases.

Plate 1.1: A polluted field at a black Pb mining area in Northern China.
Source: Fortune, 2014.

The report from the Technical Advisory Board of the Blacksmith Institute (2014), on the most polluted places in the world in 2013, is shown in **Table 1.1**, which indictment criteria includes the size of the affected population at risk, the severity of the toxins involved, and reliable evidence of health problems associated with the contamination.

Again, the *Jishui* River is among the main tributaries of *Le'an* River located in the Jiangxi province, China. Along the *Jishui* River, many non-ferrous metal mines have been exploited for decades, such as the *Yinshan* Pb-Zn mine, *Fujiawu* copper mine, *Huaqiao* gold mine and *Jinsham* gold mine. This has terribly impaired the water quality of the *Jishui* River and the aligning arable lands (Teng et al., 2010). This may need a sustainable remediation intervention, for her ecological restorations.

Table 1.1: Most contaminated places in the world in 2013 by Blacksmith Institute.

Region	Country	Problem	Pollutant exposure	Population	Contamination pathway
Agbogbloshie	Ghana	Pb	E-waste	40,000	Soil & air
Chernobyl	Ukraine	Radiation	Radioactive dust & heavy metal	10 million	Soil, air & water
West Java	Indonesia	Pb, Cd, Cr	*Citarum* River	5 million	Soil & water
Dzerhinsk	Russia	Chemicals	Toxic by-products	300,000	Soil & water
Hazaribagh	Bangladesh	Cr	Tanneries	160,000	Soil & water
Kabwe	Zambia	Pb	Pb-mining	> 300,000	Soil & water
Kalimantan	Indonesia	Hg & Cd	Gold mining	225,000	Soil & water
Matanza	Argentina	Chemicals	Manufacturing	20,000	Soil & water
Niger River Delta	Nigeria	Petroleum	Oil operations	-	Soil & river
Orilsk	Russia	Cu, Ni	Mining	-	Soil & ingestion
Mexico City	Mexico	Pb & MTBE	Oil refining	9 million	Groundwater & soil
Peru	Peru	Pb, As, Hg & Cd	Mining	30 million	Soil & water
Dong Mai	Vietnam	Pb	Pb-acid battery	2,600	Soil & water
Mailuu-Suu	Kyrgyzstan	U & Pb	Abandoned mines	25,000	Soil & water

Source: Blacksmith Institute, (2014).

Another environmental disaster, is in the “Hengshi River” which originates from the “Dabaoshan mine site” in northern Guangdong Province, in southern China where the lands along the Hengshi River, especially in the watershed basins are contaminated by acid mine drainage (AMD). These villagers are exposed to significant health risks from heavy metals, and residents are suffering from serious cancer (Wang et al., 2011).

According to the report released by The U.S environment action group, the world’s contaminated places threaten the health of more than 10 million people in many countries (Blacksmith Institute, 2014). For example, Haina in Dominican Republic is a former site for automobile battery recycling smelter, where residents suffers from widespread Pb poisoning and also in the Indian city of Ranipet, where some 3.5 million people were affected by tannery waste which contains hexavalent Cr and azodyes (WHO, 2008).

Also, the Russian industrial city of Norilsk, which houses the world’s heavy metals smelting complex is where more than 4 million tonnes of Cd, Cu, Pb, Ni, As, Se and Zn emissions are released annually in Russian and its far-east towns of Dalnegorsk and Rudnaya Pristan, and residents suffer from serious Pb poisoning from old smelter and unsafe transport of Pb concentrate from the local Pb mining sites (Jadia & Fulekar, 2009). Over two million sites have been categorized as contaminated in Europe (European Environmental Agency, 2014) and about a sixth of that are in the United Kingdom (Ashworth & Alloway, 2008).

Another past, but remarkable disaster was the “Itai-itai” disease, the first documented occurrence of mass Cd-poisoning in the world, around 1950 in Toyama Prefecture, Japan

(An, 2004). However, mining had been prevalent in the Toyama Prefecture of Japan long before 1950 (Yukimasa, 1975). But starting all the way back in 1910, Cd was being released in tremendous amounts into the *Jinzu* River in Toyama. The name "*itai-itai*" (*Ouch*! It hurts!), originates from the fact that patients of this disease suffered severe bone pain and always cry "itai itai". Studies showed that ingesting of rice or water contaminated by Cd over a prolonged time was responsible for the "*itai, itai*" disease pandemic (Perronnet et al., 2000). As a non-essential element for living organisms, Cd has a very high mobility in soil-plant systems, with propensity to adversely effect on both human health and the functioning of ecosystems (Puhui et al., 2011).

In Indonesia, as a result of low skilled mining activities at the *Talawaan- Tatelu* district, where amalgamation approach were used for the recovery of precious metals, such as gold using Hg, had also led to ecosystem degeneration. The disposed Hg that enters the environment is estimated to be around 24 to 90 tonnes per year (Tommy & Alfonds, 2015). Report from Limbong et al., (2003) on the study conducted in May to June 2001, along coastal boundaries found that there was an increase in the levels of Hg in water, sediment, and fish in the three rivers that flow into the city of Manado which is the tributaries of the Talawaan River. Residents surrounding this mine depended their lives on farming (paddy, coconut, cloves, nutmeg) and livestock (freshwater fish) activities (Tommy & Alfonds, 2015). Definitely, this would pose both health and environmental problem to these group of people.

Furthermore, the global demand for rare earth elements (REE) is increasing annually and has led to the excessive exploitation of its ores (Chen, 2011). Consequently, more REE are entering the ecosystems through mining and processing as well as mine tailing wastes (Protano & Riccobono, 2002; Hu et al., 2006; Wang et al., 2008; Cai, 2012). Xenotime (a rare earth phosphate mineral, whose major component is yttrium orthophosphate) found in Malaysian placer deposits typically contains 2% Uranium and 0.7% thorium, and this was the main reason why the Malaysian processing industry failed and the plants were closed (British Geological Survey, 2011). The associate compounds can cause negative effects on aquatic and terrestrial organisms, as well as on humans (Paul & Campbell, 2011; Rim et al., 2013; Zhe et al., 2015). The United States International Trade Commission (USITC) reported that the global remediation services in 1996 increased from 25.7 to 29.9 billion US dollar in 2002 (Koplan, 2004).

The economic growth and industrialization in recent-time Malaysia, has led to environmental problems with ever increasing land, air and water pollution (Salman et al., 2011). In 1994, the Department of Environment (DOE) Malaysia, classified the Juru River Basin located at Serberang Perai, Penang as "very polluted" based on the water quality index (WQI). This was because of the high sediments contaminated with non-residual heavy metals, such as Cd, Cu, Pb and Zn discharged from industries along the Perai industrial estate at the stretch of the river (Tan & Yap, 2006).

Ismail et al., (2013) implicated Klang River (**Figure 1.1**), to be rated as (Class III) based on the (DOE) water quality parameter guideline assayed from 1997 to 2009, and this was reportedly caused by effluents of iron and steel industries, saw-milling, battery production, seepage of leachate from landfills, and as well petroleum spills, which may indirectly establish contacts with the horizontal soil/lands (Ismail et al., 2011). So far, the Malaysia government has allocated about RM 50 billion, equivalent to USD 17 billion, to clean-up the Klang River. Following this, initial project fund of about RM 2 billion had been disbursed in 2011 (Ismail et al., 2013).

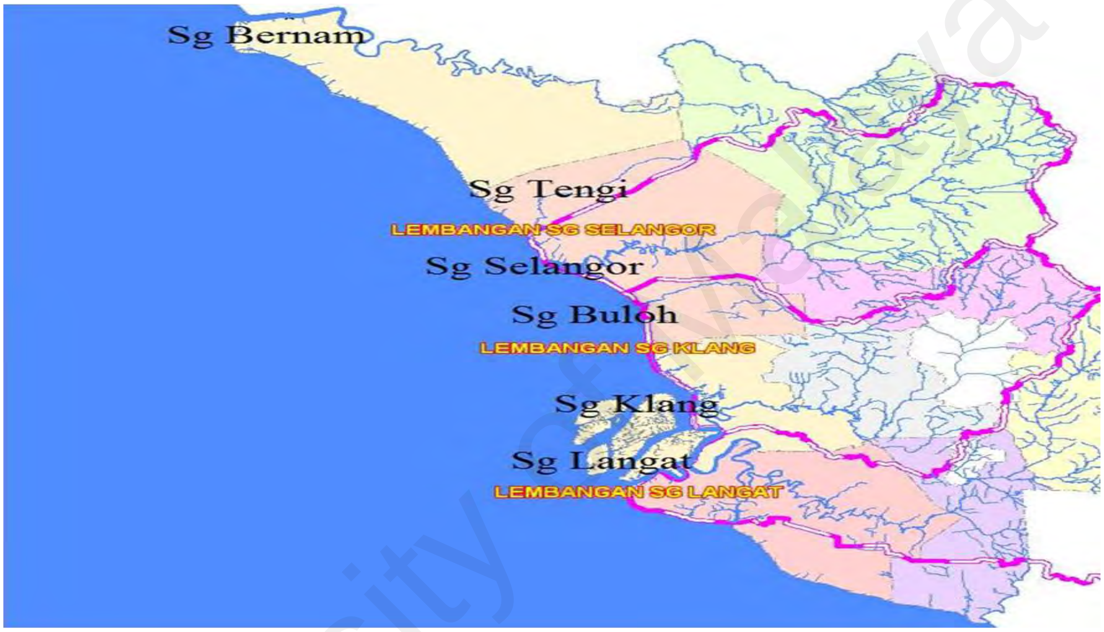


Figure 1.1: Map of Selangor showing the main rivers including the Klang River (*Sg. Klang*).

In another perspective, impacts of heavy metals from Chuping industrial district in Perlis Malaysia has been widely reported in Ripin et al., (2014), as a result of human activities which Cd and Cu were mostly indicted. Agamuthu (2001), exposed leachate contamination of arable lands, released from a 10 acres of Sabak Bernam landfill located in Selangor, Malaysia. These caused elevated level of heavy metal in soils, where agricultural activities were believed to be the predominant occupation for people in this locality. This shall unarguably introduce heavy metal into crops, leading to food chain contamination, if care was not taken.

Soil being an essential component of the earth's crust, its contamination would have a far reaching disruptive effect to our ecosystem. Hence it's imperative, to search for strategic remediation that are environmentally friendly and less capital intensive. In this study, the heavy metals were Pb and Cr, and research plants investigated were *Lantana camara* and *Pilea cadierei*. Phytoremediation and bioremediation are suggested to be an effective method.

Deterioration of soil nutrient standards as a result of heavy metal contamination, nutrients become limited to plants, their internal metabolic process are weakened to carry out remediation. Therefore, this study, supplemented organic wastes namely empty fruit bunch (EFB) and spent mushroom compost (SMC), which are cheap, available and readily seen, to enhance plant nutrient supply and support microbial activities for phytoremediation process. Inorganic fertilizers were discouraged because of its expensive nature and may re-introduce inorganic contaminants into a hitherto polluted soil.

1.5 Aims and Objectives

The aims and objectives of this study are as follows:

1. To determine the uptake rates of Pb and/or Cr in the contaminated soil using untested *Lantana camara* and *Pilea cadierei* indigenous plants, as well as evaluating Pb and Cr half-life's, metal mass balance modelling (STAN), intra-

particle or boundary layer diffusion, isotherms and thermodynamics during phytoremediation process, in addition to soil decontamination scanning electron micrograph (SEM) assay.

2. To explore the abilities of two organic wastes namely empty fruit bunches (EFB) and spent mushroom compost (SMC) used for biostimulation in promoting phytoremediation of metal contaminated soil under various metal concentrations in simulated conditions in a greenhouse.

3. To determine the heavy metal resistant bacteria (HMRB) responsible for Pb and Cr remediation in the contaminated soil (microbial activities).
4. To determine the effects of plant rhizospheric root exudates in the remediation of Pb and Cr contaminated soil.
5. To determine the phytoremediation potentials of both plants and the organic wastes in Pb and Cr co-contaminated soil.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of soil heavy metals

Metals are natural components in soil. Cd, Cr, Cu, Hg, Pb, and Zn are the most common heavy metal contaminants (Greany, 2005; Alloway, 2009; Bieby et al., 2011; Bundschuh et al., 2011; Xiaopeng et al., 2012; Xiaohong et al., 2012; Gabriela et al., 2013; Ana et al., 2014), and some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg (Kachout et al., 2009; Mackie et al., 2012; Rosas-Castor., 2014). Therefore, the understanding of their fundamental properties, environmental and health related impacts of heavy metals are very important in explaining their bioavailability, speciation, and remediation feasibility (Eugen-Schwarz & Roland, 2010; Wuana & Okieimen, 2011; Rachana et al., 2015). The pathway of heavy metals in soil, in terms of their fate and transport is determined by the forms in which the metal exists chemically and the accompanying speciation (Kumpiene et al., 2008; Abbas & Meharg, 2008; Wu et al., 2011; Guo et al., 2012; Mondal et al., 2013; Coporal et al., 2013; Paikaray et al., 2013; Ming-Li et al., 2014). As soon as heavy metals gain access into soils, initial chemistry absorbs them quickly. Followed by prolonged gradual adsorption reactions that take some days or even years to occur, and as such, they become reallocated into diverse chemical types, accompanied by variations in toxicity, mobility, and bioavailability potentials (Wuana & Okieimen, 2011). It is assumed that such reallocation are driven by certain heavy metal reactions, which take place in the soil such as (i) dissolution, and precipitation of minerals (ii) aqueous complexation, (iii) biological immobilization and mobilization, (iv) ion exchange, adsorption, and desorption, and (v) plant uptake (Park et al., 2011a).

Plumbum: In the periodic table of elements, Pb have atomic and mass number of 82 and 207.2, melting point, density and boiling point of 327.4°C, 11.4 g cm^{-3} and 1725°C, respectively. It belongs to the table group IV and period VI, and appears bluish gray in nature, and usually found in combined state with other elements like sulphur (PbS, $PbSO_4$), or oxygen ($PbCO_3$), within the concentration range of 10 to 30 mg/kg in the earth's crust (ATSDR, 2007; Zizhong et al., 2010).

However, the average worldwide Pb concentration on surface soils is about 32 mg/kg, and may vary between 10 to 67 mg/kg (Kabata-Pendias & Pendias, 2001). Industrial scaling of metal production, shows that Pb rank next to Cu, Fe, Zn, Al and approximately 50% of the Pb produced in the U.S. are used for the manufacturing of Pb storage batteries (Wuana & Okiemen, 2011). Alternative use of Pb includes soldering, plumbing, production of bearings, cable shields, manufacturing of ammunition, caulking and pigments. Certain Pb alloys exist and can be seen in antimony storage batteries (Sb), maintenance-free type of storage batteries (Ca and Sn), solder and anodes (Ag), electro-wining process (Sr and Sn), sheet and pipes used in chemical installations and nuclear shielding (Te), printing, sleeve bearing and advanced casting (Sb and Sn) (Wuana & Okiemen, 2011).

Most compounds of Pb (II) tends to be ionic (Pb^{2+} SO_4^{2-}), while Pb (IV) compounds appear covalent, example; tetraethyl lead, Pb $(C_2H_5)_4$. Some compounds of Pb (IV), in the likes of PbO_2 are strong oxidants, and many basic salts can be formed from Pb, such as $Pb(OH)_2.2PbCO_3$ (Hashimoto et al., 2009; Hashimoto, 2013). These salts, sometimes are the most generally utilized white pigment, and source of considerable chronic Pb poisoning to children with habits of eating peeled white paints. Many compounds of Pb (II) and some Pb

(IV) compounds are useful. Pb dioxide and sulphate were the most common forms and take part in the reversible reaction that occurs during the charge and discharge of Pb storage battery.

The oxides of Pb, Pb (II), and Pb- metal oxyanion complexes represent the common forms of Pb that penetrates into surfacewaters, groundwaters and finally into soil profile (Hashimoto et al., 2011). Pb (II) and lead-hydroxy complexes are the most stable Pb form, but with Pb (II) as the most available and reactive, and have the ability to form a mononuclear and polynuclear oxides and hydroxides (GWARTC, 1997). Insoluble Pb predominantly exist as Pb carbonates and (hydr) oxides (Takamatsu et al., 2010). In soil matrix, PbS is characterized of more stable solid form and increasing the sulphide concentrations enhances its formation under reducing conditions. Also, tetramethyl Pb which is volatile organo-Pb is found under anaerobic conditions due to microbial alkylation (GWARTC, 1997).

Basically, Pb exist as inorganic compounds, and some organo-Pb variants such as tetraethyl-Pb also exist. It is worthy to note the toxic and environmental effects of organo-Pb compounds, due to the impacts of tetraethyl-Pb as a gasoline additive (Wuana & Okiemen, 2011). Moreover, more than 1000 organo-Pb compounds have been synthesized, yet those of alkyl Pb (ethyl and methyl) compounds, with their salts are the ones that possess both commercial and hazardous effects.

***Chromium*:** This element is a transition metal located in the d-block group VI B of the periodic table with 24, 52, 7.19 g cm^{-3}, 2665^{o}C, and 1875^{o}C as the values of its atomic number, atomic mass, boiling and melting points, respectively. Its ground-state electronic configuration is Ar $3d^{5}4s^{1}$. It is a rare element and does not naturally exist in elemental form rather as compounds (Dhal et al., 2013). As an essential ore product, the mining of Cr occur in mineral chromite form, $FeCr_2O_4$. Cr contamination can emanate from discharges associated to electroplating processes and the disposal of waste that contain Cr (Arun et al., 2005). The stable form of Cr are the Cr (III) and the Cr (VI) species, although many other unstable valance states of Cr exist that are transient in biological systems. Cr (VI), is considered as the most toxic Cr and found in association with oxygen as chromate (CrO_4^{2-}) or dichromate (CrO_7^{2-}) oxyanions. Cr (III) is less mobile and toxic, and mainly bound to organic matters present in soil and aquatic environment but Cr (VI) is more mobile and as such exhibit greater toxicity than other forms of Cr (Arun et al., 2005; Jie et al., 2011). However, under anaerobic situation, organic matters in the soil, Fe and Se^{2-} can enhance the reduction of Cr (VI) to Cr (III), especially within deeper groundwater. Chromate and dichromate are easily precipitated in the presence of $Ba^{2+,}$ Pb^{2+}, and Ag^{+} (metal cations). At below pH 4, Cr (III) becomes the dominant form of Cr. In combination with Cl^-, F^-, CN^- , NH_3, SO_4^{2-} and OH^-, Cr^{3+} give rise to the formation of solutions and organic ligands that are soluble. With any pH less than 5, the mobility of Cr (III) is reduced by clays and oxide adsorption, just as its solubility is also low under such pH as a result of $Cr (OH)_3$ (s) formulation (Shanker et al., 2009). The major factors affecting the mobility of Cr are soil compositions, such as (electron donor availability, soil texture, competing ions, adsorption capabilities) and other conditions in the soil like (moisture content, temperature, pH and the presence of vegetation) (Banks et al., 2006). When in precipitated and/or soluble forms, transport of Cr from the surface runoff to surface waters can occur, whereas both Cr complexes (soluble and un-absorbed), can potentially get to groundwater as a result of soil leaching (Wuana & Okiemen, 2011).

2.1.1 Sources and pattern of heavy metal(loid)s into the soil and environment

Metal and metalloids are core contaminants of the aquatic systems, atmosphere, and the terrestrial environment (Cempel & Nikel, 2006; Sakultung et al., 2008; Abhijit et al., 2008; Hugh, 2009; Zamboulis et al., 2011; Keun & Tai, 2012; UNEP, 2011). They can enter the environment through a complex form of inorganic and/or organometallic species, depending on the means available, with different stabilities, which could get bioconverted and/or transformed (Vanessa & Elisa 2010; Reis et al., 2009; Santos-Francis et al., 2011; Jianxu et al., 2012; Bagherifam et al., 2014; USEPA, 2007). However, in the terrestrial ecosystem, soil is the main repository of chemical contaminants and aquatic bodies are also considered as sediment sink (Guo et al., 2008; Coman et al., 2013; Weisong et al., 2014; Huaming et al., 2014). Heavy metal or metalloids, virtually occur naturally in soil parent materials (i.e., pedogenic process), in the form that are unreadily bioavailable for plant uptake and leading to uncontamination. Unlike pedogenic inputs, heavy metals added via anthropogenic activities have high potential to be bioavailable for plant uptake, and may lead to dispersed pollution (Adriano, 2001; Bolan et al., 2014; Jay et al., 2014; Keith et al., 2015). The prevalence of heavy metals in the environment are expressed below through a mass balance model for heavy metal found in soil (Wuana & Okiemen, 2011).

$$M \text{ total} = (Mp + Ma + Mf + Mag + Mow + Mip) - (Mcr + Ml) \qquad \text{(Eq. 2.1)}$$

where M: heavy metal, p: parent material, a: atmospheric deposition, f: fertilizer sources, ag: agrochemical sources, ow: organic waste sources, ip: inorganic pollutants, cr: crop removal, and l: losses by leaching, volatilization, and so forth.

However, these contaminants come in many forms (Ali et al., 2013; Jothi et al., 2013), and may have the potential to impact toxins differently upon the environment. Significant among these sources are discussed subsequently.

2.1.1.1 Geogenic impacts

Basically, this is not unconnected with some risk, but however, it is defined as the natural occurrence of elevated concentrations of certain elements in groundwater, such as As, Fe, U and Se, through weathering of soil parent materials including igneous and sedimentary rocks, and coal (Mahimairaja et al., 2005). It has been documented that approximately 45,000 tonnes of As are released into the environment through this means annually, while 50,000 tonnes are via anthropogenic activities (Bolan et al., 2014).

Meanwhile, igneous rocks have been profiled to possess an overall As content of about 100 mg/kg, with 2 to 3 mg/kg as the average content. Whereas in sedimentary rocks it is estimated to vary from very minute amounts in lime/sand stones, to as much as 15,000 mg/kg as seen in manganese ores (Drexler et al., 2003; Bolan et al., 2014). But, in seleniferous soils, as seen in areas with Se-rich rocks, such as carbonaceous limestone and cherts, black shales, mudstones and seleniferous coal are the major routes of Se-input into soils. Similarly, volcanic actions have been implicated to mobilize natural Hg from their uninterrupted deep-earth reserves to across the atmosphere (Lamb et al., 2009). Further elaboration on the global annual emission of Hg from the mercuriferous-belts, and axis within the tectonic plates, as well as the western North America, central Europe, and the southern China zones had been estimated to be 500 Mg/year (Bolan et al., 2014). Although, in the absence of Se and As, other elements such as Cr, Ni, Pb derived via geogenic processes have limited impacts on soil as reported by Jin et al., (2011).

2.1.1.2 Anthropogenic practices

The soil and marine ecosystems are the earth's most productive and foremost diverse entities (Liliana, et al., 2005; Hopper et al., 2005; Emma & David, 2009). The healthy biodiversity of these niches are integral to their greater stability, and may avail them with proper functioning and resilience to natural and anthropogenic (i.e., environmental pollution originating from human sources) intrusions (Abrahim & Parker, 2008; Fenger, 2009; De Zwart et al., 2009; Bolan et al., 2014). However, the diversity of these ecosystems are overwhelmingly threatened by anthropogenic stressors leading to habitat destruction and climate change (Vandana et al., 2011). Crowe et al., (2004) argued that contamination arising from anthropogenic sources are assumed to be additional burden to the ecosystem. Further anthropogenic routes are distinctively discussed below:

***Fertilizer products and applications*:** The application of phosphate fertilizers to pastoral soil are considered the major source of heavy metal input to soils, especially Cd as recorded in Australia, New Zealand, and many Asian countries (Haynes et al., 2009; Bolan et al., 2014). These phosphate-rich Cd-compounds are imbedded in phosphate rocks, which needed to be chemically processed via calcination, to removed Cd from phosphoric acid, in view of recovering a P-fertilizer (Wuana & Okiemen, 2011). However, due to the expensive nature of this approach, and the observed decrease in the reactivity of the recovered phosphates thereafter, it is considered less suitable as a source of P. Hence, the less treated phosphate rocks approach, remains the way forward, and Cd elevation in soil are randomly sustained through this inadequacies. Efforts to reduce Cd-accumulation in soils, through the selective use of phosphate rocks with low Cd-contents have been explored in New Zealand. For example, the reduction of Cd-content in P-fertilizers from 340 mg Cd kg^{-1} P observed in

1990s, to 280 mg Cd kg^{-1} P by the year 2000 had been achieved, via a superphosphate fertilizer industry that focuses on selective phosphate rock approach, combined with pre-treatments (Jin, et al., 2011). But contrary to this development, many countries still source for high-Cd content phosphate fertilizers, due to reasons bordering around practical, technical, and economic limits, hence soil metal contamination continue to surge.

***Biosolids and municipal/industrial waste water*:** These are nutrient-rich organic materials and/or amendments, from the treatment of sewages. When used as soil nutrient supplements on consistent basis, can induce heavy metals elevation, such as Pb, Ni, Cr, Cu, Zn, Cd, and As to the soils (Haynes et al., 2009; Alaribe & Agamuthu, 2010). It has also been reported that biosolids application of about 250 kg N ha^{-1} $year^{-1}$ to agricultural soil, resulted in metal loading of approximately 6 mg kg^{-1}, 2 mg kg^{-1} , 5 mg kg^{-1} , and 0.2 mg kg^{-1} dry weight of Zn, Cu, Pb, and Ni (Jin, et al., 2011). These metals induced by biosolids, are said to be typically immobilized in soils, and can be toxic to soil micro-flora, and may accumulate in plants and grazing animals (Jin, et al., 2011). Therefore, Kao et al., (2006) reported that the reduction of microbial biomasses from biosolids application and/or impacts on soil, indicated that microbial activities were disrupted by heavy metals. Globally, wastewater is used to irrigate over 20 million hectares of agricultural lands (Wuana & Okiemen, 2011). In fact, agro-based wastewater irrigation accounts for 50% of vegetable supply in most urban areas of Africa and Asian cities (Bjuhr, 2007). While having much interest in high crop yields and profit maximization, most farmers show no regards for environmental benefits, and practices that mitigates hazards. Although, the concentrations of metals are presumed to be relatively low in wastewater effluents, yet a prolong application on land can ultimately lead to accumulation of heavy metals in the soil (Alaribe & Agamuthu, 2010; Wuana & Okiemen, 2011; Weiping et al., 2013; Huang et al., et al., 2015). Furthermore, about 6400 and 1600 tonnes of tannery and timber treatment effluents, are generated annually in New Zealand, which are believed to be loaded with Cr, and this pose as the major source of Cr into the terrestrial and aquatic environments (Zhitkovich, 2011; Bolan et al., 2014; Choppala et al., 2015). In India alone, about 2000 to 32,000 tonnes of elemental Cr from tannery industries escaped annually into the environment (Choppala et al., 2015). This has implicated the leather industry to have contributed about 40% influx of Cr into the biosphere (Arun et al., 2005; Zhitkovich, 2011). In extension to this, timbers treated with large scale of Cu-Cr-As (CCA) in vineyards, have been reported to discharge Cu, Cr, and As to the soil environment in Australia (Schwer III & McNear, 2011). However, Jin et al., (2011) argued that advancement of technology in sewage water treatments, through the isolation of industrial wastewater from sewage treatment plants has steadily resulted in the decline of metal content in biosolids.

Pesticides: Most of the extensively applied agricultural and horticultural pesticides in earlier days contained huge amounts of metals. For instance, it was reported that about 10% of the chemicals, previously approved for use in the United Kingdom as fungicides and insecticides contain Cu, Hg, Mn, Pb or Zn (Wightwick et al., 2013). *Bordeaux* formulations, mainly containing copper oxychloride were used as fungicidal spray, and also as a growth promoter in piggery and poultry units (Wightwick et al., 2013). However, the elevation of Cu in agricultural soils, resulting from the incessant application of Cu-fungicides have been reported in many countries, and further formation of bare sterile patches in orchards are one of the excessive impacts of Cu accumulation (Wightwick et al., 2010; Zhou et al., 2011). Problems can emanate from such contamination, especially in situations where such sites are reutilized for either agricultural or other non-agro related activities.

Manures: Repeated application of manures to soil has increasingly being identified as an alternative pathway for metal input to soils (Huang et al., et al., 2015). For example, in England and Wales, the addition of animal manures alone to agricultural lands, which represent about 25 to 40% source of metal contamination of soils annually, have recorded 225 Mg, 1821 Mg, and 5247 Mg of Ni, Cu, and Zn respectively, as yearly inputs facilitating soil pollution (Nicholson et al., 1999).

In China, the concentration of Cu measured in chicken, cattle, sheep, and pig manures showed Cu loads were 81.8 mg kg^{-1}, 31.8 mg kg^{-1}, 66.85 mg kg^{-1}, and 699.6 mg kg^{-1}, respectively. This would likely be a source of heavy metal contamination to agricultural lands in China, when repeatedly applied (Xiong et al., 2010). A similar kind of study in Sydney Australia, implicated poultry manure as the cause of Cd and Zn rise in vegetable soils (Jin, et al., 2011). Also, sludge applied to soil in New Zealand from dairy pond effluents, based on N concentration of 150 kg N ha^{-1}, was reported to induce between 31.5 kg Cu ha^{-1} to 73.7 kg Cu ha^{-1} (Bolan et al., 2003a).

In extension to this, Martinez & Peu, (2000) accounted that within 8 years, about 183 kg and 266 kg of Cu and Zn got accumulated at soil surface, through swine manure application. Therefore, animal ingestion of metal contaminated soils during grazing, manure collection and handling, and the addition of metal supplements and/or additives, such as Fe, Zn, Se, Cu, As, and Co to livestock and poultry feeds, to improve their metabolic efficiencies, may not be unconnected to the incidence of heavy metal present in soils via manures (Papaioannou et al., 2005; Mondal et al., 2007; Li et al., 2007; Bolan et al., 2014).

***Metal mining, smelting and processing of industrial waste*:** Metal mining has been implicated to be the second most prominent source of heavy metal soil-contamination following sewage sludge (Ashraf et al., 2011). This encompasses the deposition of large volumes of mining waste and/or tailings on the soil, ground and surface waters. Hence, propelling unfavourable conditions to the natural vegetations, thereby inducing acidity, low water retention capacity and less nutrients for plants (Fujii et al., 2008; Malakootian et al., 2009; Guo et al., 2010;Wan, 2014; Huang et al., 2015; Alaribe & Agamuthu, 2016). A typical scenario is shown in **Plate 2.1** of a mining contaminated area.

Plate 2.1: A mining impact contaminated land/soil in Shangdon, China.
From Liu et al., (2011).

Tailings are therefore unavoidable by-products of metal mining/smelting, and are basically from crushed mixtures of rocks, concentrators and/or washeries (Csavina et al., 2012), processing fluids from mills after extraction of economic metals, minerals, and coal from mine resource (Kossoff et al., 2012). A typical scenario of chrome-ore industrial processes is given **Figure 2.1**. Generally, tailings have bulk density ranging from 1.8 to 1.9 t m^{-3} with a specific gravity of about 2.6 to 2.8. In a fluvial environment, due to its comparative particle size, tailing grains are further dispersed to the downstream, polluting greater area of the floodplain, and perhaps causing sediment accumulation (Vandeberg et al., 2011; Anawar, 2013). Hence, allowing it to be kinetically prone to oxidation, and the subsequent release of incorporated contaminants and/or heavy metals into a previously pristine ecosystem (Kossoff et al., 2012; Kapusta & Sobczyk, 2015). Examples of environmental impacts of tailings dam failure reported, include the "May 1998" *Aznalcóllar* breech, where area of about 4286 ha was contaminated, and 2557 ha of this fraction was basically agricultural land with vast economic crops rendered unsafe (Foulds et al., 2014). Also, Pepin & Jullien (2000) reported that between January and March 2000, there were two separate tailings dam burst, that occurred in *Baia Mare* and *Baia Borsa* regions of Romania, which resulted in 120 tonnes of cyanide and metallic elements (Pb, Cu, and Zn) into the catchment areas, hence, contaminated the environment and led to fish deaths with cross-border international consequence affecting Hungary, Serbia, and Bulgaria. Also in northern Tunisia, Souissi et al., (2013) reported several tonnes of Pb-Zn ore processing waste, were abandoned as tailings piles without concern of environmental issues. This infiltrated into local watercourse, and caused elevated metal concentration in the affected farmlands (Daldoul et al., 2015).

Figures 2.2 and **2.3**, specifically elucidates the percentage distribution of major soil pollutants, and sectors contributing to it in Europe (Dhal et al., 2013).

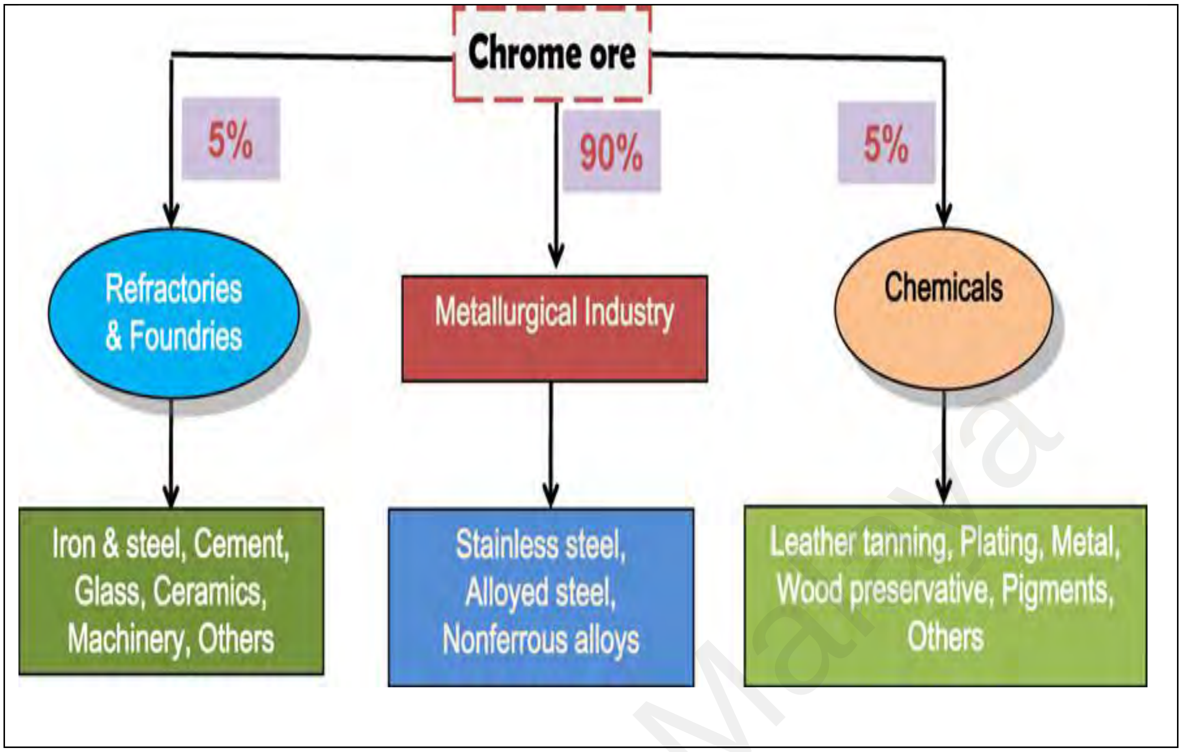


Figure 2.1: A typical scenario of a chrome ore industrial process leading to sources of environmental contamination.
Source: Dhal et al., (2013).

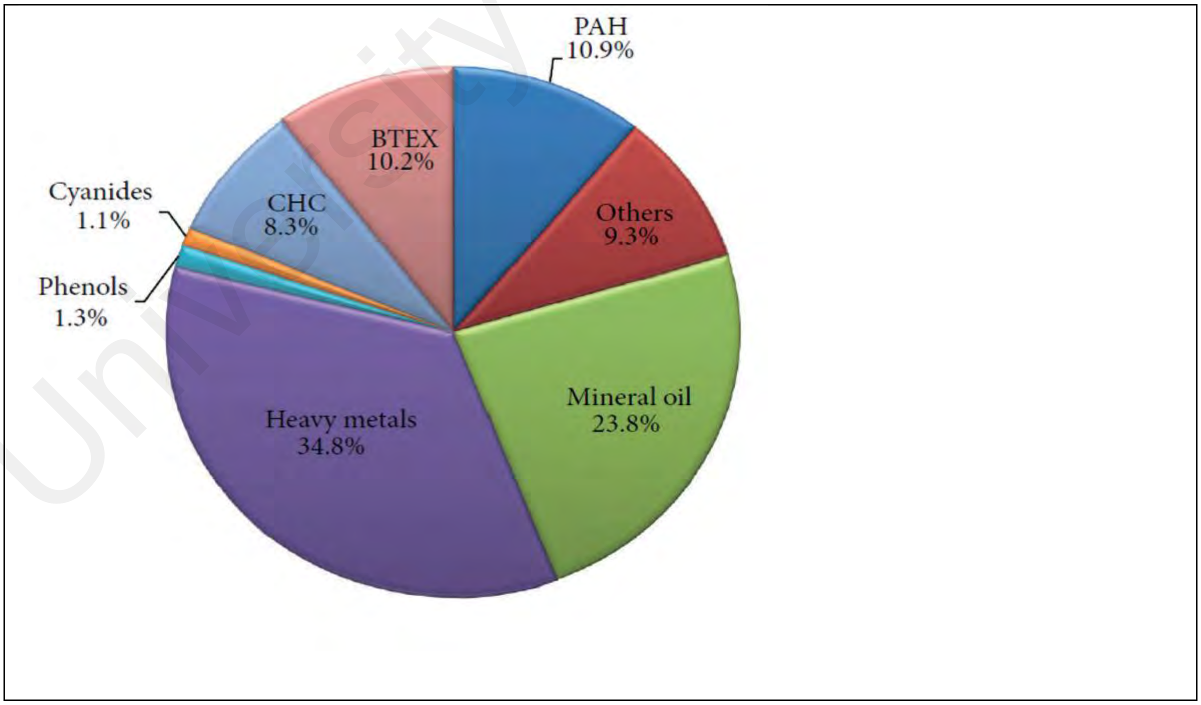


Figure 2.2: Dispersion of core pollutants affecting European soils.
Source: Dhal et al., (2013).

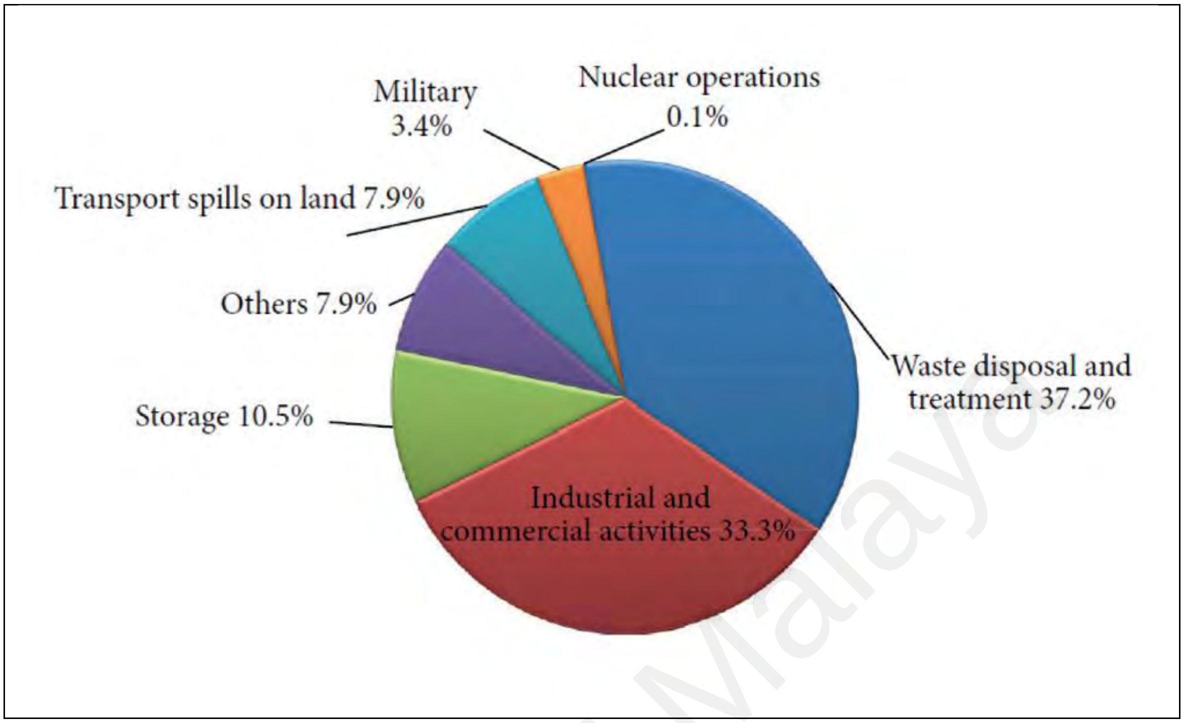


Figure 2.3: Segmentation of European sectors fueling-up soil contamination.
Source: Dhal et al., (2013).

Air-Borne Origins: The activities of integrated steel works emit polluted dust particles that are characterized to possess incrust heavy metals (Sawidis et al., 2011), and the abatement of these particles is almost impossible because of the fugitive emissions produced (Hlies et al., 2013; Lim et al., 2010). In extension, during sintering process, stack emissions are generated and hence constituting about 45% particle matter emission of heavy metals such as Pb in steelmaking processes (Sammut et al., 2010). Hence, Hlies et al., (2013) stated that irrespective of the high efficiency of the electromagnetic precipitator, with relatively high off-gas flow rate of about 1500 to 2500 Nm^3/t sinter, generates particulate emission of about 40 to 559 g dust/ t sinter, into the environment.

Since the dawn of industrial revolution, large scale contamination has been prevalent due to utilization of fossil fuels which often contain some heavy metals (Lim et al., 2010). The ensuing activities allow solid particles, such as smokes from fires, and other emissions from factory chimneys to be deposited on land and sea (Szopka et al., 2013). Moreover, the combustion of petrol containing tetraethyl-Pb promotes aerial emission of Pb (Wuana & Okieimen, 2011; Li et al., 2010), and in Shanxi China, gas emission from the coke-oven plants during coke making, have been implicated to cause about 40% air pollution in the whole of that province (Li et al., 2012).

Moreno et al., (2004) also found elevated amount of iron condensates, which is above 30 percent of the total number of air particles in Port Talbot, United Kingdom - a city near to a steel plant. Furthermore, municipal solid waste incineration have been reported as a source of anthropogenic metal contaminants into the atmosphere (Li et al., 2010), and also the indispensable role of landfill in waste disposal has indicted it as a potential source of atmospheric heavy metal pollution, because landfill sites acts as effective bioreactors that could convert inorganic-metals, to methyl-metals, and this is considered as the most toxic form of some elements that is air-borne (Li et al., 2010).

The summary of global sources of heavy metal soil contamination and quantification are presented in **Table 2.1,** and geoaccumulation index in **Table 2.2,** respectively**.**

Table 2.1: Various sources of heavy metals contamination of soils and its world annual quantification (1000 tonnes per annum).

Sources	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
Food waste and agriculture	0 ~ 0.6	0 ~ 0.3	4.5 ~ 90	3 ~ 38	0 ~ 1.5	6 ~ 45	1.5 ~ 27	12 ~ 150
Farmyard manure	1.2 ~ 4.4	0.2 ~ 1.2	10 ~ 60	14 ~ 80	0 ~ 0.2	3 ~ 36	3.2 ~ 20	150 ~ 320
Municipal wastes	0.09 ~ 0.7	0.88 ~ 7.5	6.6 ~ 33	13 ~ 40	0 ~ 0.26	2.2 ~ 10	18 ~ 62	22 ~ 97
Logging and timber Industry wastes	0 ~ 3.3	0 ~ 2.2	2.2 ~ 18	3.3 ~ 52	0 ~ 2.2	2.2 ~ 23	6.6 ~ 8.2	13 ~ 65
Municipal sludge	0.01~ 0.24	0.02 ~ 0.34	1.4 ~ 11	4.9 ~ 21	0.01 ~ 0.8	5.0 ~ 22	2.8 ~ 9.7	18 ~ 57
Organic wastes	0 ~ 0.25	0 ~ 0.01	0.1 ~ 0.48	0.04 ~ 0.61	-	0.17 ~ 3.2	0.02 ~ 1.6	0.13 ~ 2.1
Coal ash	6.7 ~ 7	1.5 ~ 13	149 ~ 446	93 ~ 335	0.37 ~ 4.8	56 ~ 279	45 ~ 242	112 ~ 484
Fertilizer	0 ~ 0.02	0.03 ~ 0.25	0.03 ~ 0.38	0.05 ~ 0.58	-	0.20 ~ 3.5	0.42 ~ 2.3	0.25 ~ 1.1
Solid waste & metal processing	0.01~ 0.21	0 ~ 0.08	0.65 ~ 2.4	0.95 ~ 7.6	0 ~ 0.08	0.84 ~ 2.5	4.1 ~ 11	2.7 ~ 19
Commodity impurities	36 ~ 41	0.78 ~ 1.6	305 ~ 610	395 ~ 790	0.55 ~ 0.82	6.5 ~ 32	195 ~ 390	310 ~ 620
Marl	0.04 ~ 0.5	0 ~ 0.11	0.04 ~ 0.19	0.15 ~ 2.0	0 ~ 0.02	0.22 ~ 3.5	0.45 ~ 2.6	0.15 ~ 3.5
Atmospheric deposition	8.4 ~ 18	2.2 ~ 8.4	5.1 ~ 38	14 ~ 36	0.63 ~ 4.3	11~ 37	202 ~ 263	49 ~ 135
Total	52 ~ 112	5.6 ~ 38	484 ~ 1309	541~1367	1.6 ~ 15	106 ~ 544	479 ~ 1113	689 ~ 2054

Source: Su et al., (2014).

Table 2.2: Categorization of soil geoaccumulation index under CEPA standard.

Geoaccumulation index	Categorization	Extent of soil contamination
$5 < Igeo \leq 10$	6	extremely serious
$4 < Igeo \leq 5$	5	Strong to extremely serious
$3 < Igeo \leq 4$	4	Strong
$2 < Igeo \leq 3$	3	Moderate to strong
$1 < Igeo \leq 2$	2	Moderate
$0 < Igeo \leq 1$	1	Light to moderate
$Igeo \leq 0$	0	Non contamination

Sources: Su et al., (2014); CEPA, (1995); Wang et al., (2007).

In further details, geoaccumulation index (Igeo), is obtained by comparing the prior soil-metal contamination values with its current contaminated level as thus;

$$\text{Igeo} = \text{Log } 2\,\left(\frac{Cn}{1.5\,Bn}\right) \qquad \text{(Eq. 2.2)}$$

where, C_n is the measured mass fraction of metal in contaminated soil (mg/kg), B_n is taken to be the background mass fraction of metal (mg/kg) prior to pollution (Wang et al., 2007). This computation is used to evaluate the extent of heavy metal contamination in soils.

2.1.2 Harmful effects of specific heavy metals on human health

It is well recognized that heavy metals can cause severe physiological harm when exposed to them, but however some are needed by humans such as Mn, Fe, Cr, and Zn in specific measurable amounts (Ali et al., 2013). Meanwhile, it is very important to note, that any of these heavy metals required by human system, for certain metabolic functions can as well destroy life when it exceeds the acceptable limits (Gosh, 2010) **(Table 2.3)**. The body mechanism responsible for metal-homeostasis can no longer operate, hence leading to organ disruptions (Mudipalli, 2008).

Table 2.3: Specific heavy metal harmful effects on human health.

Heavy metal	Harmful effects	References
Zn	Elevated dosage can result to fatigue and dizziness	Wuana & Okieimen (2011)
Hg	Autoimmune disease, drowsiness, body imbalance, fatigue, hair loss, insomnia, anxiety, depression, irritability, loss of memory, recurrent infections, vision disturbances, tremors, temper out-burst, ulcers and brain damage, kidney and liver impairment.	Gulati et al., (2010) Ainza et al., (2010)
As	Interferes with essential cellular functions, such as oxidative phosphorylation and-ATP synthesis	Tripathi et al., (2007)
Pb	It causes developmental impairment among children such as dyslexia, renal failure, and promotes risk of cardiovascular diseases.	Iqbal (2012)
Cr	Causes hair loss	Wuana & Okieimen (2011)
Cd	Mutagenic and carcinogenic, obstructs calcium regulation in biological system, causes-renal failure and chronic anaemia.	Awofulo (2005)
Cu	Elevated concentration causes kidney and brain damage, chronic anaemia and liver cirrhosis, intestinal and stomach irritation.	Bilal et al., (2013)
Ni	Allergic dermatitis referred to as Ni-itch, causes cancer of the lungs and nose when inhaled, and as well sinuses, hair loss, cancer of throat and stomach, hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, and hepatotoxic impacts.	Das et al., (2008) Khan et al., (2007) Ali et al., (2013) Khlifi & Hamza-Chaffai (2010)

2.1.3 ASEAN challenges and efforts on environmental pollution issues

Undoubtedly, Asia remains the world's largest and populous continent. Covering about 8.6% of the Earth's total surface area (or 29.4% of its land area), with over 4 billion people, that is approximately 60% of the world's unfolding human population (Fenger, 2009). Within the past three decades, tremendous industrial and economic growth has occurred in Asia. It has equally opened the region's natural environment to unprecedented risk (Fenger, 2009). However, Association of Southeast Asian Nation (ASEAN) "an entity in Asia", comprising of Brunei, Myanmar, Cambodia, Laos, Malaysia, Indonesia, Philippines, Singapore, Thailand, and Vietnam is one of the vulnerable regions in the world to environmental degradation, and her ecological debt should not be overlooked (Lee at al., 2013).

For instance, the draining of swampy peatland, usually intended for agricultural purposes in South East Asia, has caused land to subside, become highly acidic, and hence, be unfit for any use (ASEAN, 2011). Beyond peatlands, an array of problems, including intensive farming, and mining has contributed to high rates of decline in agricultural soil quality, particularly in Vietnam, Thailand, and Indonesia (ASEAN Biodiversity, 2013). The Food and Agriculture Organization, estimated that in two-third of ASEAN nations, 40% of the land is suffering either from severe or very severe degradation due to human activities (FAO, 2011). Hence, mitigating and reversing peatland degradation and associated impacts requires an integrated approach to address the root causes which are common across many countries in Southeast Asia.

There are also consistent challenges in the acceptable levels for certain heavy metals in food supplements. Concentrations can vary between 30 times lower in one ASEAN region to another (Food Industry Asia, 2013). For example, the limit for Sn allowed in flavor is 40 mg/kg, but 250 mg/kg variation has been reported (Food Industry Asia, 2013), Cd range from 0.2 mg/kg to 1.5 mg/kg, and Pb inexplicably range from 1.0 mg/kg to 7 mg/kg, while the maximum permitted level for As in one particular flavour used in syrup can be as high as 3 mg/ kg in one country and as low as 0.5 mg/kg in another ASEAN region (Food Industry Asia, 2013).These variations restrict some corporations ability, especially SMEs, to sell certain ingredients across borders due to the high cost of compliance. If probably un-complied to, means introducing elevated heavy metal products into another region, hence exceeding another regions metal compliance limit.

These fluctuations, also exist globally with reference to soil heavy metal standards as indicated in **Table 2.4**. The regulatory institutions includes but not limited to the United States Environmental Protection Agency-USEPA, Tasmania Environmental Protection Agency EPA-Australia, The Dutch Standards (Netherland), The Department of Environment (DOE) Malaysia, and the National Institute for Soils and Fertilizers (NISF) Vietnam.

However, within ASEAN, mitigating efforts have being deployed, via policy and principles of common but differentiated responsibility to minimize these impacts. Such as the ASEAN Framework for Peatland Management for Vision 2020 (adopted in 1997); envisions “a clean and green ASEAN with fully established mechanisms for sustainable development, to ensure the protection of the regions environment, sustainability of its natural resources,-

Table 2.4: Heavy metal target concentration and intervention values for soil remediation with respect to different regulatory institutions and two ASEAN regions.

	US-EPA		**TASMANIA-EPA**		**DUTCH STANDARD**		**DOE-MALAYSIA**		**VIETNAM STANDARD**	
	[NBC	**IV]**	**[NBC**	**IV]**	**[NBC**	**IV]**	**[NBC**	**IV]**	**[NBC**	**IV]**
Metals	(mg/kg)		(mg/kg)		(mg/kg)		(mg/kg)		(mg/kg)	
Pb	200	400	300	1200	85	530	0.18	36.00	1.97	43.31
Cr	22	230	50	500	100	180	0.02	14.40	1.14	145
As	0.11	0.40	20	200	29	76	1.1	43.0	1.57	43.9
Ni	72	1600	60	600	35	100	0.70	28.90	0.67	68.5
Cd	0.43	70	3.0	40	0.8	13	0.09	11.90	0.004	0.293
Zn	1100	23600	200	14000	140	720	6.9	54.3	3.53	111

Notes: **NBC**= National Background Concentration **IV**= Intervention Value

References: USEPA (2008); EPA-Tasmania (2012); Dutch values (2009); DOE-MY (2009; ***www.doe.gov.my***); NISF-Vietnam (2001) and high quality of life for its people" and addressing environmental degradation and trans-boundary pollutions (ASEAN, 2013).Which flourished alongside with "Basel Convention on the Control of Trans-boundary Movements of Hazardous Wastes and their Disposal" of 5th May 1992 (Agamuthu & Victor, 2013).

The Basel Convention had most profound global and environmental harmonization on hazardous wastes, especially on E-waste pollution control (Terazono, et al., 2006; UNEP, 2007; Agamuthu & Victor, 2013). Progressing in that direction, integrated plan aimed at protecting human health and the environment, from adverse impacts resulting from the generation, management, trans-boundary movements, and disposal of hazardous and/or toxic wastes were sustained (WHO, 2007; Thai et al., 2009; Nguyen et al., 2009; Li et al., 2011; Agamuthu & Victor, 2011 and 2013; Shinkuma & Managi, 2010; Chi et al., 2011).

2.2 Chemistry of heavy metals and dynamics in soil

Complex interactions exist between heavy metals and soil, hence it has been reported that metals bond strongly to the organic and/or inorganic (Ceriotti & Amarasiriwardena, 2009), as well as, the colloidal constituents of the soil (Alloway, 2010). Hence, the potential at which these heavy metals are retained in soils include but not limited to one or more of the following: (i) the residual properties attributed to the metal source, (ii) the intrinsic affinity of the metal ions to the soil adsorption surfaces and soluble ligands, (iii) suits of adsorption surfaces such as humus, metal oxides, alumina silicate-clays etc., (iv) transient features such as pH, temperature, biological activity, redox potential, water content, and salt concentration, importantly again is (v) soil-metal contact time (Alloway, 2010; Violante1 et al., 2010). However, synchrotron-based spectroscopy (EXAFS; XANES), electron microprobe, and diffuse-gradient technology have further elucidated the bonding structures of these metals to soil substrates (Xue et al., 2013). Therefore, before returning to soil-metal chemistry, it is imperative to reveal the three dynamic metal-states found in soil matrices as thus:

Inert-metal state: This happen when metals in soil, are held in a form which only respond to change in solution conditions over periods of years possibly via organic alterations, redox changes, and mineral weathering (Alloway, 2010). For instance, resistant primary (e.g. sulphide) or secondary (e.g. phosphate or carbonate) metal compounds dissolution over extended periods.

Non-labile state: This represents metals that are non-reversibly held, and hence exhibits apparent sorption hysteresis, (**Figure 2.4)**. In other words, kinetically constrained and respond to changing conditions slowly between days to months, in soil solids or probably in suspension linked to ligands such as humic acid (Gustavsson et al., 2006). Hence, desorption may occur without variation in redox configuration or decomposition of the absorbing substrate. For example, mixed metal compound that are in solubility equilibrium with the soil solution (Alloway, 2010).

Labile state: These are category of metal ions, which instantaneously respond reversibly (adsorption or desorption) to changes in solution equilibrium without exhibiting adsorption hysteresis (Alloway, 2010), (**Figure 2.4)**. They are found in wide range of chemical forms, such as soluble ligands complexes. Examples are exchangeable held hydrated metal ions in clay interlayers.

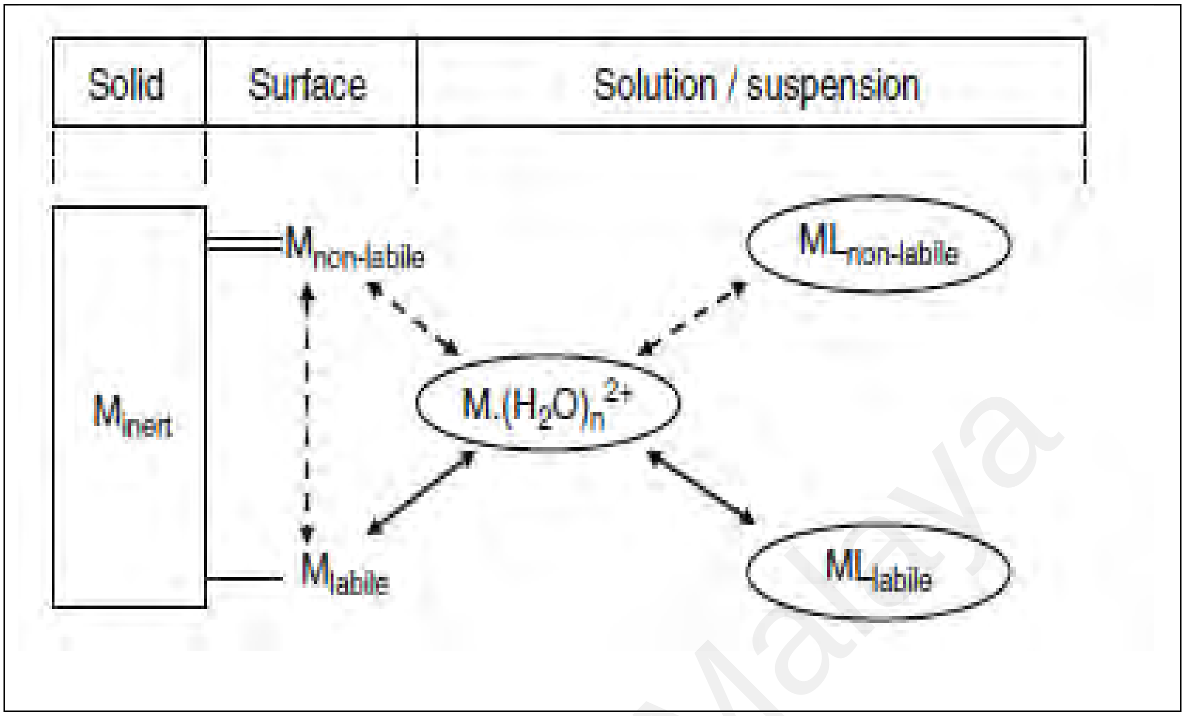


Figure 2.4: Schematic flow of three-phase divalent metal ion equilibria in soils. Broken and solid reversible arrows represent kinetically constrained and instantaneous reactions respectively.
ML indicates metal ions bound to soluble ligands and M denotes metal.

Source: Alloway, (2010).

2.2.1 Affinities and adsorption reactions of heavy metals on soils

Basically, metal ions are retained on the colloidal soil surfaces by adsorption and complexation, precipitation, and oxidation/reduction (Park et al., 2011b). These are further dependent on both soil properties and environmental factors (Alloway, 2010).

Adsorption and complexation: The formation of surface complexes which can either be physical bonding of metal ions and/or molecules onto the surface of another constituent, hence, grouped into specific or non-specific retentions (Vega et al., 2010; Park et al., 2011b; Komárek et al., 2013). The phenomenal balance of charges between the adsorption solute and the sorbent (i.e., soil), through electrostatic attraction, thereby displacing other "like-charge" ions from the soil surface is known as non-specific (Covelo et al., 2007; Merdy et al., 2009), whereas the formation of chemical bonds between the solutes and the functional groups at the soil surfaces, are typical of specific adsorption (Zenteno, et al., 2013; Bolan et al., 2014).

Nevertheless, heavy metal affinity to soil is highly dependent upon soil components such as silicate clays, organic matter, Fe, Al, manganese oxides, and as well innate soil ligands (Shahid et al., 2012; Shaheen et al., 2013; Bolan et al., 2014). The following authors, Ferrand et al., (2006); Kumpiene et al., (2008); Quenea et al., (2009); Violante et al., (2010) and Yib et al., (2010) expressed the general affinity order for heavy metals to organic matters in soil to be $Cu^{2+} > Hg^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+} >$ As (V) > As (III). Hence, when metal ions directly bound to the surface functional groups of soil particles, the ensuing complexes formed are referred to as inner-sphere complexes, and its more stable due to covalent bond (Park et al., 2011b; Quizhong et al., 2013), but when water molecules interposed between the surface functional group and the metal ions, outer-sphere complexes are formed (Bolan et al., 2014). In other words, known as multi-dentate complexes which enhances chelation bond stability. Some examples of natural ligands are humic and fulvic acids, while anthropogenic ligands include ethylenediaminetetraacetic (EDTA), and nitrilo triacetic (NTA) acids (Evangelou et al., 2007; Barrutia et al., 2010; Kovacs et al., 2010; Shahid et al., 2012). Basically, chelation with EDTA or NTA complexes in soil can promote metal mobility in view of phytoremediation in soil metal removal (Andra et al., 2009; Sun et al., 2009; Xu et al., 2013; Barrutia et al., 2010; Kolodynska, 2011). However, triple components (metal- ligand- and organic acid in solution) formation, known as ternary complex, may enhance elevated mobilization of metals and possibly leach into groundwater reserves (Kolodynska, 2011; Park et al., 2011a).

Precipitation and dissolution: This is considered to be a predominant mechanism in heavy metal immobilization process, especially when anions such as sulphates (SO_4^{2-}), carbonates (CO_3^{2-}), hydroxides (OH^-), and phosphates (HPO_4^{2-}) are present in soil, under a high pH and elevated metal conditions (Hong et al., 2007; Ok et al., 2010; Shaheen et al., 2013; Bolan et al., 2014). For instance, P has been reported to decrease the leaching of Zn, Cd, and Pb. Hence, diammonium phosphate have been implicated to decrease the concentration of metal in solution by forming metal-phosphate precipitate in activity-ration diagram (Adriano, 2001; Barrow, 2008), while liming application have reportedly induced metal retention in soil (Hong et al., 2007; Park et al., 2011a). Furthermore, Lu et al., (2011) reported co-precipitation in the presence of Fe and Al oxyhydroxides, altered the chemical features of the soil, thereby enhancing Pb removal in aqueous systems.

Oxidation /reduction: Metals such as Cr, Se, Hg, and As, are usually exposed to microbial oxidation and/or reduction reactions in soils (Bolan et al., 2014), which by extension determines their speciation and mobility functions. Redox reactions of metal in soils, are purportedly grouped into assimilatory and dissimilatory mechanisms (Madigan & Brock, 1991; Holden & Adams, 2003). Under assimilatory perspective, the metal soil-substrate acts as the terminal electron acceptor, hence promoting bacterial growth (Bolan et al., 2014). Whereas, in the dissimilatory mechanism, the metal soil-substrate has devoid specific function with regards to the metabolic process of the microbial species initiating the reactions, but rather, propels redox reactions indirectly (Park et al., 2011a).

Drawing instance from the above illustrations, Cr (VI) can be reduced to Cr (III) in soil where Fe (II) is available as the source of electron (Barren-Diaz et al., 2012). The microbial reduction of Cr (VI) occurs when organic matter is expressed as an electron donor, through both abiotic and biotic mediated processes (Cheung et al., 2007; Choppala et al., 2015; Clarholm, et al., 2015). However, the reversible oxidation of Cr (III) to Cr (VI), can enhance mobilization and bioavailability of Cr in soil (though more hazardous in nature), through abiotically mediated oxidizing agents such as Mn (IV) and Fe (III) (Kumpiene et al., 2008; Sun et al., 2009; Barren-Diaz et al., 2012; Bachate et al., 2012). Some anaerobes utilized Se (VI) as their terminal electron acceptors for growth purposes, thus enhancing Se (VI) reduction to Se (0), which is essential for the precipitation of Se in contaminated environment (Park et al., 2011a; Bolan et al., 2014). Lie et al., (2006) isolated *Bacillus sp.* from Cr-contaminated landfill soil, and demonstrated that toxic Cr (VI) was reduced effectively into comparatively less toxic Cr (III).

In continuation, interplay of soil redox potential (Eh), can also define the chemistry of heavy metals in soil (Alloway, 2010; Hafsteinsdȯttir et al., 2015). For example, under a distorted condition, caused by wetness of the soil, it impedes the ingression and/or diffusion of oxygen into the soil (Salvarredy-Aranguren et at., 2008; Őstman et al., 2008; Frohne et al., 2011), which creates alternative electron acceptors (i.e., carbon substrates, etc.), as the organic matter continue to decompose, under a progressive anaerobic/anoxic conditions, has been implicated to cause heavy metal solubility in soils (Kaplan & Knox, 2007; Frau et al., 2009). Redox potential can vary seasonally, being higher in wet season ranging from +150 to +562, and +22 to +531 for dry season, which affect the fate, concentration and mobility of edaphic contaminants (Salvarredy-Aranguren et al., 2008).

Soil pH value: Transient soil conditions such as pH, has greater influence on heavy metals chemistry than any other factors (Alloway, 2010). Soil pH indicates the hydrogen ion activity of soil solution. The solubility of metal oxides, hydroxides, and carbonates (MCO_3) can predict this phenomenon. For example, MCO_3 (s) + $2H^+ \leftrightarrow M^{2+}$ + CO_2 (g) + H_2O showed a reaction stoichiometry ($M^{2+} \leftrightarrow H^+$) exchange, usually < 2 needed for precipitation reactions (Degryse et al., 2009), and hence, indicating the significance of specific adsorption in determining metal ion solubility. The change in soil pH has several important, and independent effects on cationic metals which influences the strength of adsorption on soil solids (Unuabonah et al., 2008; Alloway, 2010). Impacts of pH values > 6 in lowering free metal ion activities in soil has been linked to rise in pH-dependent surface charge oxides of Mn, Fe, and Al, chelation by organic matter and/or metal hydroxides precipitation (e.g., Pb $(OH)_3$) (Bolan et al., 2014). In extension, heavy metal activity (e.g., Pb and Cd) in naturally acidic soils medium, has been reported to decrease with increasing pH (Violante et al., 2010), in association to increasing cation exchange capacity (CEC) (Nigussie et al., 2012).

Meanwhile, Lu et al., (2011) presented co-precipitation of Pb (II) at pH 4 with ferric oxyhydroxides, which demonstrated efficient elimination of Pb (II) from aqueous medium than adsorption, at standardized pH condition. Sharma, (2012) referred soil pH scale as logarithmic and not linear, therefore soil with a pH of 8.0 is ten times more alkaline than a soil with a pH of 7.0, and soil with pH of 5.0 indicates 100 times more acid than a soil with a pH of 7.0 (Sharma, 2012).

2.3 Mechanisms of natural nutrient acquisition and transport from soil to plant system

Soil being a product formed through natural decomposition, chemical, and physical weathering forces, acting upon native rocks, vegetation, and animal matter over an extremely long period of time contains elements, that supports plant metabolism (Brevik, 2013; Mark et al., 2013). It is well known that plant root absorbs water and inorganic nutrients from the soil. These are further delivered onto the shoots via the xylem functions (Pandey et al., 2013), either through the apoplastic (passive) or symplastic (active) adsorption pathways (White & Broadley, 2011). About 17 nutrients are essential for most ecological plants (**Figure 2.5**), but 14 of these are basically obtained from the soil, while oxygen, carbon, and hydrogen come from air and water (Rogiers et al., 2006; Yan et al., 2006; Plaster, 2011). Furthermore, cation exchange is believed to be responsible for plant nutrient uptake, because hydrogen (H^+) is pumped through the root hairs into the soil, displacing cation bonded to negatively charged soil particles, and hence renders the cation available for root uptake (Wenzel et al., 2003).

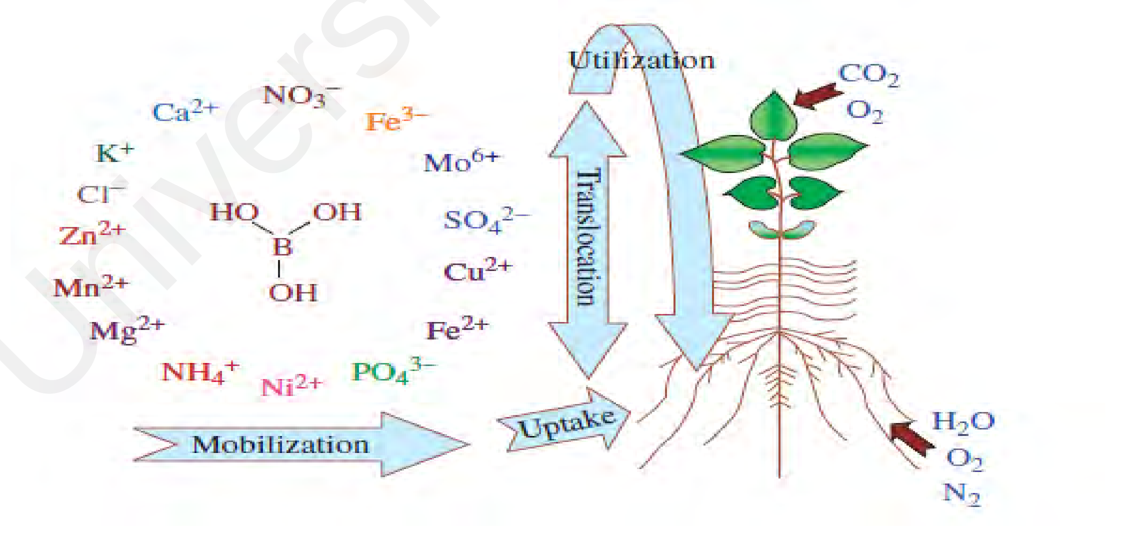


Figure 2.5: The nutritional processes in higher plants, indicating essential elements acquired from the soil or air in various forms and the processes of mobilization, uptake and utilization.
Source: Yan et al., (2006).

Whilst negative charges are observed in the root cell walls (Foster et al., 2013), cation accumulation and repulsion of anions are prevalent in the roots apoplast, particularly for di- and polyvalent ions (Sattelmacher, 2001). Haynes, (1980) argued that this could imply difference in the K: Ca ratio among plants species, while Marschner, (1995) suggested a preferential uptake of certain metals against another, such as Zn and Cu in ionic forms, rather than in chelated states. But reduction of the nutrient translocation activity into the symplast, and decrease in xylem loading, may cause restricted nutrient absorption in the root (Sattelmacher, 2001; Plaster, 2011). Thus, these assimilated nutrients are modified into numerous organic molecules, through photosynthetic and/or biochemical processes (Sage & Sata, 2015), and associated mechanisms at the shoot region. The formed organic compounds are redistributed from the leaf-source, to the different plants components; roots, flowers, nascent leaves, and onto the fruits, collectively known as the nutrient sinks, through phloem activities (Christin et al., 2011; Pandey et al., 2013).

While the intake of soil inorganic nutrients by roots is assumed to be directly and/or inversely proportional to the shoot assimilation potentials (Wilhelm & Jakob, 2011; Foster & Miklavic, 2013), the foliage organs (i.e., leaves, stem, inflorescence and fruit), may also absorb nutrients through foliar-applied spray, or other catchment sources. However, MacAdam, (2009) said it all depends on the leaf surface tension, polarity, and light etc., in addition to plant phonological stage (i.e., periodic biological phenomena) and morphological traits, such as leaf-shape and fuzzes, and also nutrient mobility determines the overall nutrient dissemination efficiency in foliar. In a different view, Wilding & Kelly, (1993) underscored water and ion uptake by plants as a parameter dependent on at least two soil forming conditions, namely; climatic (i.e., temperature and precipitation) and topography (i.e., slope impacts), and further expressed that temperate climates with high precipitation, may generally exhibit higher tree growth rates than predicted from mineralogy alone. Therefore, the beneficial effects of increased water availability has been linked-to increased uptake of N for photosynthesis and growth (Wagai et al., 2011; Clarholm & Skyllberg, 2013), and enhanced translocation rates, through soil organic matter decomposing more rapidly under favourable and moist condition (Giesler et al., 1998; Baribault et al., 2010; Groffman et al., 2006). However, there seem to be additional indirect effects contributing to this, such as the actual evapotranspiration (AET), as opined by Taiz & Zeiger, (1991). In extension, Couteaux, et al., (1995) referred AET to be responsible for cycling of base cations (Ca, Mg, Na, and K) between soil and plants. Pierzynski et al., (2005), in a short summary states that equilibrium in soil is rare (**Figure 2.6)**, due to microbial activity, plant uptake, weathering, and leaching and/or soil-plant biota processes (Kutschera et al., 2009).

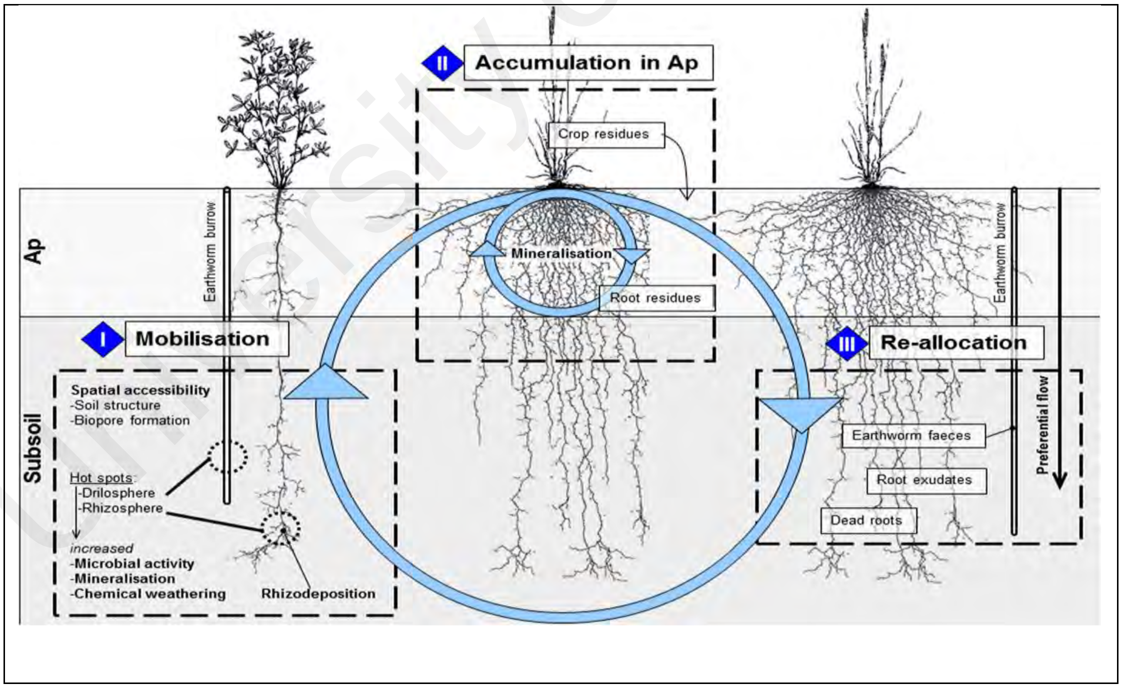


Figure 2.6: Conceptual biota model for nutrient acquisition from subsoil and topsoil in plant nutrient cycling.
Source: Kutschera et al. (2009).

In explaining topographic impacts on soil-plant nutrient mechanism, Kutschera et al., 2009 argued that relatively uninclined flow of discharged alkaline, bicarbonate (HCO_3^-), and base cations loaded groundwater, induced high concentrations of Ca and Mg at topsoil, hence, increased the plant growth rates. Whereas, fluctuating groundwater table, offering undulating water platforms for plants, yielded mosaic soil types, with reduced plant production rates despite a homogenous mineralogy. Respective plant elemental needs and/or functions are listed in **Table 2.5**.

2.4 Techniques in soil remediation

Contaminated soils are common knowledge to environmental threats. Most regions confronted with soil pollution usually have difficulty in addressing it, due to inadequate awareness, policy and regulations, and technological knowhow (Kidd et al., 2009; Reimann & Garrett, 2005). These undermines the hitherto ability of soil to protect groundwater, air quality, and the sustenance of plants, as well as animal/human productiveness, known to be the core functions of the soil (Maxted et al., 2007; Kabata-Pendias, 2001; USEPA, 2014).

Table 2.5: Nutrients and their respective functions in supporting ecological plants

Nutrients	Functions
Major	
N	Proteins and chlorophyll synthesis and plays an essential role in plant growth, also feeds microorganisms in the soil.
P	Root growth, young plants stabilization, maturing in plants, promote photosynthesis, respiration, and as well overall plant growth.
K	Moves through the plant. Promotes the movement of sugars, turgor and stem rigidity and overall resistance.
Secondary	
Ca	Enhances plant structure, especially cell walls formation, and development of the root system and plant maturity.
Mg	Promotes chlorophyll, fruit ripening and seeds germination. Reinforces cell walls and the absorption of P, N and N in plants.
S	A component of several proteins, enzymes and vitamins. Helps chlorophyll formation and absorb K, Ca, and Mg in plants
Micronutrients	
Fe	Essential for chlorophyll activities. Also enhances the formation of some enzymes and amino acids.
B	Important in overall plant health and tissue growth. Supports fruit formation and the absorption of H_2O.
Mn	Promotes plant development, and photosynthesis via chlorophyll production. Propels N assimilation and protein formation
Mo	Essential for N assimilation by plants, and N- fixation by bacteria. Important in the production of N-based proteins.
Cl	Induces photosynthesis.
Cu	Activates numerous enzymes, and enhances chlorophyll production.
Zn	Supporting role in the synthesis of proteins, enzymes and growth hormones.
Ni	Induces enzymes involved in N-metabolism and biological N-fixation.
Beneficial elements	
Si	Strengthens cell walls, energy transfer and drought resistance. Reduces water loss and prevents fungal infection.
Co	Important for N-fixation
Na	Na substitutes K in certain functions in halophytes plants. For C4 plants having dicarboxylic photosynthetic pathway
V	Supports green algae metabolism

References: Tisdale et al., (1997); Xu et al., (2013); Sing et al., (2013); White & Brown, (2010); Soetan et al., (2010); Hirschi, (2009); Marschner, (1995); Chen et al., (2013)

Hence, the remediation of contaminated soils are absolute necessity, if pristine environment must be advocated. However, it is not surprising when Thassitou & Arvanitoyannis, (2001) expressed that pollutant inputs to the soil-ecosystems, has obviously overwhelmed its self-cleaning capacity, and as a consequence, resulted in massive accumulation of pollutants.
In 2014, about 23 out of 28 Member States of the European Union had established inventories for contaminated sites, either managed at national or subnational levels (Bromba et al., 2015). However, tentative number of contaminated sites that likely required urgent remediation in the whole of Europe were estimated to be 340,000 sites (Van Liedekerke et al., 2014; Bromba et al., 2015).

The chemical and physical treatment methods used for soil remediation, known as the orthodox approach, are lowly-effective, creates secondary pollution problems, and often capital intensive with massive labour (Sheoran et al., 2011), as well as, the irreversible changes in the soil features, and disturbances of indigenous soil microflora (Vishnoi & Srivastava, 2008; Sheoran et al., 2011). But, alternative technologies and/or practices, such as plant, and microbial-based approaches has widely been recommended in recent times (Maxted et al., 2007; Montinero et al., 2008; Kumari & Singh, 2011; Bolan et al., 2014). This was not only because of their advantages over the conventional systems in pollutant reduction/removal, but also their extended ability to stabilize biogeochemical imbalances (Kalve et al., 2011; Ali et al., 2013), especially plants utilization of CO_2, which minimizes carbon dioxide inputs to global warming (Sarma, 2011; Singh & Prasad, 2011; Vithanage et al., 2012). Therefore, recent techniques incorporates the utilization of potential plants, and the stimulation of aerobic bacteria populations that can mineralize the contaminants in soil, by nutrient addition to the contaminated zone, which propels enhanced oxygen circulation (Bolan et al., 2014). However, all remediation techniques used in pollutant removals, either conform to *in-situ* or *ex-situ* systems (Ali et al., 2013). Reports from the Office of Technology Assessment, indicted that conventional methods have only shown 15 % recovery potentials in oil spilled soils (Zaheed et al., 2006), whereas biological techniques have so far recorded above 90% decontamination rate, on various pollutants ranging from heavy metals, polycyclic aromatic hydrocarbon (PAHs), and diesel oil between 3 to 15 months of observation (Jacques et al., 2008; Abioye et al., 2010; Kavamura & Esposito, 2010). **Figure 2.7** and **Table 2.6** the difference treatment methods applied in soil remediation. In this context, plants have been described to be solar-driven, which can actually remove these contaminants from the environment. Even in some cases, a particular contaminated site may require joint-remediation procedures, for optimum recovery of the debilitated site.

Most remediation technologies are site specific and the selection of a suitable technique is often difficult, but hugely imperative to achieve a successful remediated site (Khan et al., 2007; GWRTAC 1997). Clarification from Khan et al., (2007), expressed that the successful remediation of any contaminated site, is a function of proper selection, design, and adjustment to the right remediation operations, in relation to the contaminant and/or soil properties, and also the efficiency of system. Following that, the office of soil and groundwater remediation program, United States Department of Energy, have in recent times focused on delivering techniques that are highly environmentally friendly to reduce risk and cost. However, **Plate 2.2** shows a hypothetical contaminated field under the above stated technique earmarked for restoration.

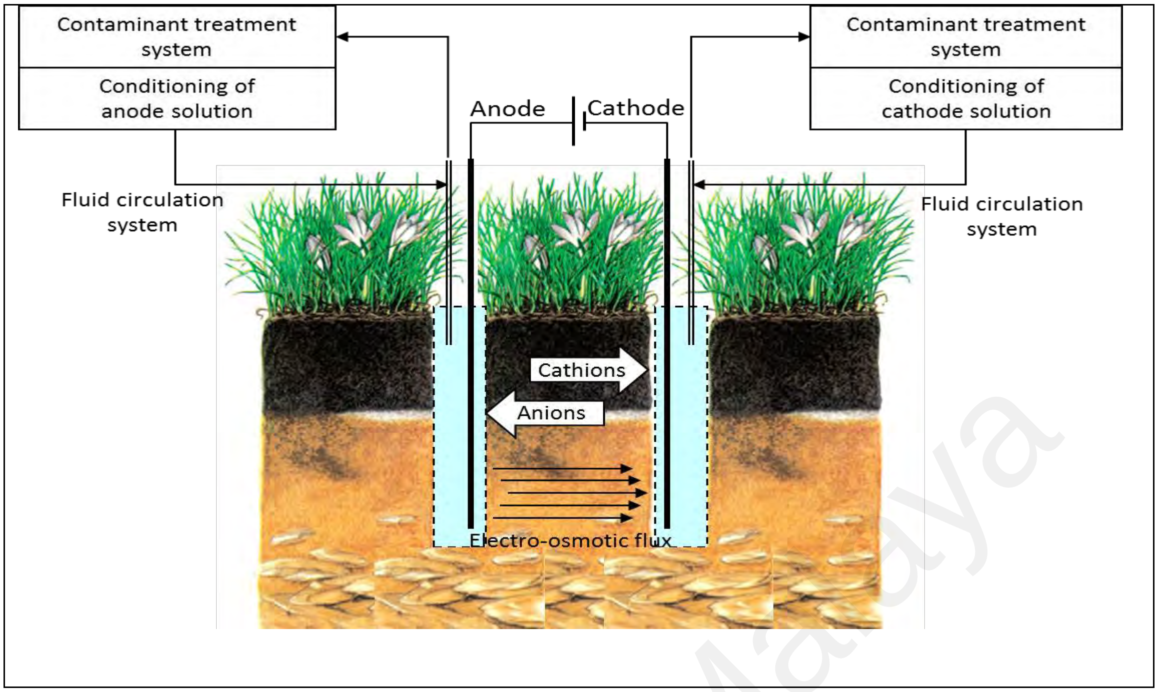


Figure 2.7: Application of electrokinetics in remediation of heavy metal contaminated site. Source: Cang et al., (2011).

Table 2.6: Treatment methods applied in soil remediation

Methods	References
Chemical treatments	
Photo degradation	Jia & Chu, 2009; Villa et al., 2010; Lau et al., 2014; Lv et al., 2009
Oxidation	Ferguson et al., 2004; Lemaire et al., 2011; Lu & Zhu, 2012
Chemical immobilization	Saad, 2009; Tampouris et al., 2001; Ou-Yang et al. 2010
Peroxide remediation	Qi et al., 2004; Khan et al., 2007; Hong et al., 2002; Yab et al. 2012
	Tokunaga & Kakuta, 2002; Li et al., 2009; Jiang et al., 2009
	Mahar et al., 2015; Wu, 2008; Harper et al., 2003; Falta et al., 2008
Physical treatments	
Soil washing	Conte et al., 2005; López-Vizicaino et al., 2012; Gome et al., 2013
Capping	Melanie, 2005; Sun et al., 2010; Robinson et al., 2015
Stabilization	Fleri & Whetstone, 2007; Yao et al., 2012; Zhou et al., 2006
Electro kinetics	Reddy et al., 1999; Wait & Thomas, 2003; Cange et al., 2011
Biological treatments	
Phytoremediation	(Salazar & Pignata, 2014; Alaribe & Agamuthu, 2015)
Bioremediation	(Agamuthu et al., 2010; Dadrasnia & Agamuthu, 2013)

Plate 2.2: A typical heavy metal contaminated site earmarked for restoration program in North America.
Source: USEPA, 2007.

2.4.1 Biological remediation techniques

This technique depend on microbial activities to breakdown and mineralize the contaminants, or biotransformed into less toxic and/or mobile compounds with reference to heavy metals (Tomei & Daugulis, 2013). Biological methods describes the mechanism in which wide range of contaminants are degraded/reduced in the environment through the metabolic potentials of microorganism and other indigenous floras (Khan et al., 2004). The remediation of heavy metal contaminated systems can either be achieved through biological or physico-chemical means. However, because of the negative ecological consequence arising from physico-chemical approaches, biological options are now plausibly explored (Okoh, 2006; Tomei & Daugulis, 2013), as summarily presented in **Table 2.7**.

Table 2.7: Biological remediation methods, modus operandi, and selected applications

Methods	Modus operandi	Application instances	References
Biostimulation	Nutrients addition to stimulate indigenous microbes.	Metal/diesel oil contaminated soil.	Kimura et al., (2006)
Bioaugmentation	Input of microbes with recalcitrant degrading ability.	Remediation of metals from fly ash/soil.	Kumari & Sing, (2011)
Bioleaching	Microbes promoting metal solubilization, e.g *T.thiooxidans*	Soil contaminated with heavy metals.	Kumar & Nagendran, (2009)
Phytoremediation	Decontamination of pollutants using plants.	Heavy metal and diesel polluted soils.	Gupta & Sinha, (2006)
Rhizoremediation	Plant exudates enhanced contaminants degradation.	Soil polluted with (PAHs)/heavy metals	Maila et al., (2005)
Landfarming	Polluted soil in piles, turned to stir microbes for degradation.	Soil immiserized /impoverished by Oil.	Jacques et al., (2007)
Composting	Mixed nutrient input to spur O_2 for indigenous soil microbes.	Creosote-contaminated soil.	Ahtiainen et al., (2002)
Bioventing	Combination of venting with bioremediation to soil.	Hydrocarbon-contaminated soil	Lee & Swindoll, (1993)
Biosorption	Use of biological materials for adsorption of metals	Biosorption of Pb (II) and Cd (II)	Sari & Tuzen, (2009)
Biopilling	Aerated piled substrates to be treated with nutrients.	Soil polluted with hydrocarbon	Mohn et al., (2001)
Nat. attenuation	Native microbes capable of degrading the pollutants.	Diesel-oil/ PAH-contaminated soil	Conte et al., (2005)
Biofilters	Use of bacteria in filters for decontamination of pollutants	Waste contaminated by Cd^{2+} and Cu^{2+}	Loutseti et al., (2009)

Note: Nat. means Natural

Besides cost and/or bioeconomy advantage, biological approach has also been reported as a permanent solution, which may led to complete mineralization of some pollutants (Tomei & Daugulis, 2013). It has been applied for the removal of pollutants in soils, groundwater, sludge, wastewater/industrial wastewater systems, and also gases (Okoh & Trejo-Hernandez, 2006). In many cases, the biodegradation processes involve a particular microorganism that attacks a specific molecular site or pollutants, and convert them into harmless products (Boopathy, 2000; Sharma, 2012). It also generates no liquid waste and present no risk of site contamination.

In addition, it can minimize the transfer of contaminants to another medium, because it is mostly done *in-situ*, and creates minimal exposure to on-site workers towards the contaminant, and possible reduction in the remedial scheduled time (Okoh & Trejo-Hernandez, 2006). Furthermore, its non-invasive technique, that leaves the ecosystem intact (Leavin & Gealt, 1993; Perelo, 2010). There are three functional units given for biological classified remediation by Leung (2004) including (i) *biotransformation*; the alteration of contaminant molecules to either mild or non-hazardous, (ii) *biodegradation*; the disintegration of organics into smaller molecules, and (iii) *mineralization*; the complete reduction and/or biodegradation of organic materials into inorganic components such as carbon dioxide or H_2O.

In conjunction, bioremediation has been implicated with some limitations, such as restricted impacts to only biodegradable compounds (Harekrushna & Kumar, 2012), and compounds containing radionuclides and heavy metals are hardly biodegraded under this technique (Boopathy, 2000). The soil must possess high penetration features, for ideal *in-situ* bioremediation, and it tends to be slow, when equated to excavation methods. This approach may not be feasible always, because microbes are mostly sensitive to contaminants, and also site conditions in retrospect. Nowadays, numerous biological techniques are in vogue, which can either be on and/or out of the sites framework (Sayara, 2010).

***In-situ* bioremediation**: This utilizes microorganisms to degrade contaminants in their original site, which seek to realize harmless compounds as end products (Jim et al., 2005), without excavation of the affected soils (Chien et al., 2010). It is frequently applied for degradation of contaminants in saturated zones. Effectively less costly, but deeply depend upon the external, physicochemical, and chemical conditions of the site, which are the fundamental considerations for *in-situ* options. Such as dissolved oxygen for aerobic processes, moisture contents, pH, and nutrient availability, especially N and P, temperature, soil composition and concentration of the pollutants (Guimaráes et al., 2010; Tao et al., 2015). The depth of the soil in association with oxygen diffusion is important for effective *in-situ* bioremediation, because Dobos & Puia (2010) reported 30 cm soil depth and below as the desirable soil-oxygen diffusion zone, whereas depths higher than 60 cm had reportedly been observed in some cases (Vidali, 2001), implying that further studies are needed to ascertain the disparity. However, different *in-situ* bioremediation approaches are detailed below:

Bioventing: This method remediates contaminated soil under aerobic condition by enhancing oxygen delivery and/or nutrients to microorganism in soil, through air injection into the polluted soil matrix (Shukla et al., 2010). It has similar *in-situ* principle with soil vapour vacuum extractions, but with exceptional remediation potency than the later. Because it uses reduced volume of air to stimulate microbial activity and allows for treatment of less permeable soils (Khan et al., 2004; Shukla et al., 2010; Sui & Li, 2011).

Biosparging: Benner et al., (2002) referred to this as the remediation of pollutants dissolved in groundwater, sorbed to the saturated soil zones, and trapped in the pores of the saturated zone. This *in-situ* process involves injecting atmospheric air, under pressure along the saturated zone (Adams & Reddy, 2003), to volatilize the groundwater contaminants and advance its biodegradation through elevating subsurface oxygen densities (Wen et al., 2015). Air channels are formed through the contaminated plume as it flows upwards through the saturated zone and into the vadose zone (Zheng et al., 2014). The injected air volatilizes the pollutants in the flow channels, and transports them to the vadose zone where they are either removed by soil vapour extraction system or biodegraded. It offers a means of remediating contaminated soils and groundwater, and eliminating the need for active groundwater pumping (Mohamed et al., 2007).

Natural Attenuation and Monitoring: Under natural condition, some physical, chemical, and biological factors/processes in soil can slowly breakdown contaminants into less hazardous components, thereby reducing the contaminant concentrations to acceptable limits (Verginelli & Baciocchi, 2013). This is also known as passive and/or intrinsic remediation, and the enabling processes includes dilution, volatilization, biodegradation, and adsorption (Majeti & Rajendra, 2012). Usually applied if tendency exist that the cleanup may pose threats to people, plant, and animals when other treatments methods are impractical or impossible (Khan et al., 2004). Regular monitoring of the system is important in ascertaining contaminant reduction and effectiveness (DEC-Alaska, 2009). Natural attenuation is a proactive approach, with the perspective of exploring natural remediation processes, order than the complete reliance on engineered process.

Methylation: Progressing our thoughts further on natural attenuation, methylation is a biological mechanism for the removal of toxic metal(loid)s suchlike As, Hg, and Se by first transforming them into methyl derivatives, and their subsequent elimination through volatilization (Bolan et al., 2014). Methylation of metals occurs through both abiotic and biotic process (i.e., biomethylation), considered as the major process in soils and aquatic metal detoxification. Methylated derivatives are excreted readily from cells, and often volatile and less toxic, for example organoarsenicals. Microbes in sediments and soils acts as active biomethylators (Bolan et al., 2014), either through trans-methylation (i.e., transfer of methyl donor group to another methyl acceptor) or by fission methylation (i.e., splitting of a methyl source to enable a reduced realignment capture of the methyl group) (Loseto et al., 2004; Frankenberger & Arshad, 2001). Although, organic matter provides the methyl-donor source for both biomethylation and abiotic methylation in contaminated soils and sediments (Ravichandran, 2004).

Ex-situ bioremediation: This process involves the direct or indirect removal and/or excavation of the contaminated soil from the primary point to elsewhere for remediation, which comprises the following:

Landfarming**:** Technically, it is the above-ground remediation approach, which reduces the level of pollutants from distressing the soil (DEC-Alaska, 2009). Excavated contaminated soils are placed in a biocell, which consist of a liner grounded berm (**Figure 2.8)**. This setting improves the aerobic microbial activity within the soils, and sometimes consortia microbes are deployed (Khan et al., 2004). System supplementation with nutrients, minerals, water and/or moisture are also well enhanced. This is basically ideal for hydrocarbon contaminated soil, and soils are regularly mixed (manually or mechanically) in order to increase the contact between the pollutants, microbes, and the oxygen circulation required for degradation (Hejazi et al., 2003; Tomei & Daugulis, 2013). Contaminated soils, are however added at intervals during this process, depending on the degradation rates of the soil-pollutants in the loaded biocell (DEC-Alaska, 2009). Hence, water that seeps through the system-cells are collected via perforated pipes to avoid cross-contamination, using installed indicator-gauge monitoring wells.

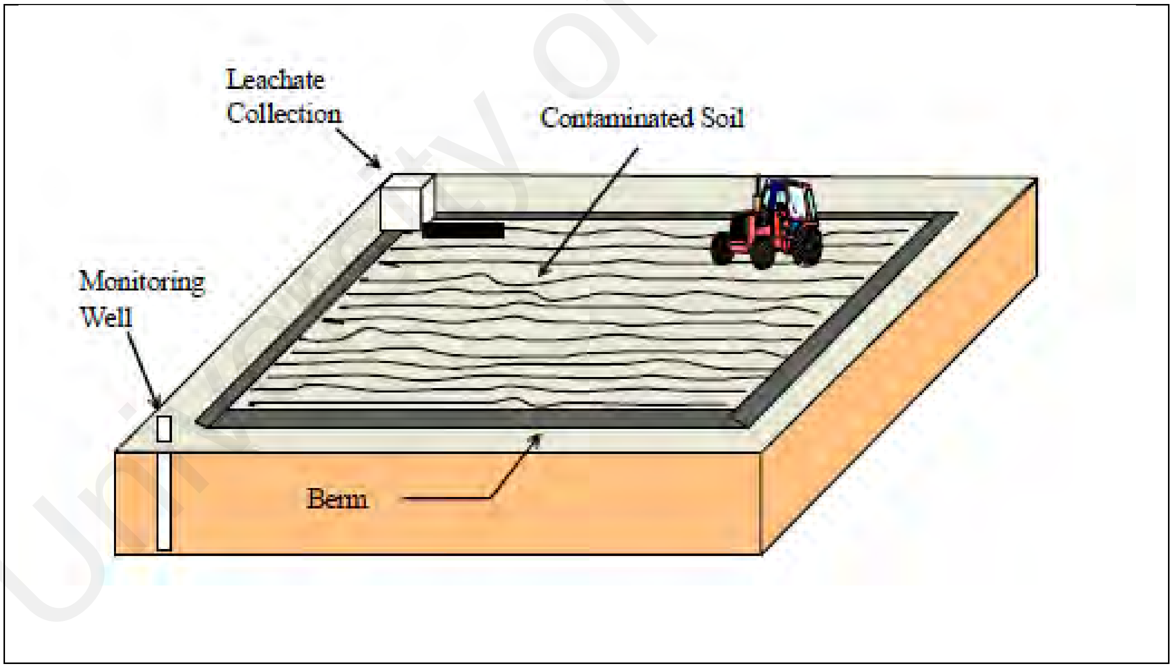


Figure 2.8: A Landfarming soil-remediation system model.
Sources: (DEC-Alaska, 2009)

Landspreading, which is related to landfarming, involves tilling of the contaminated soil surface layers and letting natural biological actions do the cleanup (DEC-Alaska, 2009).

However, landfarming is more active and enhanced than landspreading in soil cleanup practices.

Biopiling: This method involves piling of mostly petroleum-contaminated soil into heaps, and inducing aerobic microbial activity by aeration, and the addition of minerals, nutrients, and moisture (Khan et al., 2004; Gallego et al., 2011; Chemlal et al., 2012). Widely known to be a hybrid of composting and landfarming, and effective in vast range of soil organic-pollutants treatments within 4 months to 2 years (De la Torre et al., 2006; Chemlal et al., 2013). About 70% reduction level have been reported (Chemlal et al., 2012). The cost for this method varies, depending on the nature of the contaminant, or if applied in a pre and/or post treatment scenario. The estimated remediation cost per cubic yard, ranged between 130 to 260 US dollars, under this very technique (FRTR, 1991). In extension, piles have underground system through which air passes, can be up to 6 meters, and supposedly covered to prevent runoff, volatilization and evaporation, and hence promote solar heating (Chemlal et al., 2013).

Bioslurry systems/Bioreactor: This is accomplished by mixing an excavated contaminated soil with water, and several other additives *ex-situ* (Tomei et al., 2013). Also incorporated are bacteria selected for breaking down the contaminant in an engineered containment system/vessel (Janbandhu & Fulekar, 2011; Moscoso et al., 2012). Furthermore, Robbles-González et al., (2008) remarked bioreactors as an efficient bioremediation options for recalcitrant-polluted soils under controlled environmental condition. The mixing increases the soil bioremediation rate, induces slurry formation on pollutants, and subsequent degradation by indigenous organisms. Tomei et al., (2013) recorded 67 to 85% pollutant removal efficiency in a two-phase partitioned bioreactor during soil decontamination. Although, operational cost and soil excavation have reportedly being a limitations to this approach. Utilization of bioreactors for contaminated soil remediation were further elaborated in Rehmann et al., (2008) and Wang et al., (2010). **Table 2.8** present some limitations and benefits of *in-situ* and *ex-situ* bioremediation in concise format.

Table 2.8: Concise list of merits and demerits of *in-situ* and *ex-situ* bioremediation processes.

Technique	Examples	Benefits	Limitations
In-situ	Bioventing	Treats soil and water	Monitoring difficulties
	Biosparging	Most cost efficient	Extended treatment time
	Bioaugmentation	Relatively passive	Environmental constraints
		Natural attenuation process and noninvasive	
Ex-situ	Composting	Low cost	Need to control abiotic loss
	Biopiling	Can be done on site	Space requirements
	Landfarming	Cost efficient	Mass transfer problems

Source: Vidali, 2001.

2.4.2 Strategies to accomplish strategic bioremediation

In accomplishing a successful bioremediation task, the following fundamental strategies may be required:

Monitored natural recovery**:** Monitored natural recovery (NMR) is increasingly being advocated by the US Environmental Protection Agency, as an alternative for managing contaminated site (Magar & Wenning, 2006). Perelo, (2010) stated that (NMR) is the only applied strategy for sediment remediation management. Well, this entails leaving a contaminated polluted site, to undergo a natural transformation either through *in-situ* immobilization or degradation, and subsequently reducing bioavailability. Hence, (NMR) is most effective for low risk sites with low level of contamination, this techniques requires long term monitoring (Perelo, 2010).

Biostimulation/soil amendments principles**:** This operation involves the introduction of selected organic waste, fertilizers and/or harmless organic amendments as (i) metabolic source of energy and/or nutrient to stimulate the proliferation of indigenous species (microbes or plants) to degrade pollutants (ii) mobilize, and (iii) immobilization of heavy metals in contaminated soils (Kavamura & Esposito, 2010; Bolan et al., 2014).

Widada et al., (2002) argued that amendments are intentional stimulation of resident xenobiotic degrading bacteria. By using electron acceptors, water, nutrients and/ or electron donors. Usually aimed at improving the N and P quotient of distressed soils (Lee et al., 2007; Sutherland, 1992; Schaefer & Juliane, 2007). However, carbon sources tends to deplete essential soil-inorganic nutrients, suchlike N and P especially in hydrocarbon polluted soils. That is the reason C/N ratios are estimated in contaminated sites before strategizing remediation. Nitrogen is the most commonly applied nutrient in a bioremediation projects, primarily because it supports biosynthesis of (NH_4^+ and NO_3^-), or as an optional electron acceptor to oxygen (Moore & Chiu, 2001). Therefore, Hsu et al., (2009) and Chiu et al., (2009) holds similar opinion that organic amendments pivotally reduces metals, by providing a source of electron donor and carbon substrate for resistant microbes in polluted soils. **Table 2.9** lists organic amendments on metal-substrates from selected sources. **Tables 2.10** and **2.11** gives empty fruit bunch and spent mushroom compost properties.

Table 2.9: Selected references on organic amendment dynamics with metal on soil and water.

Amendments	Metal	Substrate	Observations	References
Biosolids, compost, SMC	Cr (VI)	Soil	enhanced the rate of reduction of Cr, due to dissolved carbon	Bolan et al. (2003b)
Soybean meal and rice bran.	Cr (VI)	Soil	Reduced resin extractable Cr (VI), and Cr owing to decomposed matter.	Chiu et al., (2009)
Composted cow manure	Cr (VI)	Soil	Cr leaching reduced in soils because of elevated organic matter.	Banks et al., (2006)
Carbon added-lactate & acetate	Cr (VI)	Soil	Bacteria reduced Cr (VI) in anaerobic conditions.	Pichtel & Bradway, (2008)
Sewage sludge & poultry litter	As	Soil	Mixture of both reduced As (V) to more mobile and toxic As (III).	Jackson & Miller, (1999)
Casein amino acids	Se	Soil	Produced dimethyl selenide & removal of Se from soil via volatilization	Park et al., (2011a)
Rice straw	Se	Water	Selenate reduced to selenite, as rice straw carries Se-reducing bacteria.	Bolan et al., (2014)
Cattle manure	Cr	Soil	Facilitated the speciation of Cr (VI) to Cr (III)	Cifuentes et al., (1996)
Manure/ biosolid compost	Cr	Soil	Increased dry organic matter that reduced Cr (VI) to Cr (III)	Bolan et al., (2003b)
Sewage sludge	Cu	Soil	Application ensued increased solubility and shoot accumulation of Cu.	Forsberg et al., (2009)
Compost	Pb	Soil	Raised dry matter, and metal roots and shoots uptake within guidelines.	Kandil et al., (2012)
Sewage sludge	Zn	Soil	Enhances roots together with shoots uptake in plant.	Bolan et al., (2014)
Citric acid	Cd	Soil	Mobilized metal and improved phytoextraction in *S. nigrum*	Gao et al., (2012)
Biochar	Ni	Soil	Reduced leaching of Ni when compared to sewage sludge	Méndez et al., (2012)
Green waste compost	As, Cu	Soil	Increased metal bioavailability & enhanced 30% phytoextraction	Beesley et al., (2010)
Clover	Cd	Soil	Increased Cd in *Sedum plumbizincicola*.	Wu et al., (2012)
Coir fibre	Ni, Zn	Water	Its modification by oxidation with H_2O_2 enhanced metal adsorption.	Shukla et al., (2006)
Soybean meal/rice bran	Cr (IV)	Soil	Reduced bioavailability of Cr in soil due to drier organic compound	Chiu et al., (2009)

SMC means; spent mushroom compost.

Table 2.10: Dry matter and chemical composition of EFB (a source of organic amendment).

Parameters	Values
pH	6.91
Organic carbon (%)	45.10
Total nitrogen (%)	0.55
C/N ratio	82.00
Total Phosphorus (%)	0.02
Total Potassium (%)	1.28
Cellulose (%)	33.00
Lignin (%)	34.00
Hemicellulose (%)	30.00

Adapted from: Kavitha et al., (2013).

Table 2.11: Physico-chemical composition of SMC (a source of organic amendment).

Parameters	Values
C (%)	15.3
H (%)	2.1
N (%)	1.1
P (%)	0.2
C /N	13.6
Protein (mg/g)	11.2
Moisture (%)	41.6
pH	6.89
EC value (ds/m)	36.5
Lignin (%)	12.1
Cellulose (%)	29.8
Hemiscellulose (%)	7.0

Adapted from: (Lou et al., 2015)

Composting bioremediation strategy has also been reported as an aerobic process, by mixing compost materials with contaminated soils. Once the compost matures, the pollutants are degraded by the active microflora within the complex (Semple et al., 2001). Technically based on manures from pigs, cows, and chickens (Adesodun & Mbagwu, 2008; Adriana et al., 2007). About 63% TPH removal in soil contaminated with petroleum hydrocarbon and amended with raw coffee beans were recorded by Adriana et al., (2007). Abioye et al., (2009) recorded 75% loss of oil in soil contaminated with crude oil and composted with melon shells for a duration of 28 days. Whereas, Dadrasnia & Agamuthu, (2010) recorded 80% loss of oil within 3 months, using tea leaves, potato skin, and soy cake amendments in diesel oil contaminated soil. Alaribe & Agamuthu, (2015) recorded 88% and 83% Pb reductions, using (EFB) and (SMC) as soil conditioners in phytotechnology study. Lee et al., (2008) also reported 18% to 40% heavy mineral oil reduction after 105 days using sawdust and hay supplements in oil polluted soil, at initial contamination level of 7490 mg hydrocarbon kg^{-1}, whereas the unamended had only 9% reduction. Wu et al., (2012) opined that traditional organic materials suchlike rice straw, clover etc., are preferably, and environmentally friendly than chelating agent (Ethylenediamin-N,N'-disuccinic acid) in enhancing phytoremediation efficiency of Cd contaminated soil.

Metabolism of contaminants in soil, can be feasible via desorption, solubilization, chelation, and complexation reactions which facilitates the redistribution of contaminants in solid phase to solution phase (Bolan et al., 2014), hence elevating their bioavailability (Park et al., 2011a). Zupančîč et al., (2012) reported that P fertilizer inclusion to Pb-As (V) polluted soil, resulted to increased solubilization, and thereafter mobility of selenite, As (V), and Cr (V) in soils. As (V) acts as a P-analog and is taken up by plants via a P transport system (Bolan et al., 2014), and the P-induced As uptake by plants in soil medium, is attributed to the competition of P for both plant roots absorption and adsorption to soil, respectively (Bolan et al., 2015). The efficacy of chelating agents in the remediation of contaminated soils through mobilization of heavy metals for plant uptake, were extensively reported in (Chen, et al., 2004; Thayalakumaran et al., 2003; Kirkham, 2000), and further enumerated in **Table 2.12**.

The re-dispersion of contaminants in soil, from solution phase to solid phase, in order to minimize their bioavailability and movements, achievable through precipitation, adsorption, and complexation, is known as immobilization (Porter et al., 2004; Bolan et al., 2014). Meanwhile, phosphate compounds, liming materials, organic compost, metal oxides and biochar have been implicated for this mechanism (Bolan et al., 2014; Chrysochoou et al., 2007; Garau et al., 2007; Kumpiene, 2010). **Table 2.13** shows the amendment impacts, while **Figure 2.9** illustrates the schematic flow. The precipitation of metal-phosphates, has been established for immobilization of metals, such as Zn and Pb in soils (Chrysochoou et al., 2007). These metal-phosphate compounds possess low solubility over a wide pH range, which makes them suitable in managing metal- contaminated soil, if metal immobilization is the focus. Although, phosphate accumulation in soil, is ecologically unsafe, due to nutrient pollution (Havens et al., 2003; Alkorta et al., 2004; Varol & Şen, 2012).

Table 2.12: Potential values of soil amendments in the mobilization of metal in soils.

Amendments	Metals	Observations	References
Chelating agents			
EDTA, KI, Citric acids	[Hg]	Mobilized and increased plant uptake. EDTA showed leaching.	Thayalakumaran et al., (2003)
EDTA, Citric acid	[Cd, Cu, Pb, Zn]	Increased mobility of all metals in soil & enhanced accumulation in aerial parts.	Sun et al., (2009)
Citric acid, EDTA	[Cr, Ni, Ca, Fe, Mg, Al, Mn]	Increased leaching of all metals showing the dissolution of soil minerals.	Jean-Soro et al., (2012)
Tataric acid/citric acid	[Cd, Pb, Zn]	against the control citric acid mobilized 71%, 181%, and 112% of Cd, Pb, and Zn. while tartaric acid mobilized 70%, 155%, and 135% of Cd, Pb, and Zn, respectively	Vesely´ et al., (2012)
Humic acid, ETDA	[Cd]	Plant uptake improved significantly.	Evangelou et al., (2004)
Organic matter			
Rice straw	[Cd]	Increased Cd in *Sedum plumbizincicola*.	Wu et al., (2012)
Poultry manure	[Cd, Cu]	Increased bioavailability of Cd conc., and contrary to Cu over 3 year study.	Hanč et al., (2008)
Biochar	[As]	Elevated As in pore water but decreased uptake to tomato plants	Beesley et al., (2013)
Sewage sludge	[Cd, Cu, Cr, Ni, Pb]	Increased metal uptake in root & shoot of *Beta vulgaris*.	Singh & Agrawal, (2007)

Table 2.13: Potential values of soil amendments in the immobilization of metal in soils.

Amendments	Metals	Observations	References
Phosphate compounds			
KH_2PO_4	[Cd, Pb]	Enhanced immobilization, reduced plant availability.	Bolan et al., (2003b)
KH_2PO_2	[As]	Cut-down Pb concentration but not in Zn and Cd in earthworms.	Pearson et al., (2000)
Phosphate	[Pb, As]	Raise plant of soil As. No effects on soil Pb phytoavailability	Bolan et al., (2014)
H_3PO_4, Ca (H_2PO_4)	[Pb, Zn, Cu]	Raised residual fraction of Pb, Zn, and Cu, and reduced translocation from root to shoot of plants.	Cao et al., (2003)
Liming materials			
Ca $(OH)_2$	[Cd]	Transformed less mobile fractions, decreased phytoavailability.	Bolan et al., (2003b)
Lime, FBA, Bark	[Cr]	Decreased availability for plant uptake and leaching to ground water.	McGowen et al. (2001)
Red mud, lime	[Pb, Cd, Zn]	Reduced solubility of Pb, Cd, and Zn. Changes microbial communities.	Garau et al., (2007)
Cyclone ashes/lime	[Zn, Cd]	Reduced metal accumulation in plants and Zn and Cd levels in soil pore water	Ruttens et al., (2010)
Organic matter			
Biosolids	[Cd]	Decreased bioavailability of Cd.	(Bolan et al., 2003b)
Papermill sludge/SS	[Cd, Zn]	Cd uptake enhanced, but Zn decreased	Merrington & Madden, (2000)
Wood ash, K_2SO_4	[Pb, Zn, Cd]	Improved phytotoxicity of Zn and Cd, and reduced bioavailability of Pb	DeVolder et al., (2003)
CM and GWB	[Cd, Cu, Pb]	Immobilization due to boundary of metals from exchangeable to organic bound fraction.	Park et al., (2011b)
Oak-wood biochar	[Pb]	Sorbed Pb by elevating the soil pH	Ahmad et al., (2012b)
Metal oxides			
Hydrous Mn oxide	[Cd, Zn, Pb]	Reduced mobility of Zn, Cd and Pb, and uptake of metals by *ryegrass*	Mench et al., (1994)
Fe-oxide waste by-product	[Cd, Pb]	Reduced uptake of cd and Pb by maize and barley	Chlopecka & Adriano (1996)

Note: FBA, fluidized bed boiler; CM, chicken manure; GWB, green waste biochars.

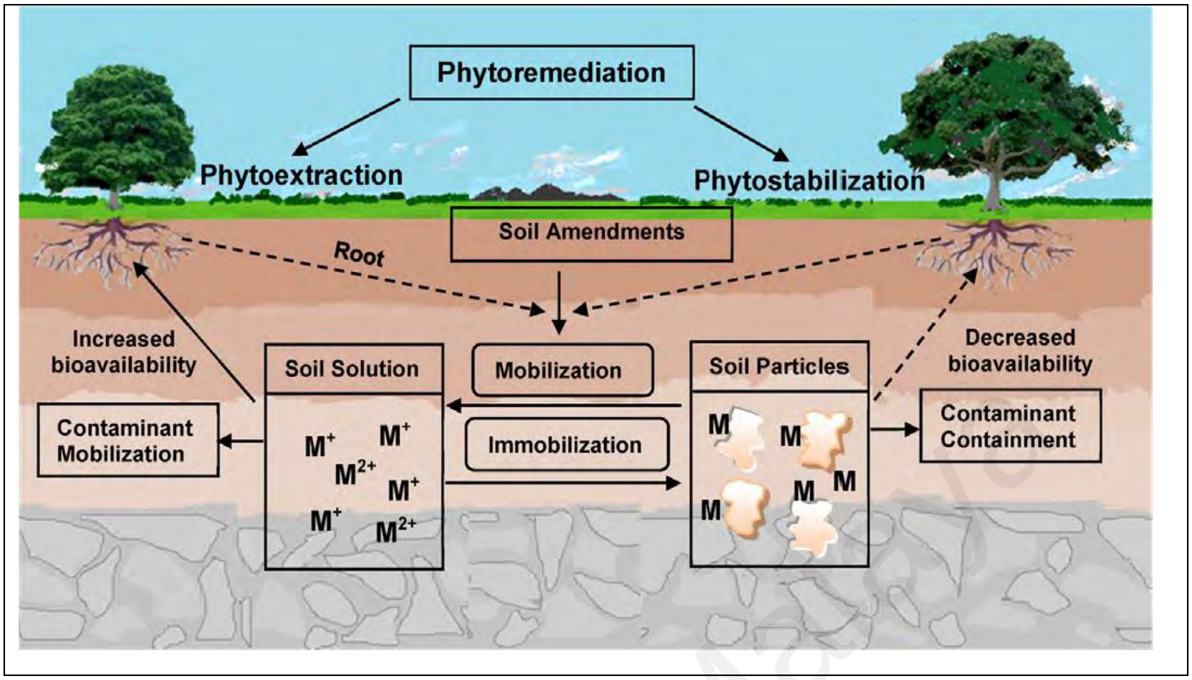


Figure 2.9: Mobilization, immobilization, bioavailability, and remediation of heavy metal contaminated and amended soil.
Source: Bolan et al., (2014).

Bioaugmentation principle: Options include the addition/reinoculation of a pre-adapted pure bacterial strain, or consortium, or genetically engineered bacteria with biodegradation relevant genes into indigenous microorganisms, for specific environmental conditions to remediate contaminated site (Sayara, 2010; Tyagi et al., 2011; Shankar et al., 2014).

This is not limited to soil alone, wastewater inclusive and microorganisms must possess specific metabolic capabilities. In situations where bioattenuation and biostimulation are inefficient, bioaugmentation is deployed especially when recalcitrant pollutants are involved (Iwamato & Nasu, 2001; El Fantroussi & Agathos, 2005).The advent of introducing exogenous microbes into the environment, was intended to speed up the rate of bioremediation. Basically, it is important to assert the fate of an introduced organism in order to ascertain its contribution to contaminant degradation and/or transformation, and also evaluate the impacts on the ecosystem (Tabak et al., 2005). While some advocate for inoculation of directly isolated microbes, others may prefer engineered organisms.

However, considering some side effects of bioaugmentation on the ecosystem, much attention accorded its application. This is because large amounts of degradative bacteria are introduced into the polluted sites, hence the impacts of such bacteria on the general environment and humans, need to be clarified in advance. Often times, the infixed microbes are conceptualized to extinct after the remediation process, this is aimed at avoiding extended negative impacts on the indigenous microbial community.

While the inoculation of bacteria on polluted-substrates for remediation purposes is thought to be progressive (Schmidt et al., 2005; Raff et al., 2003; Nies, 2003), yet the technique is besieged by some environmental uncertainties such as adverse effects on human health, survival of the specialized microorganism in the environment, ability of the microbes to thrive long in the harsh environmental conditions, in order to perform the desired clean-up task, and determination of the set risk levels acceptable to the public (Joyce, 1983).

In Japan, *Ralstonia eutropha* KT-1 was used to remediate a polluted site, where isolate was identified to be a phenol-utilizing bacteria from same spot (Iwamato & Nasu, 2001). Thereafter, re-injected without substrate addition (Iwamato & Nasu, 2001). Bioaugmentation can be interesting especially in situations where genetically modified organisms (GMOs) are utilized. A modified strain, *Burkholderia cepacia* PRI $_{301}$ was utilized for field bioaugmentation on Moffett Federal Air-field in the USA after microcosm studies in the laboratory (McCarty, 1998). The result showed that the organism effectively degraded TCE while growing on lactate and as replacement for the use of other like phenol and toluene which are toxic chemicals (Iwamato & Nasu, 2001). Bento et al., (2005) and Thompson et al., (2005) argued that indigenous microbes (pre-selected for bioaugmentation) are apparently more likely to survive and/or propagate when reintroduced into the site, as compared to transient or alien strains to such a habitat. Liu et al., (2009) evaluated the biodegradation of phenol by free and immobilized cells of *Sphingomonas* sp. FG03 and *Acinetobacter* sp. XA05 strains harnessed from activated sludge and phenol contaminated soil. Mixtures of the two strains were implicated to be more efficient than single pure cultures.

Also, Jacques et al., (2008) formed consortium from *Bacillus cereus*, *Mycobacterium fortuitum*, *Microbacterium* sp., *Gordonia polyisoprenivorans*, and *Miccrobacteriaceace bacterium* that mineralized 90% of PHAs. Several studies have also reported the need to inoculate bacteria together with nutrients to increase their degradation potential and efficiency (Mishra et al., 2001; Kavamura & Esposito, 2010). Li et al., (2009) reported the amalgamation of three bacteria; *Bacillus* sp., *Zoogloea* sp., and *Flavobacterium*, and five fungi; *Phanerochaete chrysosporium*, *Cuuninghamella* sp., *Alternaria alternate*, *Penicillium chrysogenum*, and *Aspergillus niger* consortia, which enhanced the degradation rate of aged PAH contaminated soil by 41.3%. This implicated *Bacillus* sp., to be a potential strain for bioremediation of recalcitrant polluted substrates.

Similarly, the use of traditional cultivation methods to assess the performance, or efficacy of bioaugmentation is a daunting task. This is because distinction between added inocula and the indigenous microflora requires high specificity (Jansson et al., 2000). Moreover, reports holds that after the introduction of laboratory grown strains into actual polluted sites, they always encounter stress, and probably un-culturable on agar medium (Jansson et al., 2000). Such depicts culture-based monitoring approach as lacking in specific sensitivity, upheld for precise inocula observation during bioaugmentation of contaminated sites.

2.4.3 Phytoremediation (Phytotechnology diversified)

The group of technologies that utilizes plants for remediating soils, sediments, sludges, and water contaminated with organic and/or heavy metals, is known as phytoremediation (Rámila et al., 2015; Doran, 2009; Sarma, 2011). Alternatively, the efficient ability of plants to remove, extract, sequester and/or detoxify or immobilize environmental pollutants in their growth substrates (i.e., soil) via non-intrusive and aesthetically approach (Alkorta & Garbisu, 2001; Ali et al., 2013). It can involve growing plants in the contaminated matrix, for a required growth period, to contain the pollutants and subsequently harvested, processed and disposed safely (Rascio & Navari-Izzo, 2011).

From an economic perspective, phytoremediation of polluted land would yield risk containment, phytoextraction of metals with market values such as Au, Ti, and Ni (Vangronsveld et al., 2009). Also in sustainable land management where phytoextraction gradually improves soil standards for later cultivation of crops with higher market demand (Meagher, 2000). Including fast growing and high-biomass producing plants, suchlike poplar, *Jatropha*, and willow, which have dual benefits in energy production and phytoremediation (Abhilash et al., 2012). Evans, (2002) reported that in the United States, phytoremediation market is expected to yield over $214 million in periods between 1998 and 2005. The techniques for phytoremediation comprises of the following:

Phytoextraction: Contaminant uptake from soil or water through the plant roots and subsequent translocation to the aboveground biomass i.e., shoots (Rafati et al., 2011; Alkorta et al., 2004). Alternatively known as phytoabsorption or phytosequestration, or phytoaccumulation (Ali et al., 2013) (**Figure 2.10)**. Translocation of metals to the shoot, is an essential biochemical pathway for desirable phytoextraction (Zacchini et al., 2009; Sheoran et al., 2016).

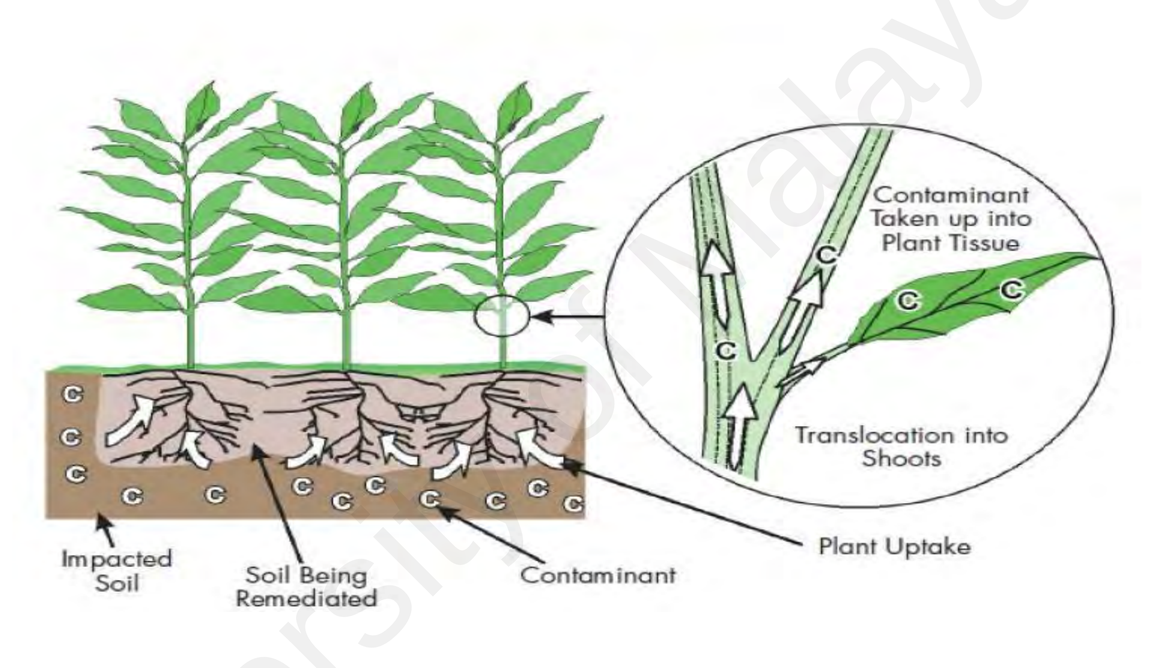


Figure 2.10: A typical phytoextraction mechanism in metal contaminated soil.
Reference: Malik & Biswas, (2012).

Suitable plants for phytoextraction ought to have the following features; (i) should be widely distributed with sophisticated branched root arrangement, and ability to grow outside their collection area, (ii) accumulation of target metals from soil, (iii) adaptation to the abysmal and/or prevailing soil conditions, (iv) elevated plant growth rate, with above-ground biomass output, (vi) toxicity tolerance of the specific heavy metals, (vii) repelling to herbivores as to avert food chain contamination, and flexible agronomic cultivation and harvest features, and

(viii) resistance to pest and pathogens (Adesodun et al., 2010; Shabani & Sayadi, 2012; Garbisu et al., 2002; Ali et al., 2013). Phytoextraction of heavy metals can either be induced (assisted plants remove of heavy metals via chelating agents) or natural (unassisted plants remove heavy metals) (Ali et al., 2013).

Phytostabilization or phytoimmobilization is the adoption of certain plants for stabilization of metal contaminants in contaminated soil (Sing, 2012). Technically reducing the mobility and/or bioavailability of the pollutants from migrating elsewhere. It does not offer a permanent solution because the heavy metals are still imbedded in the soil, only their movement are limited (Ali et al., 2013). However, Vangronsveld et al., (2009) argued that it is a management strategy for inactivating potentially toxic pollutants. Also reported via phytostabilization is the secretion of special redox enzymes (**Figure 2.11**), which systematically transforms the risky metals into comparatively less toxic ones (Yoon et al., 2006).

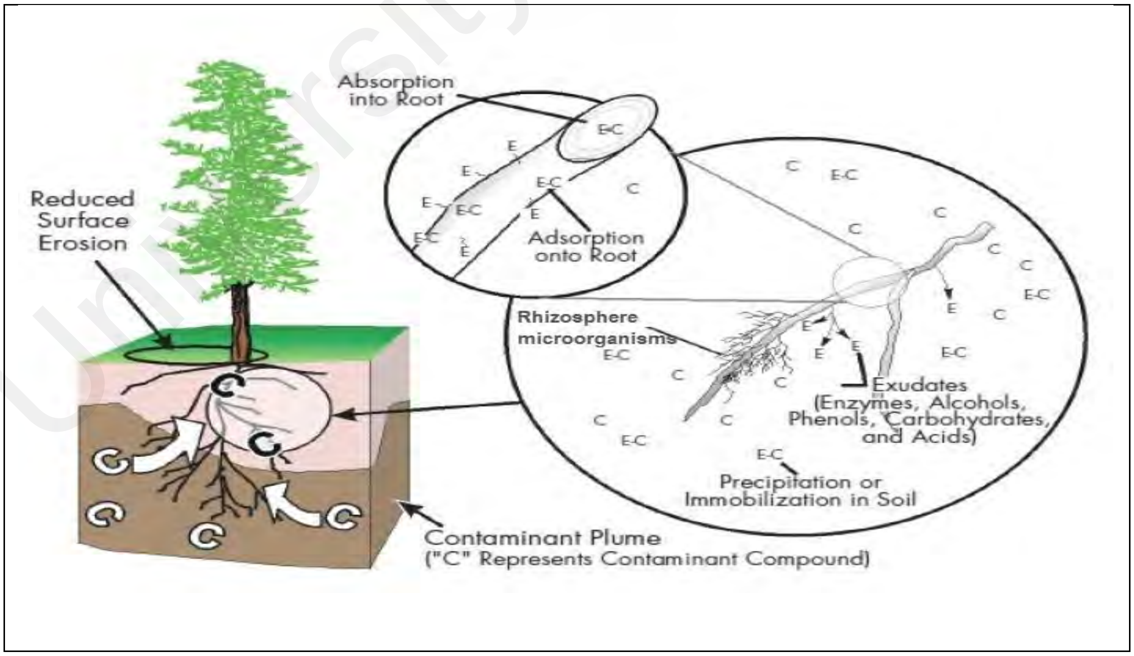


Figure 2.11: Illustration of a phytostabilization mechanism in metal contaminated soil. Reference: Malik & Biswas, (2012).

Phytovolatilization is the uptake of heavy metals such as Hg, As, and Se from soil by plants and further converted to volatile constituents and release into the atmosphere, accompanied by transpiration (Feroz et al., 2012) (**Figure 2.12).** Yet, phytovolatilization is termed as the most uncertain type of phytoremediation because it emits pollutant into the atmosphere, and re-deposited again into the environment (Padmavathiamma & Li, 2007).

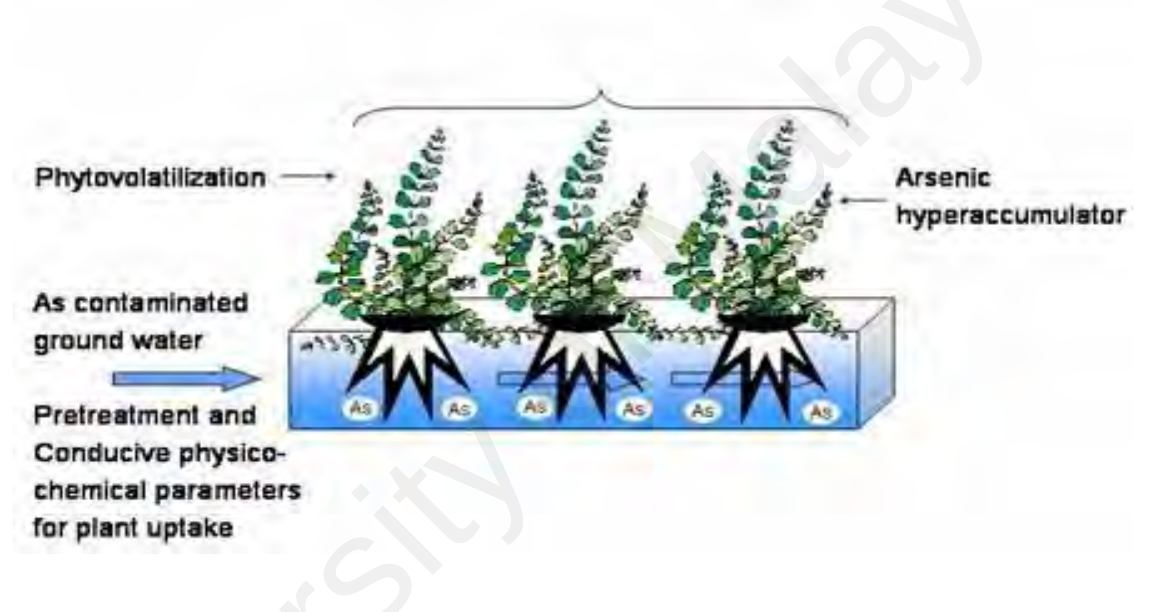


Figure 2.12: Illustration of phytovolatilization model in metal contaminated environment. Source: Padmavathiamma & Li, (2007).

Rhizodegradation implies the breakdown or transformation of pollutants in the soil by the indigenous microbial community at the rhizosphere; which covers about 1 mm circumference of the plant roots (Ali et al., 2013) (**Figure 2.13)**. Dense microbial population and metabolic activities on this region, are the main factor responsible for pollutant degradation (Boopathy, 2000). Most of the roots secretions (exudates), contains amino-acid, flavonoids, and carbohydrates (Yadav et al., 2010; Cesco et al., 2010), which serves as carbon and nitrogen sources to the soil microbes, and establish a nutrient-rich environment that stimulates their actions for rhizodegradation (Kuiper et al., 2004).

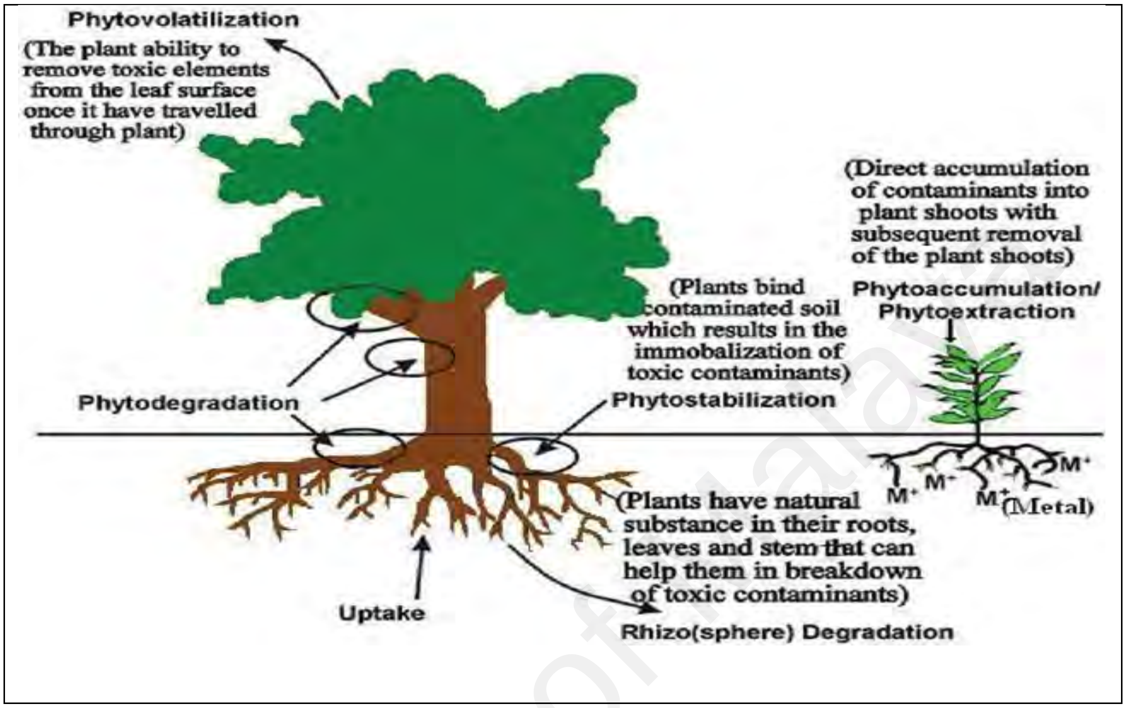


Figure 2.13: A typical phytoremediation model in metal contaminated soil.
Reference: Padmavathiamma & Li, (2007).

Phytodegradation**:** Basically, phytodegradation is the degradation of organic pollutants by plants, with the aid of enzymes such as oxygenase and dehalogenases. It is unconnected to microbial actions, but rather plants intrinsic metabolic potency to detoxify and accumulate pollutants (Vishnoi & Srivastava, 2008; Tiwari et al., 2016). Tansgenic poplars plant has been reportedly engineered for this purpose (Dorty et al., 2007).**Table 2.14** and **Table 2.15** represent the summaries of merits and/or limitations, and different techniques of phytoremediation technology.

Table 2.14: Merits and Limitations of phytoremediation technology

Merits
i. Not necessarily needs expensive equipment or highly trained personnel.
ii. It is cost effective than other conventional remediation methods.
iii. Less noisy than other remediation methods. Moreover trees mitigate industrial noise pollution.
iv. Has wide range of applicability in both organic and inorganic contaminants.
v. Flexible to implement and maintain. Because, plants are renewable, cheap, and easily available.
vi. Environmentally friendly, aesthetically pleasing, socially accepted, and low-tech options.
vii. Minimizes the amount of waste going to landfills.
viii. It can be applied in situ, and reduces soil disturbances and migration of pollutants.
Limitations
i. Limited to sites with low concentration of contaminants.
ii. It is climate and seasonal dependent. Moreover it can also lose its effectiveness if damage occurs to the vegetation from disease or pest.
iii. Supplementations and cultivation activities may have negative consequences on the contaminant mobility.
iv. It is restricted by root depth, solubility and availability of the contaminant.
v. Introduction of invasive plant be avoided, because non-native species may affect biodiversity.
vi. Contaminants may infiltrate into food chain or other ecosystems.
vii. It requires longer period for actualization.
viii. Proper disposal of plant biomass are needed because of phytoaccumulated pollutants.

References: Alkorta et al., 2004; Karami & Shamsuddin, 2010; Naees et al., 2011

Table 2.15: Summary of techniques in phytoremediation

Terms	Mechanisms of action	References
Phytoextraction	Above-ground accumulation of pollutants in harvestable biomass.	Alkorta et al., (2004); Ali et al., (2013)
Phytostabilization	Restricts bioavailability & mobility of pollutants in soil via plant roots.	Yang et al., (2005)
Phytodegradation	breakdown of xenobiotics via plant enzymes within plant tissues.	Vishnoi & Srivastava, (2008)
Phytofiltration	Plants sequestration of pollutants from contaminated water.	Meera & Agamuthu, (2011)
Phytovolatilization	Conversion of pollutants to volatile form and release to atmosphere.	Feroz et al., (2012)
Phytodesalination	Elimination of elevated salts from saline soils by halophytes.	Rabhi et al., (2010); Ali et al., (2013)
Rhizodegradation	Degradation of organic xenobiotic by rhizospheric microorganisms.	Johnson et al., (2005); Boopathy, (2000)

2.4.3.1 Actions of Metallophytes on heavy metal polluted soil

Plants adapted to growing in metalliferous soils i.e., metallophytes (Alkorta et al., 2004; Alford et al., 2010; Bothe, 2011; Sheoran et al., 2011; Ali et al., 2013), are categorized into three: (i) metal excluders, (ii) metal indicators, and (iii) metal accumulators. Ernst, (1974) elaborated plants resistant to heavy metals in soils, began due to ores outcropping pre-polluted ecological soils. Although not limited to the *Brassicaceae* family alone, they have been implicated to be dominant drivers of metallophytes (Bothe, 2011). Also, plants species have been streamlined into two main groups, based on their metal adaptation in soil: the pseudometallophytes; springs on both polluted and non-polluted metal soils (Alaribe & Agamuthu, 2015), while the absolute metallophytes; grow strictly on metal-contaminated and/or naturally ample metal soils (Dahmani-Muller et al., 2000; Bothe, 2011; Mahdavian et al., 2016). Although, *Thlaspi caerulescens* is reportedly most researched plant for phytoextraction (Wang et al., 2006), *America maritima* and *Cardaminopsis halleri* are absolute metallophytes and *Agrostis tenuis* a pseudometallophytes.

Metal excluders: Plants belonging to this category, accumulate heavy metals from soil into their roots, but disallow metal transport and/or upward flow to their aerial-region (Malik & Biswas, 2012). Example of metal excluder, is the *Festuca rubra* which is consistently used to stabilize erosion-prone metal-polluted soil (Raskin et al., 1994). However, it is presumed that alteration of their membrane permeability, and cell-metal binding capacity, conferred this potential to the excluders (Gosh & Sing, 2005), and may be ideally used for phytostabilization purposes (Lasat, 2002).

Metal indicators: Sheoran et al., (2011) referred these group of plants as having the ability to accumulate heavy metals in their aerial parts, which generally reflects the concentration of metals in their host soil/substrate (Alkorta et al., 2004). For instance, *Alyssum bertolonii* and *A. murale* are both metal indicators found in Russia, with Ni indicator-potential of about 10% concentration. *Sebertia acuminata* (found in New Caledonia), and *Becium homblei* (in Africa) with about 25% indicator-potential for Ni, and 7% for Cu respectively, in polluted soils. Moreover, they can tolerate existing concentration of metals by compartmentalizing stored metals at plants non-sensitive parts (McGrath et al., 2002; Gosh & Sing, 2005).

***Metal accumulators*:** Are known to possess intrinsic ability to accumulate metals in their aerial parts, in proportions greater than plants host "contaminated" soil (Memon & Schröder, 2009; Dickinson, 2017), example, *Pteris vittata* with profound accumulation for Se (Liao et al., 2004) **(Figure 2.14)**. Hyperaccumulators are plants (e.g., *Thlaspi caerulescens* and *Arabidopsis halleri*), that can absorb high levels of contaminants concentrated either in their roots, shoots and/or leaves (Gosh & Sing, 2005). However, Pollard et al., (2002) and (2009), affirmed that hyperaccumulators can be regarded as a special, and extreme/broader category of accumulators, which have hypertolerant for metals, and can accumulate metals in shoots between 100-1000 folds, beyond the hypertolerant non-hyperaccumulators (Rascio & Navari-Izzo, 2011). They should also show no symptoms of phytotoxicity. **Figure 2.15** shows the hypertolerant non-hyperaccumulator, and hypertolerant hyperaccumulator plant synopsis. From the elemental defence hypothesis, high heavy metal concentrations makes hyperaccumulator leaves poisonous to herbivores (Jhee et al. 2006).

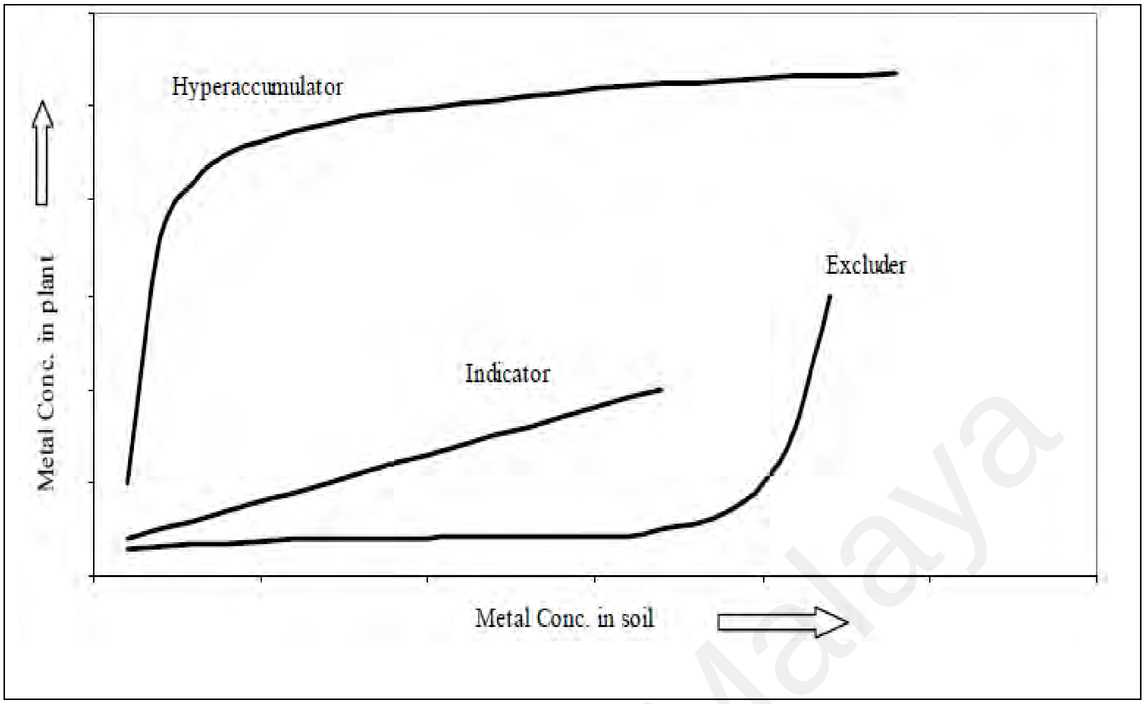


Figure 2.14: Conceptual response mechanism of metallophytes to heavy metals in soil. Source: Gosh & Sing, (2005).

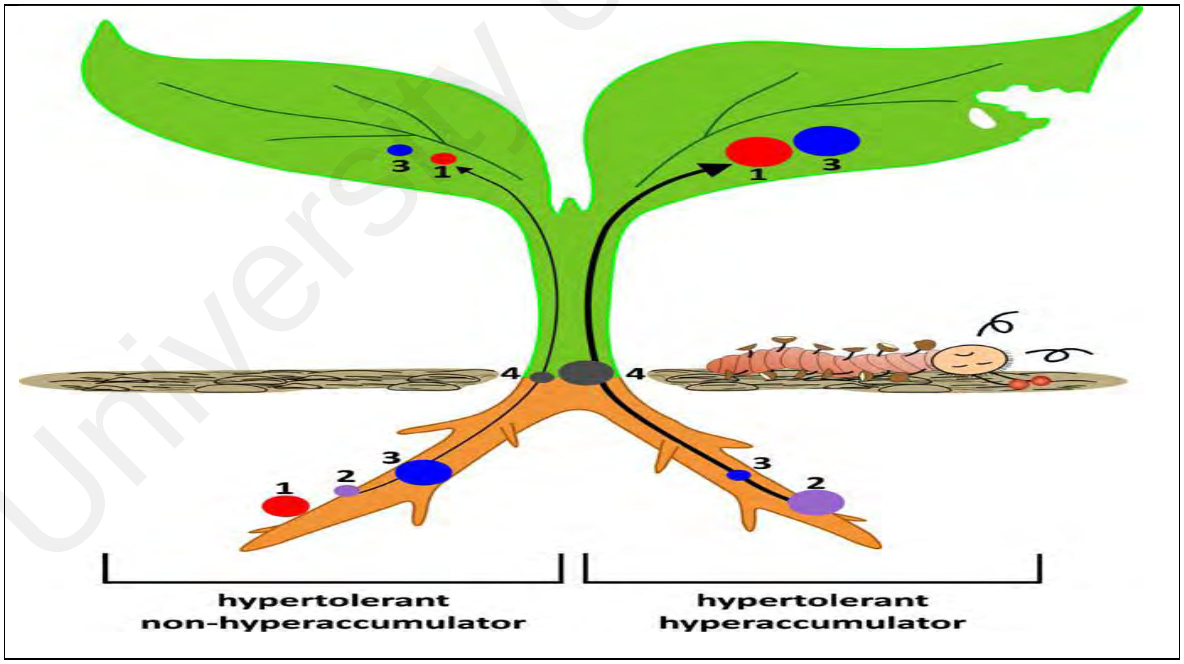


Figure 2.15: Heavy metal hypertolerance and distribution in excluder non-hyperaccumulator (left) and hyperaccumulator (right) plant. (**1**) Heavy metal binding to the cell walls/ exudates, (**2**) root uptake, (**3**) chelation in the cytosol and/or sequestration in vacuoles, (**4**) root-to-shoot translocation. The spots and sizes indicates the plant organ in which the different mechanisms occurred and the level of each of them.

Source: Rascio & Navari-Izzo, (2011).

Brooks et al., (1977) first defined metal hyperaccumulator as plants that contain more than or up to 0.1% dry weight of 1000 mg/kg of Cu, Cd, Cr, Pb, Ni, and Co, or greater than 10000 mg/kg dry weight for Zn and Mn. Reevees, (1992) further argued that for any plant to be established as a hyperaccumulator, its aboveground tissue should only be regarded as the plant foliage, and probably growing in its natural metal-rich soils (van der Ent et al., 2013; Mok et al., 2013). Ni is the only metal reported to have reached the highest concentration in plant (*Sebertia acuminate*), with about 26% on dry mass (Rascio & Navari-Izzo, 2011). Meanwhile, it has been reported that metal accumulator plants have translocation factor (TF) above 1, whereas the metals excluders have (TF) values below 1(Schmoger et al., 2000; Jamil et al., 2009). Plant bioaccumulation factor (BAF), is also an indicator for assessing hyperaccumulators, and should possess (BAF) value greater than 1 (Zhuang et al., 2007; Elekes & Busuioc, 2011; Ladislas et al., 2012), however (BAF) range of 50 to 100 has been recorded (Cluis, 2004).

There are about 420 to 500 phyto species linked to 45 plants families acclaimed to be hyperaccumulators for heavy metals (Jaffré et al., 2013; Ullah et al., 2015). Hence, only minute of them had been tested in the laboratory to ascertain their metal accumulation dynamics (Krämer, 2003). **Table 2.16** lists of some hyperaccumulator plants. For instance, two-third of the 320 species of Ni hyperaccumulators thus far discovered, are exclusively endemic in tropical regions (Proctor, 2003). This may imply that greater weathering of natural occurring ore-bodies are prevalent in those regions, hence imposing stronger selective pressures on indigenous plants (Klein et al., 2008), and that supposedly suggest the intriguing nature of metallophytes (Galeas et al., 2006).

Table 2.16: Some hyperaccumulator (HA) plants and their respective dry weight (mg/kg) heavy metal (HM) accumulated.

Metals	No. of HA sp. reported.	Plant species	HM accumulation (mg/kg)	References
Ni	320	*Alyssum bertolonii*	10900	(Li et al., 2003; Bhargava et al., 2012)
		Alyssum corsicum	18100	Li et al., (2003)
		Alyssum markgrafii	19100	Bani et al., (2010)
		Berheya coddii	18000	Mesjasz-Przbylowicz et al., (2004)
		Isatis pinnatiloba	1441	Altinozlu et al., (2012)
Cd	4	*Azolla pinnata*	740	(Rai, 2008; Sun et al., 2009)
		Eleocharis acicularis	239	Sakakibara et al., (2011)
		Solanum photeinocarpum	158	Zhang et al., (2011)
		Thalaspi caerulescens	263	Lombi et al., (2001).
As	na	*Corrigiola telephiifolia*	2110	Garcia-Salgado et al., (2012)
		Pteris vittata	8331	Kalve et al., (2011)
Cu	34	*Eleocharis acicularis*	20200	(Sakakibara et al., 2011; Wang & Zhong, 2011)
Zn	18	*Eleocharis acicularis*	11200	(Sakakibara et al., 2011; Du et al., 2011)
Pb	14	*Euphorbia cheiradenia*	1138	(Chehregani & Malayeri, 2007)
Cr	na	*Pteris vittata*	20675	Kalve et al., (2011)
Mn	9	*Schima superba*	62412.3	(Yang et al., 2008; Pollard et al., 2009)

HA, means hyperaccumulators; HM, means heavy metals; na, means not available.

Surprisingly, most researchers have focused only on identifying hyperaccumulator plants, as seen in Table 2.16. Spread attention should be given to other metallophytes (indicators), because under repeated cultivation and/or harvest, such plants can supplant scarce hyperaccumulators in some contaminated sites, to achieve desired soil cleanup. Where (TF) and (BAF) are expressed as follows;

$$\mathrm{TF} = \frac{\text{Conc. of metal (mg/kg) in plant shoot}}{\text{Conc. of metal (mg/kg) in plant root}} \qquad \text{(Eq. 2.3)}$$

$$\mathrm{BAF} = \frac{\text{Total Conc. of metal in harvested plant (mg/kg)}}{\text{Total Conc. of metal in the polluted soil (mg/kg)}} \qquad \text{(Eq. 2.4)}$$

2.4.3.2 Biochemical plant root-bacteria processes assisting soil heavy metal remediation

Metal mobility and availability in the rhizosphere are influenced by rhizospheric microbes as well as the root exudates (Zhuang et al., 2013). Excluding Fe, not much is known about active mobilization of trace elements by plants roots (Neubauer et al., 2000). However, rhizosphere acidification, exudation of carboxylates, and mechanisms promoting phosphorus acquisition enhances micronutrient bioavailability (Chaney, 2007; Badri & Vivanco, 2009; Philippot et al., 2013; Haichar et al., 2014). Most accumulated metals are bound to ligands such as organic acids, amino acids, protein, and peptides (Verbruggen et al., 2009).

Metabolites such as phytosiderophores and siderophores are organic compounds emitted by plant and bacteria, to improve their need/availability for Fe extraction, especially in plant Fe deficient soils (Ullah et al., 2015). They form stable complexes with metals suchlike Zn, Ni, Pb, Cu, and Cd (Nair et al., 2007). In dealing with plant metal stress, two most characterized metal-binding ligands in plant cells are phytochelatins (PCs) and metallothioneins (MTs) (Yadav, 2010; Bhargava et al., 2012; Shukla et al., 2013). Hartley-Whitaker et al., (2001) reported these ligands (PCs and MTs) expressions, as dominant to plants. Ligands that sorb to soil may increase ternary soil-ligand-metal complexes (Schwab et al., 2004). **Figures 2.16, 2.17, and Table 2.17** show the phytochelatins structure, complex interaction of exudates with edaphic associations, and specific functional roles.

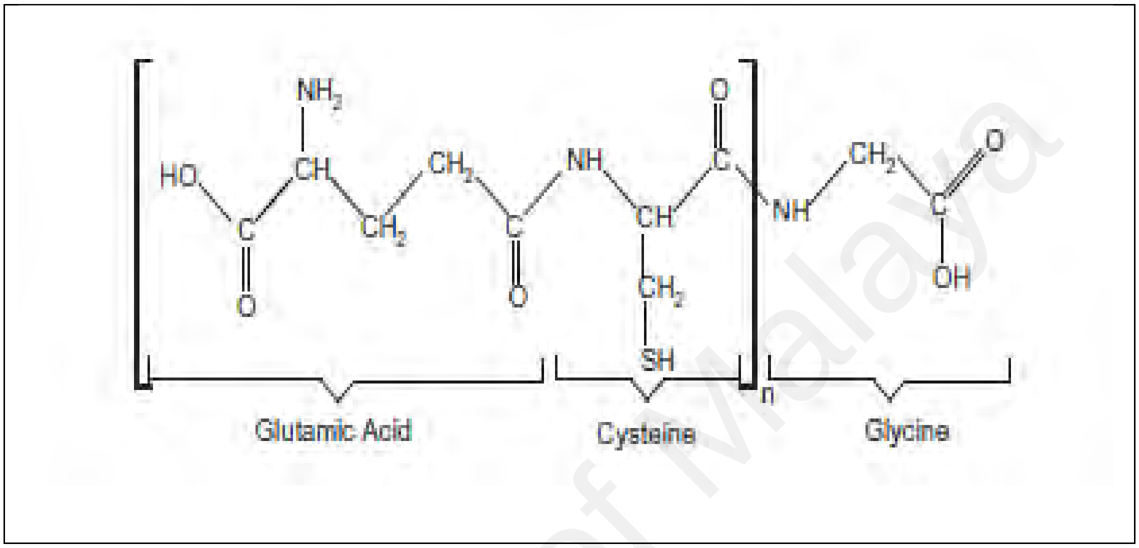


Figure 2.16: Chemical structure of plant phytochelatins.
Adapted from: (Seth, 2012; Ali et al., 2013; Shukla et al., 2013)

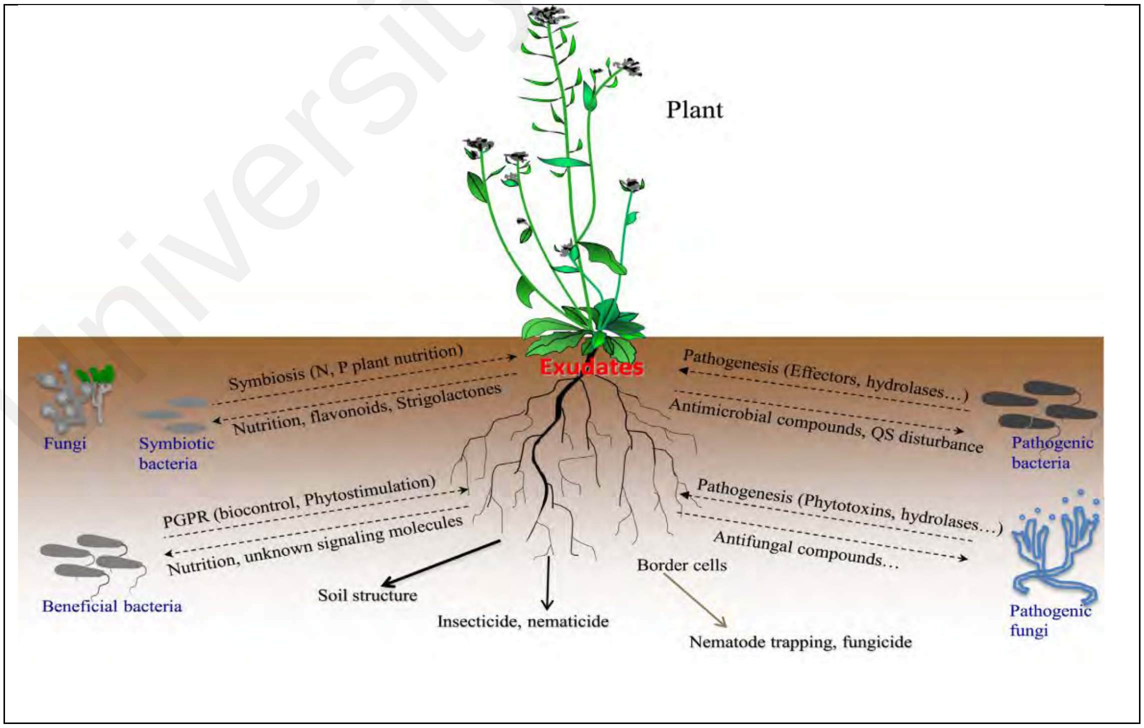


Figure 2.17: Complex interaction of root exudates with edaphic associations
Adapted from: (Haichar et al., 2014; Bais et al., 2006).

Table 2.17: Functional roles of root exudates components identified from different rhizospheres.

Exudate mixtures	Functions	Specific root exudate compounds identified.
Amino acids	Nutrient generator Mineral nutrients chelator Microbes Chemoattractant signals	α- and alanine proline, asparagine, valine, threonine, aspartate, tryptophan cystein, ornithine, cystine, histidine, glutamate, homoserine. arginine, glycine, homoserine, isoleucine, phenylalanine, leucine, -Aminobutyric acid, lysine a-Aminoadipic acid, methionine, serine,
Organic acids	Nutrient source Microbes Chemoattractant signals Chelators of less soluble minerals Acidifiers of soil Detoxifiers of Al *nod* gene inducers	Citric, glutaric, oxalic, malonic Malic, aldonic, fumaric, erythronic Succinic, ferulic, acetic, butanoic Butyric, syringic, valeric, rosmarinic, lactic, glycolic, *trans*-cinnamic, piscidic, formic, aconitic, formic, piscidic, aconitic, pyruvic vanillic, tetronic.
Protein and enzymes	Catalysts for P release from organic molecules Biocatalysts for organic matter transformations Plant defence.	Acid/alkaline, phosphatase, amylase, invertase, protease, PR proteins, lipases, β -1, 3-glucanases.
Vitamin and Sugars	Plant and microbe nutrient promoters	Glucose, desoxyribose, oligosaccharides galactose, biotin, maltose, thiamin, ribose, niacin, xylose, raffinose pantothenate, rhamnose, rhiboflavin, arabinose, fructose.
Purines & phenolics, inorganic ions & gasses.	Phytoalexins against soil pathogens Detoxifiers of heavy metals Microbes Chemoattractant signals Microbial growth promoters Resistance inducers against phytoalexins	Adenine, guanine, cytidine, uridine, HCO_3^-, OH^-, H^+, CO_2, H_2 Daidzein, 4′, 7-dihydroxyflavanone, Genistein, 4′, 7-dihydroxyflavone Coumetrol, 4, 4′-dihydroxy-2'-methoxychalcone. isoliquiritigenin, 7, 3′-dihydroxy-4′-methoxyflavone.
Root border cells	Stimulate microbial growth, and Chemoattractant Synthesize defense molecules for the rhizosphere Act as decoys that keep root cap infection-free Release mucilage and proteins.	

References: (Jones et al., 2004; Badri &Vivanco, 2009; Fosso-Kankeu & Mulaba-Bafubiandi, 2013; Haichar et al., 2014)

Most physiological and genetic studies have implicated these biomolecules (PCs and MTs), to be the critical drivers responsible for metal tolerance and accumulation in plants (Jones, 1998; Cobbeth & Goldbrough, 2002). Hence, forming stable complexes with metals at the cytosol, thereafter sequestered into the vacuole. The thiol-rich peptides of PCs consist of γ-*Glu-Cys* dipeptide repetitions, followed by terminal *Gly* with the basic structure (*γ-Glu-Cys)n-Gly*[*(PC)n*], where *n* ranges from 2 to 5(Cobbeth & Goldbrough, 2002), as seen in **Figure 2.13**.

Bacterial cells can generate and sense signal molecules, which permits the entire population to expand as a biofilm over the root surface, and become activated after a desired population density is attained. This mechanism is known as quorum sensing (Daniels et al., 2004). The rhizosphere soil may contain up to 10^6 to 10^9 bacteria per gram soil (Watt et al., 2006; Hinsinger et al., 2009; Glick, 2010; Mendes et al., 2013). Quorum sensing enhances bacteria capabilities to degrade pollutants, through volatilization, transformation and rhizodegradation (Kuiper et al., 2004; Gadd, 2010). Some plant growth promoting bacteria (PGP), are *Serratia, Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Beijerinckia*, *Flavo-bacterium* and *Gluconacetobacter* (Rosenbueth & Martinez-Romero, 2006; Ullah et al., 2015), and may simultaneously possess heavy metal resistance mechanisms, such as impermeability and efflux, complexation and enzymatic detoxification of metals from their cells. For example, is Hg(II) reduction to Hg(0) (Gadd, 2010; Kantar et al., 2008; Pavel et al., 2013). Sheng et al., (2008) demonstrated *Solanum nigrum*, inoculated with *Pseudomonas* sp. LK9, increased Cd uptake from 230 mg/kg to 292 mg/kg in shoots and roots, when compared with uninoculated plants. Hassen et al., (1998), reported enhanced biosorption of Cr by *Pseudomonas aeruginosa,* and Cu by *Bacillus thuringiensis* under citrate presence in soil.

2.4.3.3 Major metal transporters in plants

Heavy metals are translocated apoplastically into plant tissue, because of the continuum of the root epidermis and cortex (Chaney et al., 2007; Maestri et al., 2010). Hence, metals at the root cells have to pass the endodermis and casparian strip to reach the xylem (Salt et al., 2000; Xing et al., 2008). The endodermis cell wall and casparian strip constitute a barrier for apoplastic diffusion into the vascular system. For root to shoot movement, transporters carry metal ions from root symplast into xylem apoplast (Marschner, 1995), likely due to transpiration pumps (Salt et al., 1995).Therefore, understanding the molecular level for metal transport across plants is crucial for phytoremediation development.

Cation diffusion facilitator (CDF) family are heavy metals transporters associated to the transport of Zn, Co, and Cd mostly expressed in bacteria and eukaryotes (Eide, 1998). Characteristically proteins, with six transmembrane domains, N-terminal sequence, and a C-terminal cation binding domain (Maser et al., 2001). Group 1 and 3 of this family are link to plant metal tolerance and accumulation. The CDF transport gene *ZAT* was first found in *Arabidopsis*, and showed 35 to 40% similarity to the mammalian *ZnT* (Zinc transporter) gene, and functions as vascuolar sequestration of Zn (Delhaize et al., 2003). *ZAT* gene labelled as *ZTP1*, has been identified in Zn hyperaccumulator (*Thlaspi caerulescens*), and were expressed in their leave and roots (van der Zaal et al., 1999; Persans et al., 2001). The CDF transporters exhibits high variable sizes ranging from 280 to 740 residues (Paulsen & Saier, 1997).

ZIP Family: This group is involved in the translocation of divalent cations (Cd, Mn, Fe, and Zn) across membranes, and with wide range of substrate specificity. The *ZIP* proteins have about 309 to 476 amino acids, with variable regions for metal-binding and supposedly composed of histidine residues located in the cytoplasm (Manara, 2012). *ZIP* can transport Zn^{2+} and other metal ions from the extracellular into the cytosol (Maser et al., 2001). *Arabidopsis thaliana IRT1*, was the first *ZIP* metal transporter to be identified, and mainly responsible for the high uptake affinity of iron from soil (Vert et al., 2002).

NRAMP Family is the natural resistance associated macrophage protein which are responsible for transporting variety of metals, such as Ni^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} across membranes (Krämer et al., 2007). The *Nramp* gene has been identified in many other higher plants, bacteria, fungi, plants, and animals (Nevo & Nelson, 2006). In *A. thaliana*, *Nramp*1 gene has been implicated for Fe and Cd transport and homeostasis, (Thomine et al., 2000).

ABC Transporters is referred to as ATP-binding cassette (Guerinot, 2000), which function as ATP driven pumps with wide range of substrates specificity comprising of ions, lipids, peptides, pigments, sugars, xenobiotics, and heavy metals that are transported into the vacuole (Hall & Williams, 2003). Two main sub-class of *ABC* ATPase superfamily have been recognized in plants, the multidrug resistance associated proteins (MRPs), particularly active for the sequestration of chelated heavy metals, and the second is the multidrug resistance protein (MDRs), capable of mobilizing phytochelatins-Cd complexes into the vacuole via Mg-ATP-dependent manner (Ortiz et al., 1995).

CNGC Transporters is known as the cyclic nucleotide gated channels. It is basically a non-selective channel that uptakes both monovalent and divalent cations, as reportedly found in *Arabidopsis* (White et al., 2002). Some evidence suggests *CNGC* roles in heavy metal homeostasis in plants (Talke et al., 2003). For example, the overexpression of *NtCBP4* gene, a version of *CNGC* in tobacco led to improved tolerance of Ni^{2+} hypersensitivity to Pb^{2+}.

CaCA Transporters are superfamily of proteins, and membrane transporters that regulate cytosolic Ca^{2+} levels, through counter electrochemical gradient of other ions, such as H^+, Na^+, or K^+ across the tonoplast layer (Emery et al., 2012). *MHX* and *CAX* belong to the *CaCA* family, while the former is known for vacuolar Mg^{2+} and Zn^{2+}/H^+ antiporter expression in xylem-associated cells, the CAX partakes in Ca^{2+}/H^+ antiporter that also recognizes Cd^{2+}, suggesting this is an important pathway for Cd sequestration in vacuole (Salt & Wagner, 1993; Korenkov et al., 2007).

YSL Family: The *YSL* transporters are considered to mediate the uptake of metals that are complexed with plant-derived phytosiderophores (Roberts et al. 2004), and their genes have been identified in *eubacteria*, *archaea*, plants, and fungi, excluding animals. Also, indicted for metal-chelates transports in plants (Murata et al. 2006).The maize *ZmYS1* transporter gene, is the most studied among all, hence being implicated to translocate Fe, Cu, Ni, Zn, and less of Cd and Mn (Schaaf et al., 2005). Expanded details of *YSL* isolated transporters from *A. thaliana* such as *AtYSL1* and *AtYSL2*, and *AtYSL4* and *AtYSL6* from *Arabidopsis* genes are found in (Colangelo & Guerinot, 2006; Krämer et al., 2007; Conte et al., 2013). **Figure 2.18** shows the metal transporters activity flow diagram.

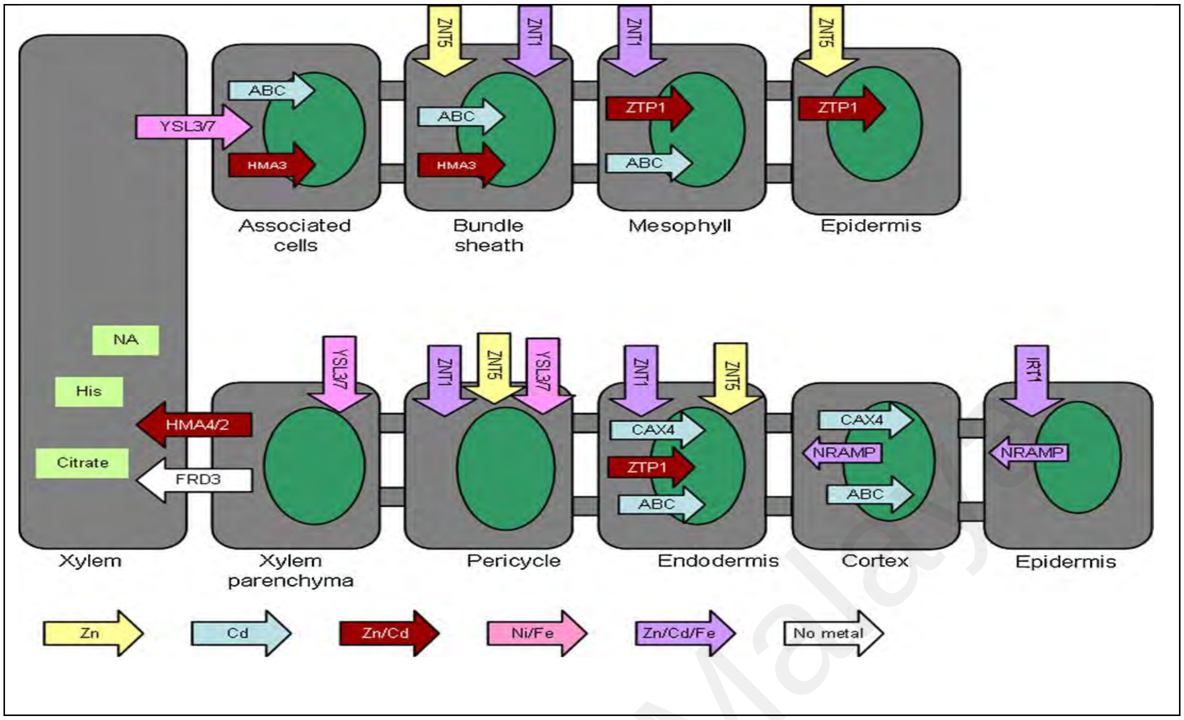


Figure 2.18: Flow model for Zn, Cd and Ni transport and sequestration in root and shoot cells of the Zn/Cd/Ni hyperaccumulator of *Thlaspi*. Metal or metal-chelate transporters are depicted as arrows located at the plasma membrane or the vacuolar membrane of tissues that take part in metal translocation. The symplast is indicated in grey, the vacuole is indicated in green. The colour of the arrows indicates the metals these proteins are most likely to transport.
Adapted from: Hassan & Aarts, (2011).

2.4.3.4 Integrated factors influencing Phytoremediation

The decontamination of soil heavy metals by plants may depend on the following, (i) soil factors; such as sorptive capacity of the soil, heavy metal content, cation exchange capacity, soil pH, and organic matter content affects metal hyperaccumulation in plants, as well as the plant age (Bhargava et al., 2012). (**Figure 2.19 & 2.20).** (ii) Environmental factors suchlike temperature, water chemistry/content in soil, and growth metabolism of plants have been reported (Yu et al., 2005). The removal rates of metals by plants are known to increase linearly with increase in temperature (Yu et al., 2010), due to transpiration. (iii) Genetic factors also exist, some plant genotypes respond positively to elevated heavy metal concentration in soil, while others may be inert, or exhibit negative growth (Nanda- Kumar et al., 1995; Hanikenne & Nouet, 2011). **Figure 2.21** shows the phytoremediation plant-genotype enhancement through genetic engineering.

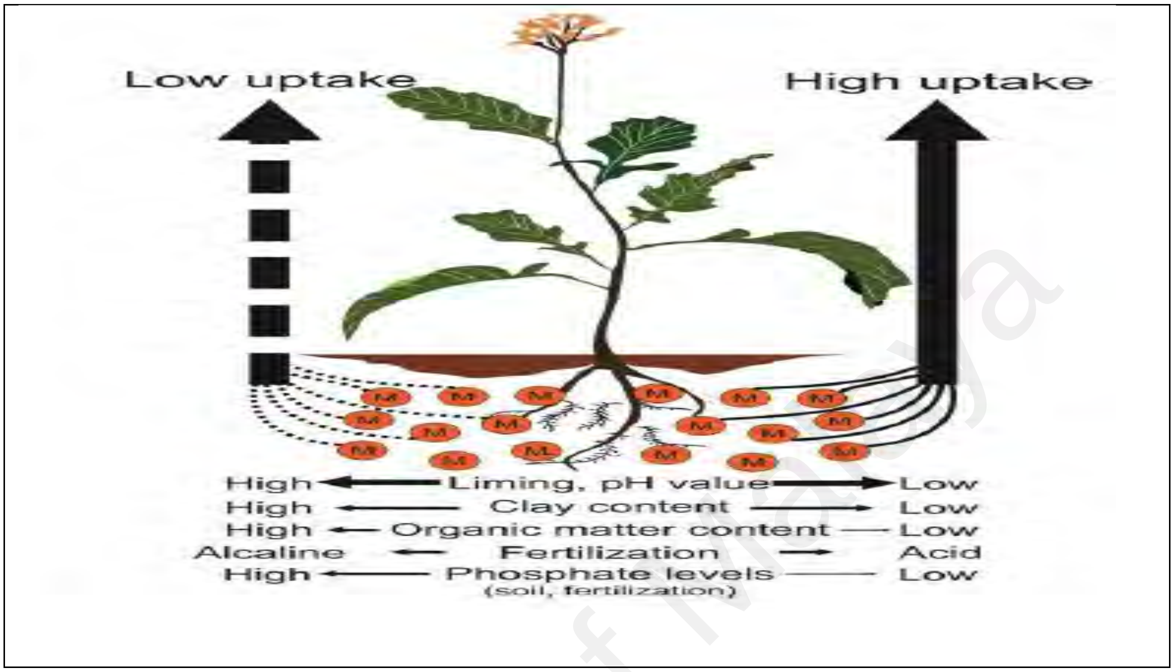


Figure 2.19: Factors influencing soil heavy metal availability. Arrows magnitude indicate relative contribution of each factor in the absorption of metals by plants. Interrupted lines indicate limited uptake. Symbol M = metal.
Adapted from: Gupta & Chatterjee, 2014.

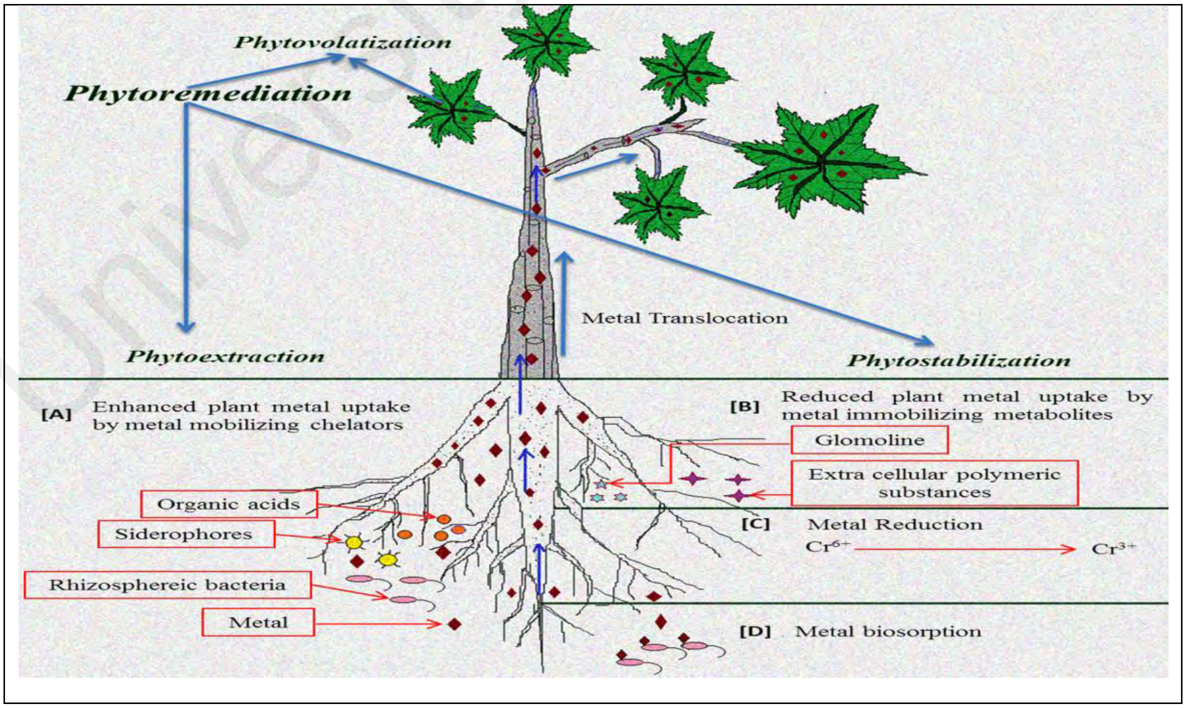


Figure 2.20: Schematic mechanisms affecting metal phytoremediation.
Source: Bauddh et al., (2015).

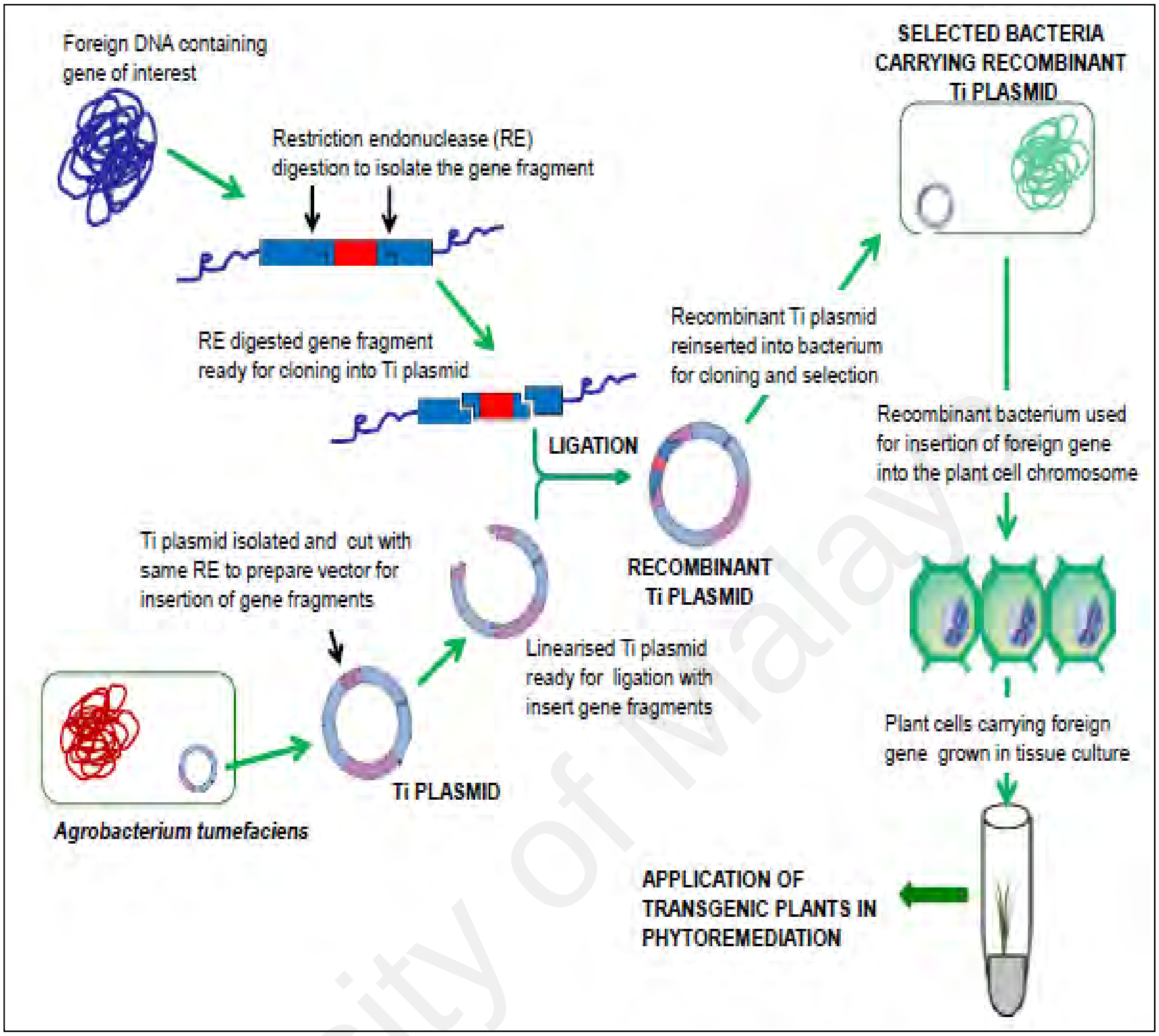


Figure 2.21: Schematic genetic engineering approach to improving plants for phytoremediation.

Adapted from: Gupta & Chatterjee, 2014.

2.5 Selection criteria for plants

Plant choice is plausibly the most important factor determining the outcome of any phytotechnology design (Team, 2001). Following the assessment of plants growth on specific sites, next step is to adopt the plant which can survive under such environment. Some fundamental literatures about the plant can assist in the project design, such as specific local names, growth habitat, tolerance of plants to diverse temperatures, moisture, and disease conditions. Indigenous plants are deemed as viable options for phytotechnology, due to their default climatic regional exposure.

Reynoso-Cuevas et al., (2008) argued that plants deserved to be native of areas in which they should be used, and be tolerant to weather and soil conditions. Several plants have been implicated for their phytoremediation capabilities. However, leguminous plants have been identified to be more useful for phytoremediation than non-leguminous species, plausibly due to their innate ability to fix N under limited availability in contaminated sites (Frick et al., 1999). Plants high in phytotoxicity, pollutant absorption, and have the ability to transfer high rate of oxygen to stem, root and leaf, are in strict sense a selectable plant (Muratova, 2003).

2.5.1 Plant species utilized for this study

This study technically undertook the selection of two indigenous plants, to decontaminate heavy metal soil. Hence, the details of the specific plants are elaborated below;

Lantana camara: *Lantana* originates from tropical and subtropical America, and presently in more than 60 countries including Malaysia (known as: *pokok bunga tahi ayam*), Philippines, Australia, India, and some African countries (Aravind et al., 2010). It is considered as weed plant in Australia, ornamental in Malaysia, and both ornamental and/or therapeutic in India (due to the presence of phytochemicals, suchlike alkaloids, flavones, lignans, catechins, etc., in its composition, Ganjewela et al., 2009). It belongs to the kingdom (*Plantae*), order (*Lamiales*), and family (*Verbenacea*) (Saxena et al., 2012).

This plant (**Plate 2.3**), can thrive under warm, and high rainfall climate with approximately 900 mm per/annum. It survives at prolong dry periods in drained and fertile soils, including rich organic, clay, and volcanic soils (NSW, 1993; Aravind et al., 2010). *Lantana sp*. has branching shrub that grows 2 to 4 m high with dense thickets, toothed leave edges with leaf dimension of about 2 to 10 cm long, hence short taproot with vast lateral root branching system, and possess flowers of multi-pigmentations. In addition, *Lantana sp*. produces strong characteristic smell when shoot components are crushed, (Rod, 2008). *Lantana sp*. was declared a class 5 noxious weed under the New South Wales noxious weeds act 1993 in Australia (www.nsw.gov.au/weeds). Meaning they are prohibited from been sold in any area of the state.

Plate 2.3: *Lantana camara*.

Pilea cadierei is also known as Aluminium plants because of its shiny-silver foliage, belongs to the family *Urticaceae*. It is evergreen, and mostly classified as herbaceous perennial with about 30 cm in height. The leaves and stem are implicated to contain mildly toxic alkaloids (Beckett, 1995). This plant (**Plate 2.4**), is a native habitat of Southeast Asia. In Malaysia, it is known as *pokok pelia*, and exclusively ornamental.

Plate 2.4: *Pilea cadierei*

However, it can flourish, and tolerates wide range of soil and climatic conditions; ranging from acidic to slightly alkaline, moist, sandy to clay soils, and warm to temperate weathers. It has been reported that *Pelia cadeirei* can form thick ground covers within two years when planted at 18-inch centres (Rauch & Weissich, 2000). Un-rooted stems may struck the ground and will propagate itself, if kept moist and mildly shaded.

2.6 Soil conditions under metal contamination and post-remediation image with SEM

During metal-soil contamination, increase in the heavy metal resident time in soil, can cause decrease in the metal mobility and bioavailability. Because of the binding affinity between the soil and heavy metals (Sayen et al., 2009). Scanning electron microscopy (SEM), are adopted to provide information (images), on the location and/or distribution of metals in soil samples within remediation context, and for evaluation purposes (Sayen et al., 2009; Vermeij et al., 2012; Vos et al., 2014). According to Shaw et al., (2004), SEM images of metal contaminated soil provide clearer contrast, and therefore applicable in numerous fields; such as in geology, agriculture, engineering, chemical, mining, and other industrial purposes. For example, geologist uses SEM micrographs from sediments size dispersion, and grain cast as provenance indicators for assessing minerals (Prakongkep et al., 2010). **Figure 2.22** shows SEM micrographs soil-metal distribution images from Prakongkep et al., (2010). In addition, it has also been reported that the clarity and distribution of metals in SEM micrographs of contaminated soils tend to diminish at post-remediation (Karimi & Gray, 2000). Hence, indicating pollutants removal and/or reduction due to remediation.

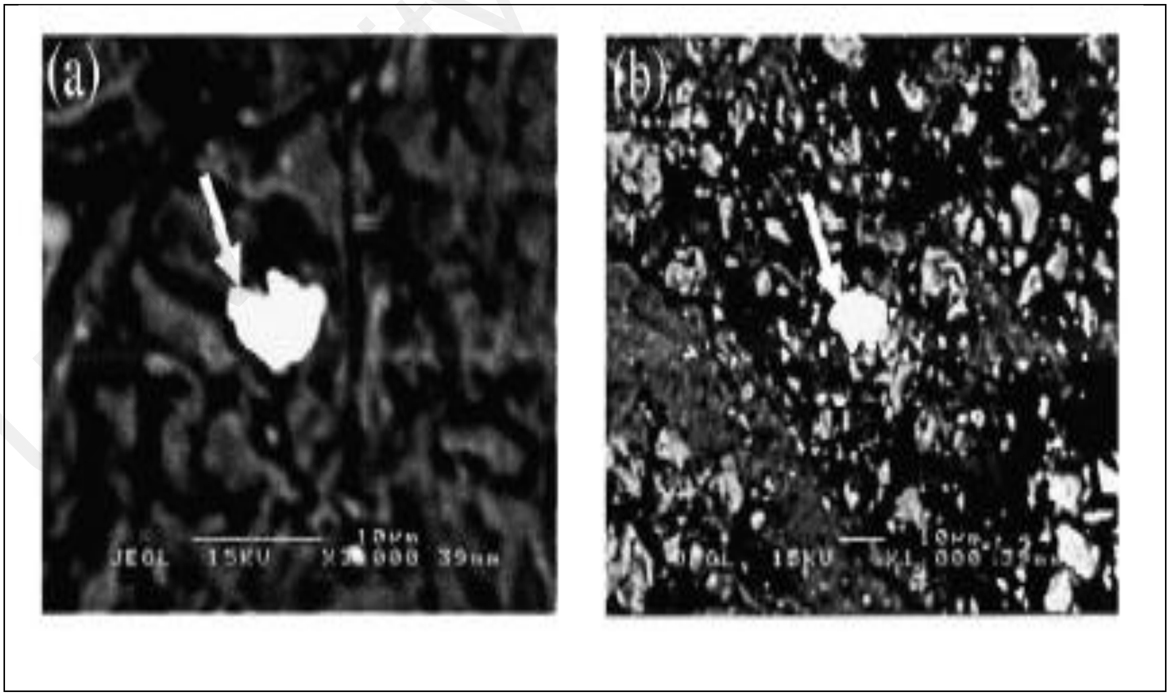


Figure 2.22: Scanning electron micrographs spectra of sand size mineral grains; shiny arrow points at (a) brass (Cu–Zn alloy) and (b) Au, indicate micrographs spots for these elements.

Source: Prakongkep et al., (2010).

2.7 Kinetic modelling of metal removal from soil during remediation

Although plants have been demonstrated to remediate heavy metal contamination in soil, and in other substrates (Yu & Gu, 2007), there are still inconclusive information concerning differences in their kinetic parameters, with respect to various treatments and models (Vrtoch et al., 2011; Ofomaja et al., 2014). Therefore, understanding the functional kinetics, can provide inherent interpretation insights, for the quantitative data obtained from bioremediation (Verma et al., 2013). Thus allowing findings and/or conclusions be deduced from the entire complex. Studies bordering on the kinetics of phytoremediation proceed in dual dimensions: (i) the initial is concerned with factors influencing the transformation of pollutants quantity with time, and (ii) seeks to unravel the curves describing the transformation processes, both in laboratory microcosm and in actual field conditions (Maletić et al., 2009).

Rončević et al., (2005) argued that researchers involved in kinetics studies do not always report whether the model they used were based on theory or experimental facts, or probably if the equation constants expressed have a physical meaning or mere fitting of parameters. Some applied studies have demonstrated biodegradation and/or reduction kinetics can be approximated with first-order kinetics (Abioye et al., 2012), as thus;

$$C_t = C_o e^{-kt} \quad \text{(Eq. 2.5)}$$

where C_t is usually the concentration (mg/kg) of pollutant at specific time, and C_o is the initial pollutant concentration (mg/kg), k is the removal rate constant (d^{-1}), and t is the time (day). Abedi et al., (2014) investigated the potential of Ryegrass on the remediation of petroleum-contaminated soil. Their findings recorded increased rate of contaminant removal until 50 days, hence reduced the soil pollutant amount after 80 days, which they suggested followed the mechanism of first-kinetic order. However, the determination of half-life ($t_{1/2}$), which is the time required for half of the original amount of pollutants present in the substrate, had been chemically transformed (Yeung et al., 1997; Fritsche & Hofrichter, 2005; Verma et al., 2013), and expressed as follows;

$$t_{1/2} = \ln 2 \,/ k \qquad \text{(Eq. 2.6)}$$

Radioactive cesium being an anthropogenic isotope, and also uranium and plutonium fission has been reported to have a half-life of 30.20 years, upon soil contamination (Benmansour et al., 2013). However, first order kinetics, as well as the profound Michaelis-Menten kinetics models, are the most frequently used equation for degradation kinetics (Pala et al., 2006). However few works have been implicated to investigate the kinetics of soil bioremediation (Abbassi & Shquirat, 2008), and the information gathered would be beneficial in determining the residual pollutants at a given time, and predicting future pollutant persistence. Hwang et al., (2001), elaborated 0.099 per day, as the first-order kinetic rate constant (*k*) of a diesel contaminated soil under compost supplementation. While Chein et al., (2010) reported 0.015 per day removal rate constant for TPH polluted soil under co-remediation with microbial inoculates. Evidently, the rates are said to be proportional to the exponent of the substrate concentration as indicated in Eq. 2.5 (Rončević et al., 2005).

2.7.1 Thermodynamic, Isotherms, and diffusion impacts on metal removals

Thermodynamic parameters focuses on engineering mechanisms that assesses the uptake of adsorbents, and also provides insight on adsorption activities (Romero-Gonzalaz et al., 2005; Safa & Bhatti, 2011; Liu & Lee, 2014). Most researchers have estimated changes in enthalpy (ΔH^0), entropy (ΔS^0), and free energy (ΔG^0) under temperature (T)-dependent equilibrium absorption quantities for thermodynamics studies as expressed in Equations **2.7 and 2.8** (Lawal & Sanni, 2010; Hercigonja et al., 2012). However, in the adsorption of Cu^{2+} at equilibrium state, Erturgay & Bayhan, (2010) noted that $\Delta H^0 = -11.64$ KJ/mole, and $\Delta S^0 = -35.5$ KJ/mole, but identified a low ΔG^0 (-0.2 to -1.29 KJ/mole) and argued that the system was a physical adsorption. Whereas, Yao, (2010) applied chestnut shell to adsorb Cu^{2+} and recorded $\Delta H^0 = -17.423$ KJ/mole, and $\Delta S^0 = -54.667$ KJ/mole and argued that the adsorption was exothermic and not driven by entropy.

$$\Delta G^o = \Delta H^o - T\,\Delta S^o \qquad \text{(Eq. 2.7)}$$

$$\Delta G^o = -R\,T \ln Kc \qquad \text{(Eq. 2.8)}$$

where R is the universal gas constant (KJ/mol/K), and *Kc* is the absorption equilibrium constant.

However, the number of adsorbate-adsorbents studies remain infinite, but understanding the absorption mechanism provide ideas on how a system responds to thermodynamic processes and/or changes, and also gives insight on the efficiency of an existing remediation system (Ertugay & Bayhan, 2010; Hercigonja et al., 2012; Kikuchi & Tanaka, 2012; Kanmani et al., 2012). The quantities and rates of adsorption/absorption are the most significant indices in assessing any adsorption system (Liu & Lee, 2014). Moreover, solute uptakes are function of interaction between the solute and adsorbent surfaces (Volesky, 2007).Yang et al., (2016) reported that for better absorption performance, the pore size of a porous adsorbent should match the adsorbates atomic/molecular size.

Isotherm mechanisms are inalienable in adsorption efficiency. This indicates how the molecules are distributed between the liquid and solid phases, when equilibrium is attained during adsorption. Some of the isotherm models are Freundlich, Langmuir, Temkin, Redlich-Paterson, and Sips equations (Febrianto et al., 2009). Biosorption energy calculated for heavy metals using Dubinin-Radushkevich isotherm model suggested that biosorption of Se^{4+} and Se^{3+} on biomass was a function of ion exchange mechanism (Tuzen & Sari, 2010; Uluozlu et al., 2010).Another important factor in pollutant absorption is the diffusion (Sakar et al., 2003).

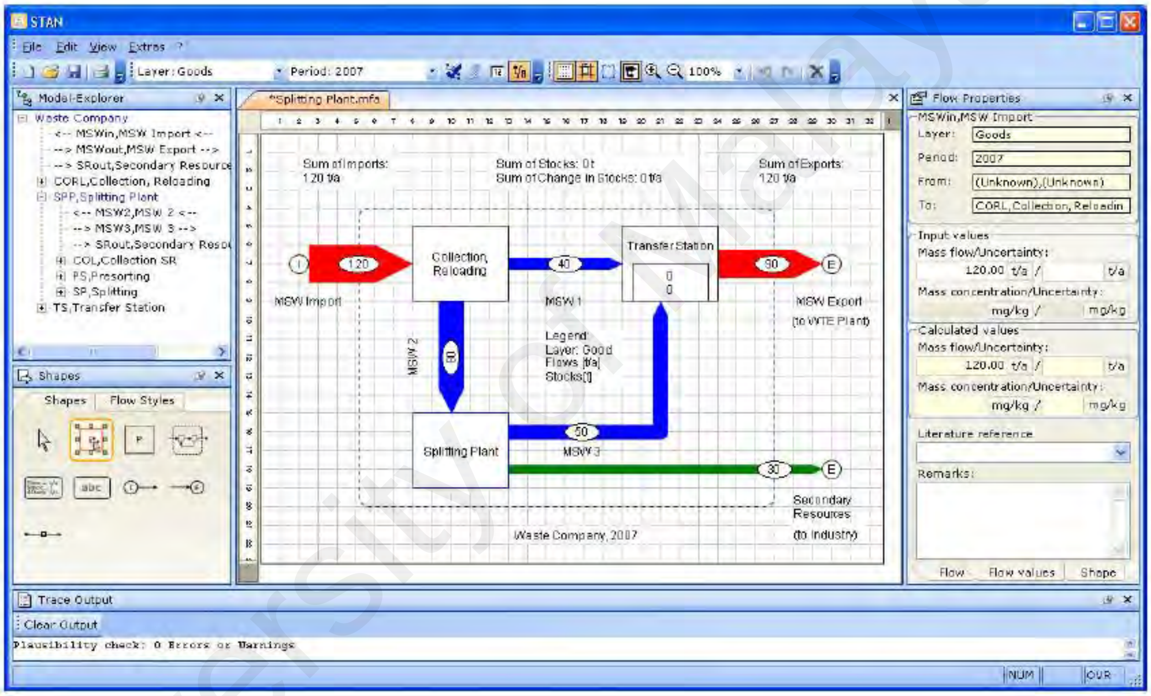
2.8 Schematic flow using STAN 2.5 in phytoremediation-metal mass balance

In phytoremediation of metal contaminated soil, it is assumed that metals are not degraded but rather are transformed and/or transferred either biologically or abiotically to less toxic compounds, into the plant or to the surrounding environments during the process. However, mass balance is the practical application of the law of conservation of mass for chemical degradation or synthesis (Mackaplow, 2010), to monitor whereabouts of substances. It has wide industrial and agricultural application (Bonnin et al., 2013; Guyonnet et al., 2015). During phytoremediation, mass balance calculations are used to assess the yields and efficiency of the metal removals from substrates. Hence, it indicates a multi-step substance loss mechanism (Baertschi et al., 2013). Mass balance is defined by the International Conference on Harmonization (ICH), as the system of summing together the assay value and levels of degradation products to ascertain how closely these add up to 100% of the initial value, in cognizance of margin of analytical error (ICH, 2003; Baertschi et al., 2013). It is mathematically expressed as;

$$\sum N_{\text{inputs}} = \sum N_{\text{outputs}} + \Delta N_{\text{storage/stock}} \qquad \text{(Eq. 2.9)}$$

where mass of N (mg/kg) entering the system should be equal to the mass of N leaving, plus the change of N stock over a given period (Baertschi et al., 2013).

STAN is the acronym for “subsTance flow ANalysis”, a free software that supports the performing of material flow analysis (MFA) (Baccini & Brunner, 1991; Brunner & Rechberger, 2004; Lederer et al., 2015). (**Figure 2.23)**. Moreover, it has widely been applied in modelling municipal solid waste (MSW). However, this can only be used for schematic depiction in phytoremediation studies when data are inputted into the STAN flow system, hence this software basically conforms to the law of conservation of mass.



MSWin,MSW Import
Layer: Goods
Period: 2007
From: (Unknown),(Unknown)
To: CORL,Collection,Reloadin

Input values
Mass flow/Uncertainty: 120.00 t/a / t/a
Mass concentration/Uncertainty: mg/kg / mg/kg

Calculated values
Mass flow/Uncertainty: 120.00 t/a / t/a
Mass concentration/Uncertainty: mg/kg / mg/kg

```
Plausibility check: 0 Errors or Warnings
```

Figure 2.23: Graphical user interphase of STAN.

Adapted from: Cencic & Rechberger, (2008).

2.9 Cost of phytoremediation

The generals cost of phytoremediation for one hectare with depth of about 15 cm is estimated to be about 2,500 to 15,000 US dollars, based on 17 to 100 US dollars per cubic meter estimate. An estimate from Frick et al., (1999) put the cost at approximately 300 US dollars per m^3 per year to phytoremediate a site contaminated with oil and inorganic pollutants using deep-root plants and trees. Although this estimate might vary in recent time, but there is no comprehensive data on this at the moment to clarify this argument, but however the increasing adoption of phytoremediation still indicate that the technology is cost effective. There are various ways to reduce the cost of phytoremediation, for example, during the *in-situ* phytoremediation process (Adadzi, 2010; Sun et al., 2011), plants using solar energy as a source of energy helps to reduce the cost of phytoremediation. Sustaining this for about 10 years will help to spread the cost over the duration.

Unfolding literatures reported that at international markets, the remediation sector is estimated to be in the range 30 to 35 billion US dollars per annum. The utilization of bioremediation and phytoremediation cleanup technologies is rapidly expanding, and reported worldwide demand for these biological approaches is thought to be valued in the range of 1.5 billion US dollars per annum (Sing et al., 2013). However, the soil remediation sector has a ready market in countries such as the US, Canada, Western European countries, Japan, and Australia. Also, more developed Eastern European, Latin American and Asian countries represent emerging markets for the remediation sector, and Africa is beginning to participate actively as well. Understandably, quantifying the value of these emerging remediation markets at the moment is not easy, especially since comprehensive catalogues of contaminated sites in these countries have not been established (Sing et al., 2013). However, Glass, (1999) presented an in-depth estimate for specific treatment cost in (tonne) for phytoextraction against other approaches, including holistic US phytoremediation market cost for metals and radionuclides, for periods on or before 1999 as given in **Tables 2.18** and **2.19,** respectively. **Table 2.20** presents a direct phytoremediation cost against other technologies by Bauddh et al., (2015).

Table 2.18: Cost of different treatments for metal-contaminated soils

Treatments	Cost ($US/tonne)	Additional factors/expenses
Vitrification	75 - 425	Long-term monitoring
Chemical treatment	100 - 500	Recycling of contaminants
Landfilling	100 - 500	Transport/excavation/monitoring
Electrokinetics	20 - 200	Monitoring
Phytoextraction	5 - 40	Monitoring

Source: Glass, (1999).

Table 2.19: Estimated 1999 US phytoremediation markets for metals and radionuclides.

Contaminated substrates	Cost in million ($US)
Landfill leachate	5 - 8
Metals in soil	4.5- 6
Inorganics in wastewater	2 - 4
Inorganics in groundwater	2- 3
Radionuclides	0.5-1
Metals in wastewater	0.1- 0.2

Source: Glass, (1999 and 2000)

Table 2.20: Estimates of phytoremediation cost against the cost of other technologies

Contaminants	Phytoremediation cost	Cost using other technologies
Heavy metals	$80 per cubic yard	$250 per cubic yard
1ha to 15 cm depth (Various pollutants)	$2500 to $ 15,000	None listed
Site contaminated with Petroleum hydrocarbons	$70,000	$850, 000
10 acres Pb polluted site	$500,000	$12 million
Heavy metals	$15 to $40 per cubic meter	$100 to $400

Source: Bauddh et al., (2015).

In conclusion, this Chapter 2 conveys informative ideas, which have enhanced understanding into the existing, but inexhaustive knowledge of bioremediation and/or phytoremediation of contaminated soil and/or environment, by recalcitrants. However, the approaching Chapter 3 shall put forward the methodology under which this study was systematically conducted.

CHAPTER 3: MATERIALS AND METHODS

3.1 Collection of study samples

Soil (Mollisol) and six-months old plants (*Lantana camara* and *Pilea cadierei*) utilized for this phytoremediation study were purchased from a nursery division at Sungai Buloh Selangor. These were safely transported to the laboratory for soil analysis, and further plant observations were conducted. Organic wastes, namely empty fruit bunch (EFB) and spent mushroom compost (SMC) were collected from two different agronomic industries; Sime Darby Sdn. Bhd., Bukit Kerayong Kapar, and Gano mushroom farm, Tanjung Sepat, both in Selangor Malaysia. The plants (*L.camara* and *P. cadierei*) were selected based on the following parameters; high drought tolerance, economic, inedible, and locally found in Malaysia and other tropical and/or subtropical regions.

3.2 Plants used in this research

***Lantana camara* (Local name: *Bunga tahi ayam*)**

This plant is commonly used for ornamental purposes in Malaysia, and hence comparatively inexpensive in nursery outlets where it was procured. Prior to use, comprehensive taxonomic review was performed to identify its biological importance. Therefore, its taxonomic strata is given in **Table 3.1**, and the image is presented in **Plate 3.1**

Table 3.1: Taxonomic review of *Lantana camara*

Kingdom:	Plantae	Plants
Subkingdom:	Tracheobionta	Vascular plants
Superdivision:	Spermatophyta	Seed plants
Division:	Magnoliophyta	Flowering plants
Class:	Magnoliopsida	Dicotyledons
Subclass:	Asteridae	-
Order:	Lamiales	-
Family:	Verbenacea	Verbena family
Genus:	*Lanata*	
Specie	*camara*	

Sources: Urban et al., (2011); Ghisalberti, (2000).

Plate 3.1: *Lantana camara* (**Local name**: *Bunga tahi ayam*)

***Pilea cadierei* (Local name: *Pokok Pilea*)**

This is also a decorative plant, locally found in Malaysia. Upon identification, efforts were made to review its taxonomic and economic importance. Hence, **Table 3.2** and **Plate 3.2** represents *P. cadierei* taxonomic level, and image, respectively.

Table 3.2: Taxonomic review of *Pilea cadierei*

Kingdom:	Viridiplantae
Phylum:	Streptophyta
Subclass:	Rosids
Order:	Rosales
Family:	Urticaceae
Genus:	*Pilea*
Species	*cadierei*

Sources: (Monro, 2006; Burger, 1977)

Plate 3.2: *Pilea cadierei* (**Local name**: *Pokok Pilea***)**

3.3 Organic waste and heavy metal used in this study

The two organic wastes (EFB and SMC) were used as amendments to soil. Chemical assay revealed that these organic wastes contain appreciable quantities of nutrients, capable of sustaining the proliferation of soil microbes (bacteria) present in the soil, and as well enhance plant physiological state. In addition to their reported alternative substrates value for soil conditioning, their utilization helps to minimize environmental problems caused by disposal into landfills (Deka et al., 2011; Gabhane et al., 2012).

Heavy metals Pb and Cr were chosen because of their wide environmental pollution impacts, ranging from mining, electronic wastes, and in tannery industries etc., which had been indicted to have influenced soil contamination globally.

3.3.1 Empty fruit bunch

Empty fruit bunch (EFB) is a by-product of oil palm (*Elaesis gunineensis*) extraction (Coral-Medina et al., 2015). It is estimated that one hectare of the oil palm produced 10 to 35 tonnes of fresh fruit bunch (FFB), and EFB alone constitute about 28.5% of the total mass of waste generated from FFB (Rupani et al., 2010). Furthermore, palm oil industries generate approximately 20.2 x 10^6 tonnes year^{-1} of solid wastes which consist of oil palm EFB, fiber, and fruit shells (Purwandari et al., 2013; Arum Sari et al., 2014). EFB can potentially represent a huge, inexpensive, and renewable source of energy, and is used in paper production (Wildschut et al., 2013; Cotana et al., 2014). **Plate 3.3** shows the EFB sample used in this study. **Plates 3.4** and **3.5** show harvested FFB and active palm plantation site (above 14 years old), where these biomasses originate from, as typically seen in Malaysia.

Plate 3.3: Empty Fruit Bunch

Plate 3.4: Fresh Fruit Bunch.

Plate 3.5: A typical active palm plantation site (above 14 years old) in Malaysia.

3.3.2 Spent Mushroom Compost

The compost residue in which mushrooms were grown, are known as spent mushroom compost (SMC) (Jordan et al., 2008). **Plates 3.6 and 3.7** show SMC sample used in this study, and a typical active mushroom farm, respectively. However, mushroom production is considered to be the biggest global solid-state-fermentation industry (Soccol & Vandenberghe, 2003; Lou et al., 2015). Finney et al., (2009) and Grujić et al., (2015) reported that every 1 kg mushroom produced a corresponding 5 kg of SMC. However, SMC has been proven to be an attractive material for improving tilled-soil structure, and dry matter production in grassland soils, based on its high organic matter content and/or availability of essential plant nutrients (Ribas et al., 2009).There are numerous agricultural, industrial, and horticultural applications for SMC, thus: a potential for agricultural fertilizer (Guo, 2005); as a ruminant feed for sheep (Beattie et al., 2001); treatment for coalmine drainage (Finney et al., 2009); enzyme extraction (Jordan & Mullen, 2007; Kubicek, 2013; Grujić et al., 2015); and for bioremediation and/or biosorbent materials (Lau et al., 2003; Chen et al., 2005).

Plate 3.6: Spent Mushroom Compost.

Plate 3.7: A mushroom farm environment.

3.4 Determination of physicochemical features of soil and organic wastes

The physicochemical properties of soil and organic waste used were determined by applying the standard methods. Soil measurement was done through the hydrometric method. N-content of both soil and organic wastes used for phytoremediation was conducted using the Kjeldahl method, as described in Agamuthu et al., (2010). The organic carbon was determined using the furnace method. P and heavy metal (Pb and Cr) concentration were assessed using inductively coupled plasma (ICP-OES)-USEPA 3050B, after acid digestion with microwave system (ETHOS-1 MILESTONE), pH was determined with pH-meter (HANNA HI 8424) on 1:2.5 (w/v) soil/distilled water after 40 minutes equilibration. Triplicate determinations were made for all analysis.

3.5 Methodology used for phytoremediation studies and sampling

3.5.1 Preliminary studies on plant for metal toxicity

Prior to final soil preparation, heavy metal contaminated soil was prepared with increasing concentrations of metals from 10, 20, 40, 80, 100, and 120 ppm for Pb, and 10, 20, 40, 60, and 80 ppm for Cr **(Plate 3.8)**. The stock solutions were made and stored in different containers following the methods of Juwarka et al., (2008) from $PbCl_2$ and $CrCl_3$ metal compounds, as further illustrated in **Table 3.3,** and with **Eq. 3.1**. This was done in order to achieve much higher Pb and Cr concentrations above the legal limits (i.e., national background concentration and intervention value) for use of soil, as promulgated by the Department of Environment (DOE) Malaysia (2009). In continuation, this was done separately in each 3 kg characterized soil aliquots, contained in high density garden polyethylene bags, with *Lantana camara* and *Pelia cadierei* planted after 21 days of routinely mixed, for soil-metal stabilization.

Plate 3.8: Set-up for initial preliminary Pb and Cr toxicity probe on *P. cadierei* and *L. camara*.

Table 3.3: Heavy metal stock solution preparation.

Metal compds.	**Molar Mass**	**Mass of** Pb	**Mass of** Cl	**Molar mass** $PbCl_2$ / **Mass of** Pb
$PbCl_2$	278.1 g/mol	207.2 g/mol	70.90 g/mol	278.1/207.2 = 1.34 g/mol

Therefore, 1.34 g of $PbCl_2$ in 1 L of distilled water = stock solution of Pb at 1000 ppm.

$CrCl_3$	**Molar Mass**	**Mass of** Cr	**Mass of** Cl	**Molar mass** $CrCl_3$ / **Mass of** Cr
	158.36 g/mol	51.99 g/mol	106.35 g/mol	158.36 / 51.99 = 3.05 g/mol

Hence, 3.05 g of $CrCl_3$ in 1 L of distilled water = stock solution of Cr at 1000 ppm.

Thus, **$M_1V_1= M_2V_2$** was used to derive all desired heavy metal concentrations for Pb and Cr, from the respective stock solutions.

$$M_1V_1 = M_2V_2 \qquad \text{(Eq. 3.1)}$$

where M_1 and V_1 are initial moles per litre and vol. in litre, and M_2 and V_2 are final moles per litre and vol. in litre.

Plates 3.9 and **3.10** show the impacts of preliminary heavy metal toxicity test on *L.camara* and *P. cadierei*, respectively.

Plate 3.9: Preliminary test representation of heavy metal acute toxicity on *L. camara*

Plate 3.10: Preliminary test representation of heavy metal acute toxicity on *P. cadierei*

3.5.2 Phytoremediation and soil experimental preparation set-up in the greenhouse

After the preliminary toxicity test discussed in Section 3.5.1. The following concentrations (10, 20, 40 and 80 ppm) for Pb, (10, 20 and 40 ppm) for Cr, and a mixture of 20 ppm (Pb + Cr) were adopted to contaminate and/or co-contaminate each 3 kg soil, respectively. Similar approach were also followed by Adesodun et al., (2010). Thereafter, they were homogenized by maintaining (60% ± 2) moisture content, spontaneous air drying, and regularly mixed for 21 days as previously reported in Alaribe & Agamuthu, (2015). This methodology was adopted in order to reproduce a process of metal-soil sorption, mimicking the actual field-ecosystem. After the 21 days of metal stabilization, 6% organic wastes of shredded EFB, or air-dried SMC were introduced. Then it was mixed at regular intervals for another three days to attain further stabilization prior to plant application (transplant approach), to form (soil-metal-organic waste) phytoremediation complex. All treatments were in triplicates (n = 3), and microcosm treatments were enumerated every 14 days. **Plates 3.11** and **3.12** show the set-ups, and **Table 3.4** provides the experimental details. Average greenhouse daytime temperature measured was at 29 ± 0.2°C, and is located at the Institute of Graduate Studies Building (roof-top), University of Malaya, Kuala Lumpur, Malaysia (3°07 28.82 N, 101°39 33.86 E).

Specific controls for each of the treatment concentrations on plants, and with zero organic waste or another without plants and organic waste (soil-metal only) to monitor biotic loss was incorporated. Further control treatments comprising of autoclaved soil containing 0.5% NaN_3 was also set-up to determine abiotic loss of the target metals from the soil. The experimental plants were carefully hydrated once in every two days with distilled water at 60% moisture capacity to prevent cases of metal leaching from the nursery polyethylene bags, and monitored for 98 days beginning from the plant inception date.

Plate 3.11: Phytoremediation experimental set-up for *P. cadierei*

Plate 3.12: Phytoremediation experimental set-up for *L. camara*

Table 3.4: Experimental treatments, design, and details.

Treatments	Treatment details.
A1	3 kg soil + 80 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*
A2	3 kg soil + 80 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*
A3	3 kg soil + 80 ppm Pb +*Pilea cadierei /Lantana camara*
A4	3 kg soil + 80 ppm Pb only.
A5	3 kg autoclaved soil +80ppm Pb + 0.5%NaN_3
B1	3 kg soil + 40 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*
B2	3 kg soil + 40 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*
B3	3 kg soil + 40 ppm Pb +*Pilea cadierei /Lantana camara*
B4	3 kg soil + 40 ppm Pb only.
B5	3 kg autoclaved soil +40 ppm Pb + 0.5%NaN_3
E1	3 kg soil + 20 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*
E2	3 kg soil + 20 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*
E3	3 kg soil + 20 ppm Pb +*Pilea cadierei /Lantana camara*
E4	3 kg soil + 20 ppm Pb only.
E5	3 kg autoclaved soil +20 ppm Pb + 0.5%NaN_3
G1	3 kg soil + 10 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*
G2	3 kg soil + 10 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*
G3	3 kg soil + 10 ppm Pb +*Pilea cadierei /Lantana camara*
G4	3 kg soil + 10 ppm Pb only.
G5	3 kg autoclaved soil +10 ppm Pb + 0.5%NaN_3
C1	3 kg soil + 40 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*
C2	3 kg soil + 40 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*
C3	3 kg soil + 40 ppm Cr +*Pilea cadierei /Lantana camara*
C4	3 kg soil + 40 ppm Cr only.
C5	3 kg autoclaved soil +40 ppm Cr + 0.5%NaN_3

Continuation of Table 3.4

D1	3 kg soil + 20 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*
D2	3 kg soil + 20 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*
D3	3 kg soil + 20 ppm Cr +*Pilea cadierei /Lantana camara*
D4	3 kg soil + 20 ppm Cr only.
D5	3 kg autoclaved soil +20 ppm Cr + 0.5%NaN_3
F1	3 kg soil + 10 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*
F2	3 kg soil + 10 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*
F3	3 kg soil + 10 ppm Cr +*Pilea cadierei /Lantana camara*
F4	3 kg soil + 10 ppm Cr only.
F5	3 kg autoclaved soil +10 ppm Cr + 0.5%NaN_3

Treatments	**Co-contamination Treatments**
H1	3 kg soil + 20 ppm (Pb+ Cr) + 6%EFB+ *Pilea cadierei /Lantana camara*
H2	3 kg soil + 20 ppm (Pb + Cr) + 6%SMC+ *Pilea cadierei /Lantana camara*
H3	3 kg soil + 20 ppm (Pb+ Cr) + *Pilea cadierei /Lantana camara*
H4	3 kg soil + 20 ppm (Pb+ Cr) only.
H5	3kg autoclaved soil +20 ppm (Pb+ Cr) + 0.5%NaN_3
Z	3 kg soil without metals + *Pilea cadierei /Lantana camara*

Note: 1ppm = 1 mg/kg

3.5.3 Determination of the residual soil and plant heavy metal contents

The total metal concentration in soil and plant tissue samples was determined by ICP-OES analysis following the methods of Doumett et al., (2008), after acid digestion using microwave system (Ethos-1 Milestone). Plants were harvested after 98 days and thoroughly washed first with flowing tap water, and later with distilled water. Plants parts were separated into roots and shoots, and dried at 70ºC for 2 days and the dry weight was analysed. 0.4 g of plant samples were ground and treated with 2 ml of 30% H_2O_2 and 7 ml of 65% HNH_3. Whereas soil samples (0.4 g) were treated with 3 ml of hydrogen peroxide, 3 ml of 65% nitric acid, and 9 ml of hydrochloric acid (HCl). Following the acid digestion, both samples were topped up to 50 ml with distilled water, filtered on 0.2 µm pore size of polytetrafluoroethylene filters and then analysed for metal concentration using ICP (USEPA 3050B). The percentage phytoreduction efficiency (PE) of heavy metals in contaminated soil was calculated as follows;

$$\%PE = \left[\frac{Co-Ct}{Co}\right] X\ 100 \qquad \text{(Eq. 3.2)}$$

where C_o (mg/kg), and C_t (mg/kg) are initial and determined concentration of metals at each withdrawal time in the contaminated soil, respectively.

The percentage net -loss in phytoremediation, percentage phytoextraction difference, and biotic and abiotic loss in metals, at specific treatments are represented below:

Net % loss = % loss in Metal $_{\textbf{(plant with ow.)}}$ - % loss in Metal $_{\textbf{(control)}}$. (Eq. 3.3)

% Net phytoextraction diff. = %Metal $_{\textbf{(plant with ow)}}$ - %Metal $_{\textbf{(plant without ow)}}$ (Eq. 3.4)

Net % biotic loss = %loss in Metal $_{\textbf{(soil only)}}$ - % loss in Metal $_{\textbf{(autoclave soil)}}$ (Eq. 3.5)

Note: **ow. =** organic waste.

3.5.4 Measurement of pH, isolation and identification of heavy metal resistant bacteria

At every sampling date and/or period (14 days), replicates (*n*=3) soil samples were scooped from the rhizospheric region, including the control treatments, and enumerated for pH, using (HANNA HI 8424) pH meter on 1:2.5 (w/v) soil/distilled water after 40 minutes equilibration time.

Further 1.0 g of soil from each replicate (*n*=3) of metal contaminated soil (rhizosphere soil) and controls, were enumerated for both aerobic heterotrophic bacteria (AHB), and heavy metal resistant bacteria (HMRB), using spread plate inoculation of 0.1 ml of serially diluted sample on nutrient agar (NA). However, HMRB were specifically determined by the addition of 0.5 mM of $PbCl_2$, and $CrCl_3$ on nutrient agar for counts of Pb and Cr resistant bacteria. To inhibit fungal growth, the media was supplemented with 5.0 g fungicidin (USP, Amresco, USA) per litre after autoclaving. Plates were incubated at 30 ºC for 7 days. Colonies which appeared on the plates, were counted and expressed as colony forming units per gram of soil (cfu/g). The bacterial isolates were characterized based on culture parameters, microscopic techniques (gram staining reaction), biochemical and/or identification with IF-A (inoculation fluid) Biolog® Microstation system method USA. After sub-culturing three times pure strains were obtained (Ruan et al., 2005). Pure cultures were thereafter preserved in 50 ml agar slant bottles with large surface areas for subsequent reuse.

Using inoculation fluid (IF), inoculums of each target cell was prepared using Protocols A (IF-A catalog no. 72401) and B (IF-B catalog no. 72403) at a turbidity range of 95 to 98%. This was done using a cotton-tipped inoculator swab (catalog no. 3321) to pick up about 3 mm diameter area of the pure cell growth from the surface of agar plate, and eventually dipping it into the desired IF. To ensure uniform suspension, any cell clumps were carefully crushed against the tube wall. Ensuing cell suspension was poured into multichannel pipette reservoir, a 8- channel pipettor was used to dispense 100 µL of the suspension into each of the wells in the MicroPlate (catalog no. 1030).The wells contained 71 carbon source utilization assays (Columns 1-9), and 23 chemical sensitivity assays (Columns 10-12), hence they can be identified at the species levels based on the "Phenotypic Fingerprint" of microorganisms provided by the test panel. These Micro-Plates were placed in Omnilog reader wherein they were read using Biolog's Microbial Identification System software, and the identified microbes were recorded. The isolate/identified microbes were formulated for the bioaugmentation process required to revalidate their potential in the remediation of heavy metal contaminated soil.

3.5.5 Gas Chromatography-MS analysis of soil root exudates

Soil was sampled from the rhizosphere region, for qualitative assessment of possible plant exudates, and metabolites that enhanced organic ligand formation which aided in phytoremediation processes. Soil samples (20 g) were dried in a fume hood for 24 h and subsequently extracted by sonication with acetone-water solution (4:1 v/v) in a 200 ml glass beaker for 20 min and stirred at 105 rad s^{-1} for extraction (Belmonte et al., 2005). Following the methods of Abdulra'uf et al., (2013), the analytes were then filtered using a 0.45 µm Whatman® filter paper, and 1.5 ml of the extract was transferred into 2 autosampler amber-glass vials for gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analysis was carried out using a Shimadzu (Tokyo, Japan) QP2010 GC-MS system equipped with a Shimadzu AOC-20i auto sampler and a DB-5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) (J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas at a flow rate of 1.8 ml/min. The GC conditions were as follows: initial oven temperature 80°C for 2 min, increased to 300°C at a rate of 10 °C/min, then held at 300 °C for 2 min. Meanwhile, the injector temperature at splitless mode was 280°C.

3.6 Modelling of Translocation Factor (TF) and Bioaccumulation Factor (BAF)

The uptake and transfer of heavy metals from the bulk contaminated soil to aboveground plant tissues is mostly expressed using the translocation and bioaccumulation factors, and more details are found in Hough et al., (2003) and Maxted et al., (2007). These are mathematically defined as follows;

$$\text{TF}_{\text{heavy metal}} = \frac{\text{Total Conc. of heavy metal in the harvested plant shoot}}{\text{Total Conc. of heavy metal on the harvested plant root}} \qquad \text{(Eq. 3.6)}$$

where the total conc. of heavy metals in both harvested shoot and root parts are in mg/kg. Whereas;

$$\text{BAF}_{\text{heavy metal}} = \frac{\text{Total Conc. of heavy metal on the harvested plant}}{\text{Total Conc. of heavy metal in the bulk contaminated soil}} \qquad \text{(Eq. 3.7)}$$

where the total conc. of heavy metal on both the harvested plant and bulk contaminated soil are in mg/kg. Ashraf et al., (2012) indicated that BAF value above 1 shows plant’s potential for phytoextraction.

3.7 Kinetic studies for heavy metal removal/uptake rate and half-life

The data for metal removal by *Lantana camara* and *Pilea cadierei* from the polluted soil were fitted into the first-order kinetics model to determine the rate of Pb and Cr removal, is shown below:

$$[C]_t = [C]_o \, . \, e^{-kt} \qquad \text{(Eq. 3.8)}$$

where $[C]_t$ is the concentration (mg/kg) of the residual metals at specific time (t) and $[C]_o$ is the initial metal concentration (mg/kg). A plot of ln $[C_t/ C_o]$ versus time, yields a straight line with slope k (day^{-1}), expressed as the phytoextraction rate constant and Half-life ($t_{1/2}$) = ln 2/k, was also derived.

3.7.1 Thermodynamics, Isotherms, and Intraparticle diffusion studies

The adsorption dynamics of Pb and Cr by *Lantana* and *Pilea* for both supplemented and non-supplemented treatments were examined to fit into the experimental data. Hence, the following models were used for study validation and interpretations, as follows;

***Freundlich model*:** This isotherm model, is an empirical equation assuming that the metal adsorption process takes place on heterogeneous surfaces, and the adsorption capacity is related to the concentration of the metal at equilibrium (Adediran et al., 2009; Abdolali et al., 2016). This isotherm model is defined by the equation;

$$\text{Log } q_e = \left(\frac{1}{n}\right) \text{Log } C_e + \text{Log } K \qquad \text{(Eq. 3.9)}$$

where q_e is the metal adsorbed on the biosorbents (mg/kg); C_e is the final concentration of Pb and Cr (mg/kg) in the contaminated soil; n and K are the empirical constant that provides an indication of the adsorption capacity and intensity of biosorbents, respectively.

Standard free energy: This is the energy available in a substance to do work. This refers to the 'transformation work' involved in chemical reactions (Kitatsuji et al., 2011). The standard free energy for metal adsorption (ΔG^o) (kJ/mol/K) during phytoremediation was evaluated and expressed as;

$$\Delta G^o = - RT \ln Kc \qquad \text{(Eq. 3.10)}$$

where R is the universal gas constant (8.314 KJ/mol/K), T is the temperature in Kelvin and Kc is the equilibrium constant expressed as;

$$Kc = \frac{qe}{Ce} \quad \text{(Eq. 3.11)}$$

hence q_e is the amount of heavy metal adsorbed (mg/kg) and Ce is the final concentration in the soil (mg/kg).

Intra-particle diffusion mechanism: During phytoremoval of Pb and Cr, solute transfer were characterized by external mass transfer (boundary layer diffusion) and/or intra-particle diffusion or both. The mechanism for identifying adsorption process is by fitting an intraparticle diffusion plot. Sakar et al., (2003) expressed this as thus;

$$q_t = K_{int} .t^{1/2} + Ci \quad \text{(Eq. 3.12)}$$

where q_t is the amount adsorbed at time (t) in (mg/kg), K_{int} is the intra-particle diffusion rate constant (mg/kg/day $^{0.5}$) and Ci is the intra-particle diffusion coefficient constant (mg/kg). The plot of qt versus $t^{0.5}$ at different initial and adsorbed metal concentration gives the value of K_{int} and this may indicate a multi-linearity, which shows two or more steps occurring in the metal removal processes.

3.7.2 Mass balance of heavy metal uptake and schematic flow using STAN

The mass balance of the metal was computed in this study based on Equation 3.13. Hence, phytoremediation data were input into STAN software as depictive flow, to enhance understanding of the complexity surrounding the metal decontamination. However, it does not absolutely represent the entire mechanism of the remediation process, rather acts as an insight to knowledge.

Mass of metal in (ppm) = Mass of metal out (ppm) (Eq. 3.13)

where mass in (ppm) = total amount of metal present in soil prior to phytoremediation, and mass out (ppm) = rSM + BL + nBL + rPU + E (Eq. 3.13.1)

therefore defining designates above: rSM = residual soil stock metal; BL = biological loss; nBL= non-biological loss; rPU = residual plant uptake; and E = phytovolatilization, respectively.

3.8 Post harvest enumeration of phytoremediation plant sections and dry weight

The biomass was determined by measurements of the root and shoot lengths spatial dimensions, through destructive sampling using a 30 cm calibrated ruler with accuracy of 0.5 cm, following the procedures of Sala et al., (2015). Hence, dry weight (grams), changes over specific treatments describing the overall performance of the study plants under its immediate environment (Wuyts et al., 2015), was determined as follows;

Dry weight = (B-A)-(C-A) (g) (Eq. 3.14)

Fresh/wet weight = (B-A) (g) (Eq. 3.15)

where,

A= weight of Aluminum foil cup (g)

B= weight of Aluminum foil cup + fresh plant sample (g)

C= weight of Aluminum foil + dried plant sample (g)

3.8.1 Scanning Electron Micrograph of remediated soil and plant tissue stain

The root and shoot samples from the harvested plants (*Lantana* and *Pilea*) and controls, including metal soils were collected after the experimental duration. Stem and root samples of 5 mm length were excised from 2 cm above and 2 cm below the stem-root intersection using ultra-microtome (Leica, CM 1950), and prepared for (i) light microscopy (LM); i.e, alcohol dehydrated, paraffin embedded, ultramicrotomed and subjected to safranin (0.1%) - fastgreen (0.2%) staining for further observation and immediate fixation in formaldehyde acetic acid, and (ii) scanning electron microscopy (SEM); i.e., fixed in 2.5% glutaraldehyde, and in 0.05M potassium phosphate buffer (pH 7.1) for 8 hours and then dehydrated in ethanol series, and observed with (SEM, JSM-840A-JEOL, USA). Soil samples were set on a standard aluminium slide with carbon adhesive, calibrated with coated layers of carbon. All protocols and/or procedural methods were drawn from Sridhar et al., (2007); Shivakumar et al., (2012); Arenas-Lago et al., (2014) and Mayanna et al., (2015).

3.9 Bioaugmentation with metal contaminated soil isolates for strain revalidation

This section technically tends to bioaugment heavy metal (Pb and Cr) contaminated soil with the primary-site-persistent-microbes (bacteria only). Bioaugmentation of contaminated soil with desired microbial strains is one of the methods adopted for tackling heavy metal menace in the environment. Where some techniques were adopted and modified from previous studies, a number of procedures were designed according to researcher's discretion. Sequel to this microbial study became a routine component of this study.

Previously isolated heavy metal persistent-soil bacteria from phytoremediation set-up of this study, identified with Biolog GEN III MicroPlate™ protocols were used. The cells were freshly regrown (16-24 hours), in order to avoid lose of viability and metabolic vigor which is typical of most organisms at stationary phase.

Microbial formulation used in this bioaugmentation experiments were composed of 3 strains isolated from Pb and/or Cr phytoremediated contaminated soil. Each strain was grown as a pure culture in nutrient agar (NA) plates at 33°C for 2 days before being inoculated in nutrient broth E and grown to stationary phase in a rotating incubation shaker at 29 °C and 150 rpm. Discrete suspensions at same physiological phase (1.3 ABS at 600nm) were then pooled in equal proportions to set-up inoculums for bioaugmentation.

To set-up bioaugmentation treatments, autoclaved (1 kg) soil was contaminated according to the ASTM guidelines: known heavy metal concentration (20 ppm) of Pb and/or Cr was dispersed into the each 1 kg soil (v/w). This concentration was chosen to minimize severe toxicity on the isolates, since no external supporting nutrient source shall be added to the soil-substrate to boost bacterial metabolism. Therefore, six microcosms in triplicates were set-up under different conditions together with their controls as presented in **Table 3.5**. However, bioaugmentation with microbial formulation was performed 14 days after soil contamination (Sprocati et al., 2011), hence marked the start of the bioremediation experiments until 90 days duration.

Table 3.5: Bioaugmentation experimental set-up.

Treatments	Treatment details
T_1	1 kg autoclaved soil + 20 ppm Pb + 40 mL bacteria strain A
T_2	1 kg autoclaved soil + 20 ppm Pb + 40 mL bacteria strain A + B
T_0	1 kg autoclaved soil + 20 ppm Pb + zero bacteria strain
Z_1	1 kg autoclaved soil + 20 ppm Cr + 40 mL bacteria strain C
Z_2	1 kg autoclaved soil + 20 ppm Cr + 40 mL bacteria strain C + B
Z_0	1 kg autoclaved soil + 20 ppm Cr + zero bacteria strain

T_1, **T_2**, **Z_1**, and **Z_2** were hydrated with 100 ml of inoculums (obtained from equal volumes of pooled discrete strains) each containing about 3.2 x 10^8 cfu/g. Soil moisture content was maintained by regular watering with distilled water. Precautions were applied to avoid excess watering, since leaching was not required in the experiment in order to avoid indiscriminate metal loss. Portion of the microcosm were scooped every 30 days (until 90 days) for metal analysis, using acid digestion with 0.5 g of soil sample, HNO_3 and H_2O_2 was added before using microwave system (Ethos-1 Milestone).The elemental concentration were measured using ICP-OES. The data obtained were processed to calculate the percentage of heavy metal removal from each treatment, and the first-order kinetics rate of metal removal, by following similar equations used in Eq. 3.2, and Eq. 3.8 respectively.

3.10 Statistical analysis

Phytoremediation descriptive statistics (mean, standard error, and graphs) were performed with Microsoft Excel, while the effects and/or test of each factor against the other in the experimental treatments were done using analysis of variance (ANOVA), from SPSS version 21. If ANOVA results were significant at α = 0.05 (95% confidence level), Duncan test comparison was used to determine the difference among treatments.

This Chapter 3 has provided insights into the wide range of experimental methods and/or approach applied to execute this phytoremediation study. However, the next (Chapter 4) shall present the respective experimental outcomes in relation to the models applied, in synchronization with results and discussion.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Physicochemical properties of soil and organic waste additives

The physicochemical features of the soil and agro-industrial (organic) wastes utilized in the investigation of phytoremediation of Pb and/or Cr contaminated soil is presented in **Table 4.1**. The soil (Mollisol) had innate pH of 6.13, supposedly considered to be slightly acidic, with abject concentration of N (0.6%) compared with EFB (1.3%), and SMC (0.9%). This could be as a result of its edaphic specialization, as also subscribed by Schirrmann et al., (2011). However, Juwarkar et al., (2008) had previously reported pH 8.5 for another type of natural soil, implicating weathering as a co-factor. Progressing in the same direction, the pH of SMC was slightly acidic as well. The reason behind this may not be unconnected to fungi growth, i.e., mushroom, which appreciably proliferates in acidic environment, and therefore the initial substrate of SMC might slightly be acidic in nature (Grujić et al., 2015).

In continuation, between the two organic wastes used, EFB had elevated concentration of N; which is a limiting nutrient (Okoh, 2006), and SMC had higher P; which is also an essential element. While the soil-substrate used for remediation had C: N ratio of 16. However, Gavrilescu et al., (2010) indicted such C: N ratio to be low for effective bioremediation of recalcitrant polluted soil. Therefore, the addition of biowaste became imperative as an alternative source of nutrient-aid, to enhance soil-niche ecophysiology. Furthermore, Gavrilescu et al., (2010) argued that C: N: P=100:1:1 ratio, as the optimum value of nutrient quotient required for pollutant removal, and also to enhance microbial growth in compromised soils. Conversely, elevated rate of hydrocarbon degradation was recorded by Röling et al., (2002) in soil contaminated with oil after amended with 2.5 g/kg of N, which amounted to C: N ratio of 300, signifying the role of nutrient-ratio balancing in soil remediation.

Table 4.1: Physicochemical composition of soil (**Mollisol**) and organic wastes used.

		Organic wastes supplements	
Parameters	Soil	EFB	SMC
pH	6.13±0.51	6.21±0.02	5.57±0.35
Nitrogen (%)	0.61±0.1	1.3±0.04	0.9±0.8
Phosphorus (%)	17.7±1.2	14.2±0.2	19.02±1.3
Organic C (%)	9.5±0.7	26.02±0.1	12.7±1.5
Moisture (%)	11.8±0.4 (dry wt.)	57.1±1.05	61.03±2.11
C: N	15.57±1.2	20.02±1.5	14.1±2.02
CEC (meq/100g)	10.2	11.6	ND
Sand (%)	34.8±3.1	ND	ND
Silt (%)	17.7±1.23	ND	ND
Clay (%)	39.20±2.1	ND	ND
Texture	Clayey	ND	ND
Pb (mg/kg)	>0.06	ND	ND
Cr (mg/kg)	>0.01	ND	ND

EFB: Empty fruit bunch, SMC: Spent mushroom compost, ND= Not Detected.

The moisture contents of EFB and SMC were recorded at 57% and 63%, as opposed to 12% for soil, respectively. However, Abioye et al., (2010) reported moisture contents of 62% for SMC and 72% for brewery spent grain (BSG), which they attributed to have harbored some important microorganisms and/or biomolecules that imparted positively to the remediation of anthropogenic pollutants in soil. Therefore, their opinion may not elude this phytoremediation study; because all the utilized wastes mentioned above, are biowastes irrespective of the source, and may be comparatively related and hence intended for similar purpose. Soil textural analysis showed clay (39.2%), sand (34.8%), and silt (17.7%), thus classifying the parent materials as Mollisol (Bremmer & Genrich, 1990).

4.2 Results of phytoremediation studies using *Lantana camara* and *Pilea cadierei*

This section deals with the percentage reductions of Pb, Cr, and its dual-contamination (Pb+Cr) in spiked soils after the experimental period, and presented based on their sequence of concentration in conjunction with phytoremediation components applied. However, 80 to 10 ppm Pb and 40 to 10 ppm Cr concentrations were finally applied, because within the context of this study, 80 ppm Pb and 40 ppm Cr were the most tolerant limits for both floras.

4.2.1 Phytoreduction of 80 to 10 ppm Pb contaminated soil

Under *Pelia sp.* (plant), the concentration of 80 ppm Pb reduction in soil varied considerably across the pool. Therefore, at the end of the treatment period of 90 days, 80 ppm Pb contaminated soil recorded 44.83%, 42.60%, and 20.73% Pb reductions when amended with EFB, SMC, and *Pilea* alone treatments at [F: ($p<0.05$) = 2291.69], respectively. Thus, the ratio of Pb reduction manifested as 2.20: 2.10: 1.0 from the sequence above (**Figure 4.1)**. The high Pb reduction observed in the amended soil against the unamended, were probably propelled by biowaste application and phytoextraction activities (Bolan et al., 2014). Which enhanced Pb desorption, and promoted its phytoextraction and/or phytostabilization (Bolan et al., 2014; Rafati et al., 2011; Ali et al., 2013). However, CEC of 11.6 meq/100 g recorded in EFB may be another reason why Pb reduced more under EFB, than in SMC treatments. Whereas only 3.73% biotic and 1.3% abiotic (autoclaved soil with sodium azide) Pb reduction were recorded at the 80 ppm controls, and their correlation for biotic and abiotic loss is presented in **Figure 4.2** at $R^2 = 0.9076$ and 0.6656, with a reduction ratio of 2.87: 1, due to natural attenuation ($R^2 = 0.9072$). This was also observed by García-Delgado et al., (2015) in a bioremediation study of PAH and Pb multi-polluted soil with SMC, which they inferred as net biotic and abiotic impacts.

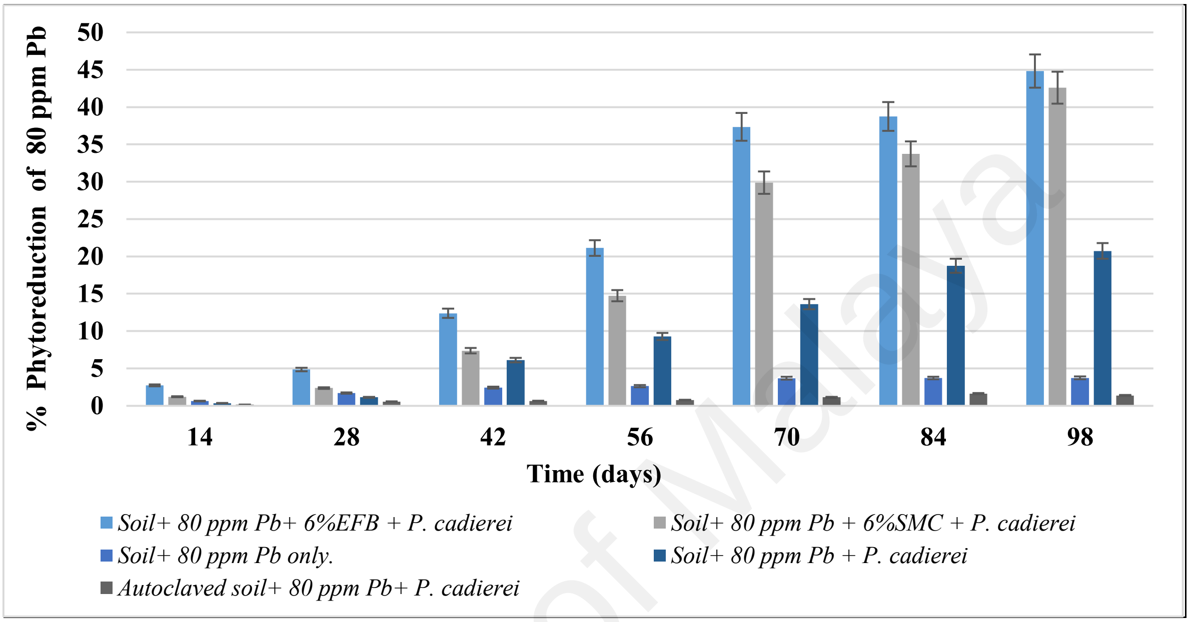


Figure 4.1: Percentage phytoreduction of Pb at 80 ppm Pb contaminated soil under *Pilea cadierei*.

Bars denotes standard error (**n**=3).

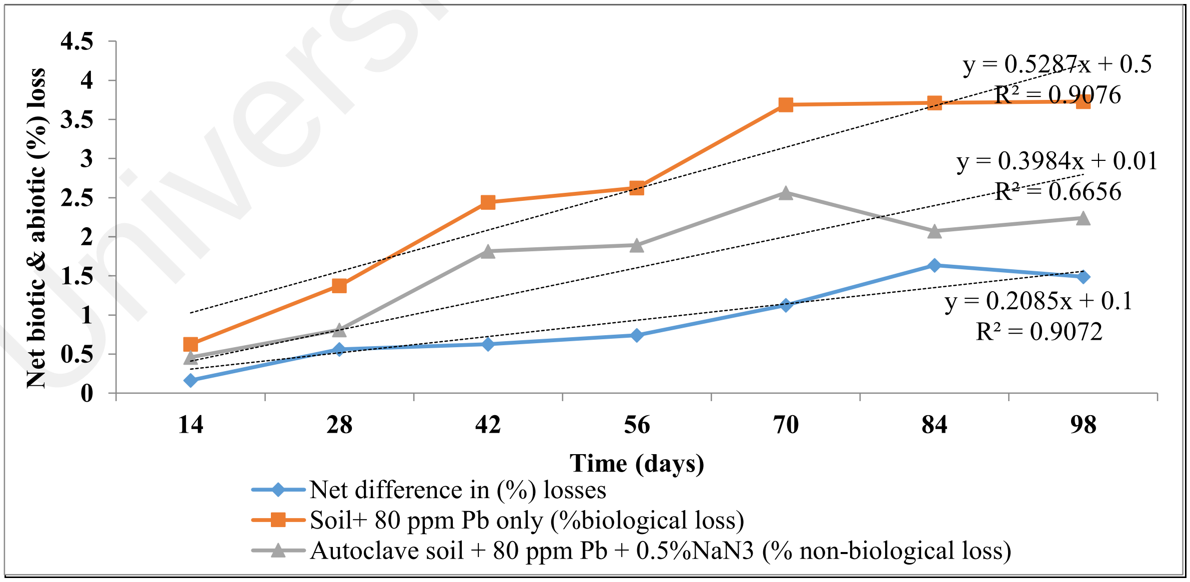


Figure 4.2: Correlation between biotic and abiotic losses with net (%) loss difference in 80 ppm Pb contaminated soil.

In another perspective of 80 ppm Pb reduction in soil under *Lantana sp.* (plant), its availability dropped by 52.46%, 45.10%, and 23.69% for EFB, SMC, and *Lantana sp.* control (unamended) with corresponding reduction ratios of 2.20: 1.9: 1.0, and significant levels of [F: (p<0.05) = 2291.69], respectively were recorded ensuing 98 days monitoring (**Figure 4.3)**. Contrary to this, Tang & Yang, (2012) recorded 89% and 86% reduced bioaccesible and leachable Pb via fixation in contaminated tailing substrate, using orthophosphate as amendments. This was because orthophosphates are inorganic compounds, and may have already assumed mobile phase, hence might react faster than biowastes i.e., EFB and SMC, but may not be ideal for a sustainable remediation option, due to environmental nutrient pollution, in case of uncontrolled escape onto non-target points.

Also, the reduction of Pb at this concentration was less when compared to 80% reduction recorded by Dadrasnia & Agamuthu, (2010) on diesel oil contaminated soil amended with other types of biowaste, suchlike tea leaves, potato skin, and soy cake. It is worthy to note, that Pb is hardly degradable because of its nature, and hence has higher binding affinity to the bulk-soil, unlike diesel oil that is degradable. However, the plants used, variation in pollutants, and also other experimental and/or environmental conditions, suchlike moisture, pH, etc., can propel contrasting results between Dadrasnia & Agamuthu, (2010) and the results of this study.

Meanwhile, EFB has shown greater Pb reduction potential against SMC at this particular concentration. This is unclear, but may not be unconnected to certain physico-chemical components (pH, CEC, total C, humification degree etc.) associated to EFB and their inherent native microbes, which was extensively argued elsewhere in Kavitha et al., (2013), as a probable advantage. However, N-variation in both biowastes (EFB and SMC) could be implicated as indicator, facilitating the discrete Pb reduction trend among them.

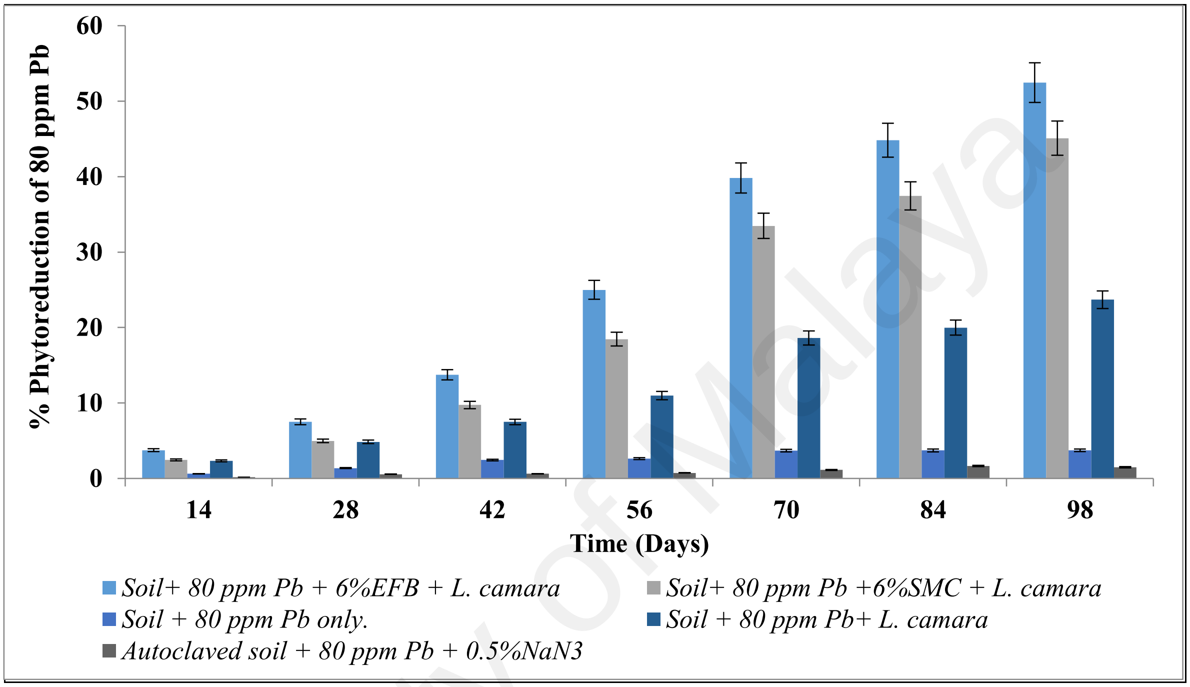


Figure 4.3: Percentage phytoreduction of Pb at 80 ppm Pb contaminated soil under *Lantana camara*.

Bars denotes standard error (**n**=3).

Further to this, treatments with agro-wastes (EFB and SMC) established optimum reduction of Pb against the unamended. Factors such as increased oxygen circulation, geared from the addition of biowaste, thus loosened the soil compacts, and fostered interstitial soil-movement of air; this was also implicated in Agamuthu et al., (2010). Hence, redox potential improved, and these inversely pushed Pb reduction in amended soils as opposed to the opposite treatments. On the contrary, the reason for the low Pb reduction at the first 28 days might be attributed to toxicity of Pb to both plant and microbes, acclimatization effects, Pb desorption from soil due to hysteresis and proton (H^+) inertia. In addition to soil been the most complex ecosystem where absolute equilibrium is rarely attained. However, this initial trend of low pollutant reduction, due to high concentration has been reported by different authors (Alaribe & Agamuthu, 2015; Chang et al., 2013; Titah et al., 2013 and Singh & Kalamdhad, 2012).

In both *Lantana sp.* and *Pilea sp.* applications at 80 ppm Pb contaminated soil, the reductions of Pb in soils where organic wastes (EFE or SMC) are affiliated to *Lantana sp.* recorded 55 to 45%, and surpassed *Pilea sp.* 45 to 43% at (p<005), under similar affiliation. This is probably because EFB and/or SMC pose more compatibility with *Lantana sp.* over *Pilea sp.*, thereby creating a better stabilization and/or complexation effects over the later. This is in agreement with Méndez et al., (2012), who reported different effects of sewage sludge and/or biochar amendments on Cu, Ni, Pb, and Zn reductions, after their application on a Mediterranean soil via phytoremediation. But interestingly, both plants (i.e., *Lantana sp.* and *Pilea sp.*) under biowaste devoid state, considerably reduced soil Pb availability of 80 ppm by the range of 24 to 21%. Therefore, the strength displayed by both plants at these instances, has profound *in-situ* bio-ecological value in searching for and/or identifying metallophytes as rightly upheld by Alkorta et al., (2004). **Appendix Tables 1 and 2** shows the detailed (%) reduction of 80 ppm Pb and its statistical significance (ANOVA) for both plants, at their respective soil treatments.

Nevertheless, in a separate 40 ppm Pb contaminated soil, under *Pilea sp.* and influenced with EFB and SMC, provoked Pb reductions of 48% and 45% in soil, while *Pilea sp.* (unamended control) recorded a decline in soil-Pb of about 21% at [F:(p<0.05) = 4677.750], respectively after 98 days. With 2.33: 2.17: 1.0 as the general reduction ratios, while 1.07: 1.0 is for specific Pb reduction at EFB: SMC respectively (**Figure 4.4**). These are clear indication of nutrient restriction; which may lead to system decomplexation, and retard plants and/or microbes abilities to metabolize Pb in the unamended soil. The difference between Pb reductions in EFB over SMC also points at appreciable N ratio, and the CEC activity of EFB, which was less seen in SMC. However, this concurred with the views of Lu et al., (2015), Brennan et al., (2014), and Puga et al., (2015) who deduced that coalesced phytoremediation with biochar (i.e., a type of organic biowaste) in soil, can raise soil biological activity and catalyze pollutant removal.

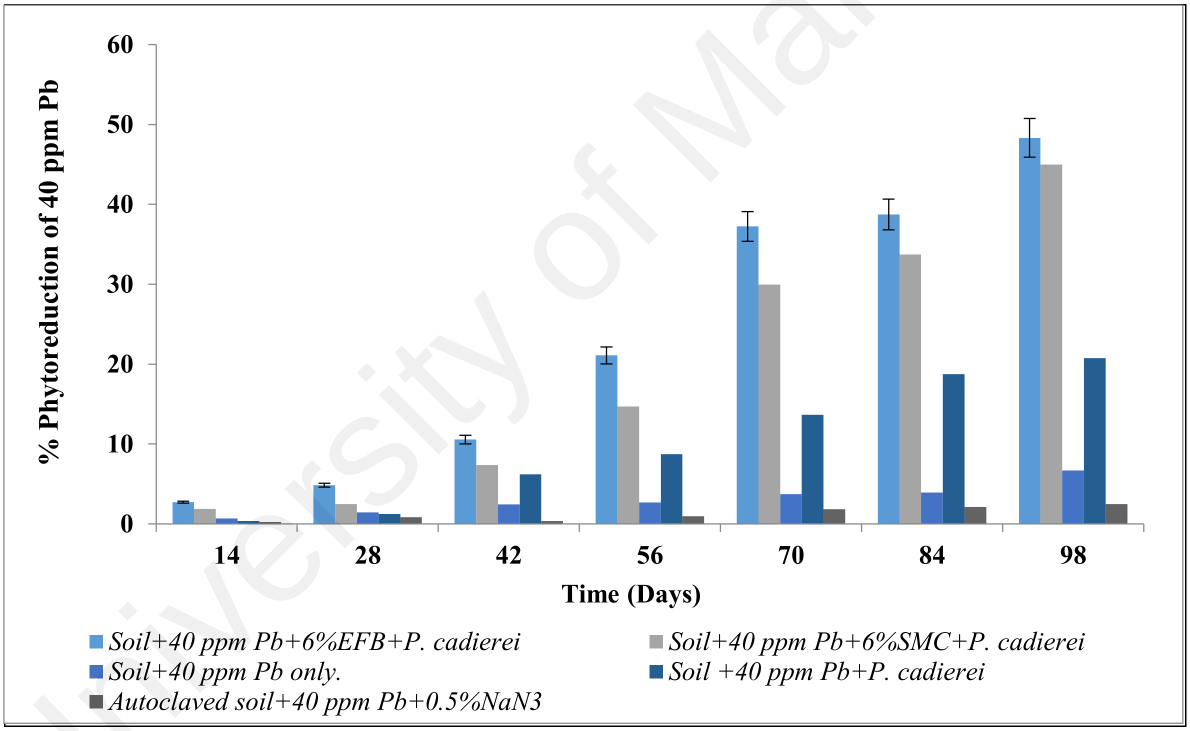


Figure 4.4: Percentage phytoreduction of Pb at 40 ppm Pb contaminated soil under *Pilea cadierei*.

Bars denotes standard error (**n**=3).

Unfortunately, same biochar application were opposed by Grossman et al., (2010) and Liang et al., (2010) because they believed it can alter the activities of beneficial soil microbes and natural biogeochemical cycles, as an unintended consequence. However, their arguments herein centered on biochar, but this present study utilized EFB and/or SMC devoid of pyrolysis. Otherwise, further investigations may be needed to substantiate the aftermath "post-phytoremediation" impacts of EFB and SMC ecologically, and compare it to biochar. Irrespective of the fact that EFB, SMC and biochar are all biomasses, they may possess certain degrees of variation. Decline in Pb availability of 6.70% for biotic and 2.49% for abiotic (autoclaved NaN_3) were recorded on 40 ppm Pb soil concentration at 2.69: 1.0 ratio (p<0.05). Hence, correlation R^2 = 0.8969, 0.456, and 0.8862 for biotic, abiotic, and their net-loss values, were respectively deduced (**Figure 4.5)**. The NaN_3 (sodium azide) used was to induce the death of microbes, purposely to differentiate losses arising from non-biological sources. This has been applied previously by a number of researchers (Szabados et al., 2004; Shahid et al., 2013), to monitor inorganic and/or organic pollutants net loss in soil remediation.

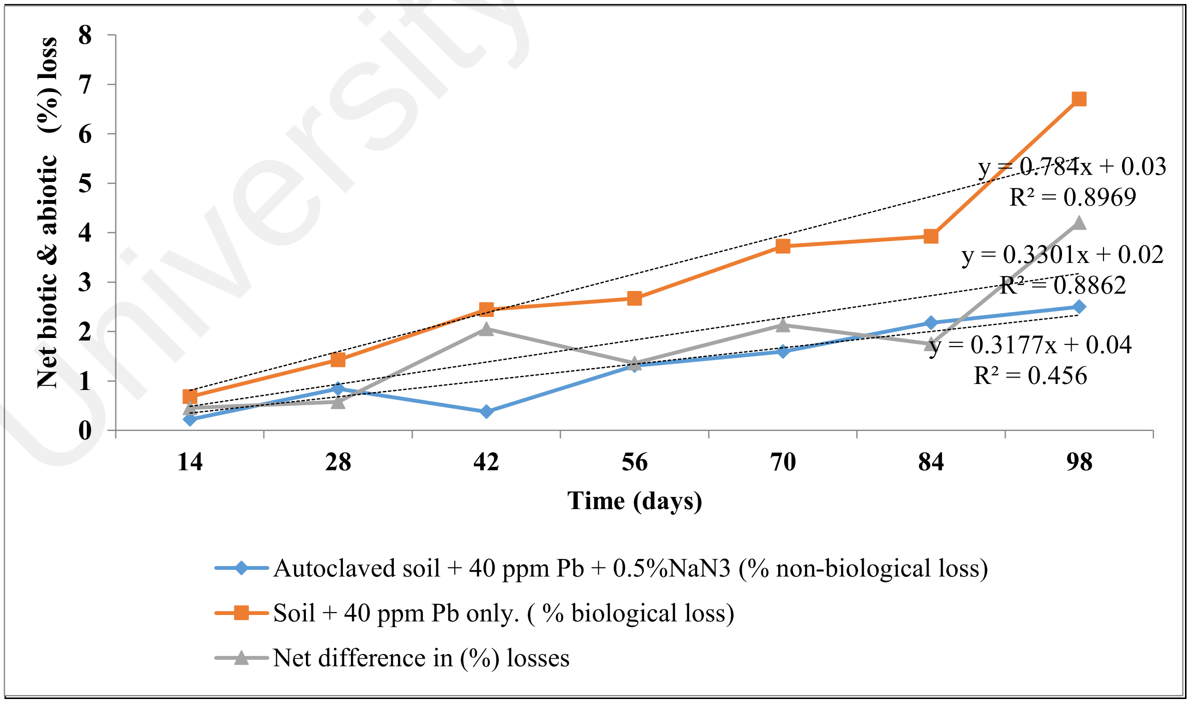


Figure 4.5: Correlation between biotic and abiotic losses with net (%) loss difference in 40 ppm Pb contaminated soil after 98 days.

For 40 ppm Pb reduction under *Lantana sp.*, significant decline of 57% (for EFB) was recorded over 47% (SMC) and 20% *Lantana sp.* (unamended) control, respectively. This occurred at inter-ratio of 2.84: 2.34: 1.0 between the amended (EFB and SMC) and unamended, after 98 days. While the amended has intra-ratio of 1.21:1 at F: (p<0.05) = 4677.750, respectively (**Figure 4.6)**. In extension to this trend, N-distribution in these substrates may have significantly imparted to the variations noticed in the reduction ratios of 40 ppm Pb in *Lantana sp.* series. Robinson et al., (2015) and Boominathan et al., (2004) reported that possibilities exist in metal contaminated soils, under similar metal concentration and plant application, but at separate enclosures, can experience fluctuation in metal reduction (i.e., variation), due to system niche modulation against the order. However, in as much as their opinion is in agreement with this result, with reference to Pb reduction in *Lantana sp.* soil, it is still unclear, and may require further investigation to unravel.

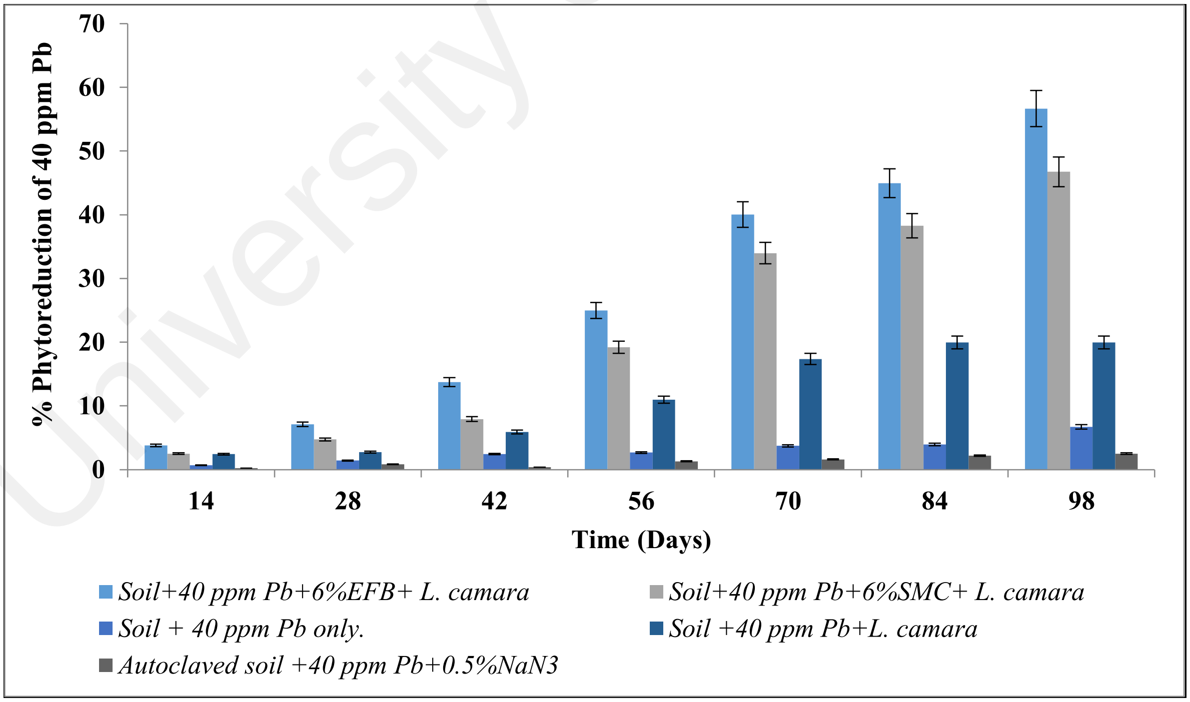


Figure 4.6: Percentage phytoreduction of Pb at 40 ppm Pb contaminated soil under *Lantana camara*

Bars denotes standard error (**n**=3).

However, spanning from *Lantana sp.* to *Pilea sp.*, the percentages of Pb reduced at 40 ppm contaminated soil for EFB ranged between 57 to 48%, and 47 to 45% for SMC, while the corresponding sequence at 80 ppm had 52 to 45% (EFB), and 45 to 43% (SMC), respectively. Pb and Zn reduction of 50% and 57% using biochar as soil amendment were also reported by Puga et al., (2015). This implied that, at lower concentration of Pb in soil, the biotas tolerance to sustain remediation 'biologically' was promoted (Wuana & Okieimen, 2011). Whereas, for (controls) unamended plant; Pb reduction from *Lantana sp.* to *Pilea sp.* ranged between 20 to 21% for 40 ppm, and correspondingly 24 to 21% for 80 ppm respectively. This may be due to the effects of concentration and/or entropy, or saturation impacts, where it is most likely that the rate of contact time between 80 ppm Pb molecules with the system components (such as plant root) may exceed that of 40 ppm Pb counterpart. In extension, this could also accelerate abiotic loss, giving rise to more percentage Pb reduction seen in the 80 ppm plants (control) soil. Surprisingly, this trend was different in Agamuthu et al., (2010), where *Jatropha* plant without organic amendments at 2.5% and 1% oil-polluted soil, recorded 57% and 68% soil oil reductions from the original concentration after 180 days, respectively. This apparent disparity may be from the oil, *Jatropha* itself and/or soil characteristics during remediation. But in this current study, Pb was used, and also another type of plant species, hence different experimental conditions. However, some authors (Ji et al., 2011; Sui et al., 2011; Sekhar et al., 2005; Lorenc-Plucińsk et al., 2013; Bauddh et al., 2015 and Bech et al., 2016), have reported such "related" trends in polluted-soils (i.e., with heavy metals), and attributed it to direct and/or inverse function of concentration phenomenon.

Appendix Tables 3 and 4 shows detailed (%) reduction of 40 ppm Pb and statistical significance (ANOVA) for both plants, at their respective soil treatments.

At a lower concentration, that is for 20 ppm Pb under *Pilea sp.*, the total Pb present in soil dropped by 49%, 45%, and 30% for EFB, SMC, and plant (unamended) control treatments respectively, after 98 days phytoremediation. Due to Pb plant accumulation, alongside with biotic/ abiotic loss, the amended treatments (EFB: SMC) reduction ratio was 1.09:1.0, while the overall reduction ratio had 1.64: 1.50:1.0, based on the indicated sequence above at F: ($p<0.05$) = 11692.259, significant level. This is presented in **Figure 4.7**.

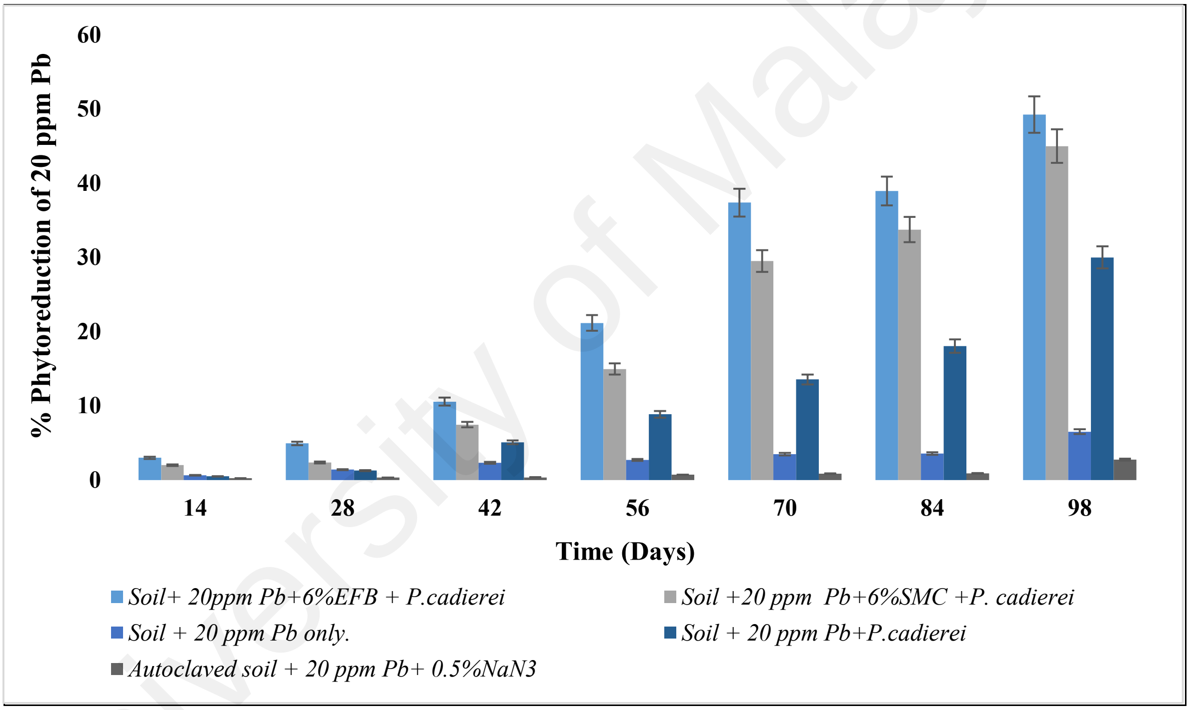


Figure 4.7: Percentage phytoreduction of Pb at 20 ppm Pb contaminated soil under *Pilea cadierei*.

Bars denotes standard error (**n**=3).

Parallel to this, 6.50% and 2.75% reductions were recorded for both biotic and abiotic (autoclaved soil with sodium azide) controls, respectively. Hence, ratios of 2.27:1 were recorded at $R^2 = 0.8725$, 0.5991, and 0.909 for biotic, abiotic, and their net loss (**Figure 4.8**).

Therefore, impingements from elevated N, and the presence of CEC in EFB may be responsible for its higher Pb reduction over SMC in phyto-applied 20 ppm Pb concentrations.

Extended Pb reduction in SMC beyond the plant (unamended) control, further implicated N to be the fixing factor. Chiu et al., (2009) observed such type of reduction from Cr, after the addition of crop residues to soil, and indicated that appreciable N in the residue, was among the metabolic drivers, indexing such effect. But they did not reveal in detail at what initial concentration was Cr prior to residue inception, to enable adequate correlation with this result.

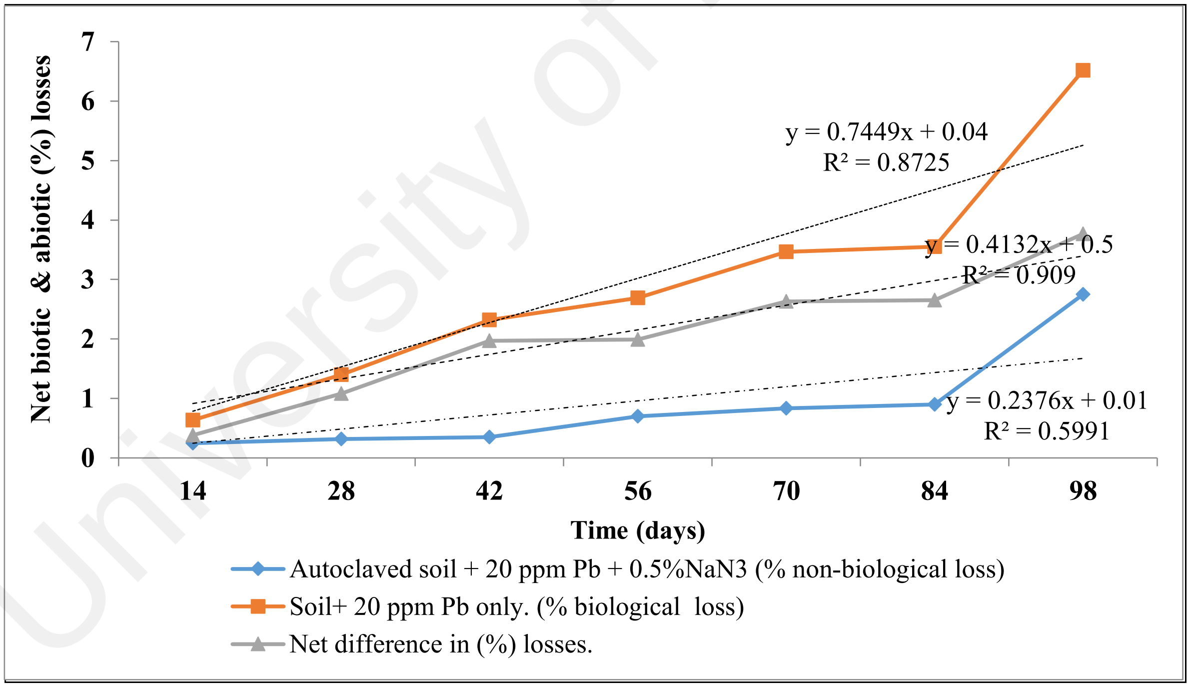


Figure 4.8: Correlation between biotic and abiotic losses with net (%) loss difference in 20 ppm Pb contaminated soil after 98 days.

Also, from the results of 20 ppm Pb polluted soil under *Lantana sp.*, it was observed that soil amended with organic wastes recorded higher percentage reductions of Pb at 57 % (EFB), 48% (SMC), and 33% (unamended) control, respectively. With corresponding reduction ratios of 1.73: 1.47: 1, while the specific reduction ratio for the amended is 1.18: 1.0 (i.e., EFB: SMC) (**Figure 4.9**). Statistical analysis showed significant difference at F: (p<0.05) = 11692.259, between the amended and unamended contaminated soil treatments, but generally EFB was most significant. Also see **Appendix Tables 5** and **6**.

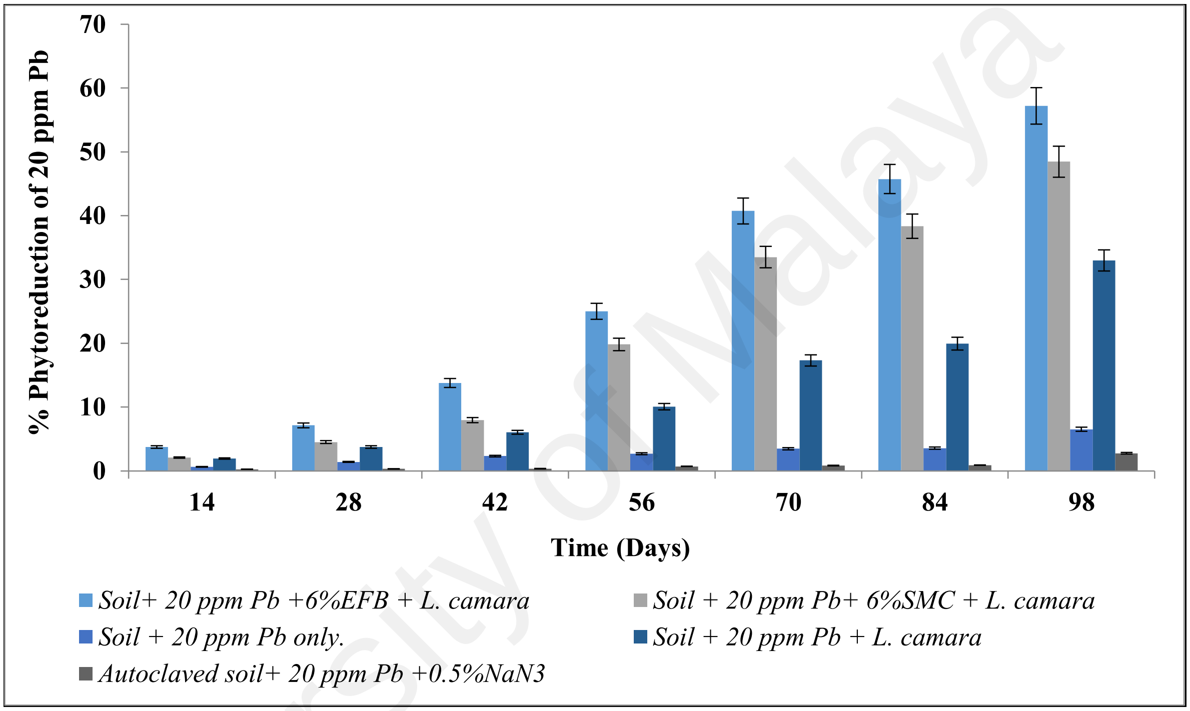


Figure 4.9: Percentage phytoreduction of Pb at 20 ppm Pb contaminated soil under *Lantana camara*

Bars denotes standard error (**n**=3).

Thus, proving the positive contribution of organic wastes to the reduction and/or removal of Pb in the soil. The reason for the observed results might be due to the difference in the nutrient capacities of the two organic wastes in stimulating the indigenous microorganisms, especially EFB. This is in contrast to the findings of Chaineau et al., (2005), who reported that low nutrient addition to crude oil contaminated soil recorded 15% increase in hydrocarbon reduction by microorganisms in contaminated soil, compared to the treatments with elevated nutrient amendments. The reason for the difference in this result and that of Chaineau et al., (2005) might be due to difference in the nutrient amendments, and the respective pollutants in question. The agro-waste utilized in this study, might not had posed toxicity to the soil microorganisms, while Chaineau et al., (2005) used mixtures of different inorganic salts, suspected to have had some degree of toxicity on the soil microbes.

This finding is supported by the reports of Lau et al., (2003) who stated that the incorporation of organic waste, such as spent mushroom compost to oil contaminated soil, reduces toxicity of the oil to the soil biota through buffering effects. This argument is in proportion to EFB and/or SMC induced imparts on Pb contaminated soil during phytoremediation.

Furthermore, this result is in partial agreement with Zhou et al., (2014) who recorded heavy metal reduction ranging from 31 to 59% (Pb), 11 to 45% (Cd), 8 to 25% (Cu), and 6 to 47% (Zn) when combined amendments (hyroxyhistidine + zeolite and limestone + sepiolite) were applied on heavy metals contaminated paddy soil. This might be justified, because more interactive mechanisms maybe needed to combat synergistic soil heavy-metals pollution. Contrastingly, Zhou et al., (2014) used joint-inorganic amendments on multiple heavy metal polluted soil, while this section of result under discuss adopted only 20 ppm Pb, and summarily recorded reduction range of 45 to 57% (amended), and it was purely agro-biowaste, unlike Zhou et al., (2014).

Generally, the percentages of Pb reduction recorded in 20 ppm contaminated soil from *Lantana sp.* to *Pilea sp.* at 57 to 49% (EFB), 48 to 45% (SMC), and 33 to 30% for plant (unamended) control, exceeded their corresponding 40 ppm Pb counterparts of *Lantana sp.*

to *Pilea sp.*, which recorded 57 to 48 % (EFB), 47 to 45% (SMC), and 20 to 21% for plant control, respectively. This is indicative, that the lesser the amount of Pb in soil, the higher the percentages and/or chances of Pb reduction and/or removal via phytoremediation. This argument is supported by similar trend that existed between 40 and 80 ppm Pb contaminated soils, at the preceding sections. These findings, however concurred with Sing & Kalamdhad, (2012) who recorded different reduction percentages of Zn, Cu, Cu, Mn, Ni, Pb, and Fe at different decreasing concentrations of metals, when sawdust and cattle manure were used in composting remediation of heavy metals, under water hyacinth plant study. However, it may not be uncommon that the pH level in *Lantana sp.* soils has been slightly higher in acidity than in *Pilea*. This might be contributing to the elevated Pb reduction in *Lantana sp.* over *Pilea sp.* soils, irrespective of the biowaste and Pb concentration involved. Therefore, on a balance of probabilities, acidic exudates from the roots of *Lantana sp.* i.e., phytosiderophores and/or bacteria siderophores might have facilitated desorption of Pb ions from soil components, than in *Pilea sp.*, thus aiding phytoextraction, and subsequent drop in soil total Pb availability. This is in agreement with Ullah et al., (2015).

Finally, the percentage reductions of total Pb in 10 ppm contaminated soil recorded significant results after 98 days. *Pilea sp.* was observed to have reduced soil Pb by 84%, 80%, and 48% for EFB, SMC, and *Pilea sp.* (unamended) applications, respectively. Notwithstanding, comparative reduction ratios of 1.74: 1.65: 1 were serially noticed. However, 1.06:1.0 Pb reduction ratio were seen at EFB: SMC, at overall phytoremediation statistical representation of F: (p<0.05) = 12048.408. These underscores organic waste impacts, in actuating and/or minification of Pb in polluted soil during phytoremediation with *Pilea sp.* plant. View **Figure 4. 10**. This significant reduction recorded may be attributed to the impacts of N, deemed to be the limiting factor, and the low Pb amount in soil. However, plant immobilization of Pb in soil through roots sorption, precipitation and complexation, in addition to Pb valence reduction in the rhizosphere may be a contributing factor. This notion was subscribed by numerous authors, suchlike Wuana & Okieiman (2011); Ali et al., (2013); Gosh & Sing (2005); Yoon et al., (2006) and Tao et al., (2015), whereas, the reduction of Pb in soil due to biotic and abiotic loss recorded 12% and 6%. With Pb reduction ratio and net loss correlation of 2.11: 1 and R^2 = 0.9255, 0.9060, and 0.9240 (**Figure 4.11**). More significant Pb reduction were recorded at the biotic set-up, this may have been influenced by the activities of microorganisms and/or biological tolerance. This was also posited by Shahid et al., (2013).

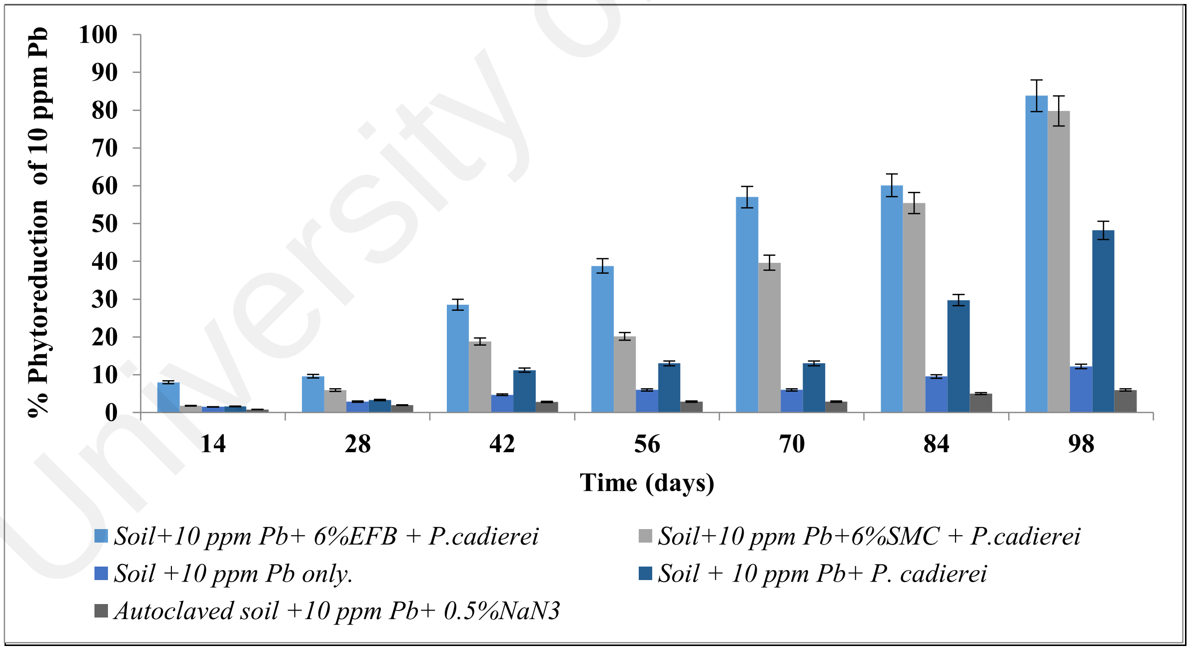


Figure 4.10: Percentage phytoreduction of Pb at 10 ppm Pb contaminated soil under *Pilea cadierei*.

Bars denotes standard error (**n**=3).

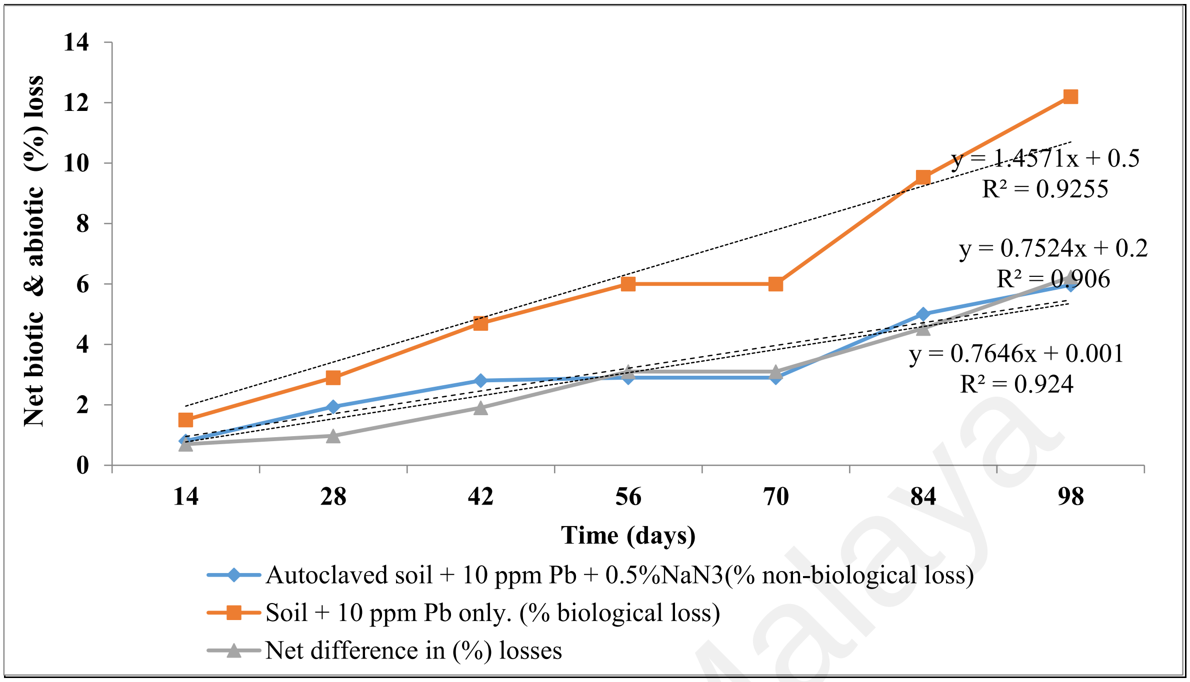


Figure 4.11: Correlation between biotic and abiotic losses with net (%) loss difference in 10 ppm Pb contaminated soil after 98 days.

In continuation, the reduction of Pb in soil under *Lantana sp.* at 10 ppm Pb contaminated soil at similar duration (98 days), recorded significant decrease. Therefore, EFB, SMC, and plant (unamended) control, claimed 89%, 83%, and 58% Pb soil reductions, respectively. **Figure 4.12** shows statistically significant difference at F: (p<0.05) = 12048.411. These consistent potentials of organic wastes, in de-escalation of Pb accumulation and/or availability in soil, indicated that both agro-wastes could jointly be used as nutrient source for the plants (*Lantana sp.* or *Pilea sp.*), and for the stabilization of soil structural properties in metal contaminated and/or degraded soils. This concurred with Alaribe & Agamuthu, (2015), and also in agreement with Juwarkar et al., (2008) who used biosludge as amendment with *Jatropha* in heavy metal contaminated soil. Conversely, Cui et al., (2011) recorded 40% reduction of Cd in a dose of 40 t ha^{-1} amended with wheat-straw biochar, in high Cd contaminated soil. More so, Fellet et al., (2014) reported Pb reduction in soil from initial 80 mg/kg to final 51 mg/kg, after 3% biochar of orchard- waste was applied to Pb polluted soil during phytoremediation. The difference between this result with Cui et al., (2011) and Fellet et al., (2014) may be due to the different amendment strata and composition applied, soil characteristics in alliance with the extent of metal contamination, together with different climatic locations. Therefore, one could conclude that N can be implicated as the limiting factor, since both the interspecific and intraspecific Pb reduction ratios in the compared soils, decreased based on the magnitude of N-availability at the respective soil-biowaste substrates.

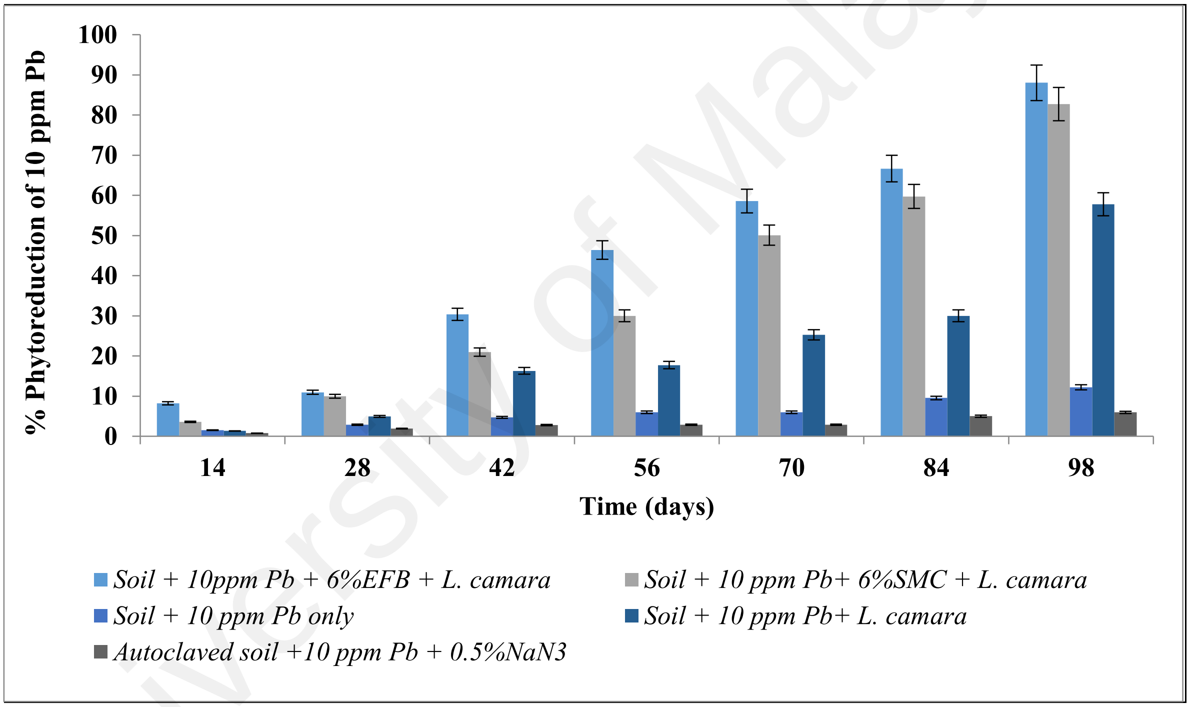


Figure 4.12: Percentage phytoreduction of Pb at 10 ppm Pb contaminated soil under *Lantana camara.*

Bars denotes standard error (**n**=3).

However, Guillamot et al., (2014) argued that substrate-induced respiration, i.e., CO_2 released when biowastes are added to metal polluted soil, can be partly regained by plant for its metabolism and/or photosynthetic activities, which may in-turn boost (plant-root-apoplast) heavy metal soil uptake strength, and subsequent decrease in soil metal load. Guillamot et al.,

(2014) view is in agreement with this result, despite them referring to Sb, while Pb or Cr are the focus metals herein (**Appendix Tables 7** and **8)**.

Summarily, the overall order of Pb reduction percentages in Pb contaminated soil for both plants and their respective applied biowaste, at various Pb-concentrations are given as thus; for *Pilea sp.* (EFB): 10 ppm Pb (84%) > 20 ppm Pb (49%) > 40 ppm Pb (48%) > 80 ppm Pb (45%), while their corresponding reductions for *Pilea sp.* (SMC), and *Pilea sp.* (control) orders were (80%) > (45%) > (45%) > (43%), and (48%) > (30%) > (21%) > (21%) at (p<0.05) respectively. In similar order, the reduction percentages of Pb in soil remediated with *Lantana sp.* are as follows; *Lantana sp.* (EFB): 10 ppm Pb (88.83%) > 20 ppm Pb (57%) > 40 ppm Pb (57%) > 80 ppm Pb (58%), while its equating *Lantana sp.* (SMC): had (84%) > (49%) > (47%) > (45%), and differently for *Lantana sp.* (control): 10 ppm Pb (58%) > 20 ppm Pb (33%) > 80 ppm Pb (24%) > 40 ppm Pb (20%) at (p< 0.05).

These results sequence, partially reflected to that of Bradshaw (1987), who reported that the extent of Pb reduced in soils; are mainly governed by the degree of metal concentration in the contaminated soil, in conjunction with the soil amendments applied, and the phytoremediating plant tolerance ability. Sequel to this, high percentage reduction recorded in EFB amended soil, might be due to its ability in contributing to increased oxygen diffusion, and mineral nutrients availability. This was previously observed by different authors (Piehler et al., 1999; Elektorowicz, 1994), as some of the characteristics seen in agro-waste residues. Hence, contributed to the carbon source quality, and act like a mechanical support surface for bacterial adsorption to the complexed Pb in soil. However, EFB like other organic wastes possibly improved the soil physicochemical characteristics to speed up microbial adaptation in heavy metal contaminated soil, and improve mineralization by indigenous microbial population (Jorgensen et al., 2000).

4.2.2 Phytoreduction of 40 to 10 ppm Cr contaminated soil

Figure 4.13 shows the level of Cr reduction, in 40 ppm Cr contaminated soil under *Pilea sp.*, and amended with organic wastes (EFB or SMC), together with control, within 98 days of phytoremediation watch. The results, recorded 48%, 38%, and 23% Cr-reductions from EFB, SMC, and plants (control) respectively. With corresponding net ratios of 2.08: 1.66: 1.0, while the amended (i.e., EFB: SMC) recorded extrapolated ratio of 1.26:1.0, and statistically significant at F: ($p<0.05$) = 283.841. The deviation in the result, might be due to differences in the composition of EFB and SMC, which was added to the *Pilea sp.* during phytoremediation study.

Also, it is unclear if (freshly) Cr contaminated soil has preference for one biowaste over the other. Agamuthu et al., (2010) recorded such disparity on BSG over SMC (organic wastes), supplemented in oil-polluted soil using *Jatropha* plant. Hence, indicating that passive selectivity for biowastes may have occurred independently between the pollutant (Cr), and the two amenders, inside the polluted soil complex, while undergoing supplemented phytoremediation.

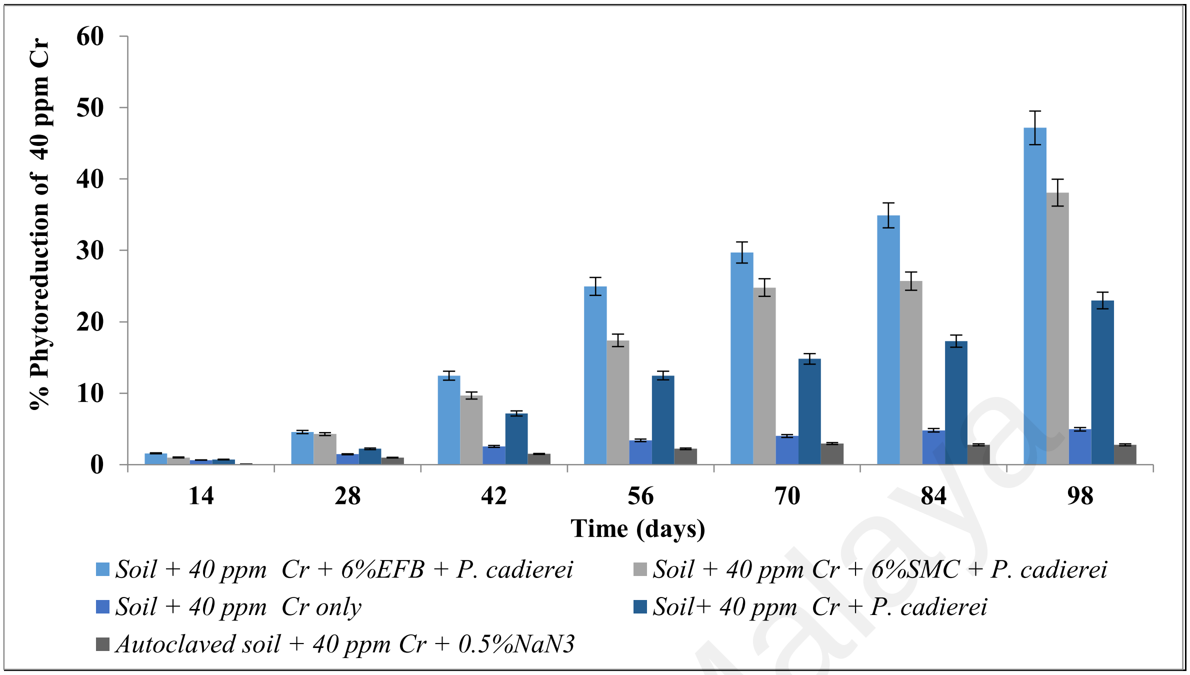


Figure 4.13: Percentage phytoreduction of Cr in 40 ppm Cr contaminated soil under *Pilea cadierei.*

Bars denotes standard error (**n**=3).

However, the potency of the unamended *Pilea sp.* control, to reduce Cr load in soil, may be purely attributed to phytoextraction mechanisms (Wu et al., 2010). Although, Bolan et al., (2014) reported that Cr could be reduced in the presence of organic matters posing as electron acceptors in soils. This assertion from Bolan et al., (2014) implicated discharges from EFB and SMC in facilitating Cr reduction in the contaminated soil. Experimental monitoring revealed lesser Cr reduction in soil between 14 to 28 days, this was probably due to initial Cr toxicity, militating against the general edaphic biota, before the inception of secondary acclimatization. Hence, limiting quorum sensing build-up for Cr tolerant soil-microbes, which supposedly should and/or indirectly participated at Cr-mineralization in the polluted soil at those given periods. This concurred with Bias et al., (2006) who stated that dense microbial population in recalcitrant polluted soils, facilitates pollutant reduction in soil via rhizodegradation. In synchronization, Wu et al., (2010) stated that microbes have a larger specific surface area in soil, hence more efficient to activate the removal of heavy metals.

Results obtained from 40 ppm Cr remediated soil, under *Lantana sp.* at 98 days phytoremediation period followed a similar reduction trend, with respect to the organic wastes applied. In contrast to the results observed in *Pilea sp.*, higher Cr-reduction of 53%, 47%, and 28% were recorded for EFB, SMC, and *Lantana sp.* (unamended) soils, respectively. This can be attributed to the following reasons, namely: the impacts of nutrient factor arising from soil supplementation, influence of soil pH and/or the production of organic acids, and the bioaccumulation potentials of *Lantana sp.*, which coordinated resistibly to push-down soil Cr-load at significant statistics of F:(p<0.05)= 283.841.This is shown in **Figure 4.14**. However, Sullivian et al., (2012) also implicated these variables stated above, as inclusive in firing range polluted soil remediation, while investigating Pb solubilization.

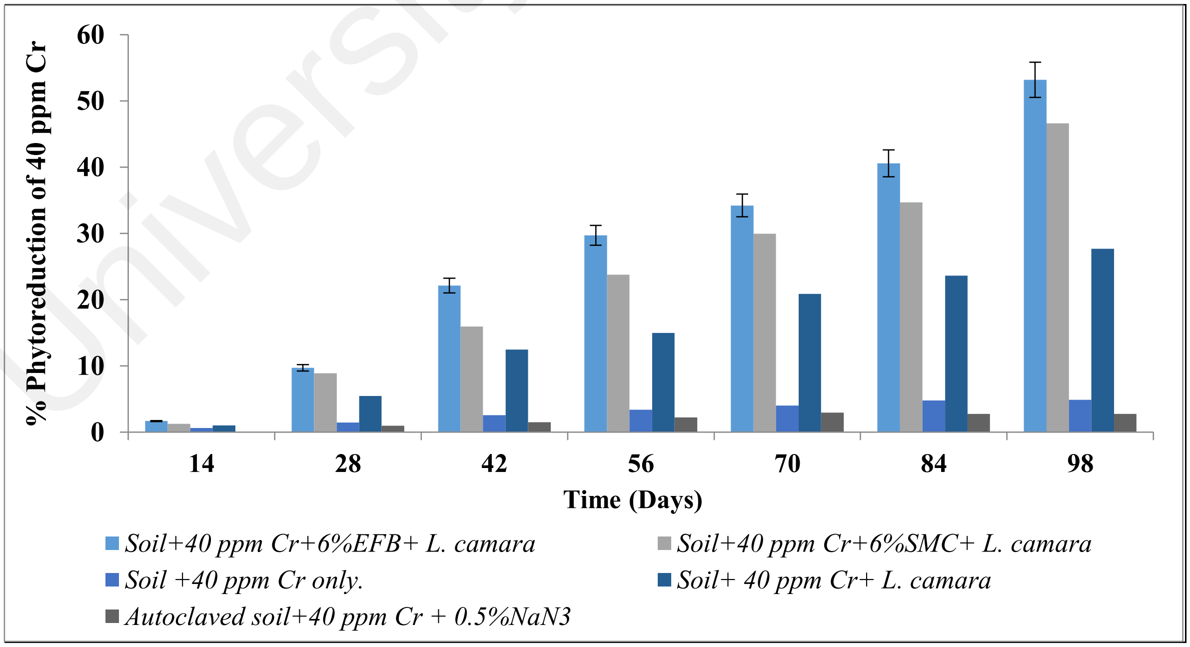


Figure 4.14: Percentage phytoreduction of Cr in 40 ppm Cr contaminated soil under *Lantana camara.*

Bars denotes standard error (**n**=3).

In continuation, it is unclear if Sullivian et al., (2012) applied or did not apply biowaste to polluted soils during remediation, which may have contrasted with this study results. Furthermore, the overall Cr-reduction ratio recorded was 1.93: 1.69: 1.0 for EFB, SMC, and *Lantana sp.* (control) respectively, at 40 ppm Cr remediating soil. Therefore, EFB showed empirical Cr-reduction ratio of 1.14:1.0 over SMC ameliorated soils, hence CEC and N potential may most likely be responsible in conferring this edge to the EFB (Purwandari et al., 2013; Cotana et al., 2014).

Contrastingly, amidst 14 to 28 days, more Cr-reduction ranging from 2 to 10% (EFB) and 1 to 9% (SMC) were recorded under *Lantana sp.*, than 2 to 5% (EFB) and 1 to 4% (SMC) observed in *Pilea sp.* counterpart. Equally, *Lantana sp.* (control) demonstrated 1 to 5% Cr-diminution over 2% seen in *Pilea sp.* (control) at the above mentioned intervals. Another possible explanation to this, is perhaps *Lantana sp.* has more initial (i.e., accelerated) secondary acclimatization over *Pilea sp.* at onset, thoughtfully due to its apoplastic physiology in Cr decontamination, and evolved mechanisms to protect itself from negative Cr stress. In extension, improved Cr-bound to-soil desorption and/or stabilization by *Lantana sp.* over *Pilea sp.* may not be indispensable, (**Appendix Tables 9 and 10)**.

Subsequently, the distribution of biotic and abiotic Cr-loss under 40 ppm Cr recorded 5% and 3%, respectively in 98 days, with corresponding correlation impact R^2= 0.9723; 0.8647, along with the net difference deduced R^2= 0.8607, as indicated in **Figure 4.15**. Robinson et al., (2015) reported that the reduction of Cr (IV) to Cr (III) is mediated through both biotic and abiotic processes in soil. Hence, the findings of Robinson et al., (2015) are in partial agreement with this result due to the involvement of Cr.

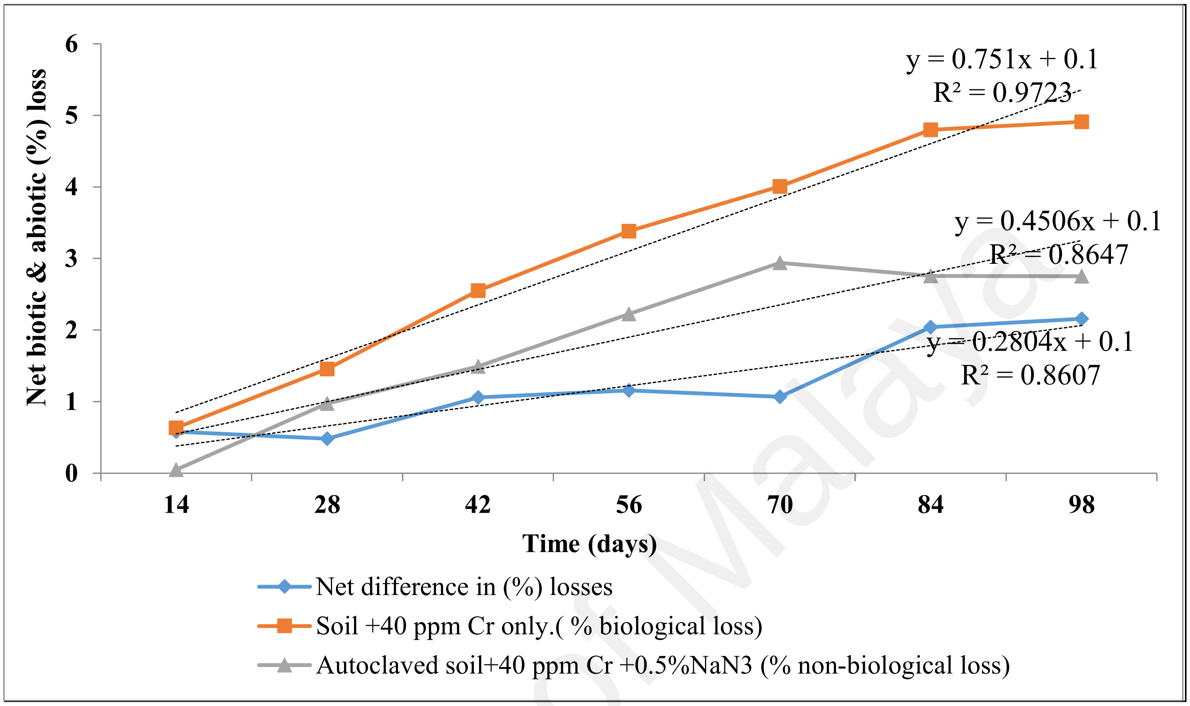


Figure 4.15: Correlation between biotic and abiotic losses with net (%) loss difference in 40 ppm Cr contaminated soil after 98 days.

Furthermore, it may be concluded from this result that both biotic and abiotic losses, contributed to marginal Cr-reduction in the amended-polluted soils. Thereby, supporting Ravichandran, (2004), Lambertsson & Nilsson, (2006), and Bolan et al., (2014) who previously upheld that biomethylation and/or abiotic methylation of heavy metals/metalloids do occur in soils and sediments, through organic matters acting as methyl donors during polluted soil remediation.

In another concentration perspective, the reduction of Cr, in 20 ppm Cr contaminated soil under *Pilea sp.* showed 48%, 39%, and 28% Cr-disappearance from soil after 98 days, at corresponding EFB, SMC, and *Pilea sp.*(control) phytoremediation spots, respectively (**Figure 4.16**). These consistent trends of EFB toppling SMC in soil Pb or Cr reduction has obviously indicted EFB to be non-invasive, and/or sustainable organic complex, hence capable of sustaining soil heavy metal phytoremediation drive (Alaribe & Agamuthu, 2015). At same time, not ignoring to applaud the demonstrated capacities of SMC towards Cr soil reduction in this study. Mathematically, 1.70: 1.38:1.0 empirical ratio for 20 ppm Cr fading in soil between EFB, SMC, and *Pilea sp.* control, were respectively recorded. While 1.23:1.0 for Cr-reduction was empiric for EFB: SMC, with high statistical significant of F: ($p <0.05 = 987.163$].

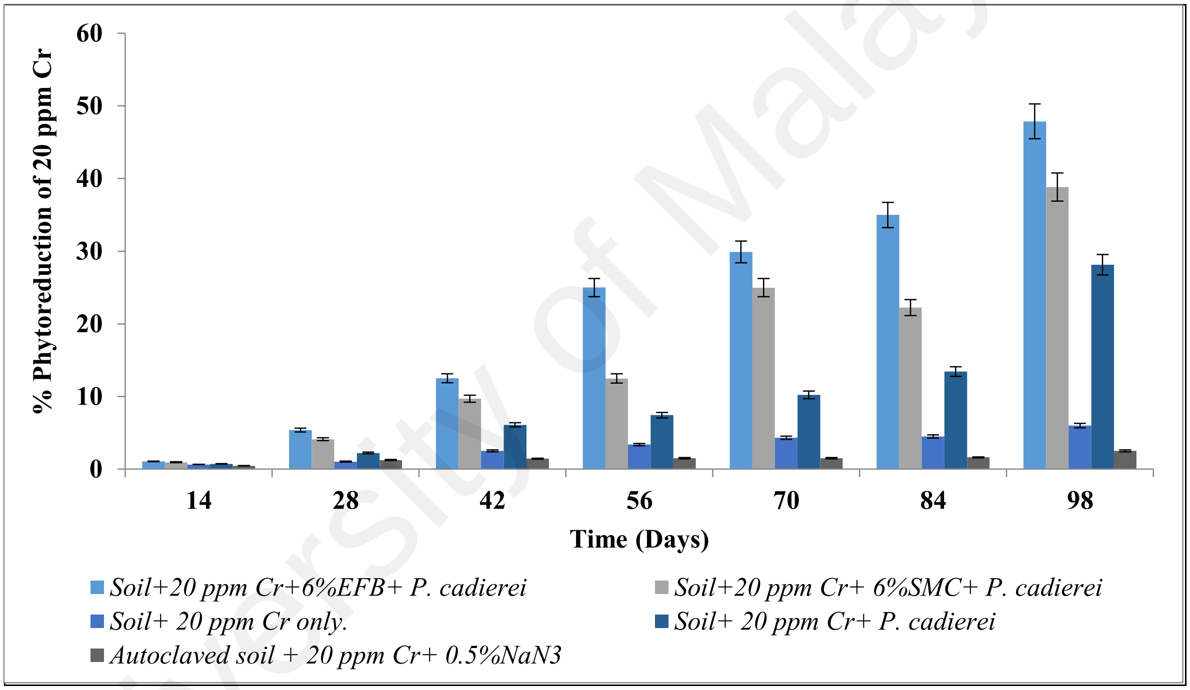


Figure 4.16: Percentage phytoreduction of Cr in 20 ppm Cr contaminated soil under *Pilea cadierei.*

Bars denotes standard error (**n**=3).

However, the unamended 20 ppm Cr soil showed less metal reduction over amended, this may be connected to nutrient deficiency and/or decomplexation factor present in the control, due to devoid biowaste supplementation. Farrell & Jones, (2010) investigated into numerous effects of different composts on the remediation of metal-contaminated soil; and categorically stated that composts decreased the level of heavy metals in soil medium. Thus, concurred with the findings of this study, especially with the spent mushroom compost (SMC). Further to this, Pérez et al., (2007) surprisingly argued that compost application increased soil pH, and reduced solubility of heavy metals. Thus, this partially contrasted with this study observation, because most pH recorded here (details in **Section 4.4**), was slightly acidic (i.e., a decreased pH range). These arising discrepancies may also be attributed to the nature of the composts materials Pérez et al., (2007) applied on the metal polluted soil in question. Add to the soil physicochemical features and/or soil metal speciation, which was unelaborated by the above author.

For the *Lantana sp.* plant, also under 20 ppm Cr concentration in soil, registered 55%, 49%, and 30% Cr- withering at EFB, SMC, and *Lantana sp.* (without supplementation) at the end of 98 days. Unarguably, nutrient gradients from organic wastes (i.e., N and P availability), may have propagated the difference seen in Cr-attenuation across the soil treatments, due to improved C: N ratio. This phenomena was also observed and reported by Schaefer & Juliane, (2007). The tallying ratio and associated agro-waste/treatment at which Cr was reduced in soil are 1.86 (EFB): 1.66 (SMC): 1.0 (*Lantana sp.* unamended) respectively, while the ratio of 1.12:1.0 for EFB: SMC represents specific Cr reduction difference under amended soils. At high significant level of F: (p <0.05) = 987.163 (**Figure 4.17)**, and extensively elaborated in **Appendix Tables 11** and **12**. Meanwhile, Beesley et al., (2010) remarked that compost may possess significant soluble P component which can displace heavy metal at soil binding sites, an effect also found in organic acids. However, the unaided reduction of Cr recorded in *Lantana sp.* (control) soil, acclaimed *Lantana sp.* to be a hypothetical metallophyte (Alkorta et al., 2004). Therefore, EFB and SMC could be regarded as emitters of organic chelators, which may bind to Cr ions in soil and render them uncharged (Wu et al., 2010).

Meanwhile, Wu et al., (2010) stated that zero-charged metals has high mobility, and easier to get through to root cellular membranes, hence this phenomena probably facilitated Cr soil decontamination as indicated in this study. However, drawing inference from Wu et al., (2010), one could still assert that EFB probably possessed more chelating prowess over SMC, thus its higher Cr reduction ratio observed when amended to *Lantana sp.* from this study findings.

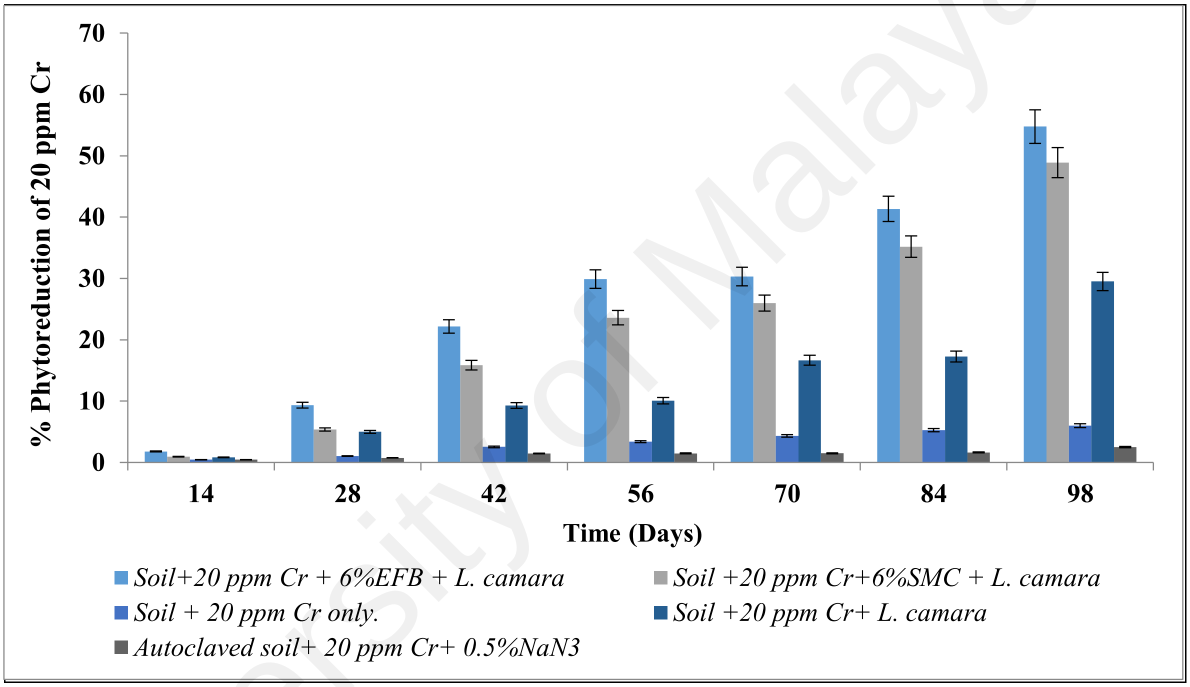


Figure 4.17: Percentage phytoreduction of Cr in 20 ppm Cr contaminated soil under *Lantana camara*.

Bars denotes standard error (**n**=3).

Progressing further, Pover et al., (2014) and Pover & Spinu, (2015) expressed that materials incorporated into metal contaminated soils, invariably speedup optimization process, leading to soil remediation, through immobilization and mediated by precipitation, complexation, and redox reactions. Therefore, this substantiated the role of EFB and SMC used in this study, as inducer bio-materials to optimize soil metal phytoremediation (Park et al., 2011a).

In continuation, **Figure 4.18** shows the correlation between the biotic and abiotic Cr loss in 20 ppm Cr soil concentration. However, 2.88% net biotic loss, arising from 4.5% (biotic loss) and 1.62% (abiotic loss) from volatilization etc., were observed respectively at $R^2 = 0.8836$; 0.9741; 0.6500. These in otherwords, measured the natural attenuation of 20 ppm Cr concentration in soil (Pover & Spinu, 2015). Although, no clear statistical significance were recorded here between Cr biological and non-biological loss reduction at ($p<0.05$). Therefore, the possible explanation to this may be that soil native-nutrient profile, was inadequate to sustain biological life (i.e., bacteria) under metal stressed condition, this may lead to less biological loss of Cr. Hence, leaving behind much residual Cr, stocked inside the soil, that is perhaps, in direct proportion to the Cr quantity adsorbed to the soil under the autoclaved Na-azide poisoned control. Thus, causing none significant reduction effect between the both Cr controls. This has also been implicated in Li et al., (2016) who investigated metal kinetics and desorption in Zn and/or Cd contaminated soils.

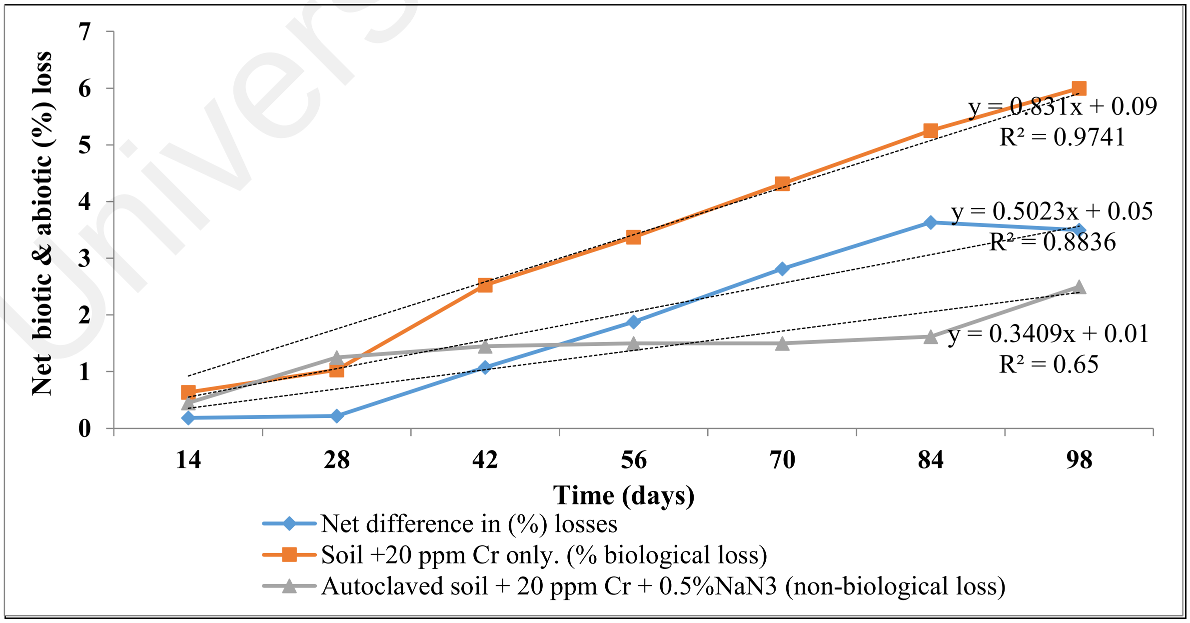


Figure 4.18: Correlation between biotic and abiotic losses with net (%) loss difference in 20 ppm Cr contaminated soil after 98 days.

However, there was a sharp contrast to the findings of Palmroth et al., (2002) who reported 70% diesel oil loss within 28 days of study in Na-azide poisoned contaminated soil. The differences in these results, might be because poisoned control in this study was autoclaved soil, mixed with 0.5% sodium azide and heavy metal (Cr or Pb)- which is non-biodegradable. Whereas Palmroth et al., (2002) used only 0.5% sodium azide, on diesel oil soil- which is biodegradable, without autoclaving the soil. Therefore, sodium azide possibly could not completely sterilize the diesel soil, hence oil loss was through indigenous microbial activities. More so, the quantity of soil investigated by Palmroth et al., (2002) was unknown, which may vary, and all put together might be insightful differential factors.

In next concentration of 10 ppm Cr polluted soil, during the 98 days phytoremediation experiment, using *Pilea sp.* plant; treatments amended with EFB, SMC, and *Pilea* (unamended) removed 72%, 67%, and 45% of soil Cr load, respectively. This recorded the highest percentage of Cr reduction, as opposed to 40 and 20 ppm Cr soils remediation (earlier discussed); with respect to *Pilea sp*. Probably, because of the low metal concentration in this particular treatment, which posed less toxic impacts on the soil biota, and thereafter enhanced the available soil Cr mineralization. Sing & Kalamdhad, (2012) and Meera & Agamuthu, (2011) had previously observed differential concentration impacts of this nature, in a hydroponic metal remediation system.

Sequel to this, ratios of 1.62 (EFB):1.51(SMC): 1.0 (*Pilea* control) were recorded for Cr reductions, and 1.07 is to 1.0 was specific for EFB to SMC compared ratio, respectively. Notwithstanding the ratio recorded by *Pilea sp.* control, it still demonstrated and/or substantiated itself to be a bioindicator plant in Cr impoverished soil. This result was investigated to be significant at F: ($p<0.05$) =294.755. More details are given in **Figure 4.19**, and expanded in **Appendix Tables 13** and **14**.

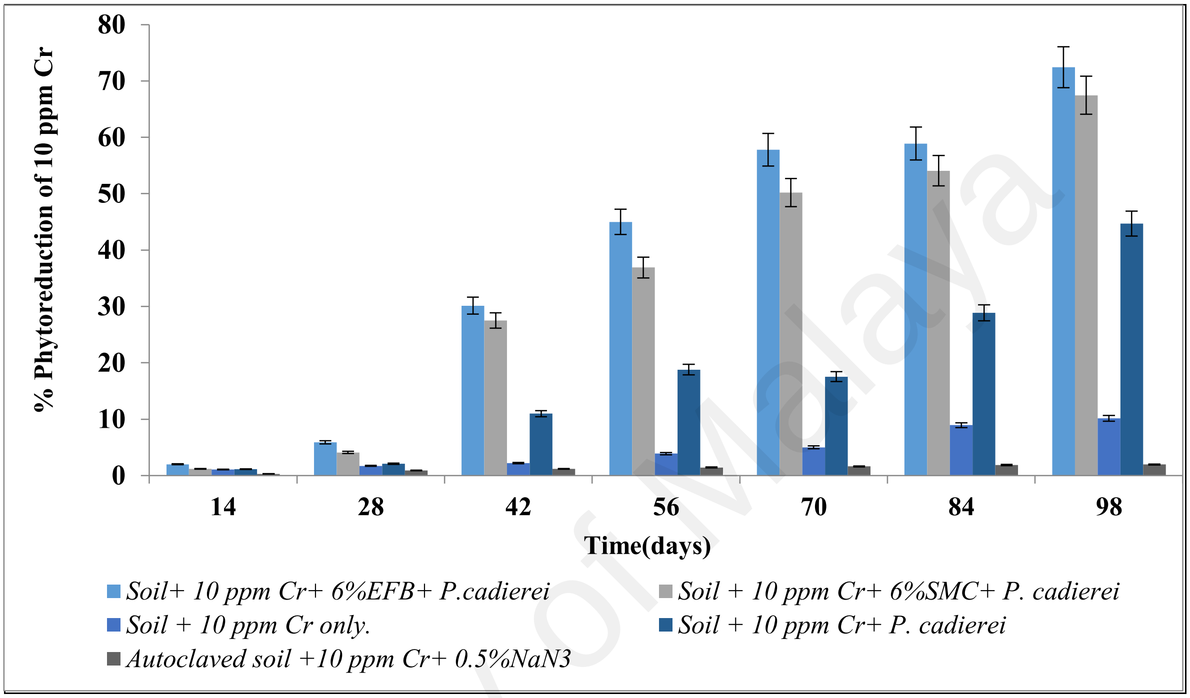


Figure 4.19: Percentage phytoreduction of Cr in 10 ppm Cr contaminated soil under *Pilea cadierei.*

Bars denotes standard error (**n**=3).

Another indicted reason for elevated "percentage" removal of soil Cr under (**Figure 4.19)** concentration (amended) - is due to appreciable N from organic wastes, which boosted *Pilea sp.* resistance and energy level to combat stress. Also, the possessed C/N ratio of both EFB and SMC contributed significantly. Because, the following authors Taiwo et al., (2016); Huang et al., (2004) and Guo et al., (2012), reported that for a compost to be qualified for use in a metal contaminated soil, the C/N ratio should be between 12 and 20. Therefore, in this study, C/N ratio of 20.02 and 14.1 for EFB and SMC was recorded (**Table 4.1**). This is also relative to the suggested compost C/N range required for agricultural soil application (Taiwo et al., 2016). This shall eventually improve the contaminated soil C/N quotient, in agreement with the reports of Jadia & Fulekar, (2008).

The fraction of Cr remediated by varying organic waste with *Lantana sp.* under 10 ppm Cr contaminated soil are broadly shown in **Figure 4.20**. A similar trend of Cr removal was observed, which translates in 76% for EFB, 72% (SMC), and 49% for *Lantana sp.* (control). The remediation ability of *Lantana sp.* affixed with organic wastes removed greater percentage of Cr than unamended treatments, at ratios of 1.54: 1.48: 1.0 in same order as above. However, the exception is that Cr showed elevated removal rate in EFB, but its difference with SMC was not significant ($p<0.05$).

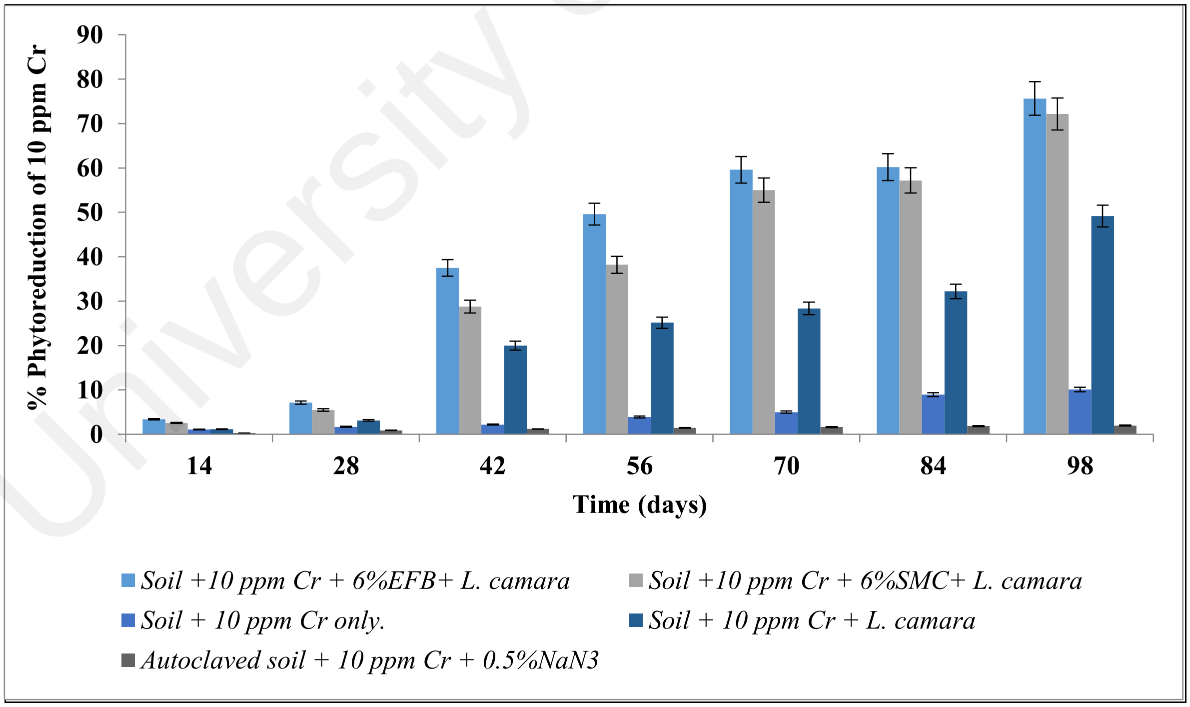


Figure 4.20: Percentage phytoreduction of Cr in 10 ppm Cr contaminated soil under *Lantana camara*.

Bars denotes standard error (**n**=3).

This may be attributed to the fact, that the *Lantana sp.* plants at separate (i.e., EFB or SMC) treatments, perhaps absorbed optimum nutrient at indexed proportionality, capable of mineralizing the bioavailable soil Cr simultaneously. Secondly, tendency exist that equilibrium in Cr mobility, and their remediation pace may be in close par with the enriched "biowaste" soils. This concurred with McConnell et al., (1993) who observed that the introduction of compost increased the soil CEC by at least 10%, and promoted the removal of heavy metal present in low metal polluted soils via biological treatments.

Eventually, under this concentration and remediation strata (i.e., 10 ppm Cr), *Lantana sp.* reiterated high diminution of Cr in soil over *Pilea sp*. Probably implicating the former to have more tolerable Cr sequestration mechanism over the later in heavy metal compromised soils. Differently, such reduction variations between *Sebertia acuminata* and *Becium homblei* plants on Ni and Cu ruined soils, has been previously reported by Gosh & Sing, (2005) and Alkorta et al., (2004).

Another study from Mangkoedihardjo et al., (2008) reported 50% removal of Cr (VI) in chromium eluted-soil using combined compost (i.e., fused organic waste), and plant technology. But this study contradicted with Mangkoedihardjo et al., (2008) because it removed 76% and 72% of Cr separately, under EFB and SMC (uncombined) with *Lantana*, at 10 ppm total Cr remediated soil. This is to prove that biowaste combination does not necessarily guarantee highest metal reduction and/or removal in the remediation of metal polluted soil, but rather, what matters is the agro-waste individual capacity, potency, environmental-friendly and its nutrient compatibility with plant, add to their complexation effects in supporting the mineralization of heavy metals in the contaminated soil. This argument on the inherent- potency of organic amendments to metal soil remediation was also re-emphasized in details elsewhere in Wang et al., (2016).

Under the 10 ppm Cr remediated soil, the biotic and abiotic Cr losses recorded 10% (R^2=0.8724) and 2% (R^2=0.8932) respectively, and having 8% (R^2=0.7796) net difference after 98 days. This is shown in **Figure 4.21**. Hence, this significant net difference recorded at $p< 0.05$ may be from the inputs of low Cr concentration applied, which did not exert absolute toxicity on the bulk soil microbe (bacteria). Sodium azide acted like a biocide (Chefetz et al., 2006), and the soil in question was thoroughly autoclaved, hence relying only on abiotic influence, which may be passive in removing the Cr subjugated to the soil matrix. These together, were used to monitor the strength of natural Cr removal and/or attenuation from polluted soil, and their kinetics as well.

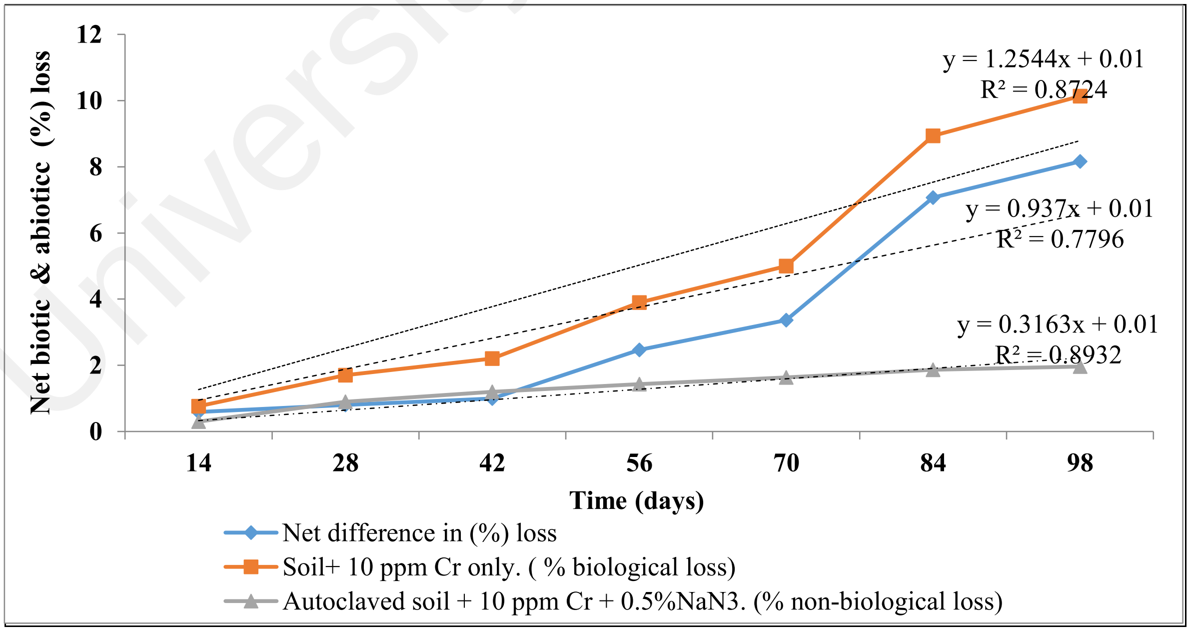


Figure 4.21: Correlation between biotic and abiotic losses with net (%) loss difference in 10 ppm Cr contaminated soil after 98 days.

Chefetz et al., (2006) revealed from their soil-pollutant sorption study, that application of biocide control was crucial since the activity of micro-organism can interfere with the composition and amount of both the sorbent and sorbate. Hence, these interferences should be eradicated to assess the real-time sorbate-sorbent interactions. However, Chefetz et al., (2006) views are not fully in-line with the frame of this study, but what could be deduced is that both biotic and abiotic losses and/or interactions are significant mechanisms needed to be investigated, if actual soil heavy metal remediation models must be advocated.

In continuation, inference indicted biotic loss at this concentration (10 ppm Cr soil) to be high, but less in 20 ppm, and lesser in the 40 ppm Cr polluted soils at expressed ratios of 2.05: 1.21:1.0, respectively. This consequence, may be directly linked to the effects of concentration (Kuiper et al., 2004), because the higher the concentration of heavy metal in soil, the higher the intensity of their toxicity to the soil biota (bacteria), hence bacteria activities are incapacitated (Gadd, 2010; Yang et al., 2016).

4.2.3 Phytoremediation of co-contaminated soil- 20 ppm (Pb+Cr)

Progressing into the dual-metal contaminated soil results, i.e., at 20 ppm each (Pb + Cr) remediated soil under *Pilea sp.* after 98 days (**Figure 4.22**). This co-concentration were chosen because it was the highest limit adapted by both plants, under mixed condition. However, it showed systematic decline of Pb availability in soil by 31% under EFB, 22% in SMC, and 18% for Pb at the (*Pilea sp.* Pb+Cr) unaided parallel control treatments. Accordingly, the co-contaminant (Cr), in a simultaneous nature recorded a decrease of 25% apropos to EFB, 17% for SMC, and 15% linked to Cr at (*Pilea sp.* Pb+Cr) unamended coupled control, respectively. Unequal ratio of Pb is to Cr reduction of 1.27:1.0 for EFB, 1.31:1.0 to SMC, and 1.23:1.0 for (*Pilea* Pb: Cr) controls were recorded, at the dual metal polluted soil. With high significant occurrence value of F: ($p < 0.05$) = 719.482.

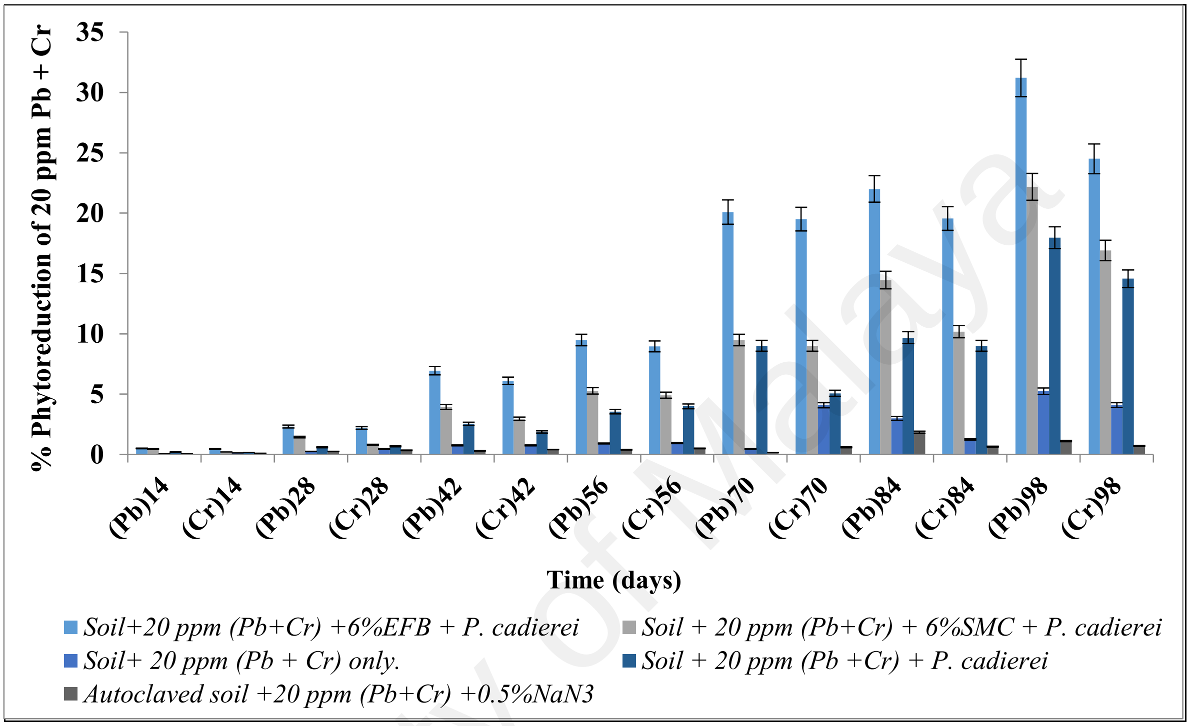


Figure 4.22: Percentage Phyto-reduction of Pb and Cr in 20 ppm (Pb +Cr) co-contaminated soil under *Pilea cadierei*.

Bars denotes standard error (n=3).

However, the same trend of co-reduction of Pb and Cr was also recorded under *Lantana sp.* Pb was noticed to have disappeared by 35.13% under EFB, 27% for SMC, and 20% for Pb in (*Lantana sp.* Pb +Cr) twin metal control soils. This was in parallel disappearance of Cr by 29% in soil amended with EFB, 21% in soil amended with SMC, and 15% for only Cr in (*Lantana sp.* Pb+Cr) control treatments (**Figure 4.23)**. Nevertheless, remote possibility for high removal of Pb and Cr in the amended co-contaminated soil, is due to electrostatic force reduction (Paunov et al., 1996), which remained visible in the unamended soil, hence retards metal phytoextraction. Therefore, simultaneous ratios (Pb: Cr), for metal reduction in soils compared gave 1.22:1.0 under EFB, 1.26:1.0 for SMC, and 1.32: 1.0 for *Lantana sp.* (Pb: Cr) controls, at significant level of ($p<0.05$), (**Appendix Tables 15** and **16)**.

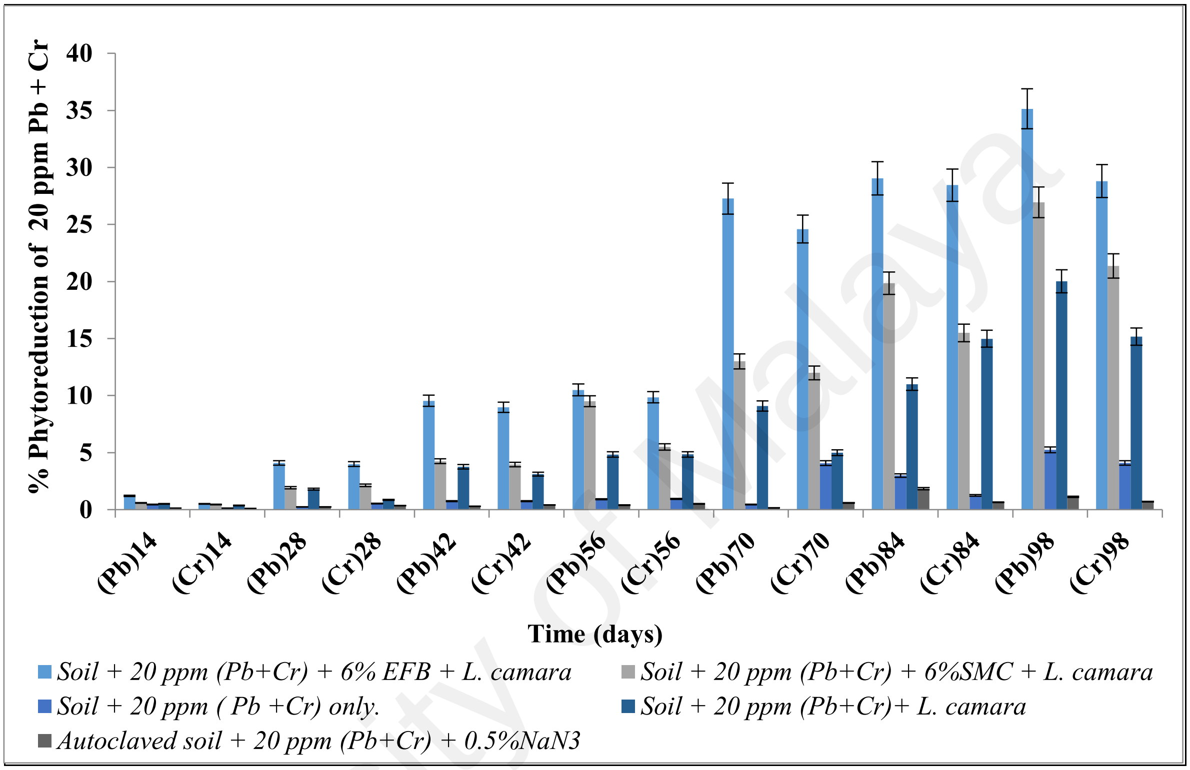


Figure 4.23: Percentage Phyto-reduction of Pb and Cr in 20ppm (Pb +Cr) co-contaminated soil under *Lantana camara*

Bars denotes standard error (n=3).

First of all, it would not be unequivocal to state that nutrient availability provided by organic wastes, and extreme plant tolerance, agitated the survival of *Pilea sp.* and *Lantana sp.* in a bi-contaminated heavy metal soil. Generally, the co-metal remediated soils for both *Pilea* and *Lantana sp.*, independently recorded high Pb removal than Cr, in both amended and as well unamended soils (retrospect from **Figures 4.22** and **4.23**). This is supposedly due to the nature of the metal ions and/or speciations thereafter. Meanwhile, Adediran et al., (2009) states that the hydrated sphere of Pb ions is smaller than that of Cr ions, and hence their absorption and/or adsorption capacities to organic substrates, depends on the size of their hydrated ions. Drawing inference from Adediran et al., (2009), one can infer that Pb was favoured to be taken-out and/or phytoextracted from the contaminated soil after complexation than Cr. Also, this conformed to the inner and/or outer sphere complex metal ion formation, argued by Quizhong et al., (2013); Bolan et al., (2014); Park et al., (2011b); Ming-Li et al., (2014), in metal polluted soils. Moreover it was observed that in both *Pilea sp.* and *Lantana sp.* treatments under 20 ppm (Pb+Cr) polluted soil, parallel decline of Pb and Cr were retarded at the initial 14 to 42 days. The possible explanation to this, may be that both metal ions were competing for binding sites, and probably undergoing complex reactions, which can affect their speciation states, required to propel inclusive phytoremediation activity (Hafsteinsdȯttir et al., 2015; Wuna & Okieiman, 2011; Yang et al., 2016). Secondly, both Pb and Cr are cations, hence may oppose and/or repel each other in the co-contaminated soil. This could lead to competing difficulty in capturing external "*Lantana sp.* or *Pilea sp.* roots" metal removal sites, due to electrostatic forces acting in-between the both cations in soil. A slightly related view on this phenomena is found elsewhere in details by Kralchevsky et al., (2011).

Figure 4.24 showed the correlation of biotic and abiotic losses after 98 days, monitored for 20 ppm (Pb+Cr) co-contaminated soil. Thus, indicated 5% and 4% of Pb and Cr were lost biologically, while 1% exited abiotically from the respective soils. Hence, 4% (Pb) and 3% (Cr) were implicated as parallel net losses at low correlation ($R^2 = 0.4425$; $p<0.05$). However, under single soil contamination at 20 ppm of either Pb or Cr; elevated biotic of 7 % to 6.0%, and abiotic of 3% to 2.5% losses were recorded on both soils. Implying that natural loss of metals are usually impeded, when multiple metals caused soil devaluation. This concurred partially with Yang et al., (2016) who indicted such factor in the remediation of co-

contaminated Cu and Cd paddy soil. However, this study proposes that more attention should be paid to soils impoverished by multiple-metals during biological remediation, due to factors bordering within ecological and/or geochemical uncertainties.

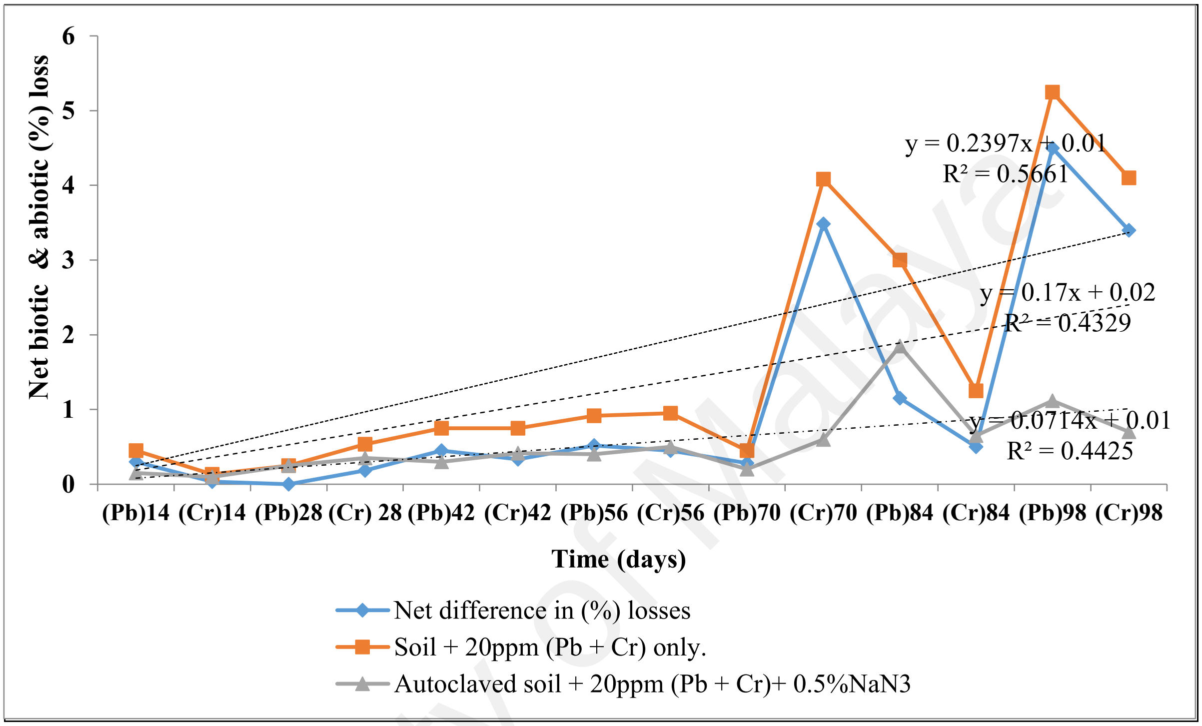


Figure 4.24: Correlation between biotic and abiotic losses with net (%) loss difference in 20 ppm (Pb +Cr) contaminated soil after 98 days.

4.3 Microbial enumeration in phytoremediation studies

Heavy metal presence in soil, and its unprecedented adjustments induced on the edaphic microfauna (specifically bacteria), are undeniably interlinked. Hence, this section is fragmented to buttress these tolerance dynamics as follows:

4.3.1 Microbial counts in soil contaminated with Pb

The counts of aerobic heterotrophic bacteria (AHB) in soil contaminated with 80 ppm Pb under *Pilea sp.*, and amended with EFB ranged between 22.3 x 10^6 to 81.0 x 10^6 cfu/g, while soil amended with SMC recorded 20 x 10^6 to 72.0 x 10^6 cfu/g, and 14.67 x 10^6 to 38.3 x 10^6

cfu/g for *Pilea sp.* (unamended) control. In extension, biotic and abiotic control soils recorded AHB orders of 13.3 x 10^6 to 31.0 x 10^6 cfu/g, and 1.0 x 10^6 to 1.6 x 10^6 cfu/g, respectively. At overall high significant level [F: (p <0.05) = 321.62] (**Figure 4.25)**. This obvious differences noted at the microbial biomass of amended and unamended treatments, may possibly be from the presence of carbon, via organic waste input. This was substantiated by Pérez-de-Mora et al., (2006) who stated that biosolid compost addition induced a shift in bacterial biomass in a metal contaminated soil. Moreover, Rathnayake et al., (2013) also supported this result, by way of stating that the toxicity of metal ions, on soil microbial habitats are greatly reduced upon the addition of organic components.

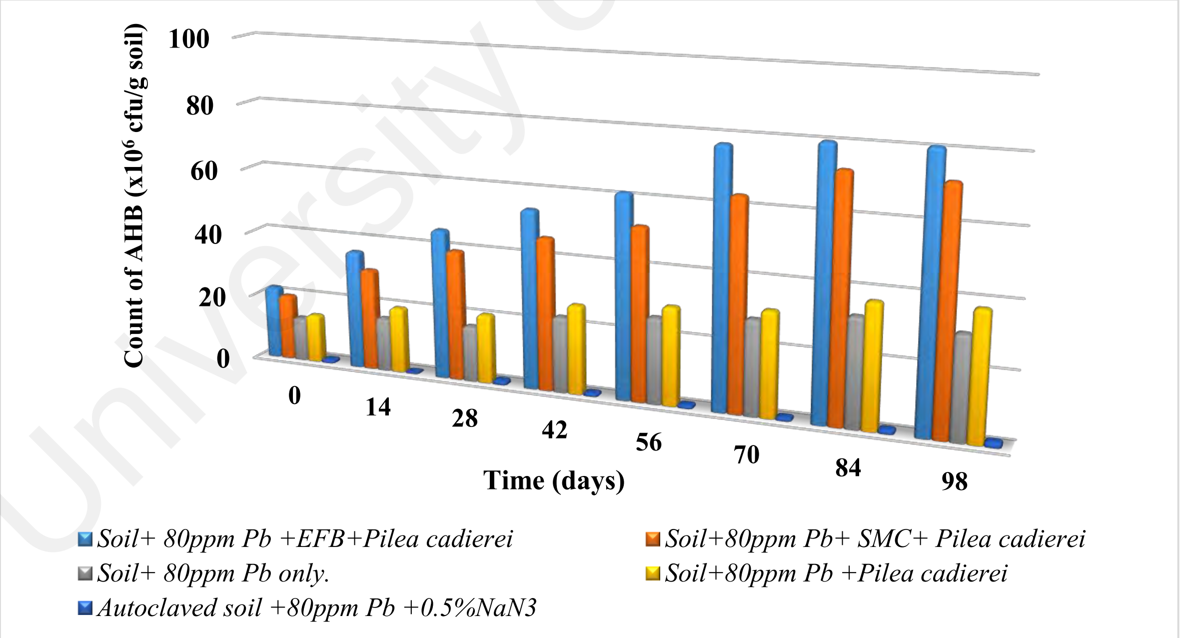


Figure 4.25: Counts of aerobic heterotrophic bacteria (AHB) in 80 mg/kg Pb contaminated soil under *Pilea cadierei* at 98 days.

Note: readings were taken at n=3.

García-Delgado et al., (2015) observed increase in the numbers of heterotrophic bacteria (1.29×10^7 MPN g^{-1}) after 63 days bioremediation of Pb and PAHs co-polluted soil, but amended with spent mushroom. Also, this study recorded AHB increase at EFB or SMC 80 ppm Pb remediated soils after 98 days. But contrasted with García-Delgado et al., (2015) because Pb and PAHs were present as co-pollutants, and may likely exert more and/or less toxicity on the soil microbial ecology, irrespective of the amendments applied. Whereas the Pb-soil investigated in this study were unmixed with any persistent organic pollutant (i.e, PAHs) as seen in García-Delgado et al., (2015), hence this could cause variations.

Furthermore, in 80 ppm Pb remediated soil under *Pilea sp.*, the counts of culturable heavy metal resistant bacteria (HMRB) recorded 6.0×10^6 to 51.0×10^6 cfu/g for EFB, 4.3×10^6 to 39.0×10^6 cfu/g for SMC, and 3.3×10^6 to 29.3×10^6 cfu/g for unamended soil, respectively. At high significant level F: ($p < 0.05$) = 411.22. Meanwhile, 3.0×10^6 to 21.0×10^6 cfu/g, and 0.6×10^6 to $1.3.0 \times 10^6$ cfu/g HMRB were recorded for biotic, and abiotic controls under same concentration (**Figure 4.26**). The HMRB counts from EFB amended soil, was about 10.4% and 18.35% higher than SMC amended and *Pilea sp.* (unamended) control soils. While soil amended with SMC was about 7.8% higher in HMRB counts than the unamended *Pilea sp.* control soil. Obviously, these differences are quite unclear, but Boshoff et al., (2014) adjudged that metal related stress-responses by soil microorganisms are extremely dynamic. Also, imbalance in catabolic evenness, and non-uniformity in nutrient composition of the soil substrates, may have possibly caused functionality loss at the heavy metal resistant bacteria under SMC and *Pilea sp.* (unamended) control soils, with respect to the EFB soil (Chapman et al., 2013).

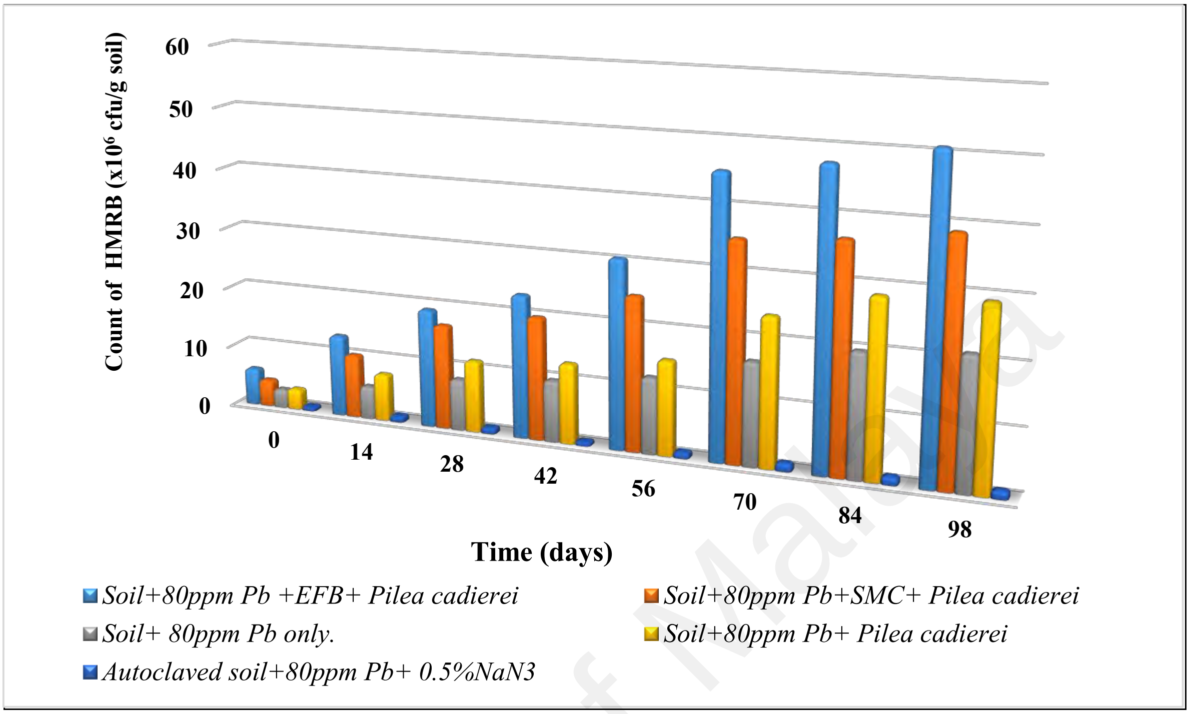


Figure 4.26: Counts of heavy metal-resistant bacteria (HMRB) in 80 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

Examining the culturable counts of AHB in 80 ppm Pb remediated soil under *Lantana sp.*, populations of 23.0 x 10^6 to 83.3 x10^6 cfu/g soil, 21.0 x 10^6 to 74.0 x10^6 cfu/g, and 14.6 x 10^6 to 38.33 x10^6 cfu/g were implicated for soils amended EFB, SMC, and *Lantana sp.* (unamended) control, respectively. Alongside with AHB counts of 3.3 x 10^6 to 31.0 x10^6 cfu/g, and 1.0 x 10^6 to 1.67 x10^6 cfu/g for biotic and abiotic soil controls respectively. There was a statistically significant decrease between AHB contents (**Figure 4.27**), of organic amended soils and unamended controls at F: (p <0.05) = 613.42. However, the EFB amended soil was the most significant, followed by SMC. Therefore, the decreased number of AHB from amended soil to the unamended, up to biotic and abiotic controls, indicted loss in the ability of bacteria cells to maintain its wide range of functions due to Pb toxicity and nutrient deficiency (Chapman et al., 2013). Hence, considered as a function of decreased soil health. Meanwhile, this was not only observed in this study, but Boshoff et al., (2014) also pointed out this on Pb and Zn contaminated soil microbial assay.

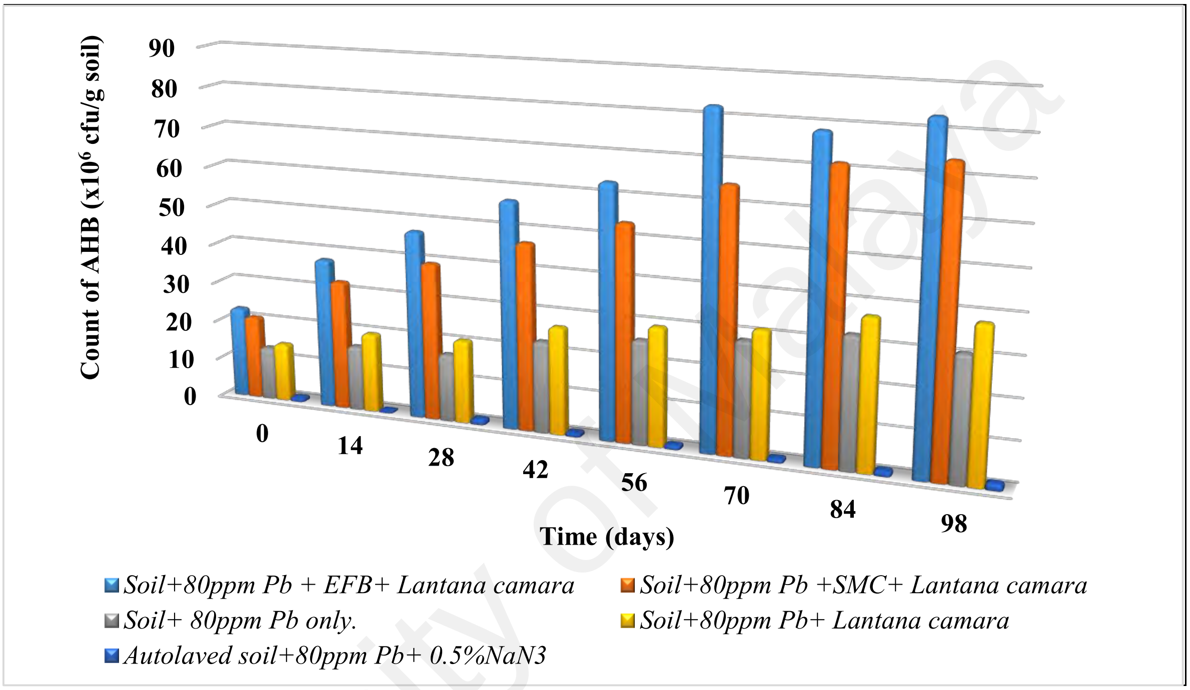


Figure 4.27: Counts of aerobic heterotrophic bacteria (AHB) in 80 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

Furthermore, analysing the heavy metal resistant bacteria (HMRM) counts under 80 ppm Pb remediated soil with *Lantana sp.*, about 8.3 x 10^6 to 56 x 10^6 cfu/g and 5.67 x 10^6 to 39.0 x 10^6 cfu/g were recorded in soils amended with EFB and SMC, respectively. While 3.3 x 10^6 to 29.3 x 10^6 cfu/g, 3.0 x 10^6 to 21.3 x 10^6 cfu/g, and 0.6 x 10^6 to 1.3 x 10^6 cfu/g of HMRB counts were evidenced in *Lantana sp.* (unamended) control, biotic, and abiotic set-ups respectively. To this effect, EFB amended soil HRMB isolates, significantly recorded more counts than other treatments when equated at F: (p <0.05) = 589.20 (**Figure 4.28**).

Comparatively, the number of AHB exceeds HMRB, because further sensitivity test are applied to the later.

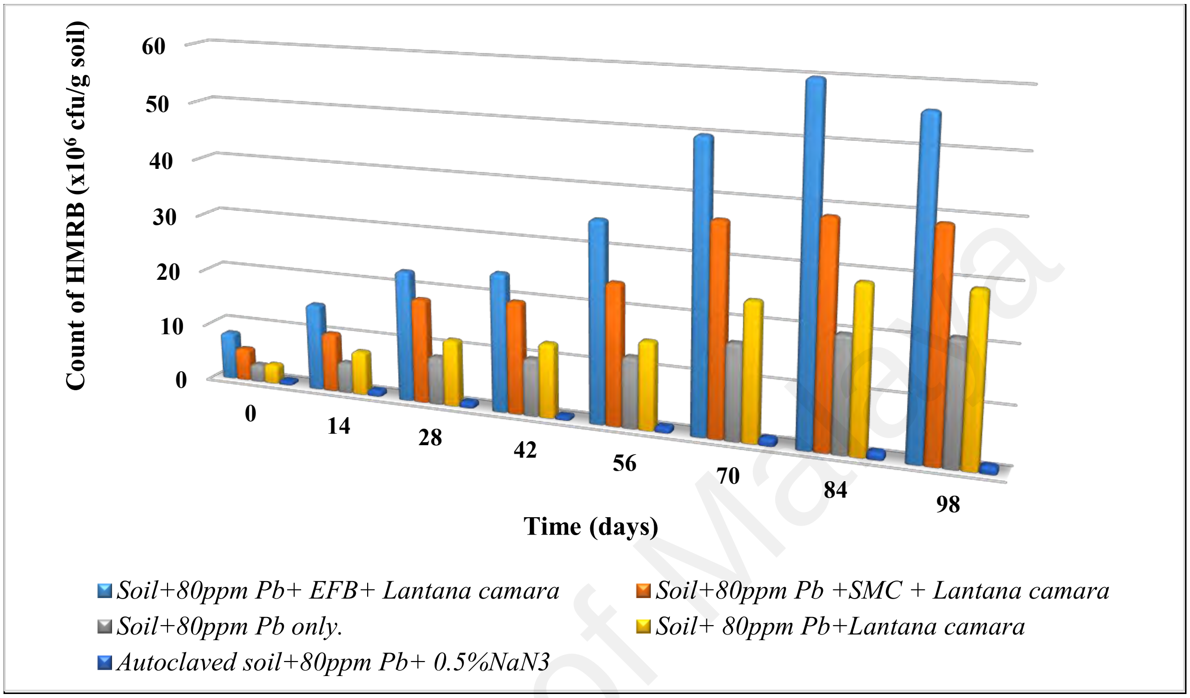


Figure 4.28: Counts of heavy metal-resistant bacteria (HMRB) count in 80 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

The reason for increase in counts of HRMB in soils amended with agro-waste, might be due to the presence of nourishments, especially N and C which enhanced the multiplication of bacteria in soil. In extension to these numbers, EFB amended soil recorded about 18.87%, 23.63%, and 37.14% higher HMRB counts than *Lantana sp.* (unamended), biotic, and abiotic controls, respectively. With 1.4:1 ratio difference between EFB: SMC counted HMRB. However, in a different but related study, on lubricating-oil polluted soil by Agamuthu et al., (2010) recorded 240 x 10^5 cfu/g elevated counts of hydrocarbon utilizing bacteria (HUB) in 2.5% oil polluted soil, amended with brewery spent grain (BSG), and fixed with *Jatropha* plant. This reflects that on a balance of probabilities, when other types of pollutants are responsible for soil ruin outside of Pb, if amended with organic waste, might probably improve bacteria tolerance and/or efficiency during phytoremediation.

Furthermore, both the AHB and HMRB counts recorded under *Pilea sp.* and *Lantana sp.* at 80 ppm Pb remediated soils, tend to be initially low between 0 to 42 days. Witter et al., (2000) described this type of phenomena as prolonged lag phase, prior to the onset of exponential growth induced by heavy metal, hence, this can retard bacterial physiological status. Therefore, Witter et al., (2000) opinion may be consented by this result, as the key factor responsible for the above argued, considering the Pb density induced to the studied soil.

In the next experiment, following the remediation of 40 ppm Pb soil affixed with *Pilea sp.*, the AHB counts ranged from 30.3 x10^6 to 95.67 x 10^6 cfu/g, 28.67 x10^6 to 74 x 10^6 cfu/g, and 18.0 x10^6 to 37.67 x 10^6 cfu/g were observed for soils amended with EFB, SMC, and *Pilea sp.* (unamended) control. This is not unconnected to the 17.3 x10^6 to 32.67 x 10^6 cfu/g and 1.0 x10^6 to 1.6 x 10^6 cfu/g witnessed at the biotic and abiotic controls (**Figure 4.29)**

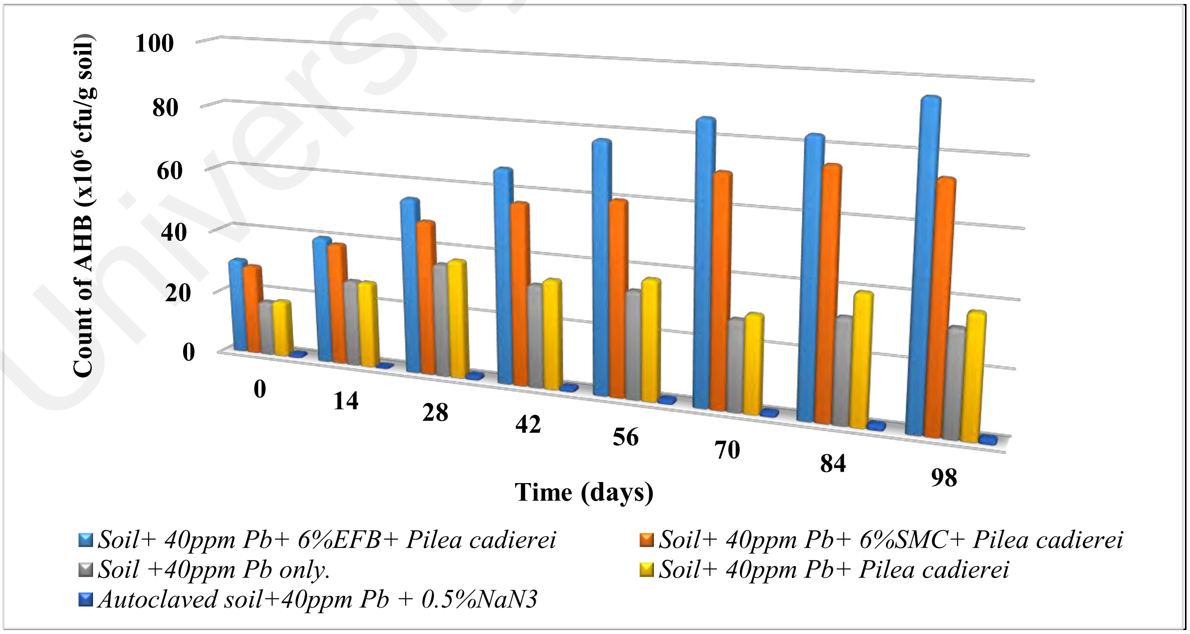


Figure 4.29: Counts of aerobic heterotrophic bacteria (AHB) in 40 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

However, the organic waste treated soils, progressed with more AHB counts at high significant level [F: (p <0.05) = 711.09]. This was possibly due to the presence of induced supporting nutrients, from EFB and SMC (Table 4.1), which promoted bacteria growth, and shielded their cells from direct negative pressure of soil metal toxicity. Consequently, the extreme low AHB population recorded at the abiotic soil, was because of sodium azide soil-poison (Chefetz et al., 2006), and autoclaving effects. Which significantly hampered bacteria cell proliferation.

Similarly, the HMRB counts isolated under same *Pilea sp.* (40 ppm Pb) remediated soil, had orders of magnitude 19.0 x 10^6 to 61.3 x10^6 cfu/g and 10.0 x 10^6 to 48 x10^6 cfu/g for EFB and SMC amended soils add to 4.3 x 10^6 to 28.3 x10^6 cfu/g obtained from *Pilea sp.* (unamended) control, respectively (**Figure 4.30).** However, several AHB upper limit counts against the HMRB showed ratios of 1.56:1.0 (for EFB soil), 1.54:1.0 (for SMC soil), and 1.33:1.0 (*Pilea sp.* unamended) soil respectively, at significant level F: (p <0.05) = 741.11.

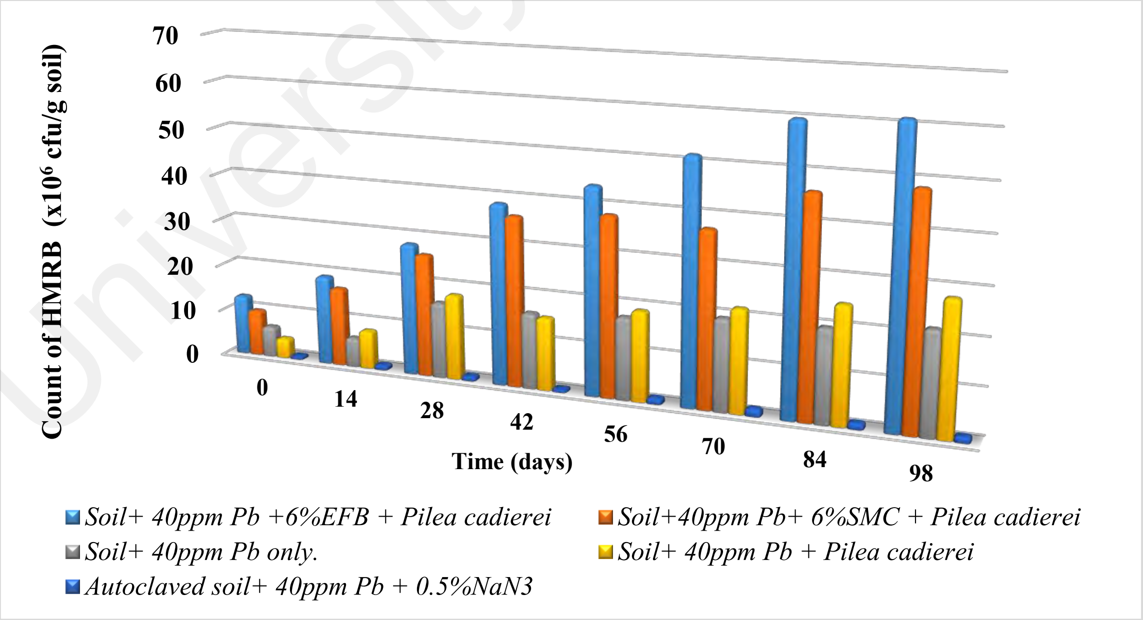


Figure 4.30: Counts of heavy metal-resistant bacteria (HMRB) in 40 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

This indicated that in any given bacterial population present in recalcitrant (Pb) polluted soil, only a tolerant and/or resistant bacteria may most likely be the core-primary drivers to heavy metal transformation. Moreover, higher counts of AHB and HMRB were recorded in soils contaminated with 40 ppm Pb when compared to 80 ppm Pb soils, this might be attributed to the 50% less in concentration of Pb at the former. In otherwords, this may imply that high percentage of Pb in the contaminated soil might be poisonous to some of the indigenous bacteria population, hence, inhibiting their growth. This was also reported elsewhere by Witter et al., (2000) who observed lethal effects of elevated concentrations of other heavy metals, suchlike Cu, Zn, and Cd on soil bacterial community in their study.

Figure 4.31 expresses the rhizospheric AHB aggregates at 40 ppm Pb remediated soil using *Lantana sp*. Soils amended with organic wastes reckoned 25.0 x 10^6 to 88.67 x 10^6 cfu/g, and 23.0 x 10^6 to 76 x 10^6 cfu/g for EFB and SMC, respectively. While enumerations from *Lantana sp.* (unamended), biotic, and abiotic soil controls had AHB counts of 15.3 x 10^6 to 39.3 x 10^6 cfu/g, 17.3 x 10^6 to 32.67 x 10^6 cfu/g, and 0.6 x 10^6 to 1.7 x 10^6 cfu/g, respectively. Edaphic variation in nutrients, may have in overall facilitated these remarkable AHB differences, seen statistically at F: (p <0.05) = 589.51, under 40 ppm Pb *Lantana sp.* remediated soil. Meanwhile, the AHB numbers recorded at rhizospheric zones of amended soil of *Pilea* and *Lantana sp.* similitudes (i.e., 40 ppm Pb remediated soils), showed not much of differences at some points. Probably, due to the relative cushion effects provided by organic supplementation to soil microbial entity. Although, inflections in counts of AHB under *Pilea sp.* and *Lantana sp*. remediated soils occurred at various points between 0 to 98 days. This may be attributed to bacteria physiological adjustment to their changing soil niche, according to Bolan et al., (2014).

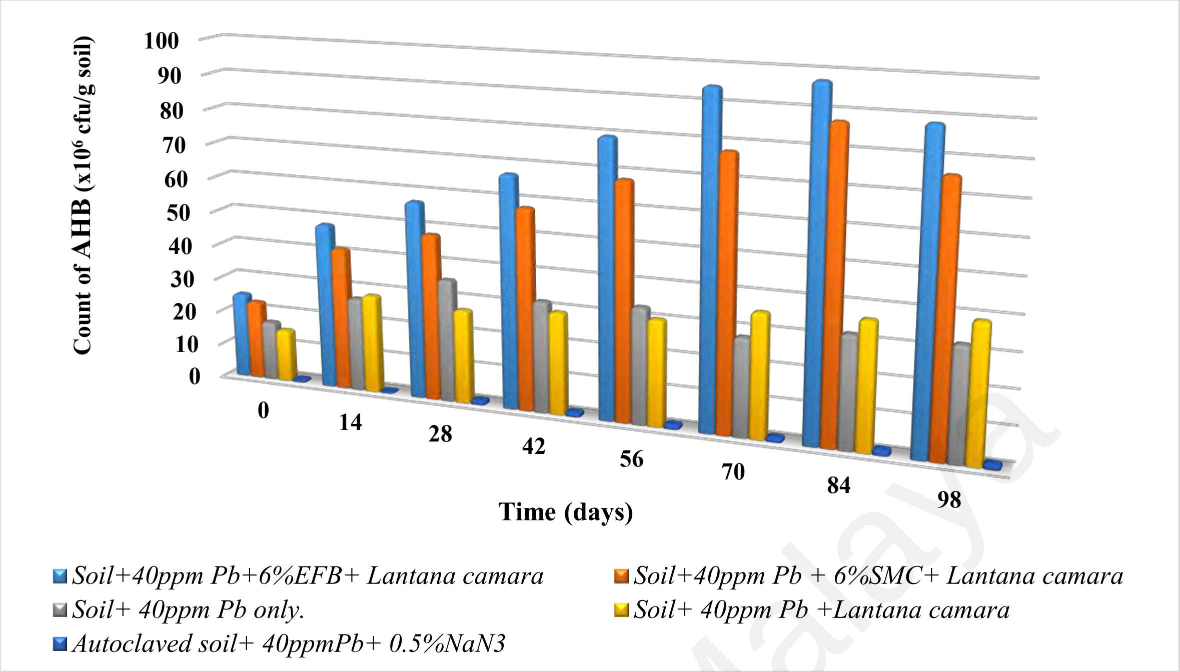


Figure 4.31: Counts of aerobic heterotrophic bacteria (AHB) in 40 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

Tao et al., (2015) reportedly said that increase in the amount of soil organic constituents, via organic materials, it's essentially a mechanism to increase the overall size, and activity of soil microbial biomass. However, the obvious rhizospheric suppressive number of AHB recorded in *Lantana sp.* (unamended) treatment, may be as a result of increased competition for space and/or limited resources induced by Pb. Wakelin et al., (2013) whose opinion is very important to this result, opined that such induction may trigger functional and phylogenetic changes in soil microbial ecology.

It is worth mentioning that the HMRB counts in 40 ppm Pb remediated soil under *Lantana sp.* showed very similar graph shapes with Figure 4.30, thus having 15.0 x 10^6 to 66.3 x 10^6 cfu/g and 11.67 x 10^6 to 39.0 x 10^6 cfu/g, and 7.33 x 10^6 to 29.3 x 10^6 cfu/g for EFB and SMC amended soils, and *Lantana sp.* (unamended) control, respectively. As well as 6.67 x

10^6 to 22 x 10^6 cfu/g and 0.6 x 10^6 to 1.33 x 10^6 cfu/g for biotic and abiotic controls. In which there were minor and major inflection points, and statistically significant at F: (p <0.05) = 802.32. **Figure 4.32** represents graph in triplicate average of this findings.

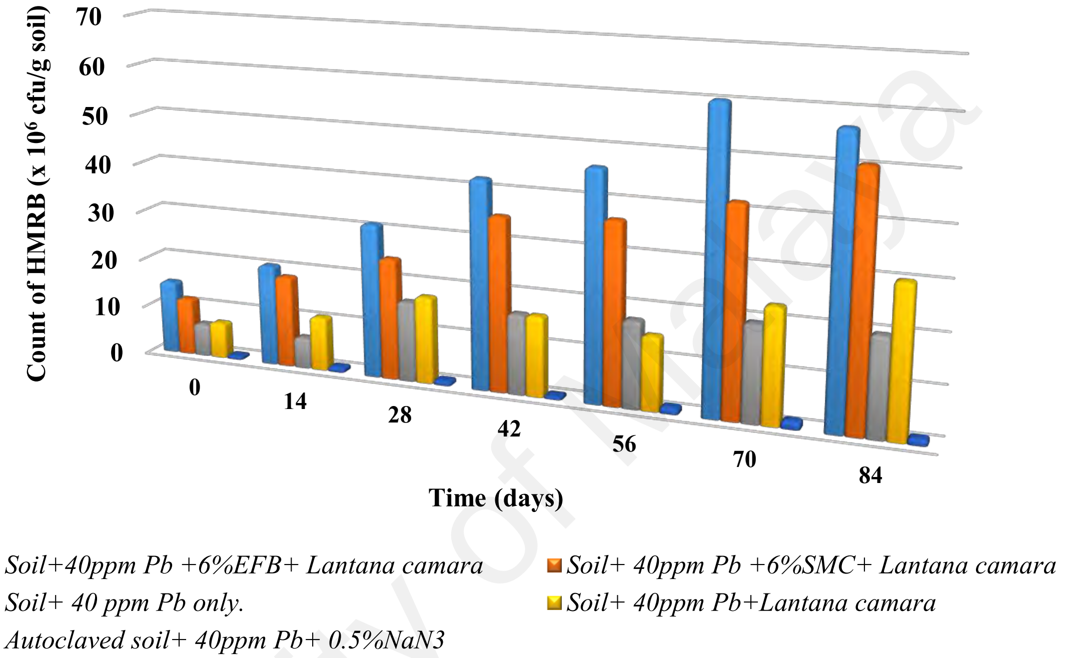


Figure 4.32: Counts of heavy metal-resistant bacteria (HMRB) in 40 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

In extension, the general ratio of AHB against HMRB i.e., 1.34: 1.0 for EFB amended soils, and 1.97: 1.0 for SMC amended soils under this concentration implicated AHB to outnumbered HMRB. Hence, depicting the principles of actively-resistant few bacteria, which by strict sense, are the actual ones doing the soil heavy metal mineralization task and/or aiding the phytoremediation process in contaminated soils. Although, some authors suchlike Bolan et al., (2014), García-Delgado et al., (2015), Cheng et al., (2011), and Agamuthu et al., (2010) should have mentioned about this particular phenomena in their studies, but it was omitted or probably an oversight, and this contrasted our views. However, Stefanowicz et al.,

(2009) partially recognized my arguments, but rather coined it to be pollution-induced community tolerance (PICT).

In continuation, the experimental results presented in **Figure 4.33**, shows increase in rhizospheric zone AHB counts under *Pilea sp.* at 20 ppm Pb soil remediation. This were believed to be driven by less Pb load and/or organic substrate impacts on the polluted soil. However, AHB counts ranged between 44.0 x 10^6 to 97.67 x 10^6 cfu/g soils, 40.0 x 10^6 to 82.3 x 10^6 cfu/g soil, and 27 x 10^6 to 38.0 x 10^6 cfu/g soil for EFB, SMC, and *Pilea sp.* (unamended) soil were recorded, respectively. Even, the biotic and abiotic AHB registered 21.0 x 10^6 to 33.7 x 10^6 cfu/g soil, and 1.67 x 10^6 cfu/g soil, respectively. At general statistical significant difference of [F: (p <0.05) = 661.11].

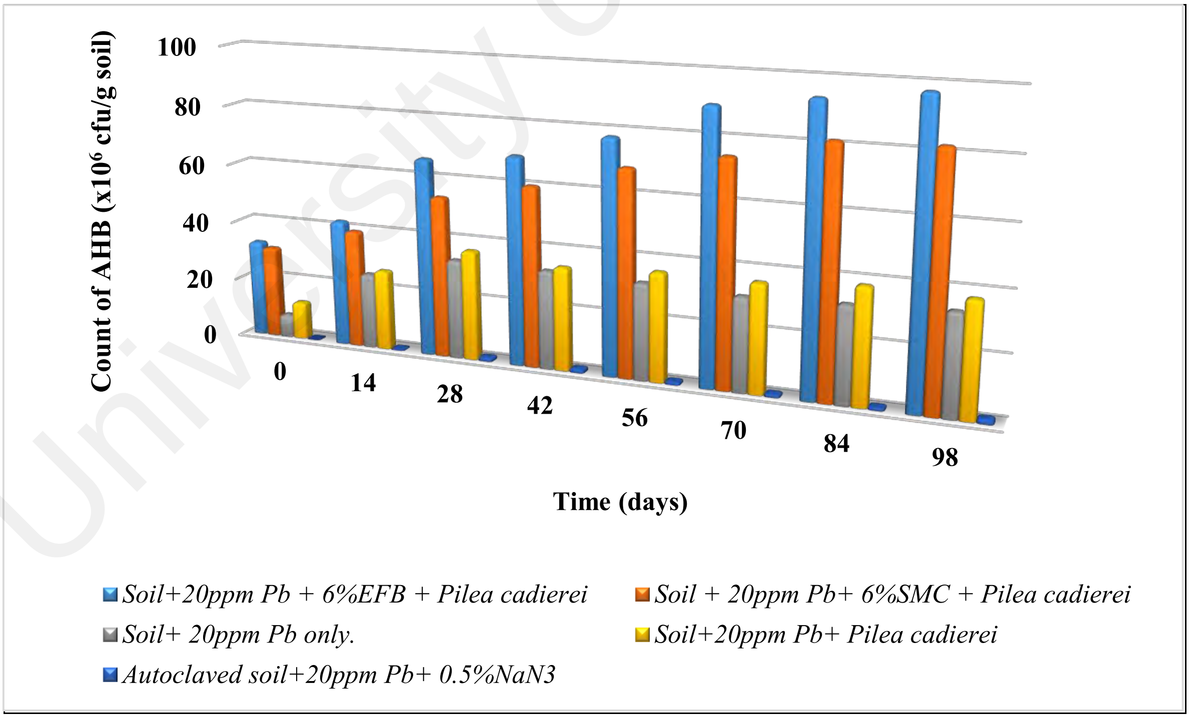


Figure 4.33: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

Therefore, it is possible to deduce from this result, that in low Pb polluted soils, increase in the heterotrophic bacteria, can lead to greater competition for resources; such as nutrients and habitat. However, the AHB ratios for 20: 40: 80 ppm Pb rhizospheric sites (upper range), gave 1.21: 1.17: 1.0, and 1.14: 1.02:1.0 for EFB and SMC amended soils, respectively. This findings is partially supported by Marschner et al., (2003), who reported that low and/or moderate rates of organic amendments in soil, could increase bacterial biomass, while having no effects on other soil micro-floras, in biological restoration of contaminated soil.

Alternatively, the HMRB counts for *Pilea sp.* 20 ppm Pb remediated soil, showed increased numbers of resistant strains, this could be associated to cell vigor, and acquired traits due to prolong metal-exposure. Conferred through less Pb-density in soil, and the external (organic) nutrient added. Hence, the effective resistant strain count revealed 14.67 x 10^6 to 66.3 x 10^6 cfu/g soil, 11.0 x 10^6 to 57.3 x 10^6 cfu/g soil, and 6.3 x 10^6 to 27.7 x 10^6 cfu/g soil for EFB, SMC amended, and *Pilea sp.* (unamended) control soils, respectively. This is graphically shown in **Figure 4.34**, and the statistical significant derived from this is F: (p <0.05) = 633.01. Surprisingly, the effective HMRB counts at 20 ppm Pb biotic control soil, had 1.1% and 2.1% elevated units of resistant strains over 40 and 80 ppm Pb corresponding counterparts. This may have partly led to the high percentage disappearance of Pb at 20 ppm Pb soil remediation strata, due to increased mineralization by resistant strains, as opposed to the results obtained in 40 and 80 ppm Pb remedied soils. Such trend, had also been reported by García-Delgado et al., (2015) on soil PAH-degrading bacteria after 28 days of incubation. Although, no significant difference was observed in the abiotic HMRB counts between 20 ppm Pb control soil, and the corresponding 40 and 80 ppm Pb soils, respectively. Probably, due to extreme autoclaving and equal application of sodium azide.

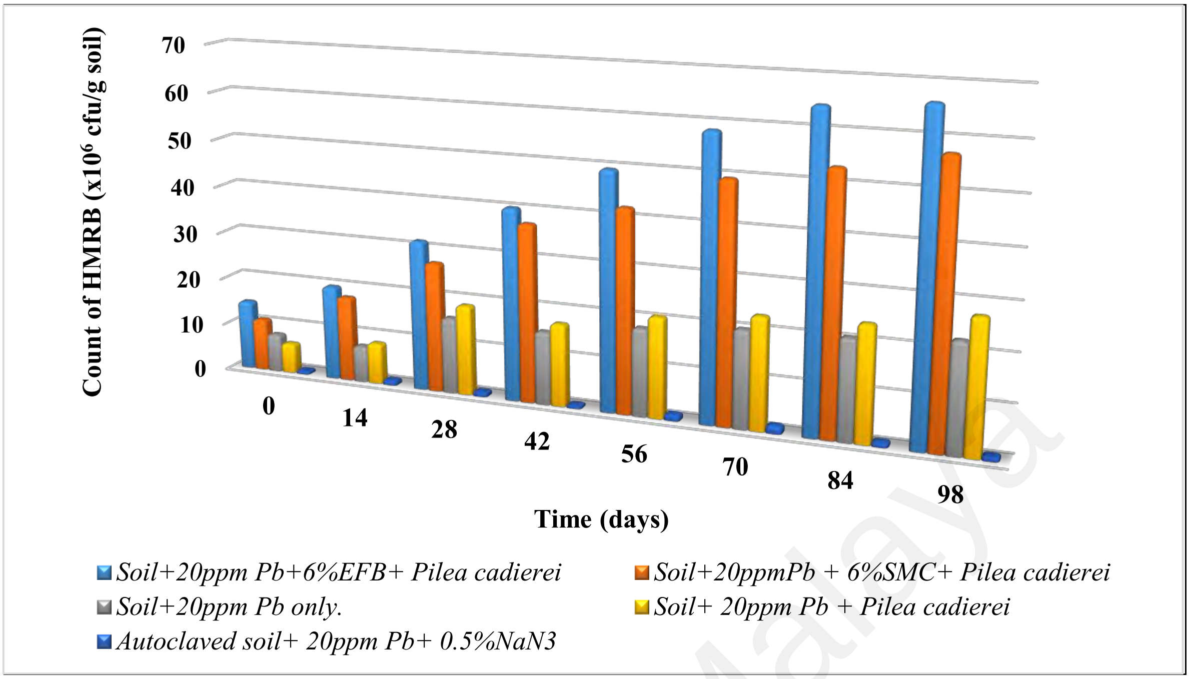


Figure 4.34: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

Progressing into another trend of AHB under 20 ppm Pb soil remediation, with *Lantana sp.* in perspectives. Noticeable AHB counts were recorded, ranging from 26.3 x 10^6 to 88.7 x 10^6 cfu/g, 24.3 x 10^6 to 76.0 x 10^6 cfu/g, and 15.0 x 10^6 to 39.0 x 10^6 cfu/g for EFB, SMC, and *Pilea sp.* (unamended) soil control, respectively(**Figure 4.35)**, and at statistical validation of F: (p <0.05) =901.11. As consistently observed, the HMRB counts under this status-quo, were less in number than AHB. This was not strange but rather anticipated due to inhibitory factors. Meanwhile, HMRB counts of 15.7 x 10^6 to 70.67 x 10^6 cfu/g, 12.0 x 10^6 to 60.3 x 10^6 cfu/g, and 7.6x 10^6 to 32.0 x 10^6 cfu/g for EFB, SMC, and *Lantana sp.* (unamended) soil control, were respectively recorded (**Figure 4.36**), at statistical significance F: (p <0.05) =891.31.

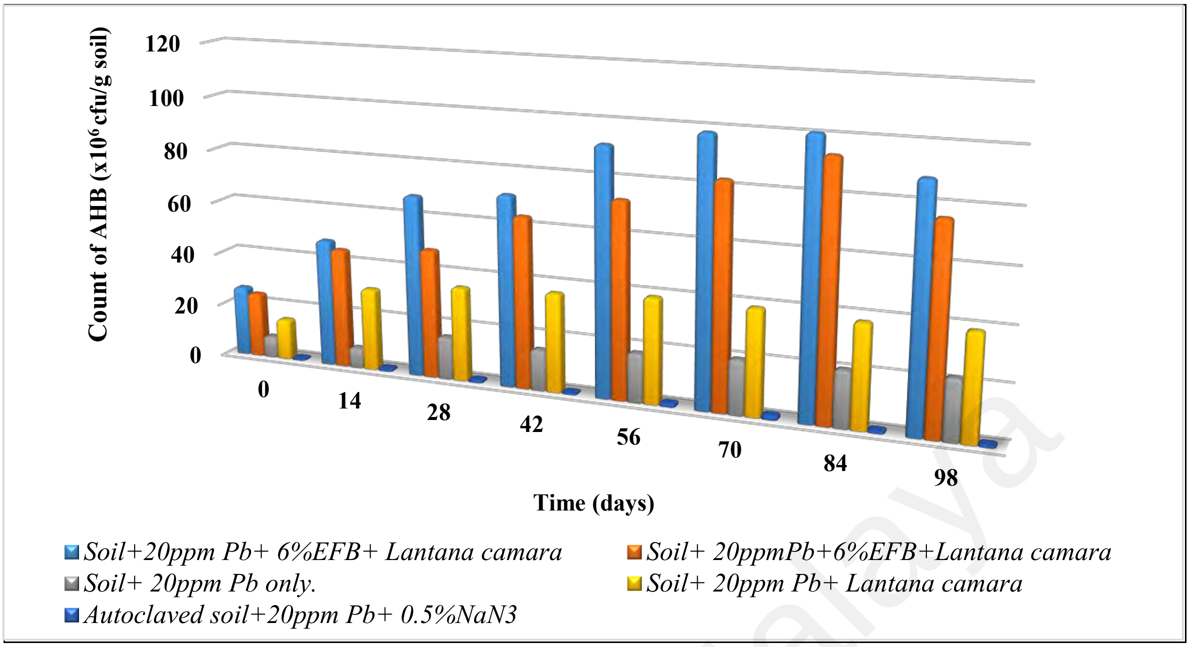


Figure 4.35: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

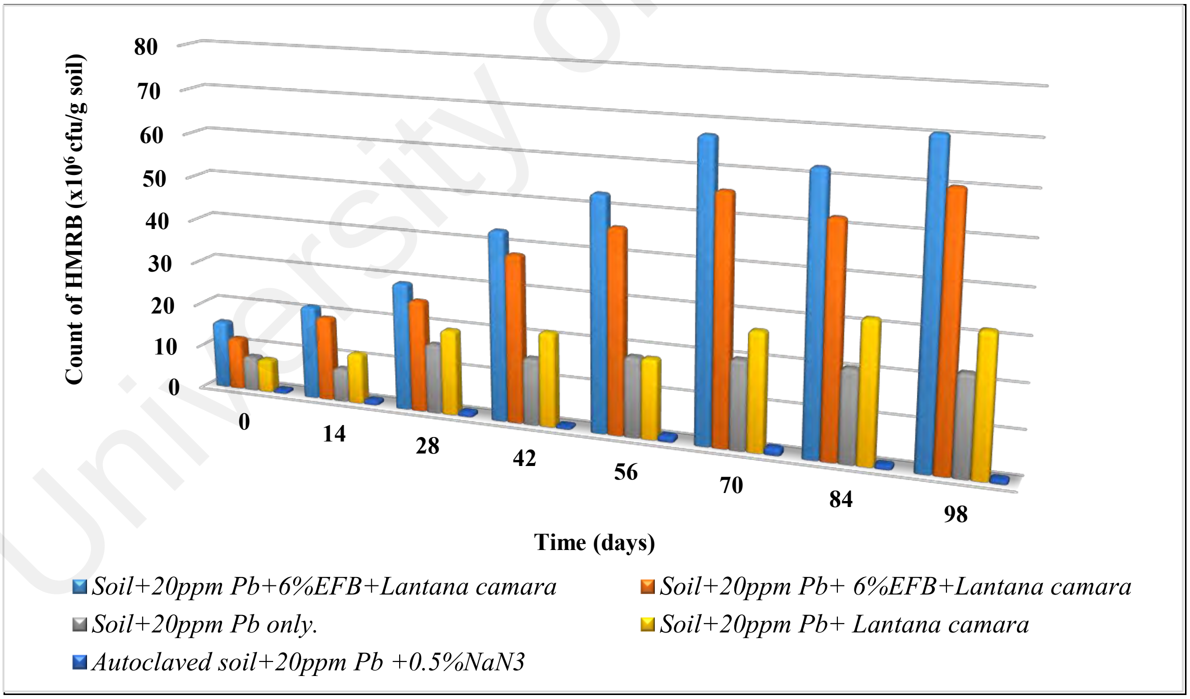


Figure 4.36: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

For both scenarios, that is AHB and HMRB counts, the inherent organic nutrient supplemented, propelled strain vigor for proliferation and combating of toxicant effects. Hence, ratios of AHB is to HMRB were modelled to be 1.16:1.0, 1.26:1.0, and 1.22:1.0 for EFB, SMC, and *Pilea sp.* (unamended) soil control, respectively. Instigating that, in rhizosphere region of metal polluted soil, both the AHB and HMRB strains co-exist, but only the later is established to be the shock-absorber, which drives the soil medium metabolism.

Furthermore, Piotrowska-Seget et al., (2005) inserted that metals can impose toxicity effects on microorganisms living at close edaphic associations, through various means. But, only those metal-tolerant bacteria could survive in these habitats, and possibly be isolated and selected for their potential use in bioremediation of contaminated soil and/or sediments. This concurred with this current study. However, AHB and HMRB numbers in biotic treatments, recorded 22.60 x 10^6 cfu/g soil and 22.67 x 10^6 cfu/g soil, respectively. Whereas, the abiotic comparison showed no significant difference at ($p<0.05$).

Finally, at the least and/or lowest Pb concentration (i.e.10 ppm Pb) remediated soil; under *Pilea sp.*, recorded distinct outcomes of bacterial biomasses. Here, the AHB accounted between 43.67 x 10^6 to 105.6 x 10^6 cfu/g, 40.67 x 10^6 to 95.67 x 10^6 cfu/g, and 22.0 x 10^6 to 38.0 x 10^6 cfu/g towards EFB, SMC, and *Pilea sp.* (unamended) control soils, respectively. As well as 20.67 x 10^6 to 36.3 x 10^6 cfu/g, and 1.66 x 10^6 cfu/g for the biotic and abiotic soil AHB densities, respectively (**Figure 4.37**). Meanwhile, the rhizosphere AHB value, obtained from EFB amended soil, was most significant to others at F: ($p<0.05$) =1121.44.

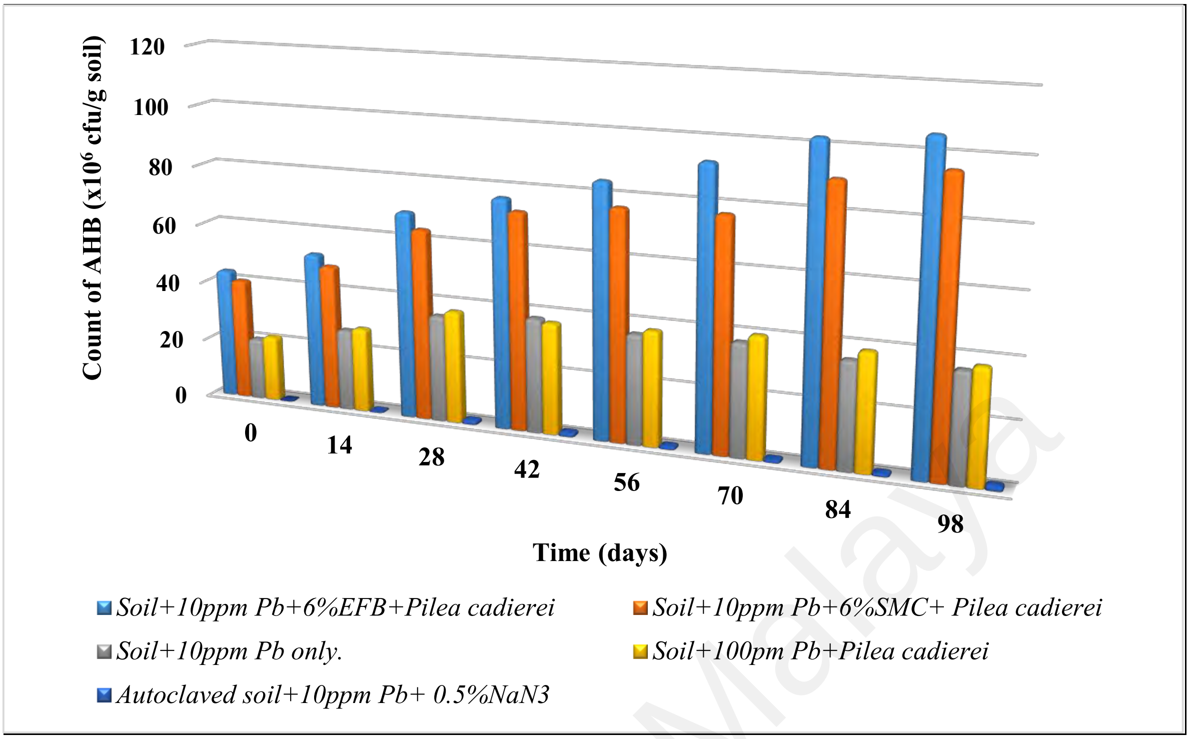


Figure 4.37: Counts of aerobic heterotrophic bacteria (AHB) in 10 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.
Note: readings were taken at n=3.

In a similar manner, the HMRB recorded appreciable integers of resistant strains, in orders of 20.3 x 10^6 to 72.0 x 10^6 cfu/g, 15.67 x 10^6 to 62.3 x 10^6 cfu/g, and 13.0 x 10^6 to 28.0 x 10^6 cfu/g for the EFB amended soil, SMC inclusive, and *Pilea sp.* (unamended) control soil, respectively (**Figure 4.38)**. However, the dual effect of increased numbers of AHB and HMRB recorded, may be as a result of enhancement of soil quality. Brought about, via addition of organic wastes, which positively influenced microbial development and activity (Bastida et al., 2007). Thereby, buffering the toxicity impacts at the hitherto low (10 ppm) Pb remediated soil. Unlike the 80, 40, and 20 ppm Pb remediated soils, which had relatively higher soil Pb-pollution index. Therefore, it is logical to resolve, based on this study; that the degree of heavy metal present in soil, is perhaps directly and/or inversely proportional to the bacterial densities striving in a particular contaminated soil, at that given moment.

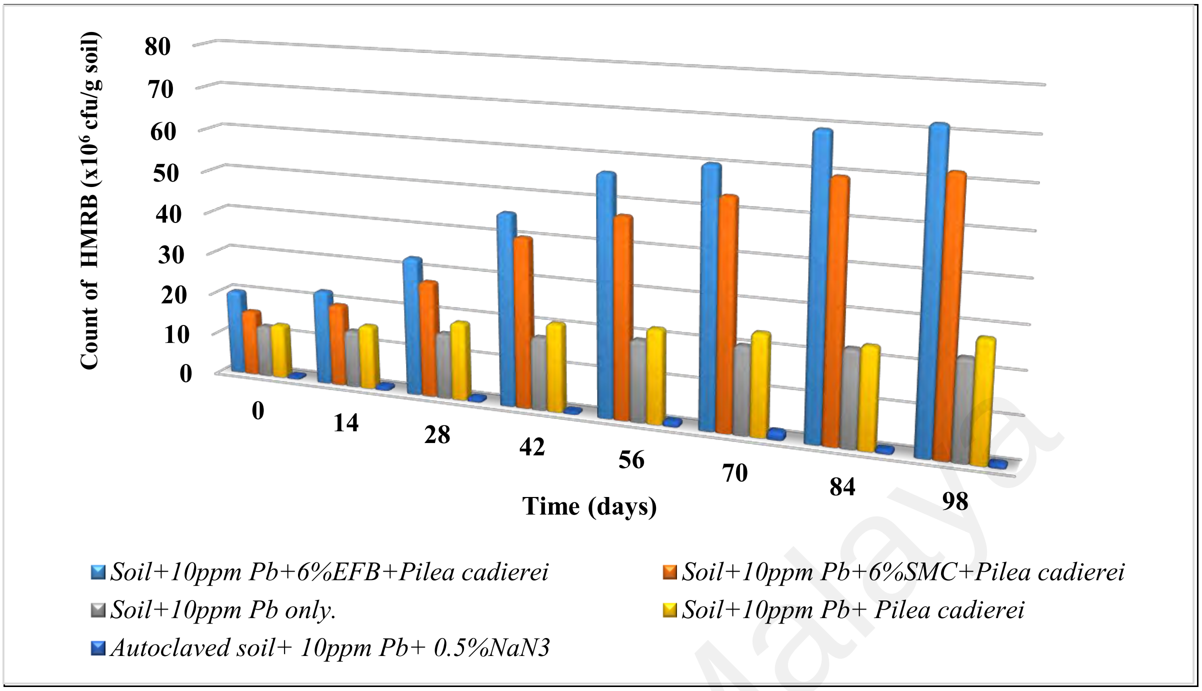


Figure 4.38: Counts of heavy metal-resistant bacteria (HMRB) in 10 mg/kg Pb contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

At 10 ppm Pb remediated soil under *Lantana sp.*, the prevalent rhizosphere AHB counts toppled their *Pilea sp.* counterparts. However, nominal ratio of 1:1 were obtained at their compared abiotic counts. In continuation, 39.67 x 10^6 to 109.0 x 10^6 cfu/g, 37.3 x 10^6 to 99.66 x 10^6 cfu/g, and 21.67 x 10^6 to 40.0 x 10^6 cfu/g counts of AHB were obtained at sites amended with EFB and SMC, and *Lantana sp.* (unamended), respectively. Meanwhile, soils for biological monitoring of Pb-loss recorded between 20.7 x 10^6 to 36.36 x 10^6 cfu/g counts of AHB (**Figure 4.39**). The amended-soil counts, demonstrated high statistical AHB post-hoc-variance, especially with the EFB soil at F: (p <0.05) = 1302.01. Although, EFB has not been widely reported in amending metal polluted soil, this study implicated it to be sustainable and/or viable. Hence, enforcing bacterial growths, and aiding the mineralization of Pb in soil.

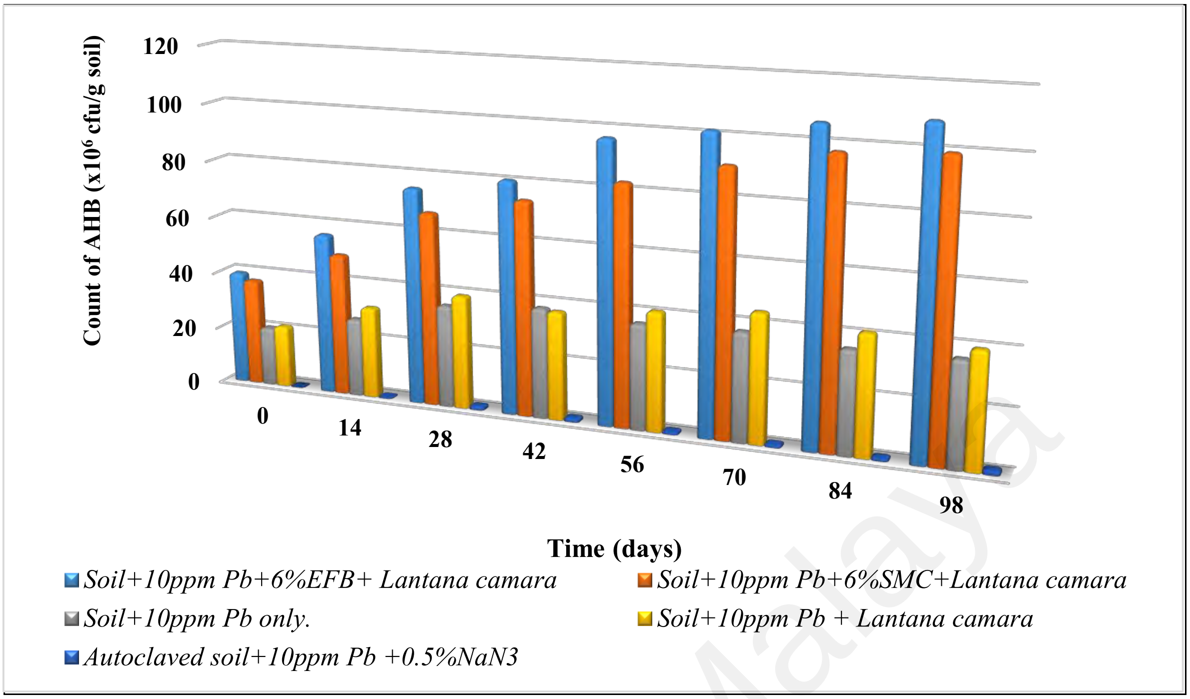


Figure 4.39: Counts of aerobic heterotrophic bacteria (AHB) in 10 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

In the absence of EFB and SMC apparently adopted for this study, some other organic wastes, suchlike; tea leaf, potato skin, banana skin, and rice straw etc., have been reportedly utilized for biological remediation of both diesel oil, and heavy metal (Cd, Cu, and Zn) polluted soils by Dadrasnia & Agamuthu (2010), Agamuthu et al., (2010), and Wu et al., (2012). This was ostensibly seen to be sustainable “ecologically”, in decontamination of polluted soils, evidenced from these authors’ documented results.

Moreover, the HMRB counts (**Figure 4.40**), were correspondingly less, and had upper limit ratios (i.e. AHB: HMRB) of 1.37:1.0, 1.57:1.0, and 1.25:1.0 for EFB amended soil, SMC, and *Lantana sp.* (unamended) control, respectively. This indicates, that not much of the bacteria population present in Pb-contaminated soils, are 100% obligate Pb-resistant in nature. However, the HMRB specific counts following the sequence above, numbered between 21.3 x 10^6 to 79.67 x 10^6 cfu/g, 18.0 x 10^6 to 63.3 x 10^6 cfu/g, and 12.0 x 10^6 to 32.0 x 10^6 cfu/g, respectively, at 10 ppm remediated soil, and statistically significant at F: (p <0.05) = 978.06.

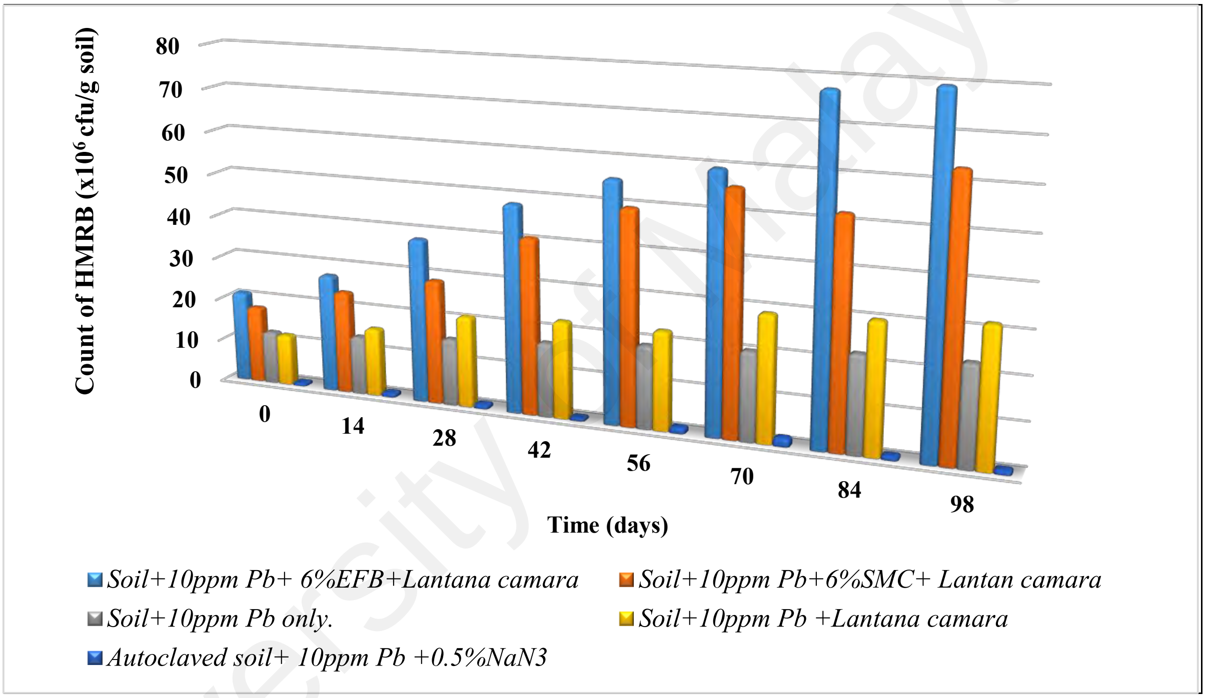


Figure 4.40: Counts of heavy metal-resistant bacteria (HMRB) in 10 mg/kg Pb contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

More HMRB units were seen in **Figure 4.40** remediated soils, than in 80, 40, and 20 ppm *Lantana sp.* counterparts. The possible explanation to this, might be that the presence of elevated concentration of heavy metal, i.e., Pb, interfered with soil microbial colonization (García-Delgado et al., 2015).

4.3.2 Microbial counts in soil contaminated with Cr

Under 40 ppm Cr-remediated soil using *Pilea sp.*, the amount of AHB and HMRB recorded varied. However, the former had values ranging between 21.0 x 10^6 to 75.0 x 10^6 cfu/g, 18.3 x 10^6 to 60.1 x 10^6 cfu/g, and 16.97 x 10^6 to 34.30 x 10^6 cfu/g for EFB amended soil, SMC-aided soil, and *Pilea sp.* (unamended) controls, respectively (**Figure 4.41**), at post-hoc significant F: (p <0.05) = 772.88. Correspondingly, the HMRB layouts ranged from 9.0 x 10^6 to 50.04 x 10^6 cfu/g (EFB soil), 7.97 x 10^6 to 41.03.07 x 10^6 cfu/g (SMC soil), and 3.9 x 10^6 to 23.07 x 10^6 cfu/g for (*Pilea sp.* unamended soil) respectively, at F: (p <0.05) = 699.50 significant point (**Figure 4.42**). These enumerations above, showed that EFB originated-soil counts, were the most significant.

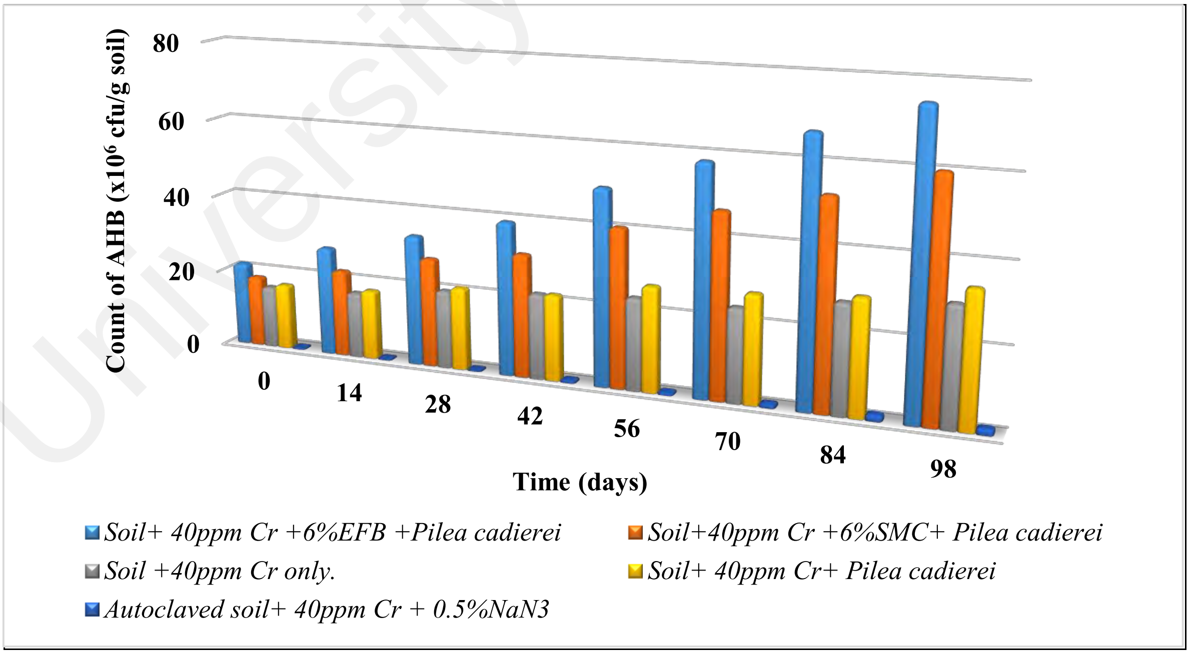


Figure 4.41: Counts of aerobic heterotrophic bacteria (AHB) in 40 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

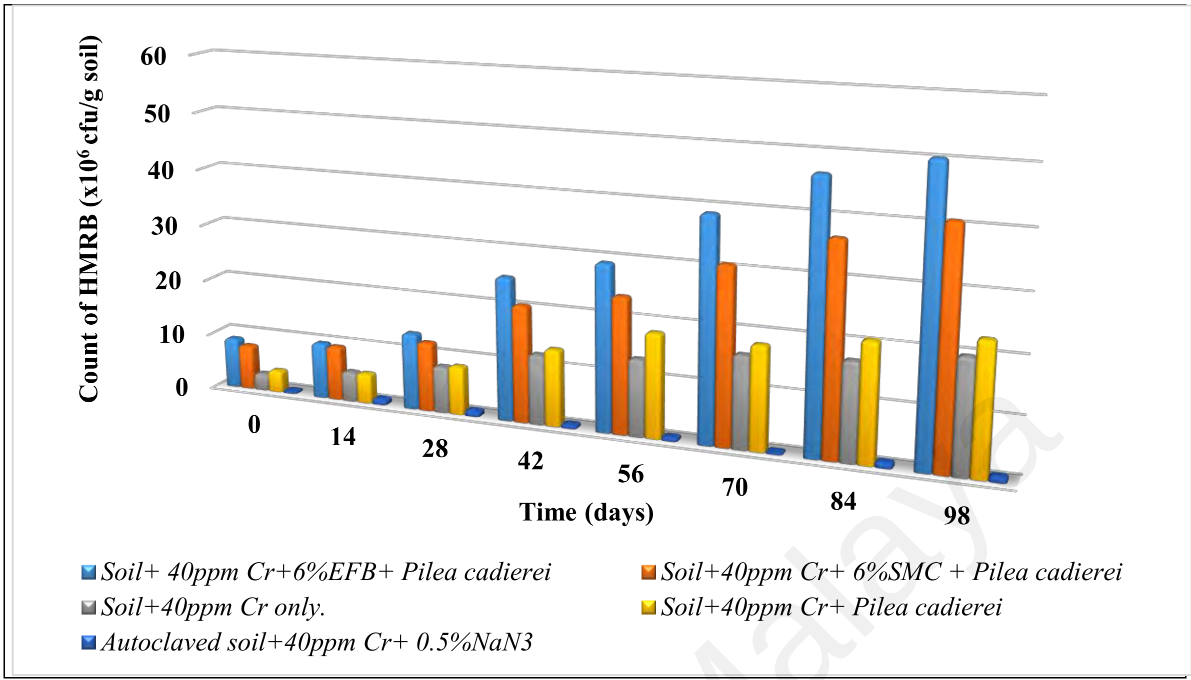


Figure 4.42: Counts of heavy metal-resistant bacteria (HMRB) in 40 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

However, the deviation at AHB and HMRB count points, may signify selective pressure on Cr-tolerant strains, when exposed to Cr rich-sediments. Alternatively, ability of the unamended *Pilea sp.* control, to sustain rhizosphere Cr-resistant microbes, indicates that the plant or bacteria itself, might have some metabolic-exudates (i.e., compounds), considered to be nutritive and/or supportive in nature, as to survive in a distressed soil ecosystem.

Furthermore, at *Lantana sp.* (40 ppm Cr) remediated soil, the compared ratios of AHB is to Cr-HMRB counts for *Lanata* against *Pilea*, recorded 1.06:1.0 and 1.11:1.0 (for rhizosphere EFB soil), 1.04:1.0 and 1.11: 1.0 (for rhizosphere SMC soil), and as well 1.10 :1.0 and 1.09:1.0 (for rhizosphere *Lantana sp.* and *Pilea sp.* unamended soil), respectively. Meanwhile, the specific counts attained by AHB and HMRB, under 40 ppm Cr-remedied

Lantana sp. soil, registered between 23.67 x 10^6 to 79.67 x 10^6 cfu/g and 9.33 x 10^6 to 55.97 x 10^6 cfu/g for (soils amended with EFB), 19.67 x 10^6 to 62.67 x 10^6 cfu/g and 7.66 x 10^6 to 45.63 x 10^6 cfu/g for (soils amended with SMC), and finally 15.3 x 10^6 to 37.67 x 10^6 cfu/g and 5.33 x 10^6 to 25.3 x 10^6 cfu/g for (*Lantana sp.* unamended soil), respectively. Statistically, the AHB counts were significant at F: (p <0.05) = 941.63, and expressed in **Figure 4.43**. While the HMRB equally recorded a post-hoc significant level of F: (p<0.05) =911.33, and graphically displayed in **Figure 4.44**.

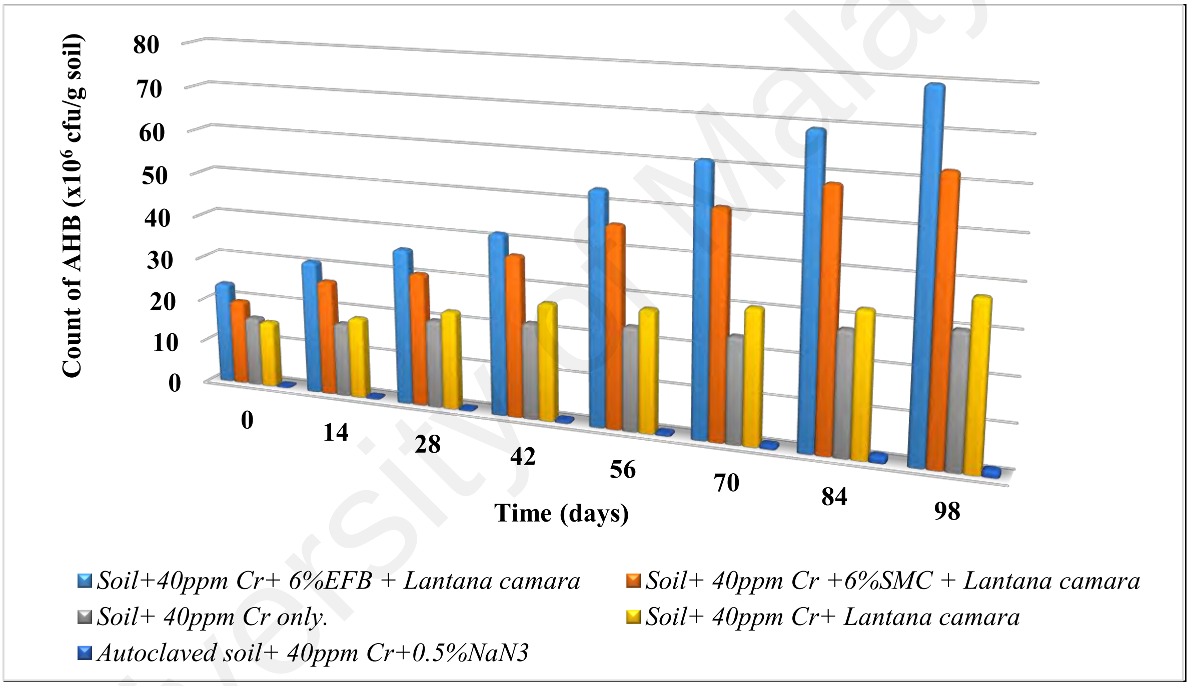


Figure 4.43: Counts of aerobic heterotrophic bacteria (AHB) in 40 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

Following the trends of these counts above, it is most probable that the presence of elevated Cr-density exerted selective pressure on the soil microbial ecology, and favoured more resistant Cr-bacteria at the *Lantana sp.* and/or *Pilea sp.* rhizosphere region, where it is assumed that soluble Cr concentration were high, due to dynamic (plant) root association.

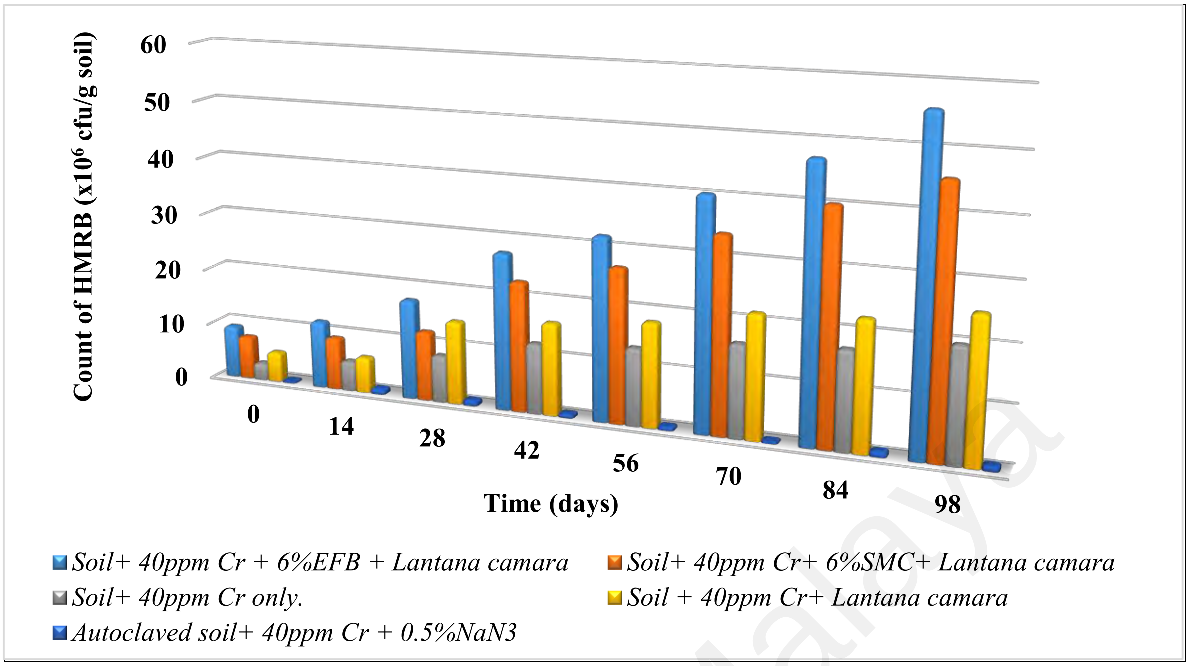


Figure 4.44: Counts of heavy metal-resistant bacteria (HMRB) in 40 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

However, Pérez-de-Mora et al., (2006) have also indicated such trends, in soil microbial ecology study of heavy metal contaminated soil. While, Zhang et al., (2012) subsequently reported after long exposure of heavy metals in soil, with *Chenopodium ambrosioides* (i.e., Mexican tea plant), certain bacteria strains suchlike; *Acidobacteria*, *Bacteroidetes*, and *Verrucomicrobia* became relatively stable at the rhizosphere soil, indicating established resistance. Therefore, the findings of Zhang et al., (2012) may be partially linked to this current study, in terms of microbial resistance in heavy metal contaminated soil.

With respect to 20 ppm Cr remediated soil, rhizobacteria varied between AHB and HMRB counts under *Pilea* plant, as shown in **Figures 4.45** and **4.46**, respectively.

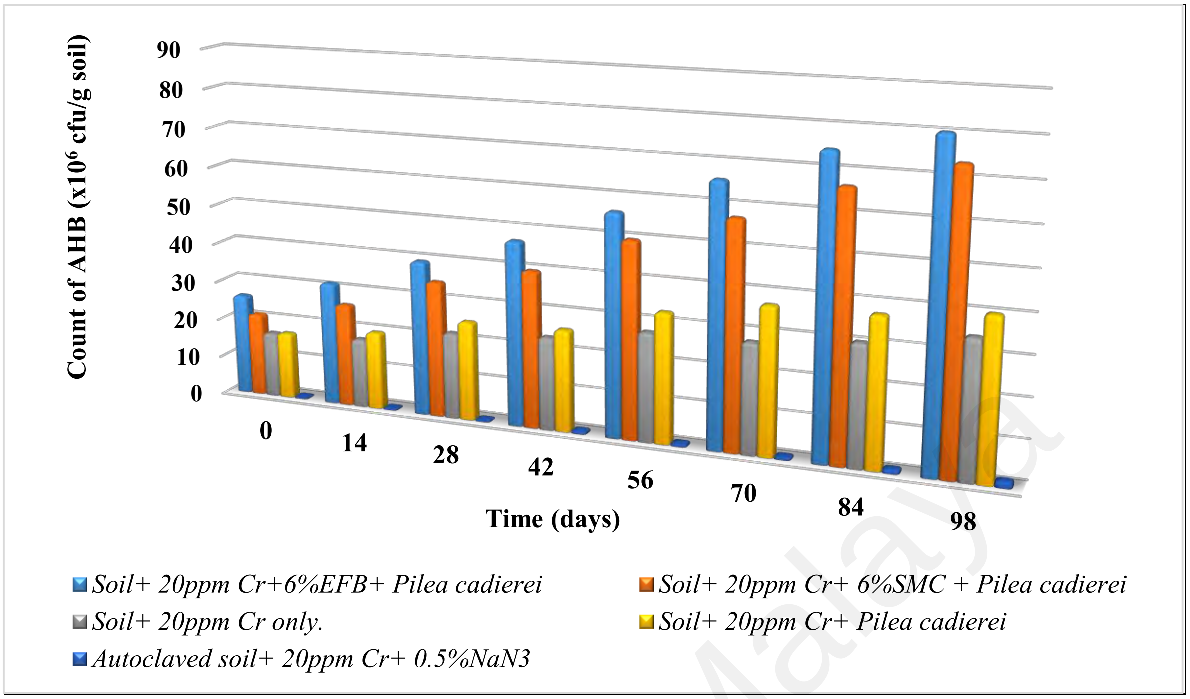


Figure 4.45: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

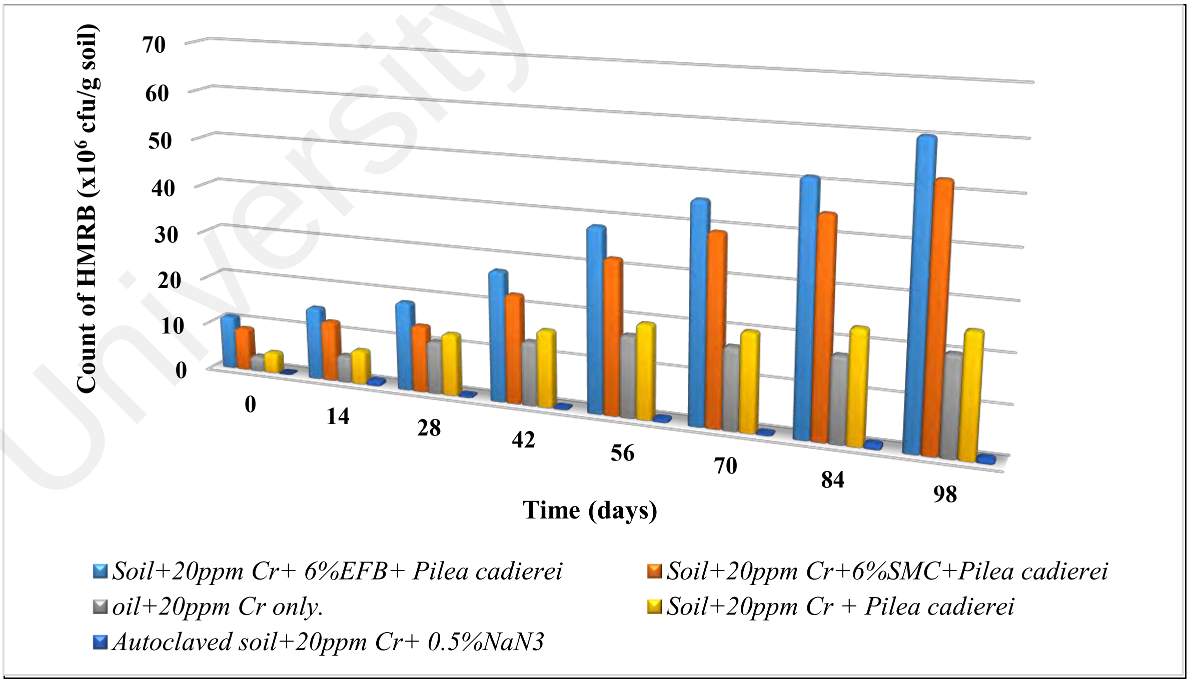


Figure 4.46: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: readings were taken at n=3.

Highest AHB counts were seen in organic wastes amended soils, and more precisely at EFB with 26.0 x 10^6 to 80.27 x 10^6 cfu/g, and statistically significant at F: (p <0.05) = 922.08. While, HMRB recorded counts between 38.0 x 10^6 to 60.33 x 10^6cfu/g under EFB-aided soil, at post-hoc significant F:(p <0.05) = 889.67, over the rest treatments assayed. Most apparently, the added agro-wastes boosted the nutrient profile of the Cr-soil, and supported microbial growth. Even at that, toxicity still played-down on their survival rate, and this manifested through the reduced HMRB numbers, consistently expressed.

Similarly, the AHB and HMRB counts recorded under *Lantana sp.* (20 ppm Cr) remediated soil, followed same order (see **Figures 4.47 and 4.48**). Statistical post-hoc significant of F: (p <0.05) = 861.08, and F: (p <0.05) = 840.21 for AHB, and HMRB counts, respectively, mostly in favour of EFB than SMC amended soils (probably due to nutrient variability). But one major contrasting difference *Lantana sp.* had with *Pilea sp.*, is the emergence of more AHB and HMRB units under *Lantana sp*. The reason, may be that some bacteria had probably established long association with the *Lantana sp.*-rhizosphere than with *Pile*a *sp*., hence this. However, He et al., (2010) demonstrated such interaction between Cu-resistant rhizobacteria associated to plant, in addition to Ma et al., (2011) rhizosphere study on metalliferous soils, respectively.

It was further reported by Mengoni et al., (2001) that *Pseudomonas* and *Arthrobacter* spp., mainly had higher relative abundance in the rhizosphere of Ni-hyperaccumulating plant (*Alyssum bertolonii*). However, this result may slightly differ from that of Mengoni et al., (2001), because of the different plants involved and/or application.

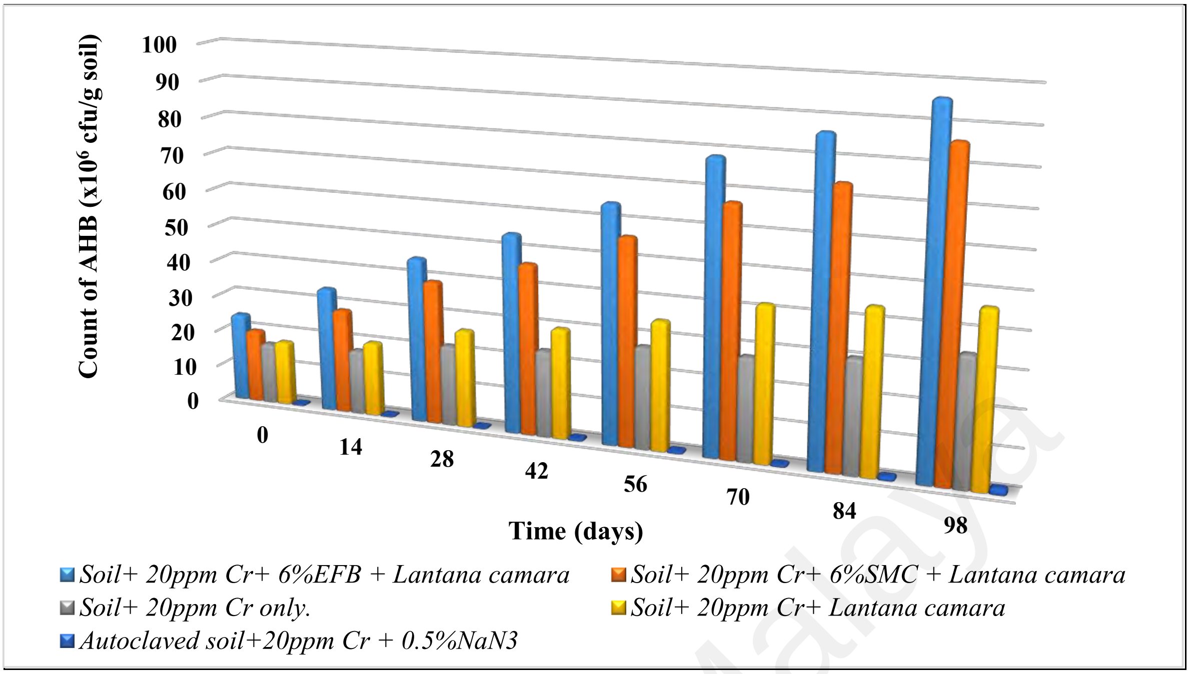


Figure 4.47: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

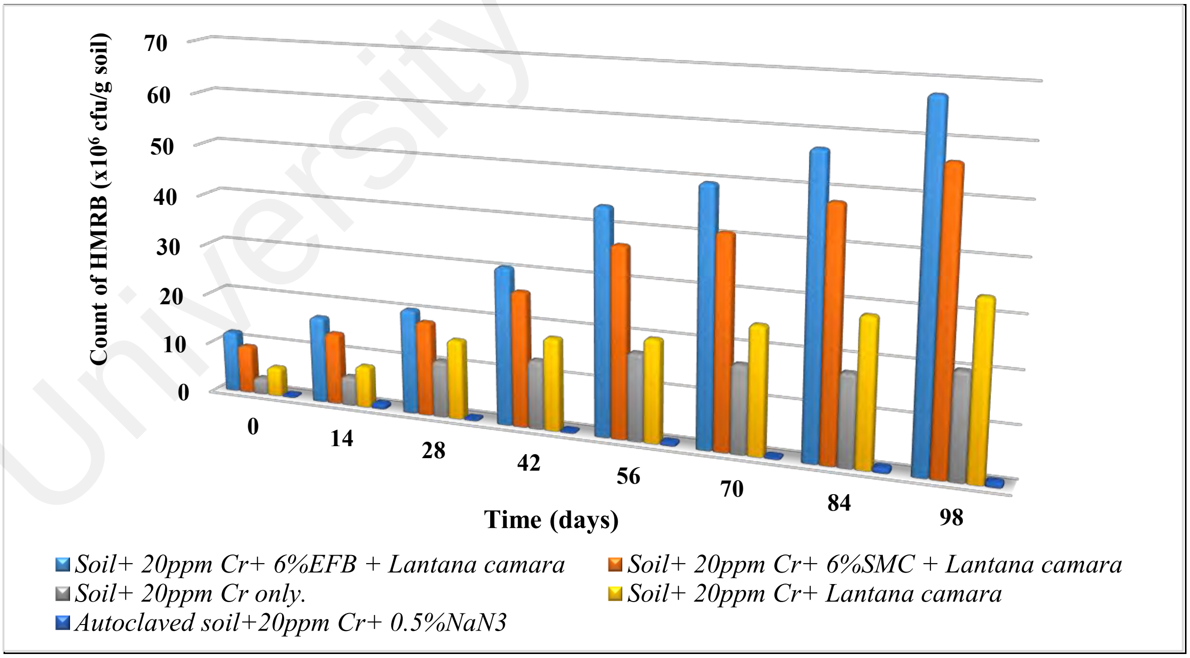


Figure 4.48: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: readings were taken at n=3.

Lastly, the microbial population (i.e., AHB and HMRB) in *Pilea sp.* and *Lantana sp.* rhizosphere of 10 ppm Cr remediated soils evaluated, varied proportionally. Both AHB and HMRB counts, implicated under *Pilea sp.* ranged between 34.03 x 10^6 to 99.77 x 10^6 cfu/g and 18.3 x 10^6 to 66.17 x 10^6 cfu/g (for EFB amended soils), 27.53 x 10^6 to 86.33 x 10^6 cfu/g and 13.0 x 10^6 to 57.5 x 10^6 cfu/g (for SMC aided soils), and 21.03 x 10^6 to 47.0 x 10^6 cfu/g and 9.13 x 10^6 to 26.0 x 10^6 cfu/g for (unamended 10 ppm Cr *Pilea sp.* and *Lantana sp.* soils), respectively. Layouts of AHB counts are shown in **Figure 4.49**, with significant level F :($p<0.05$) =1110.05.While HMRB counts (**Figure 4.50)** had post-hoc significant F: ($p<0.05$) = 993.54.

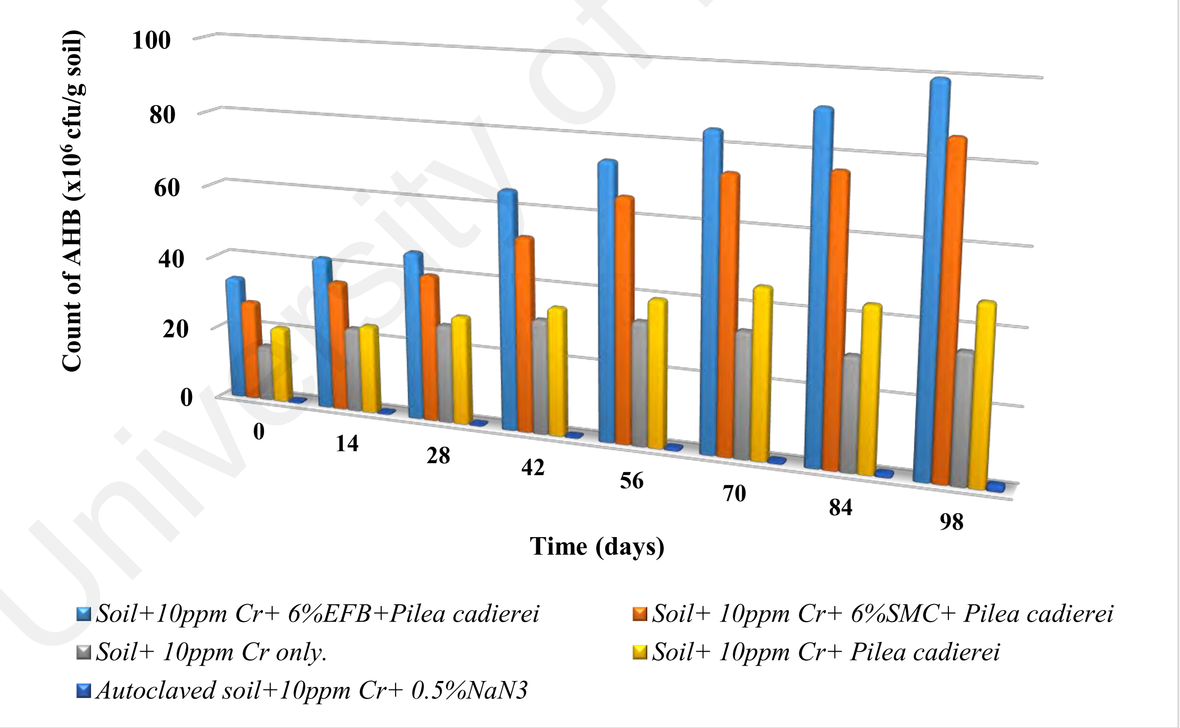


Figure 4.49: Counts of aerobic heterotrophic bacteria (AHB) in 10 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: average readings taken at n=3.

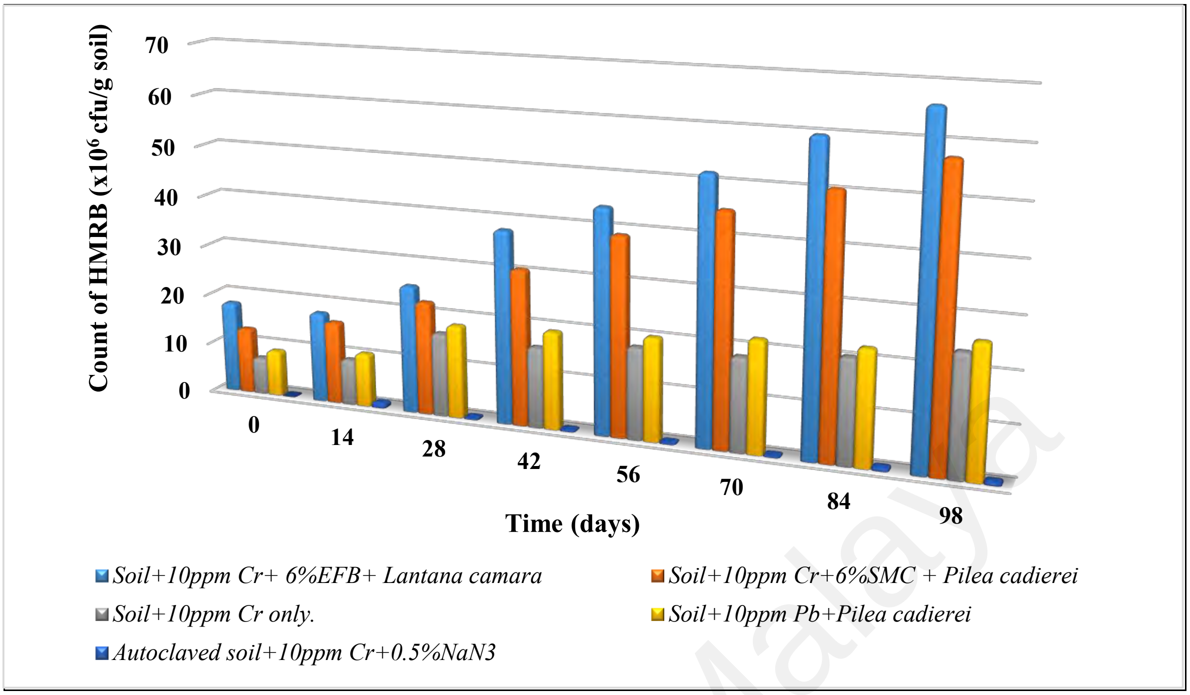


Figure 4.50: Counts of heavy metal-resistant bacteria (HMRB) in 10 mg/kg Cr contaminated soil under *Pilea cadierei* after 98 days.

Note: average readings taken at n=3.

However, under *Lantana sp.* 10 ppm Cr remediated soil, the AHB (**Figure 4.51**), and HMRB (**Figure 4.52**) recorded highest units, thus at amended soils. Hence, displaying 36.63 x 10^6 to 104.5 x 10^6 cfu/g, for (AHB counts); [F:(p<0.05) =1110.05], and 19.3 x 10^6 to 71.0 x 10^6 cfu/g for (HMRB counts); [F:(p<0.05) =1110.05], and mostly significant under EFB amended soil. The reason behind this low count ratios recorded in HMRB against the AHB, in Cr-polluted soils, may be due to ecological selective-pressures, to uncover the emergence of effective Cr-resistant strains. Although, possibility exist that HMRB strains, could also be plant growth promoting bacteria (PGPB), however this is outside the scope of this current study. Meanwhile, Ahemad, (2015) reported Cr-resistant *Bacillus* species PSB10, and *Pseudomonas* sp. RNP4, isolated from the rhizospheres regions of *Brassica* spp., and *Cicer arietinum* L, that concurrently functioned like PGPB, and also accelerated the remediation of metalliferous soils.

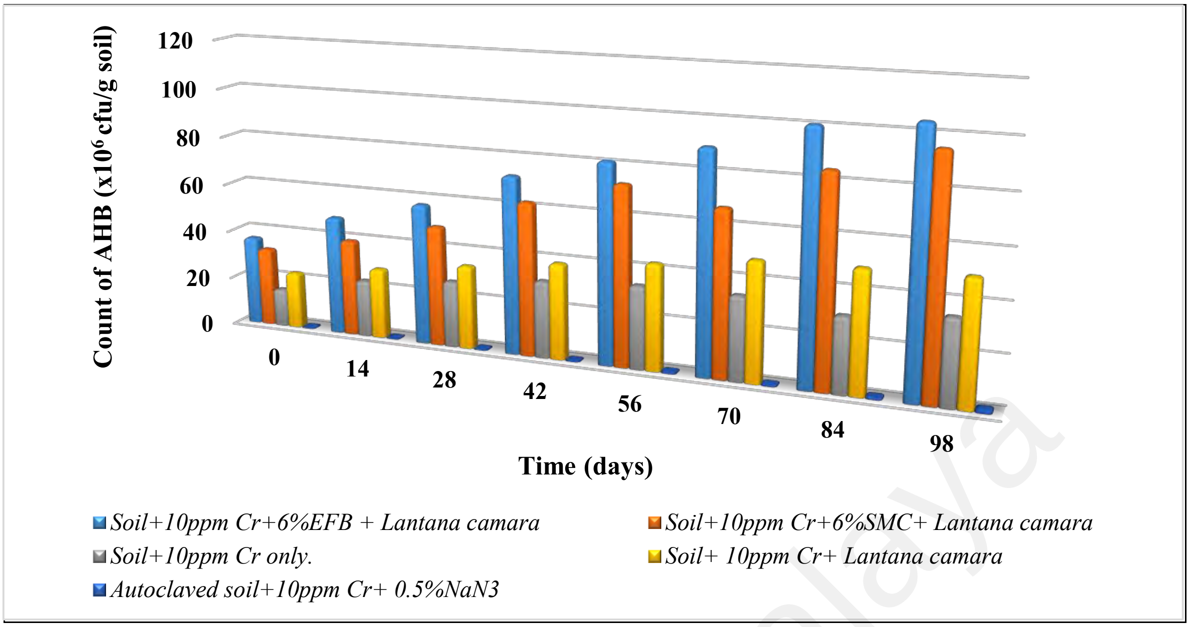


Figure 4.51: Counts of aerobic heterotrophic bacteria (AHB) in 10 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: average readings taken at n=3.

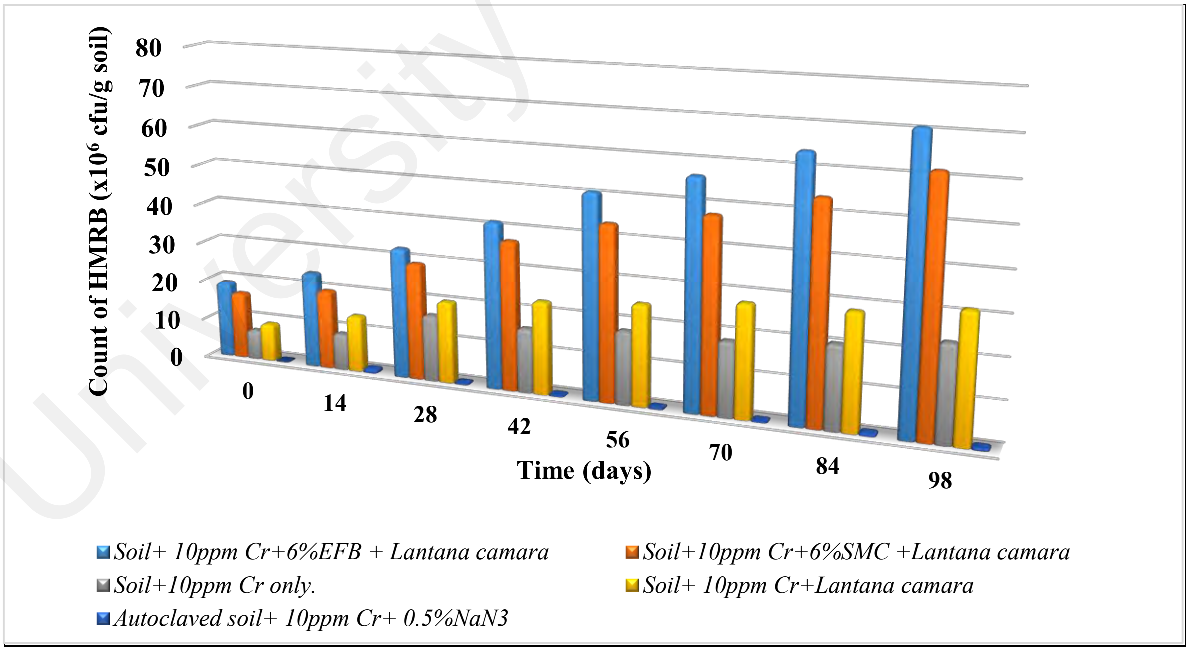


Figure 4.52: Counts of heavy metal-resistant bacteria (HMRB) in 10 mg/kg Cr contaminated soil under *Lantana camara* after 98 days.

Note: average readings taken at n=3.

Also, the elevated number of AHB and HMRB counts recorded in 10 ppm Cr remediated soil, against its 40 and 20 ppm Cr counterparts, could be as a result of relatively lower soil metal exposure (10 ppm Cr), and befitting nutrient range. Abou-Shanab et al., (2005) in their findings, reported that elevated heavy metal concentrations of 39 mg/kg Co, 3 mg/kg Cd, 79 mg/kg Ni, 114 mg/kg Pb, and 123 mg/kg Cr in dry soil, were responsible for relatively low bacteria count, at the rhizosphere of *Diplachne fusca*. Therefore, the findings of Abou-Shanab et al., (2005) can plausibly be linked to this current phenomenon.

4.3.3 Microbial counts in soil co-contaminated with 20 ppm (Pb+Cr)

In heavy metal co-contaminated soil, it is plausible to state that more toxic pressures have been exerted towards the edaphic bacteria. However, under *Pilea sp.* remediated 20 ppm (Pb+Cr) paired metal pollution, AHB and HMRB counts ranged between 23.07 x 10^6 to 69.0 x 10^6 cfu/g and 11.6 x 10^6 to 39.57 x 10^6 cfu/g (for EFB amended soil), 19.73 x 10^6 to 61.0 x 10^6 cfu/g and 8.83 x 10^6 to 36.07 x 10^6 cfu/g (soil amended with SMC), and 9.2 x 10^6 to 39.0 x 10^6 cfu/g and 4.33 x 10^6 to 21.3 x 10^6 cfu/g (unamended *Pilea sp.* soil) as shown in **Figures 4.53** and **4.54** at statistical significance F:($p<0.05$) =221.05 for AHB, and F:($p<0.05$) =201.10 for HMRB. In extension, the AHB: HMRB ratio for biological monitoring of metal loss in soil was 1.38:1.0., with insignificant abiotic loss-soil count.

In continuation, the amended co-contaminated soils, recorded higher bacteria counts when compared to the unamended. Therefore, Olaniran et al., (2013) deduced that the addition of organic substrate, might increase soil nitrate, due to N-transformation, via microbial degradation of organic waste C-N bonds, and thereafter nutrient recycling within the soil.

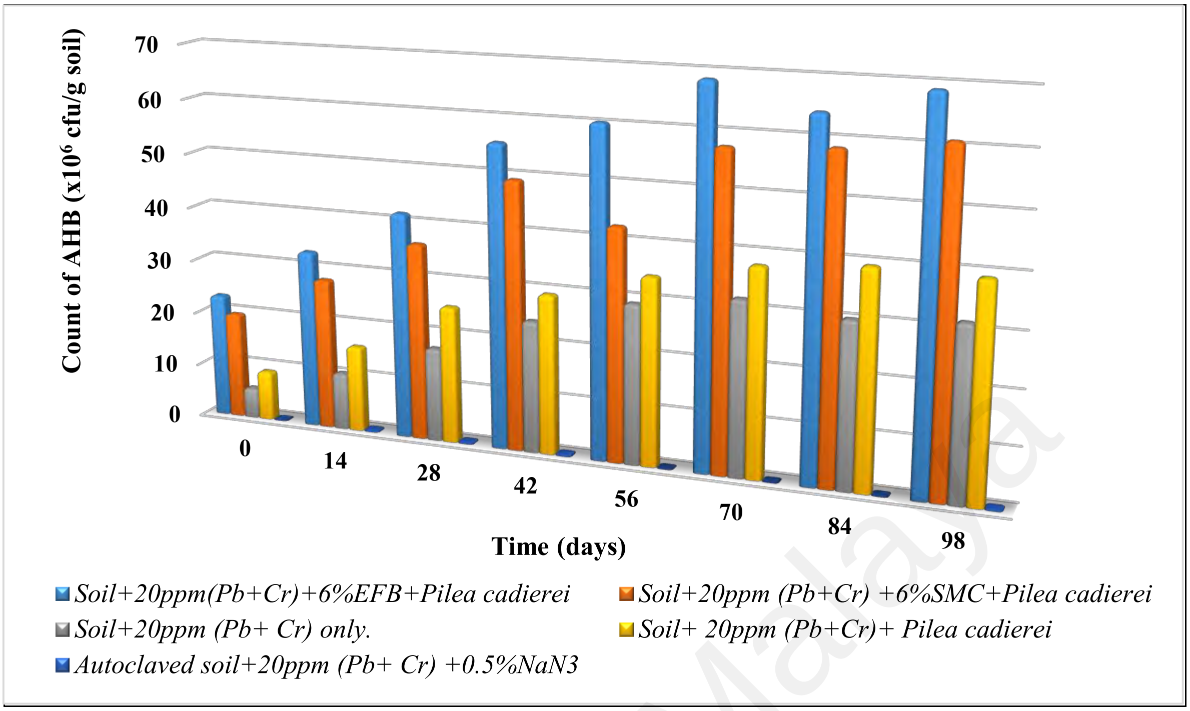


Figure 4.53: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg (Pb+Cr) co-contaminated soil under *Pilea cadierei* after 98 days.

Note: average readings taken at n=3.

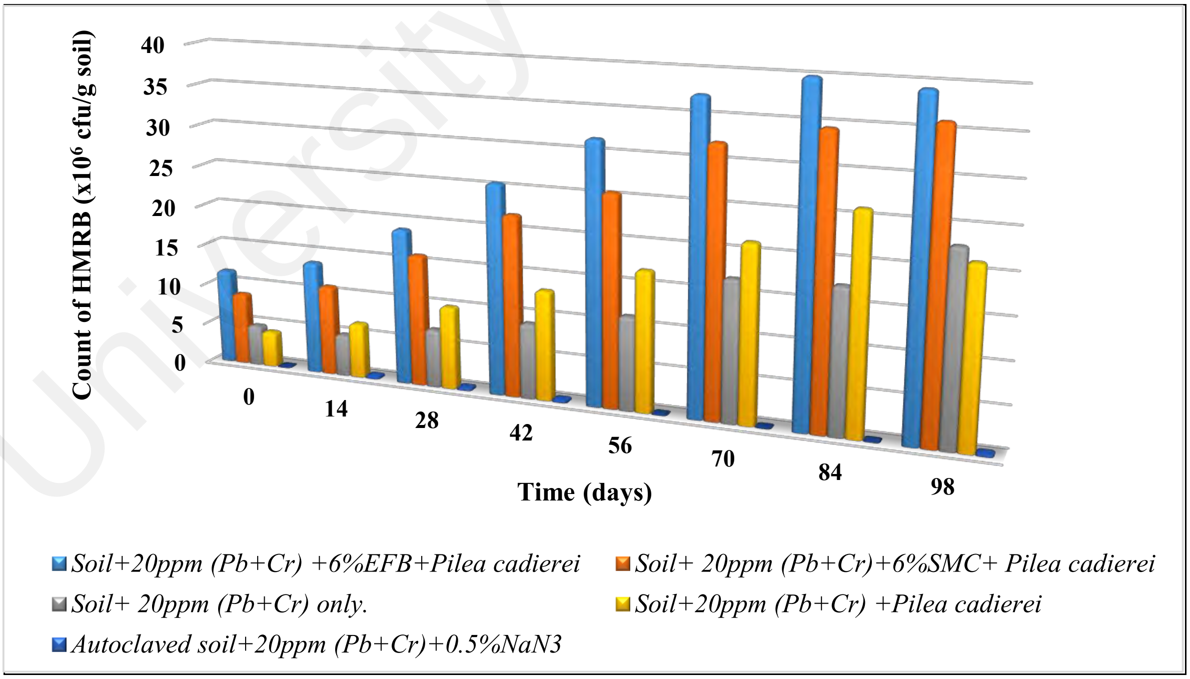


Figure 4.54: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg (Pb+Cr) co-contaminated soil under *Pilea cadierei* after 98 days.

Note: average readings taken at n=3.

At *Lantana sp.* co-contaminated soil remediation, AHB: HMRB ratios of about 1.79:1.0 (for EFB augmented soil), 1.69:1.0 (for SMC aided soil), and 1.63: 1.0 for (unamended) *Lantana sp.* soil, were obtained and graphically shown in **Figures 4.55** and **4.56**, respectively, with corresponding post-hoc significant F:(p<0.05) =327.88 for AHB, and F:(p<0.05) =299.05 for HMRB. Hence, conferring EFB amended soil, to be the most significant biowaste. Similarly, Haroun et al., (2007) reported a 50-day composting treatments that relatively increased the removal of Cr, Pb, Cd, Cu, and Zn in multiple metal "tannery waste" contaminated site; but alongside recorded significant reduction in microbial density.

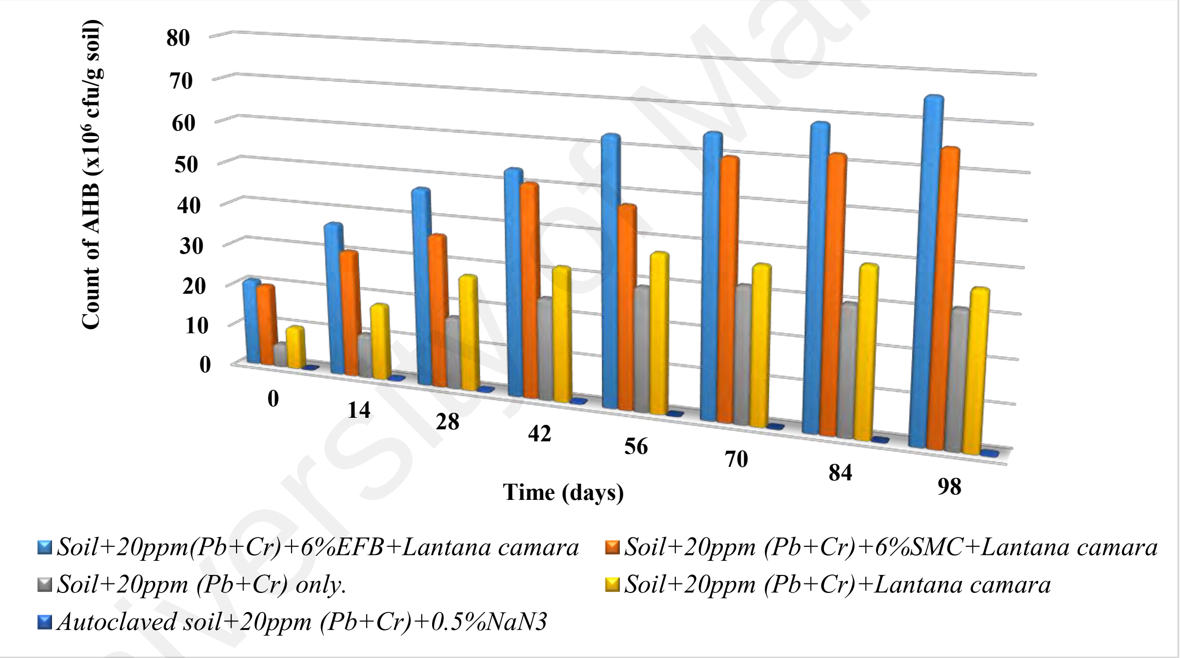


Figure 4.55: Counts of aerobic heterotrophic bacteria (AHB) in 20 mg/kg (Pb+Cr) co-contaminated soil under *Lantana camara* after 98 days.

Note: average readings taken at n=3.

Generally, lower counts of AHB and HMRB were seen at co-contaminated soil remediation, as opposed to the lone 20 ppm Pb or 20 ppm Cr soil (**Figures 4.33** to **4.36**, and **Figures 4.45** to **4.48**). This may be attributed to double "inhibitory" stress, imposed by unspecific metal speciation from Pb and Cr towards microbial cells. For example, Yang et al., (2016) reported

Co, Cd, and Pb multiple soil pollution, which triggered a cease in the metabolisms of *Bacillus licheniformis*. Therefore, it is plausible to conclude that the interaction between Pb and Cr in this current study, may have altered absorption kinetics and/or dynamics of the metals, thereby exacerbating toxicity on specific microbes.

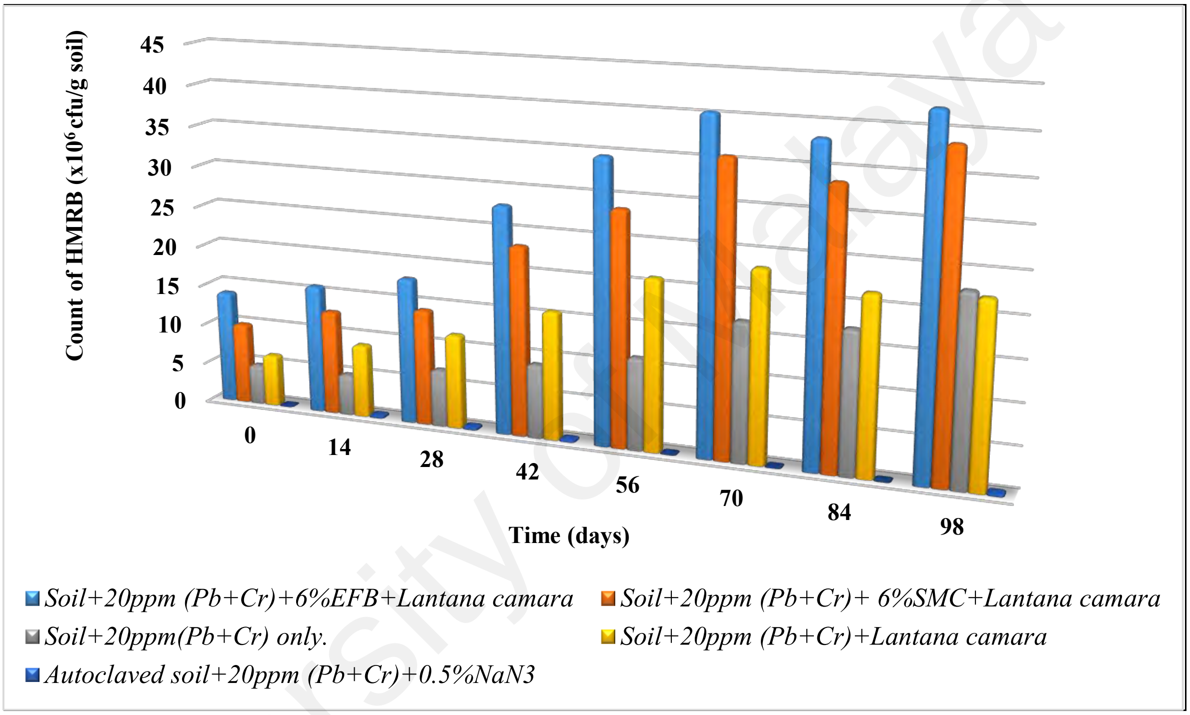


Figure 4.56: Counts of heavy metal-resistant bacteria (HMRB) in 20 mg/kg (Pb+Cr) co-contaminated soil under *Lantana camara* after 98 days.

Note: average readings taken at n=3.

4.3.4 Heavy metal resistant bacteria (HMRB)

The heavy metal resistant bacteria (HMRB) isolated from Pb and Cr contaminated soil, and identified with Biolog® micro-station were three, namely; *Enterobacter cloacea* (isolated from Pb remediated soils), *Bacillus pumilus* (isolated from Cr remediated soil), and *Lysinibacillus sphaericus* (isolated from both Pb and Cr soils), respectively. These bacteria species had been implicated in heavy metal resistant and/or tolerance by different authors (Kavamura & Esposito, 2010; He et al., 2010; Lozano & Dussán, 2013; Naik & Dubey, 2013; Ellis et al., 2003; Babu et al., 2013; Lin et al., 2016). However, the presence of *L. sphaericus* in either Pb or Cr remedied soils, implicated it to have wider resistance and/or tolerance mechanisms. Such mechanisms may include; enhanced intra and extracellular metal sequestration and/or metal efflux-pumps activity, with more complex metal-chelators, suchlike metallothioneins and/or biosurfactants (Sandrin & Maier, 2003). The actions of *L. sphaericus* can plausibly be defined as bi-spectrum heavy metal resistant bacteria, within the context of this study. Mench et al., (2009) opined that interactions of bacterial siderophores can increase labile metal pools and uptake by roots.

4.4 Soil pH analysis and associated rhizosphere exudates assessment

The rhizosphere soil pH, and its surrounding exudates are dependent factors in soil heavy metal phytoremediation. Hence, these results shall be discussed under this sub-section:

4.4.1 pH and exudates under *Pilea sp.* and *Lantana sp.* Pb and/or Cr remediated soil

The soil ab-initio pH used for phytoremediation was pH 6.13, and the organic wastes had pH 6.12 (EFB) and 5.57 (SMC), respectively (**Table 4.1**). However, the pH values within 98 days under *Pilea sp.* at 80 to 10 ppm Pb remediated soils (**Figures 4.57 to 4.60**), ranged about 6.11 to 6.77, 5.48 to 6.01, and 6.51 to 6.14 for the EFB amended soil, SMC supplemented, and *Pilea sp.* (unamended) control, respectively. But, the un-contaminated plant control treatment (i.e., *Pilea sp.* without Pb and organic additives) had pH 6.23 to 6.24, while the autoclaved sodium-azide (abiotic) control soil, recorded an elevated pH ranged between 6.85 to 8.71, due to alkaline nature of Na.

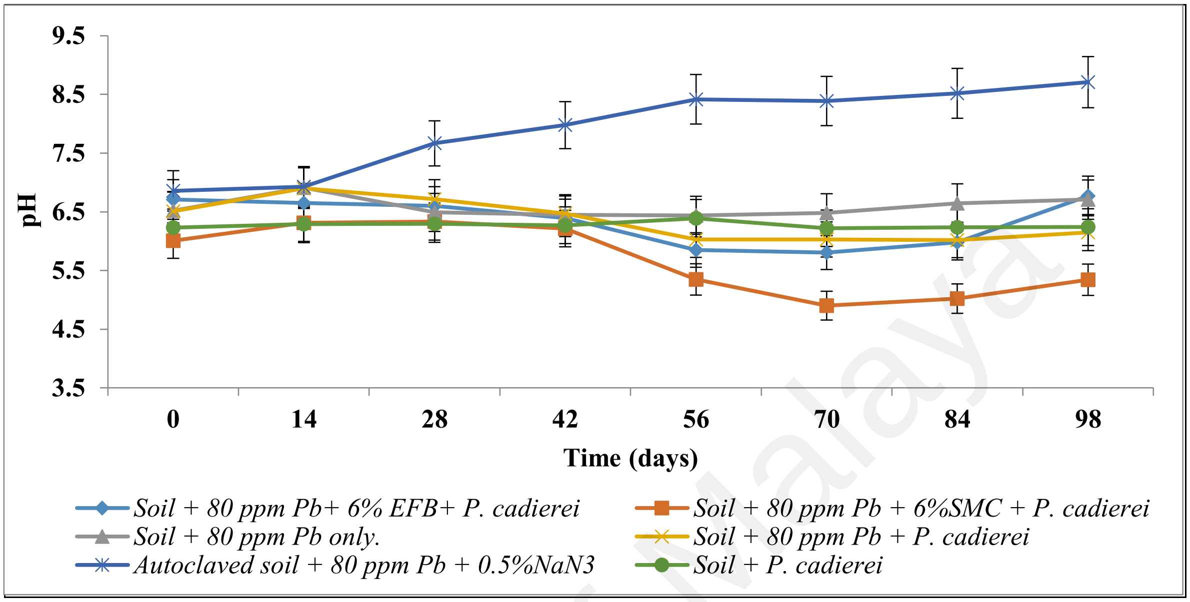


Figure 4.57: pH for 80 ppm Pb contaminated soil under *Pilea cadierei* Phytoremediation Bars denotes standard error (n=3).

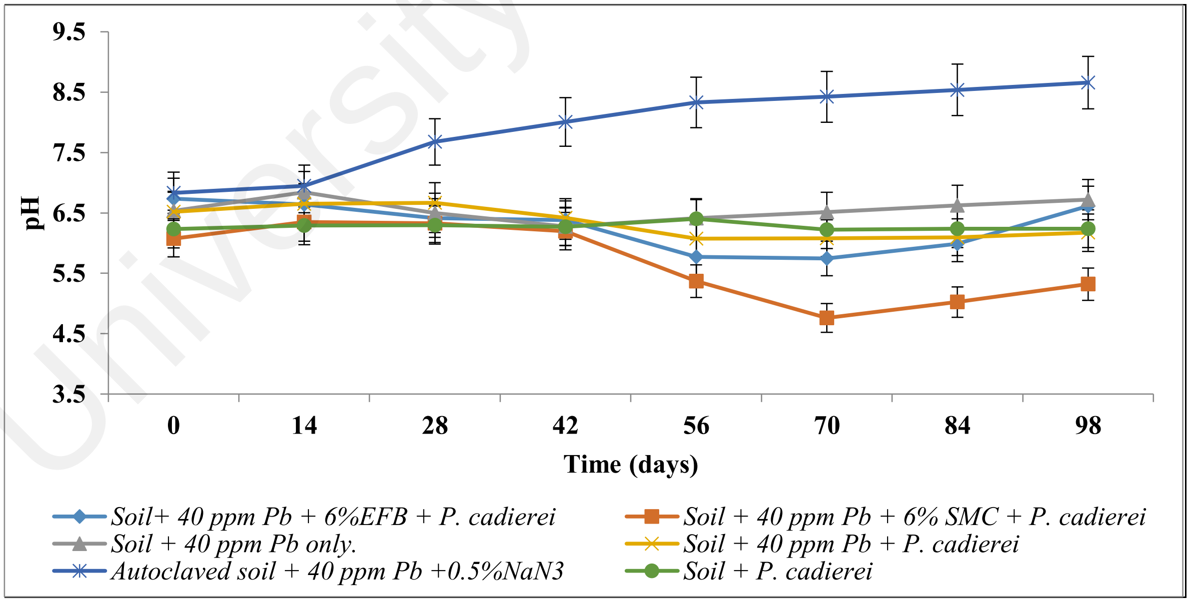


Figure 4.58: pH for 40 ppm Pb contaminated soil under *Pilea cadierei* Phytoremediation Bars denotes standard error (n=3).

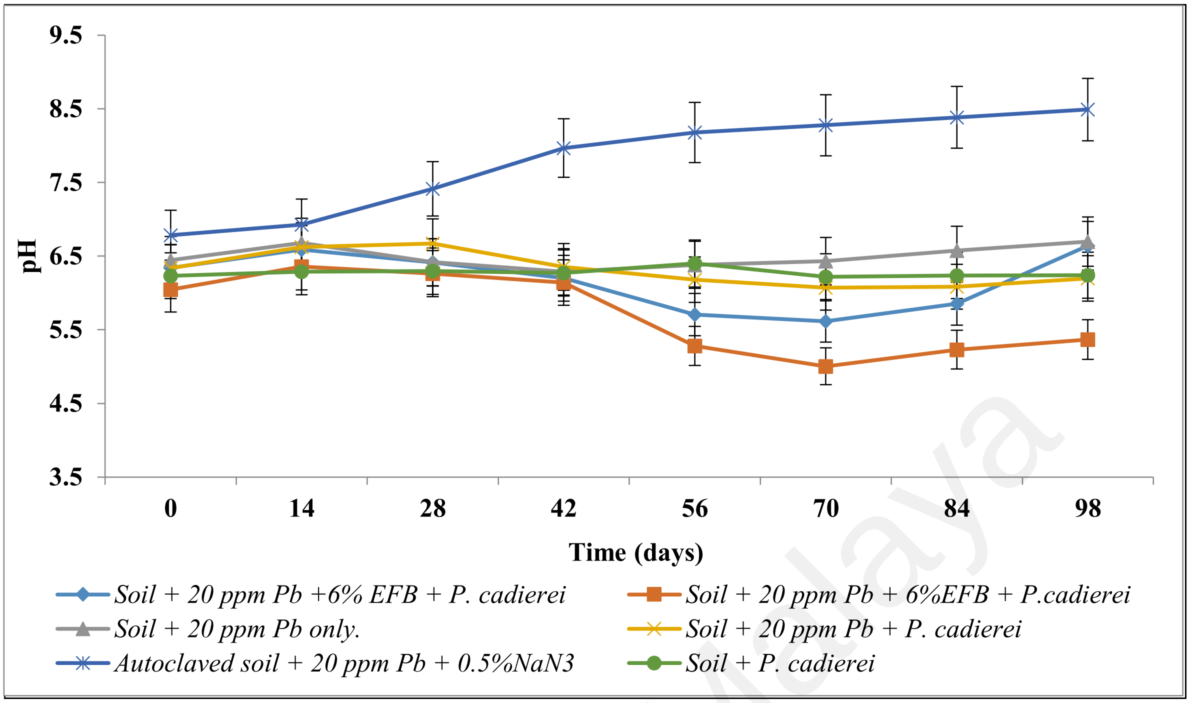


Figure 4.59: pH for 20 ppm Pb contaminated soil under *Pilea cadierei* Phytoremediation Bars denotes standard error (n=3).

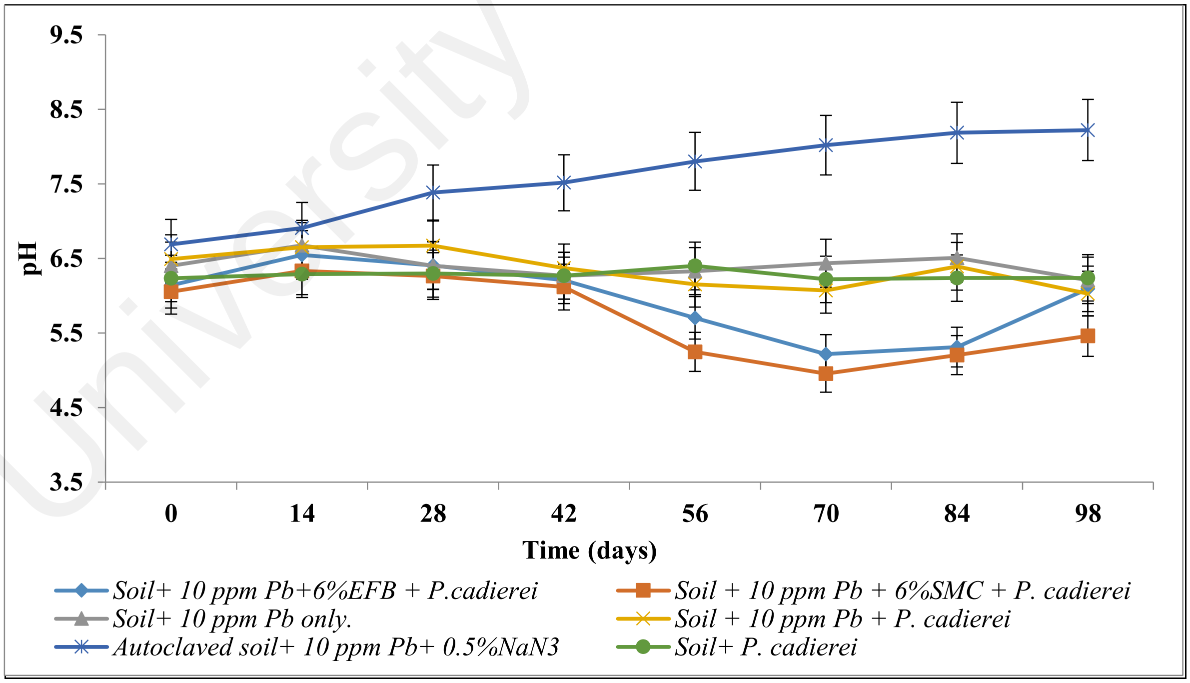


Figure 4.60: pH for 10 ppm Pb contaminated soil under *Pilea cadierei* Phytoremediation Bars denotes standard error (n=3).

Surprisingly, between 56 to 84 days, there was consistent drop in pH, ranging between pH 5.86 to 5.98, pH 5.35 to 5.02, and pH 6.03 to 6.01. At corresponding EFB amended soils, SMC supplemented, and *Pilea sp.* (unamended) control, respectively. This could be regarded as the optimum pH range for Pb phytoremediation. Because, at this specific time-intervals of pH-drop, there was between 29.85 to 65% disappearance of Pb, from the total percentage reduction of Pb in the remediated soils. However, acidification of the rhizosphere soil during phytoremediation, may be attributed to the secretion of H^+ ions by *Pilea sp.* root, thereby increasing Pb dissolution from soil (Ali et al., 2013). Because H^+ can displace heavy metal cations adsorbed to soil particles (Ali et al., 2013; Alaribe & Agamuthu, 2015), hence propelling Pb-availability, and thereafter its phytoextraction. This result concurred with Wang et al., (2006) who reported soil pH 5-6, to be the optimum for Cd and Zn uptake at the shoot of *T. caerulescens*.

Also, Chapman et al., (2013) further mentioned other plants that survived Zn contaminated soil, at relatively low rhizosphere pH, suchlike *Lolium perenne* (pH 4.3), *Sorghum bicolour* (pH 4.3), *Trifolium pratense* (pH 3.9), and *Trigonella foenum-graecum* (pH 2.0). These can all be likened to *Pilea*, under Pb soil influence. Except that, the aforementioned plant species, displayed greater acidic rhizosphere soil, probably because they discharged more organic acidic exudates. More so, different heavy metal (Pb) was used in this current study, as opposed to Zn mentioned by Chapman et al., (2013). This could instigate variation between both studies.

In continuation, at *Lantana sp.* 80 to 10 ppm Pb remediated soils, three phase of pH ranges were noticed in the supplemented soils; pH 6.64 – 6.01 (0 to 42 days), pH 5.01- 4.50 (56 to

70 days), and pH 4.79 – 5.95 (84 to 98 days) (**Figures 4.61** to **4.64**). This however, proposes that the medium was also acidic, hence in line with Chapman et al., (2013) findings.

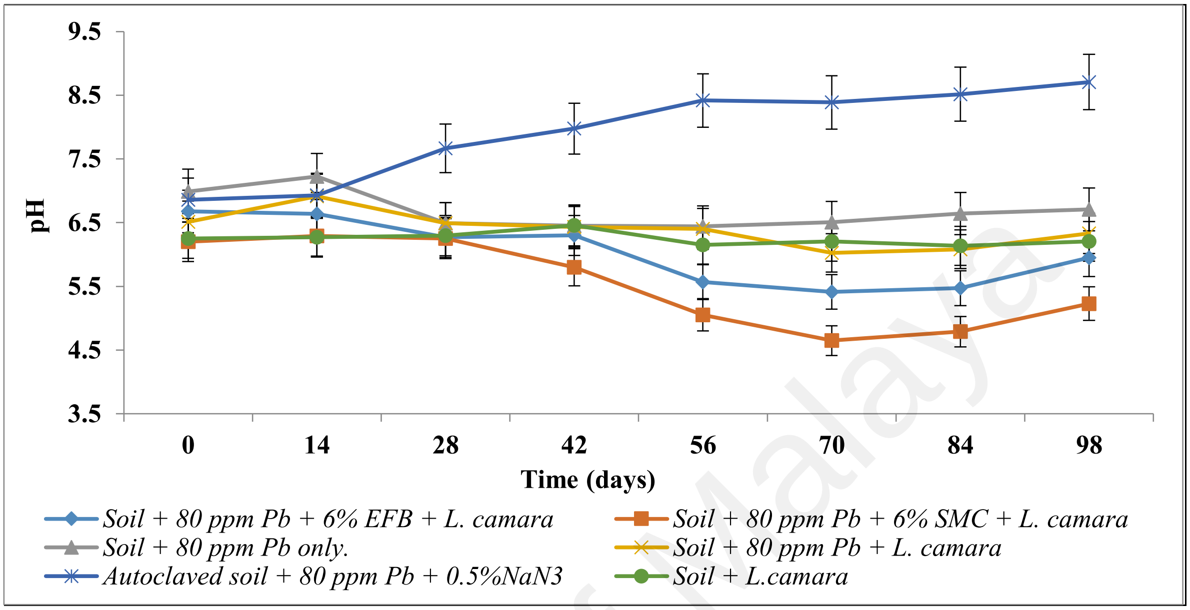


Figure 4.61: pH for 80 ppm Pb contaminated soil under *Lantana camara* Phytoremediation Bars denotes standard error (n=3).

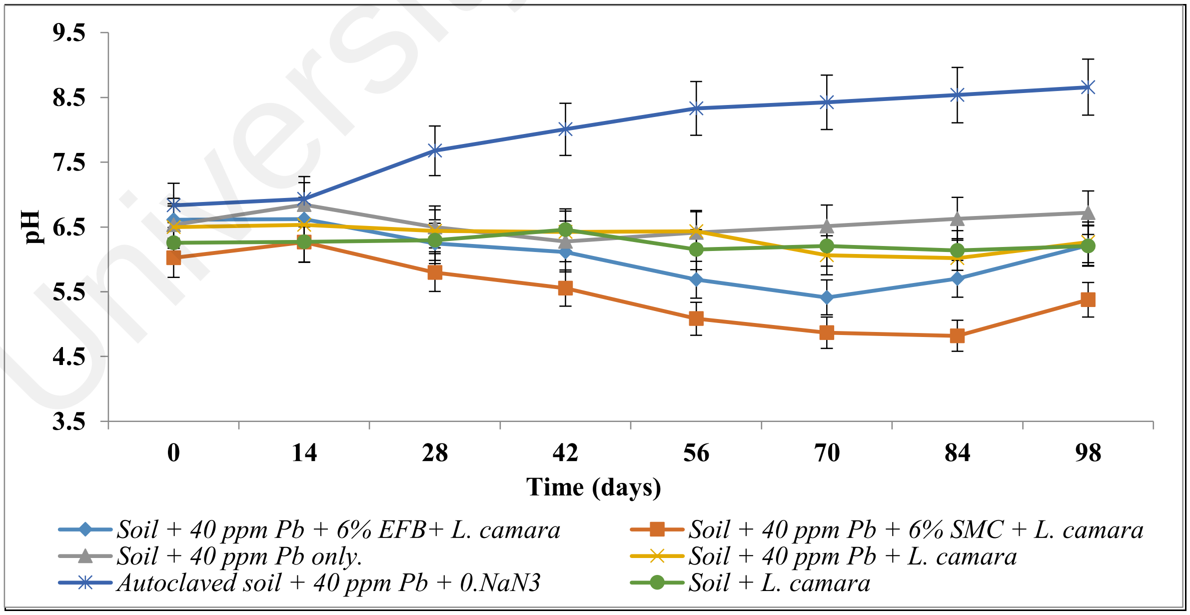


Figure 4.62: pH for 40 ppm Pb contaminated soil under *Lantana camara* Phytoremediation Bars denotes standard error (n=3).

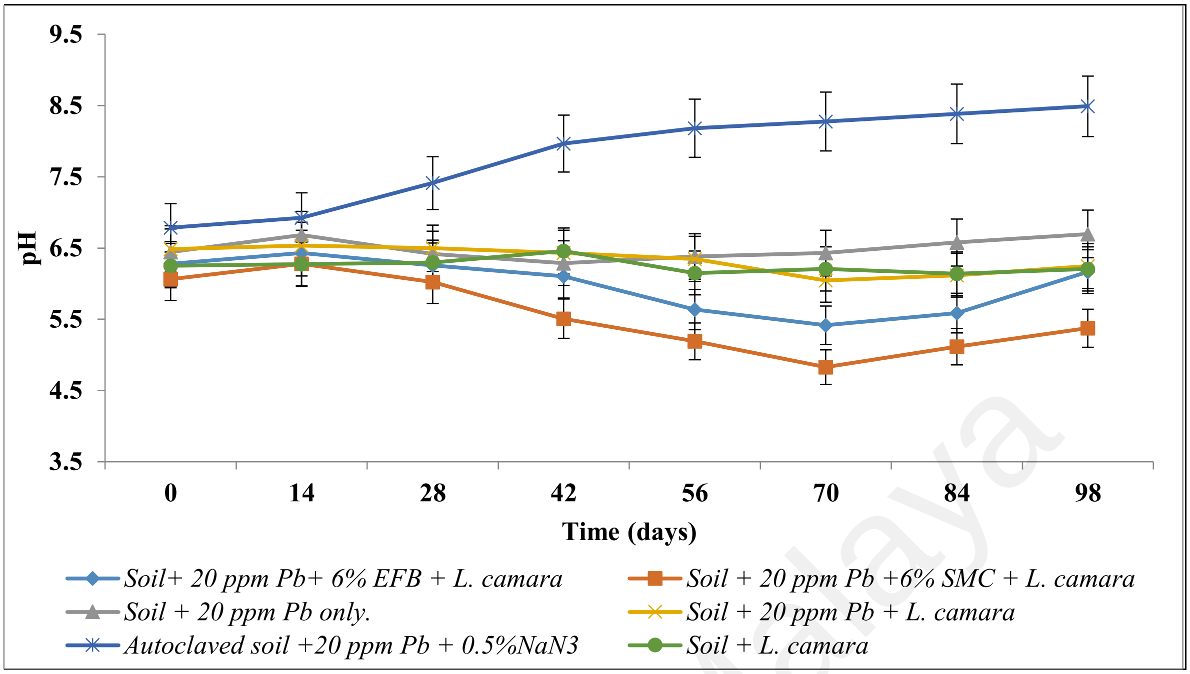


Figure 4.63: pH for 20 ppm Pb contaminated soil under *Lantana camara* Phytoremediation Bars denotes standard error (n=3).

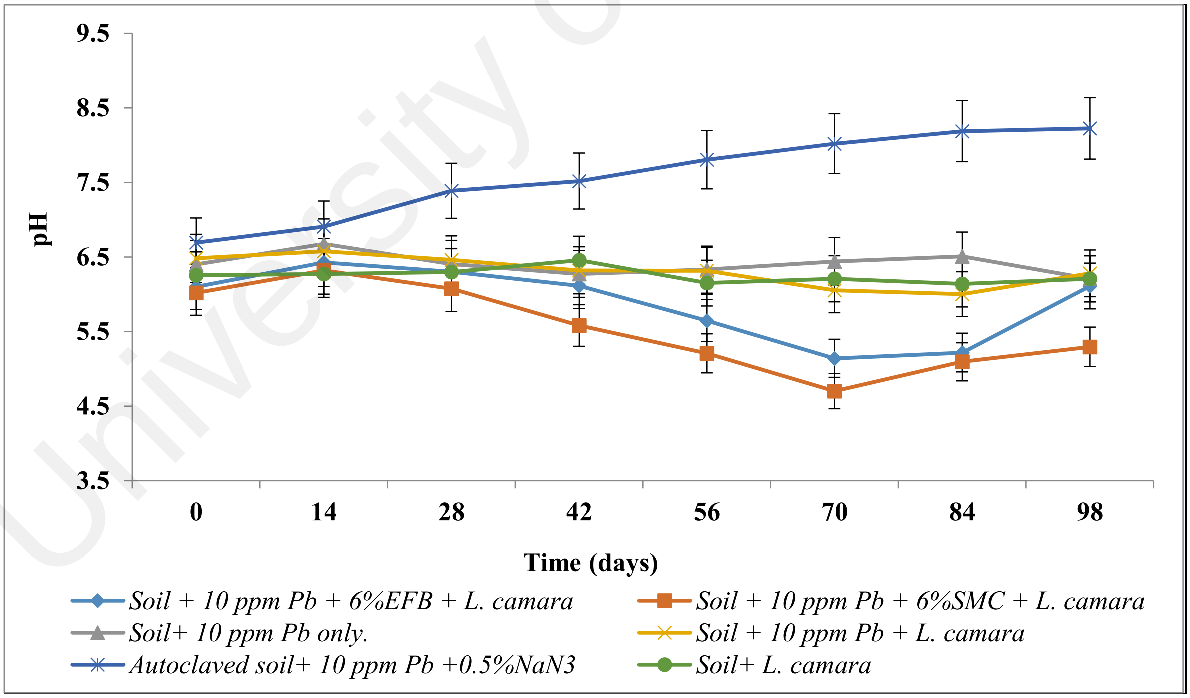


Figure 4.64: pH for 10 ppm Pb contaminated soil under *Lantana camara* Phytoremediation Bars denotes standard error (n=3).

Furthermore, this decreased pH range noticed at the *Lantana sp.* rhizosphere region, hence implicated desorption and/or displacement of Pb at their soil binding sites, due to H^+ (proton) activity (acting a displacer of Pb in soil), which thereafter enhanced Pb bioavailability, and subsequent Pb-uptake by *Lantana sp.* roots. Similar trends had been reported by numerous authors (Brown et al., 1995; Maxted et al., 2007; Alaribe & Agamuthu, 2015).

Although, the *Lantana sp.* remediated Pb-soil, recorded more acidic pH medium than *Pilea sp.* counterparts, and this may be implicated as the rationale behind more Pb-reduction in *Lantana sp.* soil over *Pilea sp.* soil series, at the end of the study duration. This correspond to Bhargava et al., (2012) findings, about insights that enhanced soil-heavy metal phytoextraction.

However, chelation of metals can be of great relevance into decontamination of soil heavy metal contents. The list of analysed metabolites implicated at the rhizosphere soils of *Pilea sp.* and *Lantana sp.* Pb and/or Cr contaminated soils, are shown in **Tables 4.2 to 4.4**. These ligands may range from being phytochelatins or methionine (i.e., peptides and/or amino-acids, or related biomolecules), and phytosiderophores and/or siderophores (Bhargava et al., 2012; Ali et al., 2013). Although, the investigation of these biomolecules beyond this particular point attained was not feasible in this study due to limited tools. Hence, may need further studies with more sophisticated analytical tools for exudate enumeration and/or classification.

Table 4.2: GCMS-QP2010 Plus; Possible metal complexing ligands under *P. cadierei* with organic wastes during phytoremediation.

All Treatments (soil rhizosphere)	Compounds found (organic ligands)	Mol. Formula	Mol. weight
Pilea cadierei **+ Pb/Cr+ EFB**	**Acetic acid, butyl ester**	$C_6H_{12}O_2$	**116**
	Decanoic acid/ Nonylcyclopropane	$C_{10}H_{20}O_2$ / $C_{12}H_{24}$	**172.3 /168**
	3-Tetradecene /4-Heptanone, 2, 3:5, 6- diepoxy-2, 6-dimethyl	$C_{14}H_{28}$ / $C_9H_{14}O_3$	**196 /170**
	Furan, tetrahydro-2, 2, 4, 4 -tetramethyl	$C_8H_{16}O_6$	**128**
	2, 2, 5, 5- Tetramethyl-4-ethyl-3-imidazoline-3-oxide	$C_9H_{18}N_{20}$	**170**
	Glucofuranose, 6-amino-6-deoxy-1, 2: 3, 5-di-O-isopropylidene	$C_{12}H_{21}NO_5$	**259**
	E-11, 13-Tetradecadien-1-ol / 2-methyl-2-hexanol	$C_{14}H_{26}O$ / $C_7H_{16}O$	**210/116**
	5-Amino-N-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine	$C_3H_5N_5O_2$	**143**
	O-Trimethylsilylpropargyalcohol	$C_6H_{12}OSi$	**128**
	Cyclobutanecarboxylic acid	$C_8H_{14}O_3$	**158**
	Isobutenyl methyl ketone	$C_6H_{10}O$	**98**
	1, 6-Anhydro-3, 4-O-isopropylidene-2-tosyl-D-galactose	$C_{16}H_{20}O_7S$	**356**
Pilea cadierei **+Pb/Cr+ SMC**	**Acetic acid, 2-methylpropyl ester**	$C_6H_{12}O_2$	**116**
	3-Hydroxy-2-methylpentanal	$C_6H_{12}O_2$	**116**
	1, 2-O-Isopropylidene-beta-1-idofuranurono-6, 3-lactone	$C_9H_{12}O_6$	**216**
	E-11, 13-Tetradecadien-1-ol	$C_{14}H_{26}O$	**210**
	5-Amino-N-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine	$C_3H_5N_5O_2$	**143**
	O-Trimethylsilylpropargyalcohol	$C_6H_{12}OSi$	**128**
	2-methyl-2-hexanol	$C_7H_{16}O$	**116**
	Cyclobutanecarboxylic acid / Decanoic acid	$C_8H_{14}O_3$ / $C_{10}H_{20}O_2$	**158 / 172.3**

Table 4.3: GCMS-QP2010 Plus; Possible metal complexing ligands under *L. camara* with organic wastes during phytoremediation.

All Treatments (soil rhizosphere)	Compounds found (organic ligands)	Mol. Formula	Mol. weight
Lantana camara +Pb/Cr+ EFB	Acetic acid, butyl ester	$C_6H_{12}O_2$	116
	Dodecanoic acid	$C_{12}H_{24}O_2$	200.3
	Nonylcyclopropane / 3 -Tetradecene	$C_{12}H_{24}$ / $C_{14}H_{28}$	168 / 196
	4-Heptanone, 2, 3:5, 6- diepoxy-2, 6-dimethyl	$C_9H_{14}O_3$	170
	Furan, tetrahydro-2, 2, 4, 4-tetramethyl	$C_8H_{16}O_6$	128
	2, 2, 5, 5- Tetramethyl-4-ethyl-3-imidazoline-3-oxide	$C_9H_{18}N_{20}$	170
	Glucofuranose, 6-amino-6-deoxy-1, 2: 3, 5-di-O-isopropylidene	$C_{12}H_{21}NO_5$	259
	E-11, 13-Tetradecadien-1-ol	$C_{14}H_{26}O$	210
	5-Amino-N-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine	$C_3H_5N_5O_2$	143
	O-Trimethylsilylpropargyalcohol	$C_6H_{12}OSi$	128
	Isobutenyl methyl ketone	$C_6H_{10}O$	98
	1, 6-Anhydro-3, 4-O-isopropylidene-2-tosyl-D-galactose	$C_{16}H_{20}O_7S$	356
	2-methyl-2-hexanol / Oxalic acid	$C_7H_{16}O$ / $C_2H_2O_4$	116 / 90
Lantana camara +Pb/Cr+ SMC	Acetic acid, 2-methylpropyl ester	$C_6H_{12}O_2$	116
	3-Hydroxy-2-methylpentanal	$C_6H_{12}O_2$	116
	1, 2-O-Isopropylidene-beta-1-idofuranurono-6, 3-lactone	$C_9H_{12}O_6$	216
	E-11, 13-Tetradecadien-1-ol	$C_{14}H_{26}O$	210
	5-Amino-N-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine	$C_3H_5N_5O_2$	143
	O-Trimethylsilylpropargyalcohol	$C_6H_{12}OSi$	128
	2-methyl-2-hexanol / Oxalic acid	$C_7H_{16}O$ / $C_2H_2O_4$	116 /90
	Dodecanoic acid	$C_{12}H_{24}O_2$	200.3

Table 4.4: GCMS-QP2010 Plus; Possible metal complexing ligands under *P. cadierei and L. camara* with zero organic waste during phytoremediation.

All Treatments (soil rhizosphere)	Compounds found (organic ligands)	Mol. Formula	Mol. weight
Pilea cadierei **+Pb/Cr**	**E-11, 13-Tetradecadien-1-ol**	$C_{14}H_{26}O$	**210**
	2-methyl-2-hexanol	$C_7H_{16}O$	**116**
	Acetic acid	$C_2H_4O_2$	**60**
	Decanoic acid	$C_{10}H_{20}O_2$	**172.3**
	Cyclobutanecarboxylic acid	$C_8H_{14}O_3$	**158**
Lantana camara **+Pb/Cr**	**Acetic acid, 2-methylpropyl ester**	$C_6H_{12}O_2$	**116**
	Oxalic acid	$C_2H_2O_4$	**90**
	2-methyl-2-hexanol	$C_7H_{16}O$	**116**
	5-Amino-N-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine	$C_3H_5N_5O_2$	**143**
	Dodecanoic acid	$C_{12}H_{24}O_2$	**200.3**

Meanwhile, under a separate experimental condition with Cr, the pH obtained at 40 to 10 ppm Cr phytoremediated soil, relatively fluctuated. Probably, due to (soil-plant-metal) niche dynamics. However, the rhizosphere pH value under *Pilea sp.* (40 to 10 ppm Cr) remediated soils, ranged between pH 6.8 to 5.90, 6.64 to 5.41, and 6.52 to 5.52 for EFB amended soils, SMC amended soils, and *Pilea sp.* (unamended) control, respectively (**Figures 4.65 to 4.67**). It is also worthy to note, pH of Cr autoclaved- sodium-azide soil (control), recorded between pH 6.87 -8.40. This was due to sodicity and/or Na-affinity to clay-particle as a basic cation, hence without extreme interference from biological factors.

In between 56 to 84 days, decrease in pH that ranged from pH 5.95 to 5.35 were observed. At this stage was where approximately 24.90 to 58.9%, out of the entire soil removed Cr gained significant elimination ($p<0.05$). Hence, very plausible to implicate this pH range as being optimal for Cr-phytoextraction and/or stabilization under *Pilea sp.* This might have been facilitated by *Pilea sp.* root-exudation, and other microbial induced metabolites (**Tables 4.2 and 4.4**), as it correctly correlated with the reviews of Kavamura & Esposito (2010) and Bhargava et al., (2010).

However, Wiseman et al., (2015) also observed a reduction in pH from 7.32 to 7.08 after compost addition in metal impoverished soil during remediation. This result, contrasted a bit with that of Wiseman et al., (2015) in terms of pH values, and this may be attributed to differences in waste and/or compost materials applied, soil type and climatic condition, as well as the duration of metal-pollutants in soil.

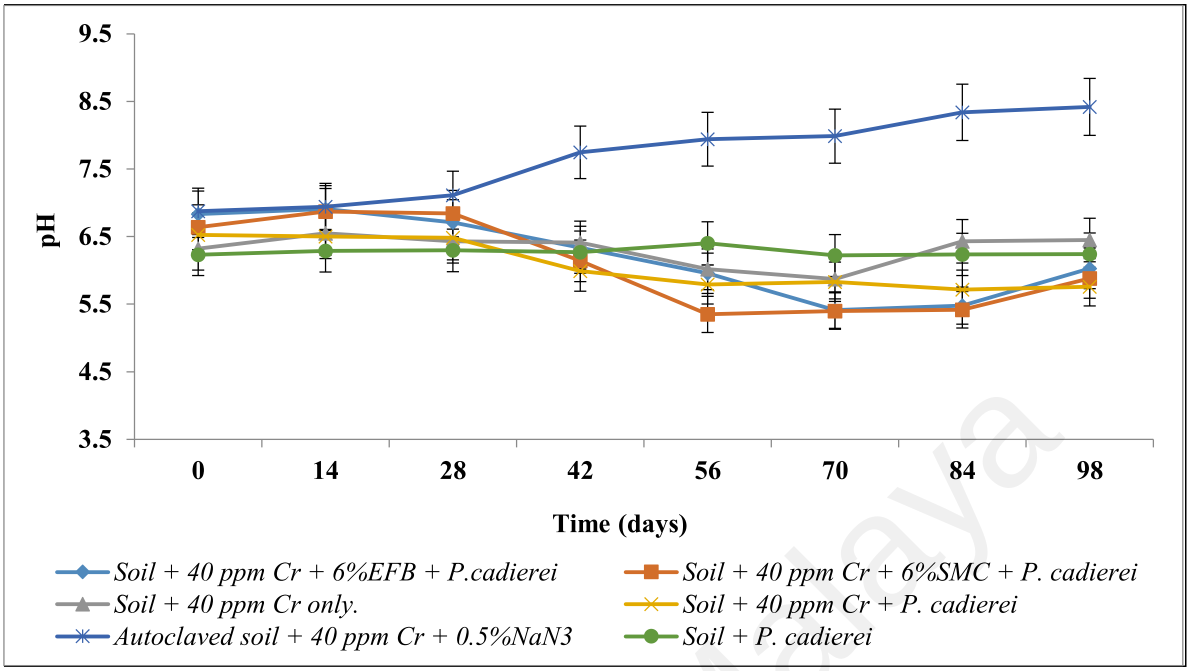


Figure 4.65: pH for 40 ppm Cr contaminated soil under *Pilea cadierei* Phytoremediation

Bars denotes standard error (n=3).

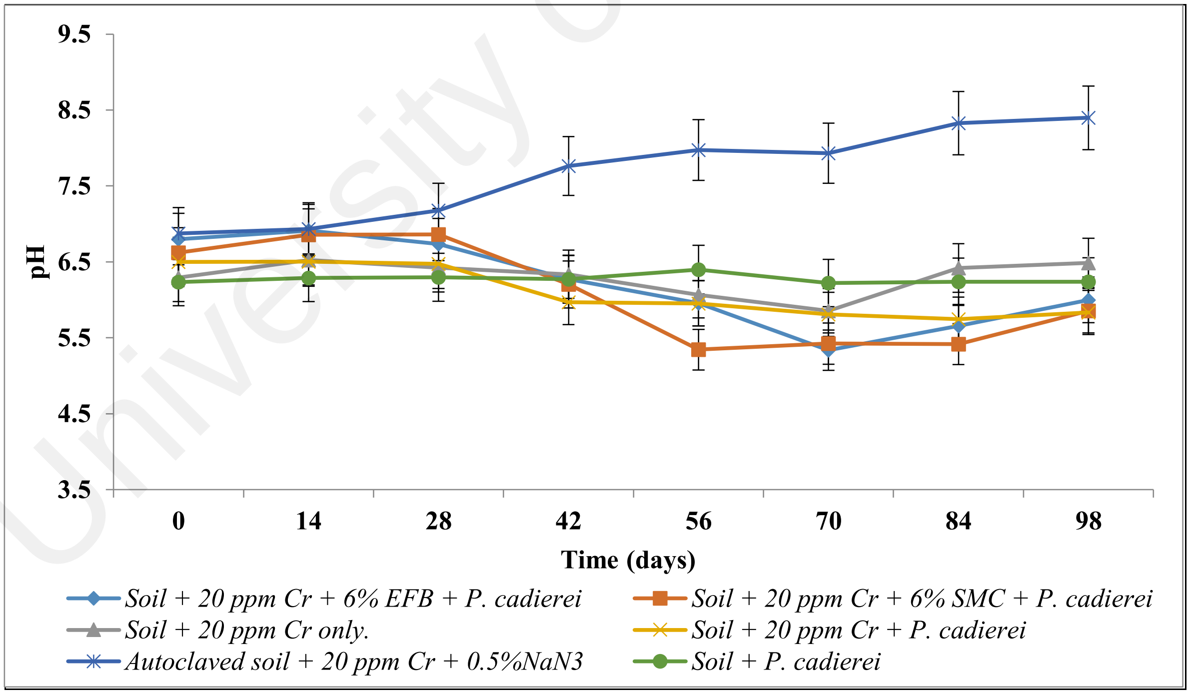


Figure 4.66: pH for 20 ppm Cr contaminated soil under *Pilea cadierei* Phytoremediation

Bars denotes standard error (n=3).

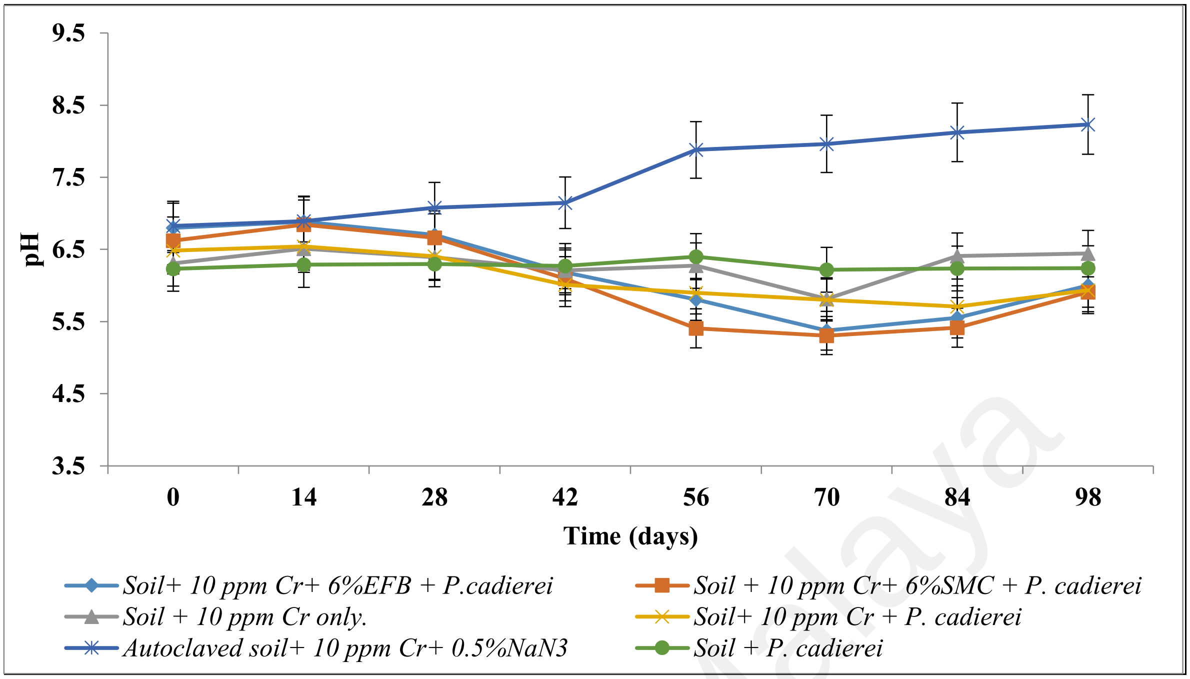


Figure 4. 67: pH for 10 ppm Cr contaminated soil under *Pilea cadierei* Phytoremediation Bars denotes standard error (n=3).

Evaluation of pH impacts under *Lantana sp.* at 40 to 10 ppm Cr remediated soil, implicated diverse pH ranges. This may be due to concentration, and/or complexity factors. Hence, pH ranges of pH 6.67-6.0, pH 6.50-5.99, and pH 6.39-6.07 were recorded at rhizospheres of soils amended with EFB, SMC, and *Lantana sp.* (unamended) control, respectively. Also, between 42 to 84 days, a reduction in pH, ranged from 5.98-5.16 and 5.99-5.50 were observed at both amended soils. These periods of pH drop, however constituted about 22.22 to 60.02% in the overall riddance of Cr from soil, in view of the 98 days cumulative time. Most probably, this seems to be the optimum pH range for Cr removal under *Lantana sp.* This also corresponded to pH 5- 6 implicated by Wang et al., (2006) for Zn and Cd soil-phytoremediation, using *Thlaspi caerulescens* (**Figures 4.68 to 4.70**). Hence, elevated removal of Cr recorded in *Lantana sp*. soils against *Pilea sp.*, may be partly linked to these variations in acidic pH, induced by organic acid exudates and/or other secondary metabolites (**Tables 4.3 and 4.4**), which displaced Cr at soil binding sites to enhance its bioavailability, and uptake.

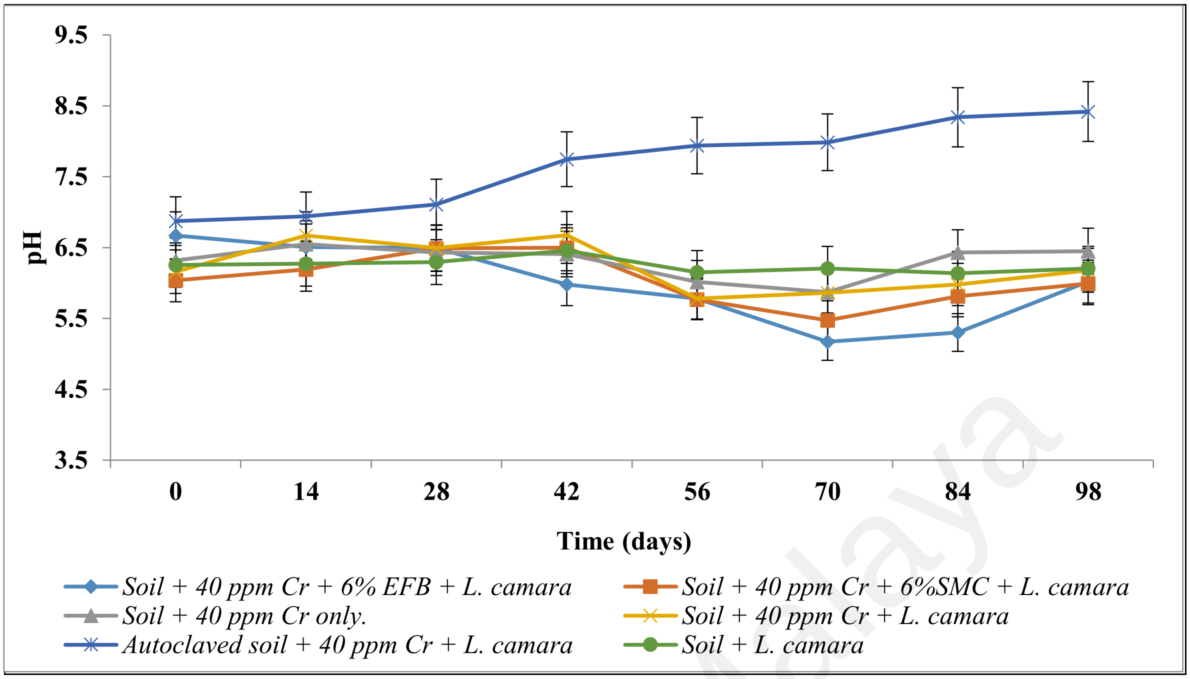


Figure 4.68: pH for 40 ppm Cr contaminated soil under *Lantana camara* Phytoremediation Bars denotes standard error (n=3).

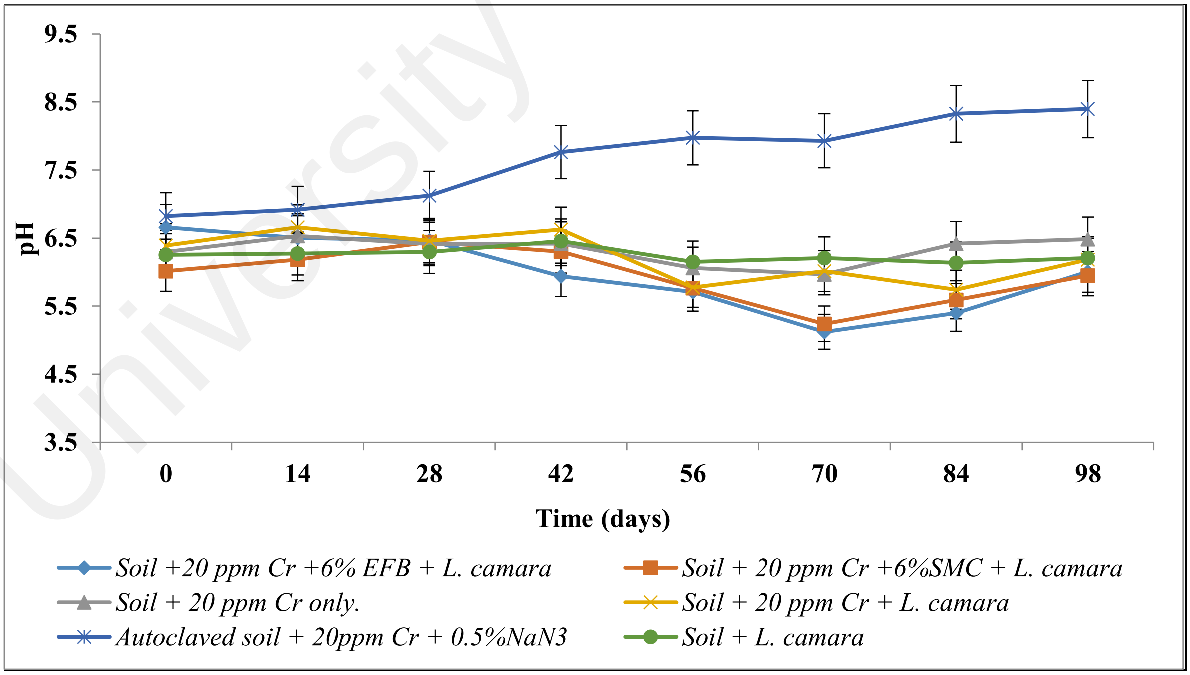


Figure 4.69: pH for 20 ppm Cr contaminated soil under *Lantana camara* Phytoremediation

Bars denotes standard error (n=3).

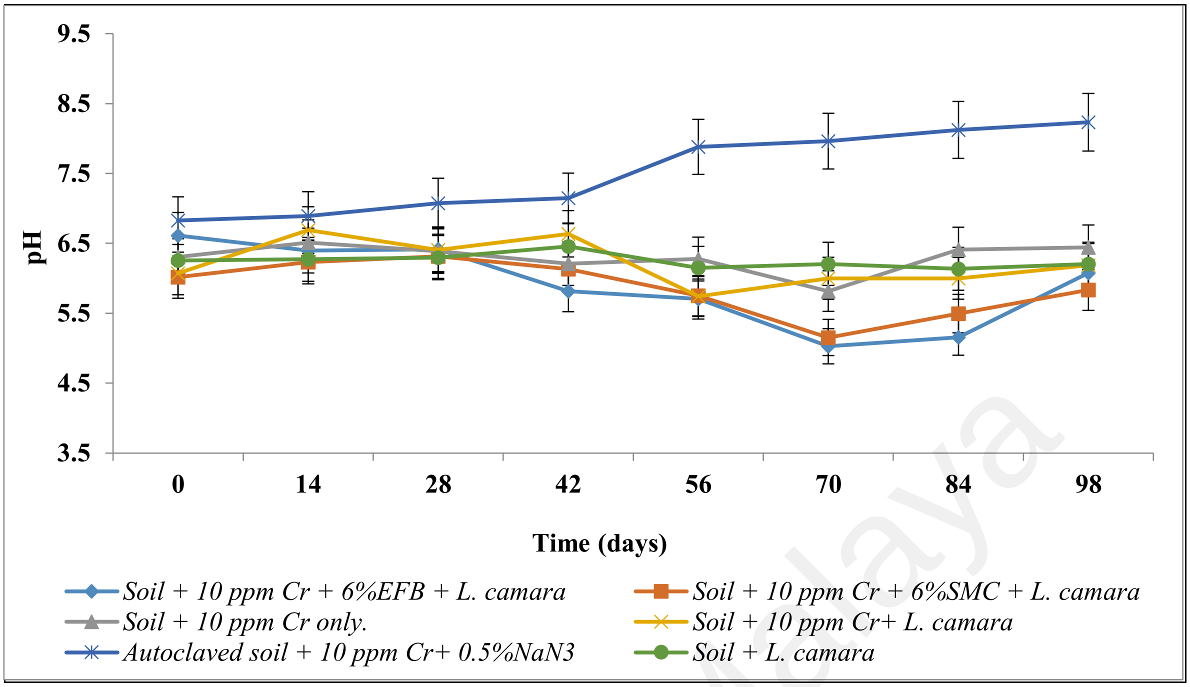


Figure 4.70: pH for 10 ppm Cr contaminated soil under *Lantana camara* Phytoremediation

Bars denotes standard error (n=3).

Meanwhile, several authors (Ali et al., 2013; Alloway, 1990; Zeng et al., 2011; Sheoran et al., 2011; Vamerali et al., 2010), have reported how root exudates can lower the rhizosphere soil pH by one or two units over the bulk soil. This is in agreement with this result. However, Thangavel & Subbhuraam, (2004) further stated that lowered pH increased the concentration of heavy metals in solution, hence promoting desorption from soil, and the addition of organic wastes further induced the complexation of Cr.

Moreover, metal salts are generally soluble in acidic media rather than in basic media (Ali et al., 2013), and this can enhance bioavailability. Therefore, conscious efforts are necessary to avoid leaching through the application of moderately-stabilizing organic amendments to soil.

Further to this, under co-contaminated (Pb+Cr) remedied soil, the *Pilea sp.* and *Lantana sp.* at respective treatments, recorded pH values that ranged from pH 6.74 to 6.01 and pH 6.47

to 6.06 for (EFB amended soil), pH 6.59 to 5.67 and pH 6.18 to 5.76 for (SMC-amended soil), and 6.49 to 5.97 and 6.42 to 6.18 for *Pilea sp.* / *Lantana sp.* (unamended soil) control. This tallied with the pH of single metal (Pb or Cr) remediated soils at between 42 to 84 days, where a pH drop in the range of pH 5.82 to 5.31 and pH 5.61 to 5.47 was observed, respectively (**Figures 4.71** and **4.72**). Meanwhile, this pH results being within acidic pH range, and where maximum Pb and Cr reductions (20.08 to 31.20% and 19.5 to 24.5%) occurred, presents this values as the optimum pH for Pb and Cr bioavailability for plant uptake.

But surprisingly, similar acidic pH range were also seen in 20 ppm Pb or Cr single-metal remediated soil, but they recorded higher elimination of Pb or Cr as opposed to jointly-contaminated soil. Therefore, one could deduce that pH probably promoted metal bioavailability at the co-metal remediated soil, but due to antagonistic effects, from the simultaneous presence of Pb and Cr within same rhizosphere radius, may however hindered both metals from clinging to *Pilea sp.* and/or *Lantana sp.* root extraction sites. Hence, this resulted in reduced elimination of Pb and Cr in the co-contaminated soil during phytoremediation. This is in agreement with Flogeac et al., (2007) on the competitive sorption of metal ions. This showed that apart from optimum pH range for metal phytoextraction, cationic antagonistic impacts, can impede phytoremediation in multiple metal polluted soils.

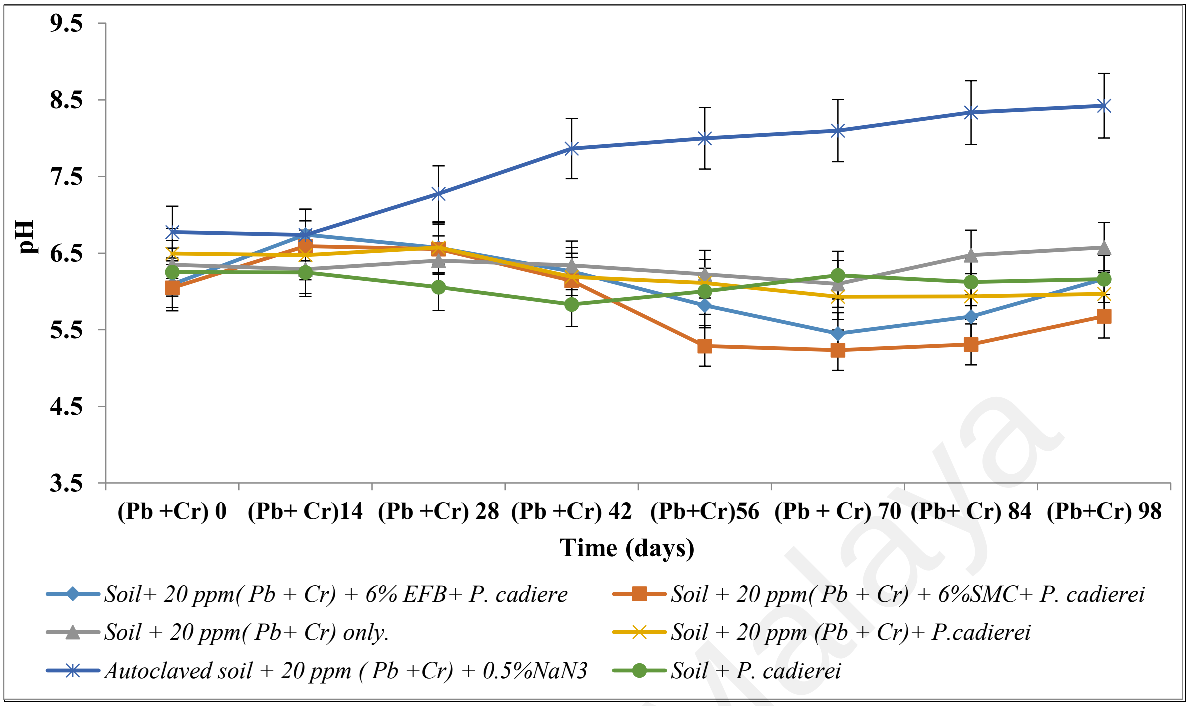


Figure 4.71: pH for 20 ppm (Pb+Cr) co-contaminated soil under *Pilea sp.* Phytoremediation Bars denotes standard error (n=3).

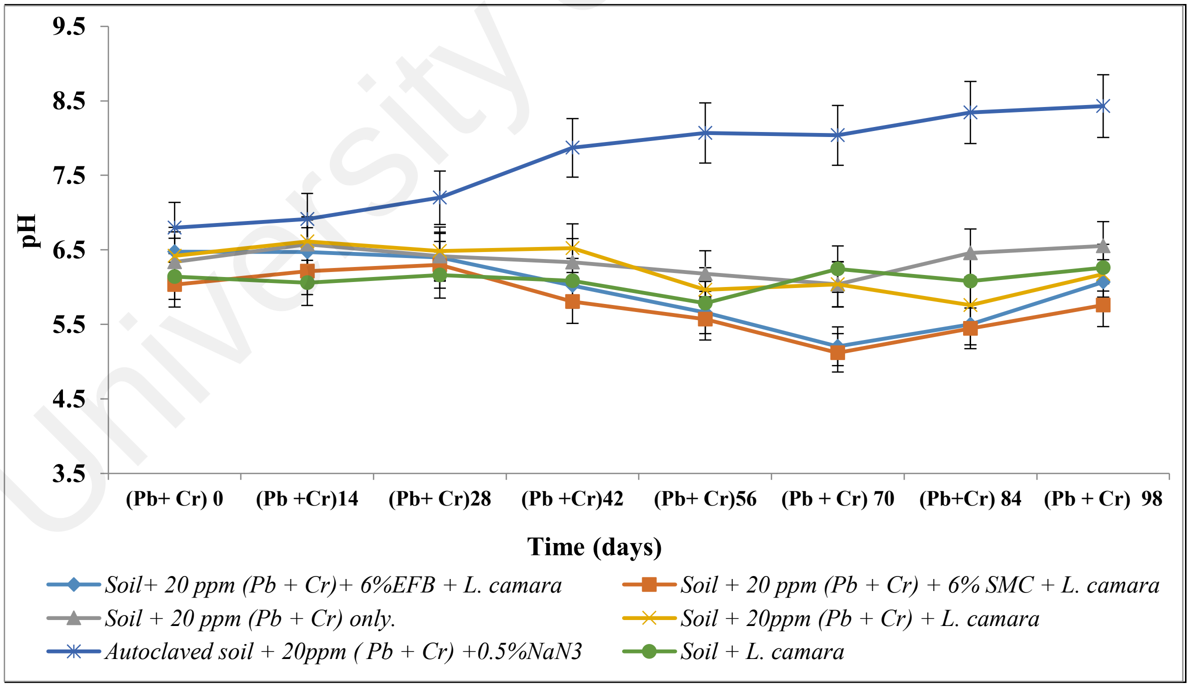


Figure 4.72: pH for 20ppm (Pb+Cr) co-contaminated soil under *Lantana sp.* Phytoremediation Bars denotes standard error (n=3).

From the rhizosphere metabolites, seen in **Tables 4.2-4.4**, the GC-MC total list of possible root exudates detected from both *Pilea sp.* and *Lantana sp.* tallied to 19 compounds. These compounds ranged from low-molecular weight primary and/or secondary metabolites, to high molecular weight compounds. Moreover, they possessed derivatives for phytochelatins, metallothioneins, phytosiderophores, as well as siderophores. Inducible under plant-soil heavy metal stress (Ali et al., 2013). However, to mention but a few among these metabolites detected; Decanoic acid ($C_{10}H_{20}O_2$), Dodecanoic acid ($C_{12}H_{24}O_2$), Cyclobutanecarboxylic-acid ($C_8H_{14}O_3$), 1,6–Anhydro-3,4-*O*-sopropylidene-2-tosyl-*D*-galactose ($C_{16}H_{20}O_7S$), 3-Hydroxy-2-methylpentanal ($C_6H_{12}O_2$), Oxalic acid ($C_2H_2O_4$), Acetic acid-2-methylpropyl ester ($C_6H_{12}O_2$), and 5-Amino-*N*-hydroxy-[1, 3, 4] oxadiazol-2-carboxamidine ($C_3H_5N_5O_2$). Therefore, Sheoran et al., (2011) agreed with this result, because they remarked that organic acids and amino acids, are ligands for heavy metal ions chelation due to the presence of donor atoms (O, S, and N) in their molecules.

Contrary to this result, Luo et al., (2015) detected 58 compounds of low molecular weight organic acids, amino acids, sugars, and alcohols from root exudates of *Sedum alfredii* hyperaccumulator under Cd contaminated soil. While, Strehmel et al., (2014) detected more than 100 compounds, mainly secondary metabolites from *Arabidopsis thaliana* (a metallophyte). These contradictory findings, between this current study with Luo et al., (2015) and Strehmel et al., (2014) may be as a result of differences in plants, and the type of heavy metal it is exposed to, in addition to soil diverse physicochemical properties.

Also, results from Luo et al., (2015) implicated *S. alfredii* to have exuded dodecanoic acid, which mobilized soil Cd and promoted its absorption by *S. alfredii* roots. This concurred with decanoic and dodecanoic acids, observed in *Pilea sp.* and *Lantana sp.* rhizosphere exudates respectively. This also implied, that both decanoic and dodecanoic acids, as well as oxalic and acetic acids exudates, indeed mobilized both Pb and Cr at *Pilea* and in *Lantana sp.* phytoremediated soils. Also, Kidd et al., (2009) reported that Ni was predominantly bound to citrate in hyperaccumulator rhizosphere of Ni polluted soil.

In another development, Jones et al., (2009) and Bertin et al., (2003) stated that approximately 10% of the photosynthetically fixed carbon is released by soil-grown plants as root exudates into the rhizosphere. This correctly correlated to this study because most exudates detected are carbon-containing compounds (see Tables 4.2- 4.4).

However, Salazar et al., (2016) reported exudates capable of extracting Pb, when *Tegetes minuta* and *Biden piposo L.* were only grown in high Pb polluted soils; indicating a behavioural response to Pb exposure. Such stimuli may equally exist in *Pilea sp.* and/or *Lantana sp.* when exposed to Pb and/or Cr contaminated soils. Furthermore, it has also been reported by Raskin et al., (1997) where *Alyssum lesbiacum* (Ni-hyperaccumulator), exudes histidine to acquire Ni mobilization in polluted soil, and its subsequent uptake.

Finally, *Pilea sp.* and *Lantana sp.* could plausibly be indicted to have responded to Pb and/or Cr concentrations in soils, by modifying their roots exudates production and/or composition. However, that may be the reason why their specific exuded-metabolites, somehow, were not identical with other defined metallophytes (*Sedum alfredii* and *Arabidopsis thaliana*), from different authors (Raskin et al., 1997; Strehmel et al., 2014; Luo et al., 2015), but only shared some similarities between them.

4.5 Bioaccumulation/ Translocation factors (BAF and TF) of heavy metal

The extraction results of Pb and/or Cr from the contaminated remedied soils, into the plants components are highlighted as thus;

4.5.1 BAF and TF of Pb in *Pilea sp.* and *Lantana sp.*

The concentration of Pb in both *Pilea sp.* and *Lantana sp.* tissues (i.e., roots and shoots), at 80 ppm Pb remediated soils are shown in **Figure 4.73**, after 98 days. Under *Pilea sp.*, about 58 to 74% and 26 to 43% of Pb was extracted from the polluted soil and is localized at the root and shoot regions, respectively, with corresponding bioaccumulation factor (BAF), and root-to-shoot translocation factor (TF) ranging from 0.36 to 0.13 and 0.75 to 0.42 computed using **Equations 3.7** and **3.6**, and derived from **Figure 4.73** shoot and root presented Pb-residual *Pilea sp*. uptake concentration (data). Correspondingly, *Lantana sp.* recorded BAF and TF values ranging between 0.40 to 0.16 and 0.72 to 0.60, respectively. However, in *Lantana sp.* and *Pilea sp.* Pb soil, amended with EFB and SMC, recorded root to shoot partitioned Pb ratios of 1.38:1.0 and 1.39: 1.0, and as well as 1.34: 1.0 and 1.38: 1.0, respectively. Meanwhile, Doucleff & Terry, (2002) reported 95% of 100 ppm As in soil, was extracted to above-ground plant tissues of *P. vittata*. This contrasted with this results which may be due to volatile nature of arsenic, together with variation in plant and habitats.

Whereas, at unamended Pb soil, both *Lantana sp.* and *Pilea sp.* tissues, had root: shoot Pb ratios of 1.06: 1.0 and 1.51: 1.0, respectively. Generally, *Lantana* recorded higher BAF-value of 0.40 against 0.36 seen in *Pilea*. At both root and shoot residual-Pb, with statistical difference F: (p<0.05) =549.432 and F: (p<0.05) =9548.287 respectively, mostly at organic additives spots, hence boosted plant energy level for extraction and translocation of Pb to the aerial parts. However, under amended conditions, more Pb were translocated from root to shoot of *Pilea sp.* at TF-values of 0.750 to 0.723, than in *Lantana sp.* with 0.723 to 0.721 TF-values. But at unamended scenario, *Lantana sp.* recorded TF-value of 0.596, which was nominal to 0.417 observed in *Pilea sp*.

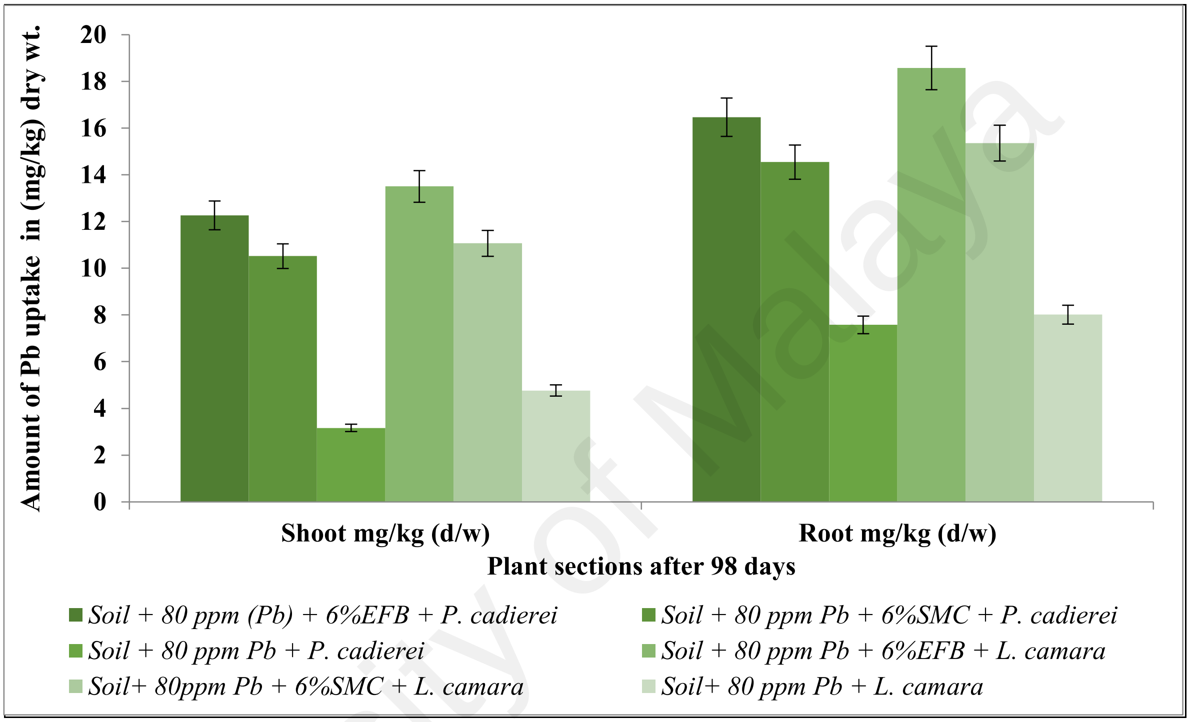


Figure 4.73: Conc. of Pb in plant tissues after 98 days at 80 ppm Pb soil phytoremediation. Bars denotes standard error (n=3).

Basically, what transpired at TF-values of amended *Lantana sp.* indicated that some metallophytes may have specific ability to extract-out Pb from polluted environment, and store them in their roots. But, it may concurrently have a slow root to shoot metal transfer mechanism. This concurred with several arguments presented by Salazar & Pignata, (2014) on the screening of native species in phytoremediation of Pb polluted soil. Also, Meera & Agamuthu, (2011) found similar trend with *H. cannabinus* in landfill leachate, for As and Fe phytoextraction.

However, the translocation of Pb from roots to aerial *Lantana sp.* and *Pilea sp.* parts was confined to below 1. But Jamil et al., (2009) stated that metal accumulator plants have TF values >1, while Baker, (1981) has also reported where metal excluders showed TF value < 1. But, van der Ent et al., (2013) concluded by arguing that, TF alone was insufficient to define hyperaccumulation, but rather it constitutes to useful evidence in profiling metallophytes. Furthermore, **Figure 4.74** presents another concentration of Pb in *Pilea sp.* and *Lantana sp.* tissues after 98 days, at 40 ppm Pb phytoremediated soil.

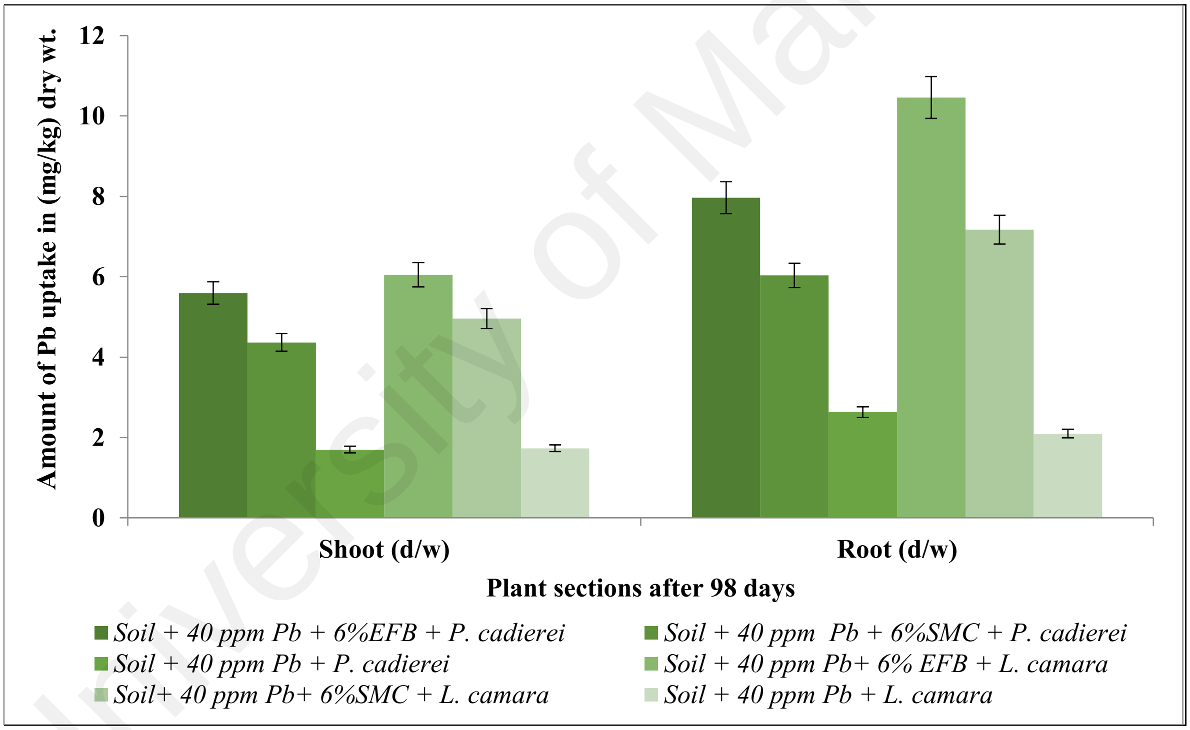


Figure 4.74: Conc. of Pb in plant tissues after 98 days at 40 ppm Pb soil phytoremediation Bars denotes standard error (n=3).

Pilea sp. and *Lantana sp.* deposited between 59 to 64% and 61 to 63% total extracted Pb to the root regions, at significant levels that favoured amended soils [F:(p<0.05)=822.932]. Correspondingly, 39 to 42% and 37 to 45% of the total phytoextracted Pb concentrations were stocked at the shoot regions of *Pilea sp.* and *Lantana sp.,* respectively, at post-hoc significant difference [F: ($p<0.05$) =826.770].

Basically, the cumulative concentration of metal absorbed is a direct function of BAF, and indirect for TF. Hence, BAF-values ranged from 0.108 to 0.384 and 0.096 to 0.413 were seen at *Pilea sp.* and *Lantana sp.,* respectively, from **Equations 3.7,** and data from **Figure 4.74**. TF-values between 0.65 to 0.723 and 0.59 to 0.82 were also computed in similar plant order using **Equation 3.6** and data from **Figure 4.74**. This was further confirmed by Pourrut et al., (2011) who argued that plants with BAF and TF <1, are useful tools for phytostabilization.

BAF has consistently favoured *Lantana sp.*, and TF has been fluctuating in both plants; probably due to translocation inertia (Bech et al., 2016). However, 40 ppm Pb remediated soil recorded higher BAF-value of 0.413 at EFB amended sites, than in 80 ppm counterpart with 0.40. This may be attributed to concentration difference, but Alaribe & Agamuthu, (2015) did extensively explain this phenomena. However, both *Pilea sp.* and *Lantana sp.* can be considered as accumulators, because of their metal absorption and tolerance abilities, but not as hyper-accumulators based on their BAF and TF values. Hence, they can be good candidates for phytostabilization as well. Salazar & Pignata, (2014) also implicated *S. halepense* plant to also possess such quality.

Furthermore, under 20 ppm Pb phytoremediated soil, *Pilea sp.* and *Lantana sp.* recorded BAF rates between 0.18 to 0.32 and 0.21 to 0.41, with corresponding TF-values arrayed from 0.39 to 0.64 and 0.48 to 0.68, respectively, using **Equations 3.7** and **3.6**. These values were deduced from the data of total deposits of Pb in *Pilea sp.* and *Lantana sp.* tissues after 98 days of phytoremediation, as shown in **Figure 4.75**.

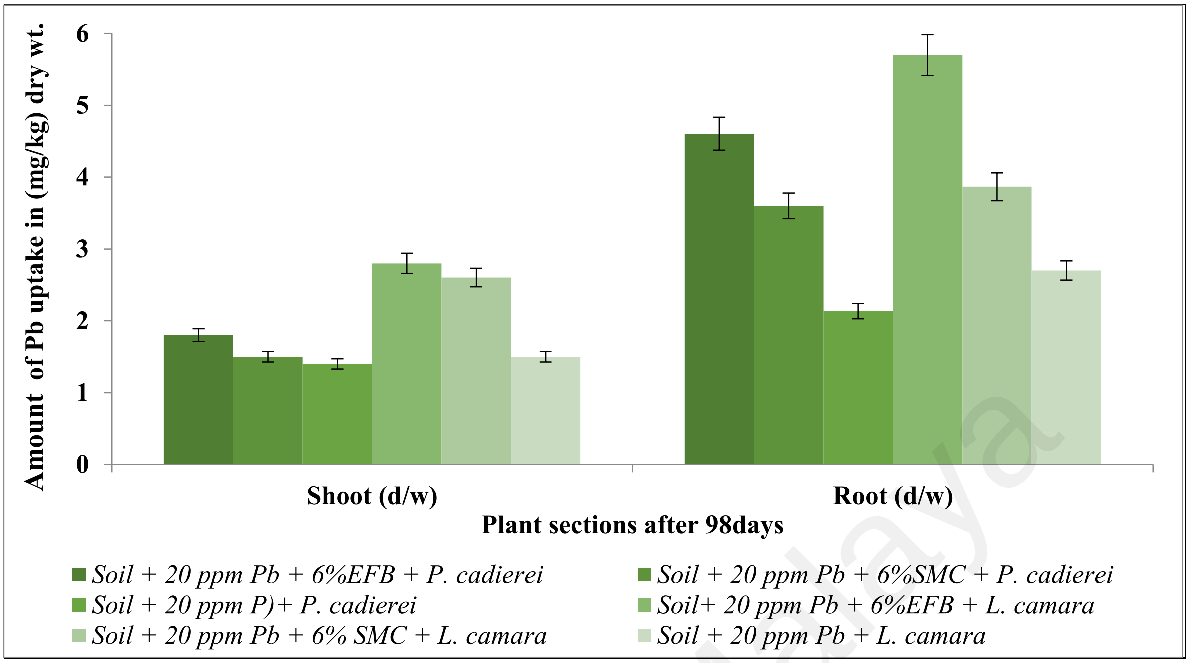


Figure 4.75: Conc. of Pb in plant tissues after 98 days at 20 ppm Pb soil phytoremediation Bars denotes standard error (n=3).

However, BAF maintained its consistent range when compared to previous 80 and 40 ppm Pb counterparts, while, TF recorded maximum value of 0.68 at *Lantana sp.* amended with SMC in soil. Hence, TF-values were observed to have reduced in 20 ppm Pb (*Lantana sp.*) amended with SMC between 1 to 3% in favour of 80 to 40 ppm *Lantana sp*. This implies that, translocation of Pb from root to shoot, were lower at 20 ppm Pb concentrations, and high at elevated Pb treatments. Despite all this, there are some possibilities that both plants may produce certain kind of metal transporters, but this is yet unclear. This agreed with the findings of Lorence-Plucińsk et al., (2013) on the capacities of *A.glutinosa* and *Alnus incana* to grow in metal-contaminated soil.

Meanwhile, more concentrations of the phytoextracted 20 ppm soil Pb, aligned between 60.34 to 71.88% and 64.29 to 67.10% were located at the root parts of *Pilea sp.* and *Lantana sp.*, with corresponding 28.12 to 39.66% and 32.94 to 40.19% at the shoot zones, respectively, at post hoc significant levels F: ($p<0.05$) =136.238 for roots, and F: ($p<0.05$) =28.762 for shoots, respectively. This coincided with Lorence-Plucińsk et al., (2013) who confirmed greater accumulation of Pb, Cu, and Cd at the root regions of *A. incana* and *A. glutinosa* at ($p=0.001$) significant level.

Following this, the range of BAF and TF for *Pilea sp.* at 10 ppm phytoremediation recorded 0.23 to 0.56 and 0.44 to 0.45, while *Lantana sp.* had 0.31 to 0.64 and 0.45 to 0.48, respectively, deduced from **Equation 3.7** and **3.6**, with data in **Figure 4.76**. At this spot, BAF raised to 0.64, as against 0.41 to 0.42 seen in 80-20 ppm Pb series. This may be as a result of lower Pb concentration with respect to the soil-substrate, which posed less burden to plant uptake mechanisms. Salazar et al., (2016) study, observed similar relationship with Pb, mostly at the root of *Tegetes minuta* L., which they said were mainly influenced by the various quantities of Pb present in soil, before phytoremediation began.

However, BAF and TF obtained in both *Pilea sp.* and *Lantana sp.* were <1. In such case, Bech et al., (2012) and Pourrut et al., (2011) recommended that such plants are useful phytostabilization tools in low heavy metal impoverished soils.

Meanwhile, between 69.64 to 70.18% and 66.61 to 68.89% concentrations of the total extracted Pb (**Figure 4.76**), got localized at the *Pilea sp.* and *Lantana sp.* roots, respectively. Correspondingly, lower range of 30.36 to 33.43% and 31.10 to 33.39% were focalized at the shoot region, in similar plant order and statistics F: ($p<0.05$) =1121.688 for root, and F: ($p<0.05$) =101.174 for shoot, respectively. Dahmani-Muller et al., (2000) however observed such trends at the root of *Armeria maritima ssp. halleri* with Pb (176 mg/kg), during phytoremediation of heavy metal contaminated soil. That means, *Pilea sp.* and *Lantana sp.* alone, may not be the only metallophytes implicated for high Pb root-concentration.

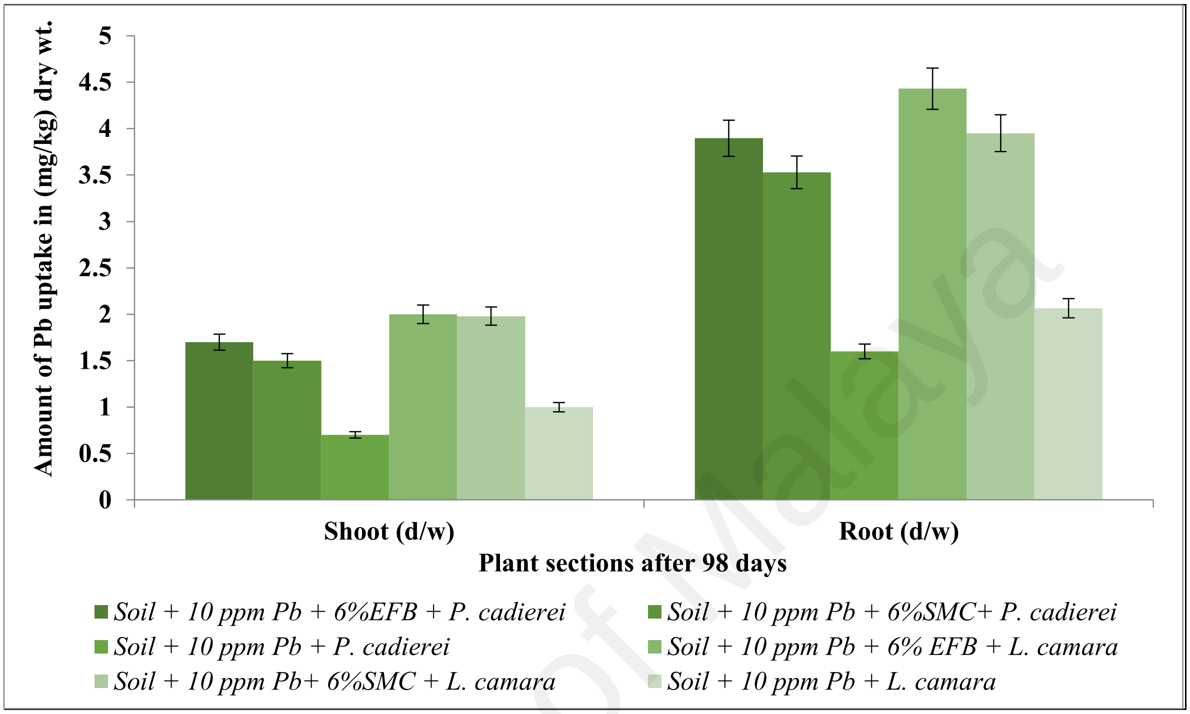


Figure 4.76: Conc. of Pb in plant tissues after 98 days at 10 ppm Pb soil phytoremediation Bars denotes standard error (n=3).

4.5.2 BAF and TF of Cr in *Pilea sp.* and *Lantana sp.*

Figures 4.77, **4.78** and **4.79** illustrate the total Cr accumulated at the aboveground tissues of *Pilea sp.* and *Lantana sp.* in 40, 20, and 10 ppm Cr phytoremediated spots, respectively. With corresponding general root and shoot post hoc significant level F: (p<0.05) =411.784 and F: (p<0.05) =1368.393 for 40 ppm Cr plants, F: (p<0.05) =1522.912 and F: (p<0.05) =216.152 for 20 ppm Cr plants, and also F: (p<0.05) =224.918 and F: (p<0.05) =107.70 for 10 ppm Cr plants, respectively.

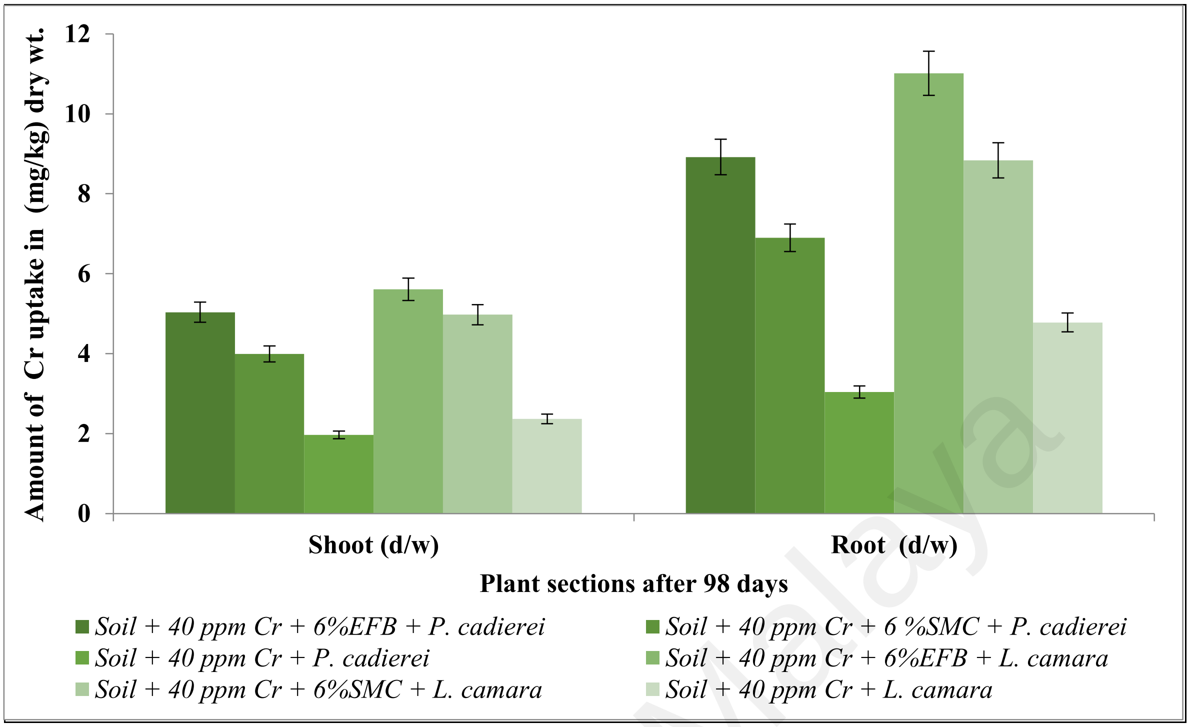


Figure 4.77: Conc. of Cr in plant tissues after 98 days at 40 ppm Cr soil phytoremediation Bars denotes standard error (n=3).

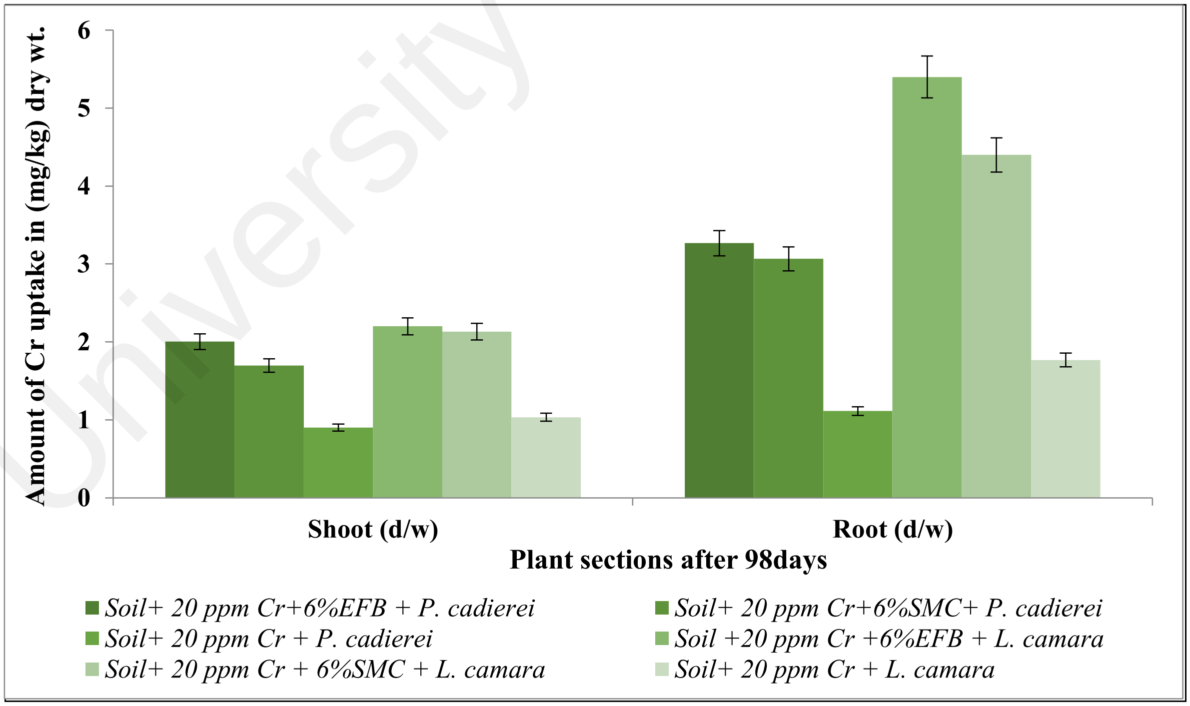


Figure 4.78:Conc. of Cr in plant tissues after 98 days at 20 ppm Cr soil phytoremediation Bars denotes standard error (n=3).

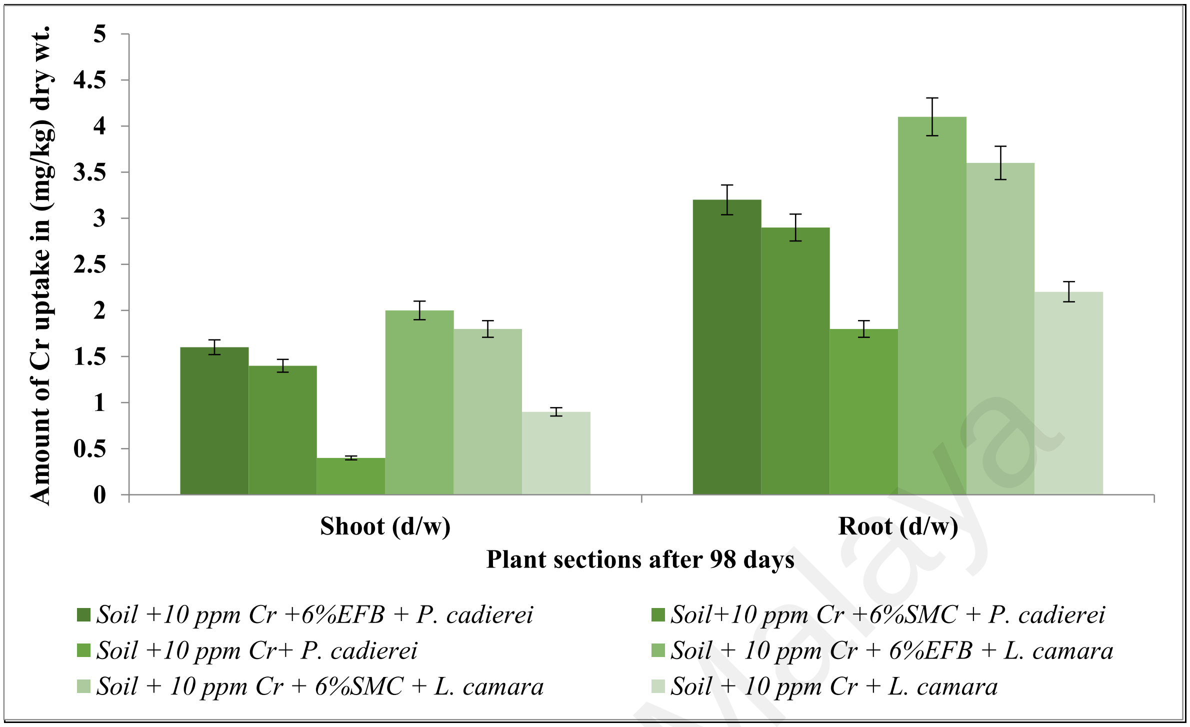


Figure 4.79: Conc. of Cr in plant tissues after 98 days at 10 ppm Cr soil phytoremediation Bars denotes standard error (n=3).

These results showed that 55.22 to 81.82% and 18.18 to 44.78% of the total extracted Cr concentration were deposited at the root and shoot sections for *Pilea sp.* spots, while 63.21 to 70.05% and 29.03 to 36.78% were piled-up at the root and shoot compartments of *Lantana sp.* applied treatments, respectively. Indication showed that un-aided *Pilea sp.* and *Lantana sp.* plants, recorded high backlogs of Cr at the roots. However, insufficient energy required to power the translocation of Cr by *Pilea sp.* and *Lantana sp.*, due to nutrient deficiency may be solely implicated for this dulling act.

Meanwhile, Satpathy & Reddy (2013) had also reported higher deposition of Pb, Zn, Cu, and Cd at the root parts of *Brassica juncea*, when compared to its shoots after 45 days. When amended with solid waste compost, (p<0.05 significant level). This imply that heavy metal have tendencies to stack-up at the root compartments of some bioindicator plants, apart from *Pilea sp.* and *Lantana sp.*

Therefore, BAF and TF ranged between 0.10 to 0.48 and 0.22 to 0.83 for *Pilea sp*, and also 0.14 to 0.48 and 0.41 to 0.56 for *Lantana sp.* were observed, respectively, hence deduced from **Equations 3.7** and **3.6**, using the result data shown in **Figures 4.77**, **4.78** and **4.79**. This means that, TF has consistently been higher in *Pilea sp.* over *Lantana sp*, and the reason for this phenomena has already been explained when discussing the Pb counterparts. This result contradicted with the BAF and TF of 1.9 to 2.2 and 1.0 to 1.8 range seen in Pb under *B. juncea* by Satpathy & Reddy (2013). This may be as a result of being different elements with Cr, or attributed to the fact that *B. juncea* is already an established hyperaccumulator metallophytes (Prasad & Freitas, 2003), while *Pilea sp.* and *Lantana sp.* are still undergoing pilot studies. Secondly, *Pilea sp.* and *Lantana sp.* also have tremendous advantage over *B. juncea*, despite recording lower BAF and TF, because they are not edible, hence does not pose a food chain risk, and also minimizes threats to global food security, unlike *B. juncea*.

Finally, **Figure 4.80** presents the concentration of combined Pb+Cr in *Pilea sp.* and *Lantana sp.* sections, at co-contaminated soil treatments. However, BAF-values were generally very low, when compared to the single 20 ppm Pb or Cr dented soils studied. By extension, TL also recorded wide fluctuating limits. Due to unspecific speciation, that impeded extraction and translocation. Obviously, the dominant concentration of Pb and Cr uptake by plants were pooled in the roots with 55 to 98.48% and 56.50 to 73.33% for *Pilea sp.* and *Lantana sp.*, respectively. Sun et al., (2011) also observed this trend in Cu and Pb co-contaminated soil using *Tagetes patula*. Therefore, BAF and TF-values under *Pilea sp.* ranged between 0.10 to 0.19 and 0.11 to 0.50. Similarly, 0.08 to 0.27 (BAF) and 0.36 to 0.66 (TF) were also observed in *Lantana sp.* following **Equation 3.7** and **3.6**, from data used in **Figure 4.80**. This simply indicate, that *Pilea sp.* and *Lantana sp.* are more suitable for remediation of single metal contaminated sites, as opposed to multiple metal contaminated soil. The above opinion is formed, based on the context of this study findings. Meanwhile, Rosselli et al., (2003) correctly correlated with this findings, where *A. incana* used for phytoremediation Cu, Zn, Cd, and Pb co-metal polluted soils, had a general BAF value < 1. Implying that, multiple-metal soil contamination poses more edaphic ecological danger (Bolan et al., 2014), and more difficult for plants to combat.

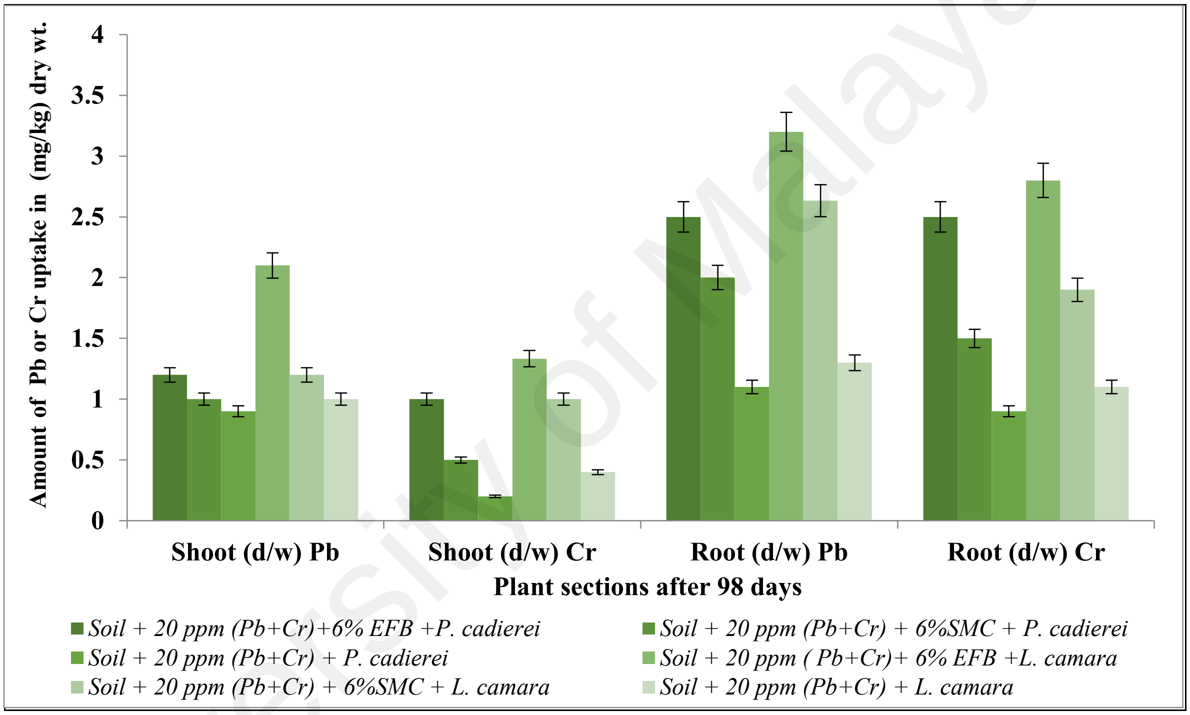


Figure 4.80: Conc. of Pb and Cr in plant tissues after 98 days at 20 ppm (Pb+ Cr) soil phytoremediation.

Bars denotes standard error (n=3).

4.5.3 Analysis of harvested plants root length and shoot height

Figures 4.81, **4.82**, **4.83** and **4.84** illustrates the roots height and/or shoots length of harvested *Pilea sp.* and *Lantana sp.* at 80, 40, 20 and 10 ppm Pb phytoremediated soils, after 98 days. However, 10 ppm Pb recorded highest on both root and shoot length, due to lower metal concentration. Therefore, no significant difference were noticed between roots and shoot lengths of EFB and SMC supplemented metal soils ($p<0.05$). Rather, differences were seen between the amended, and unamended roots when compared [F: ($p<0.05$) =240.31]. This evidenced at their respective soil Pb reductions as well. Jadia & Fulekar, (2008) have also recorded between 7.2 to 13.2 cm and 14.5 to 20 cm for both root and shoot lengths in 50 to 10 ppm Pb contaminated soil, respectively, using *Helianthus* plant, and implicating root length to be a crucial factor in Pb absorption and/or phytoextraction from polluted soils.

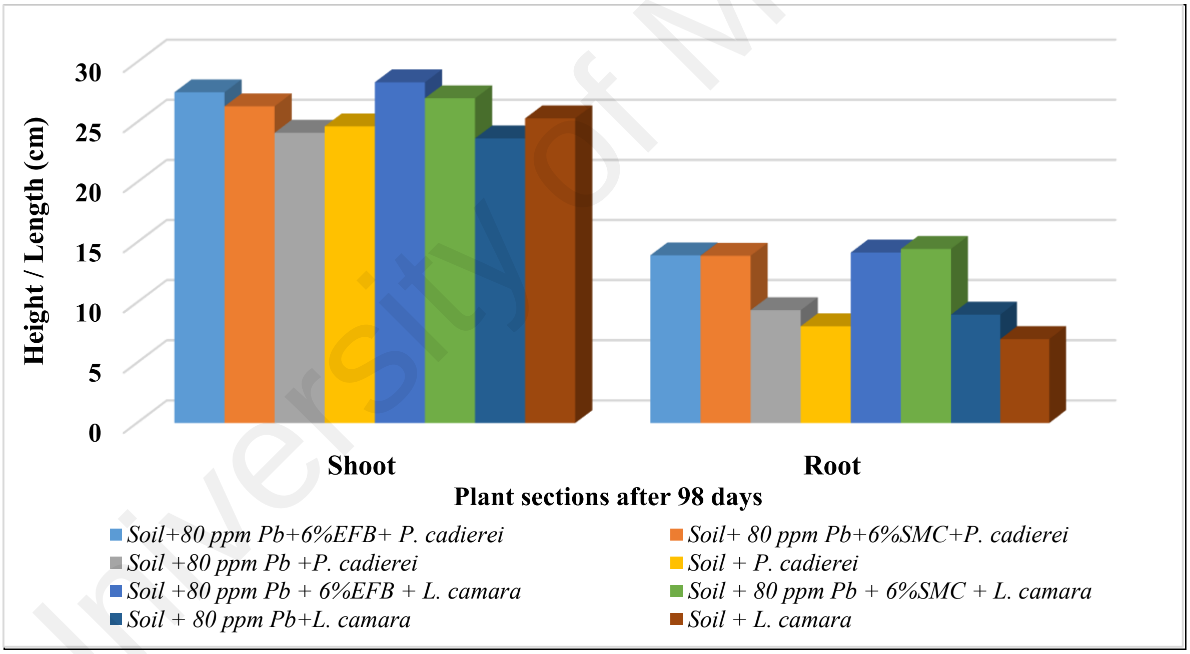


Figure 4.81: Postharvest comparison of plant shoots height and roots length exposed to 80 ppm Pb contaminated soil during phytoremediation with controls.

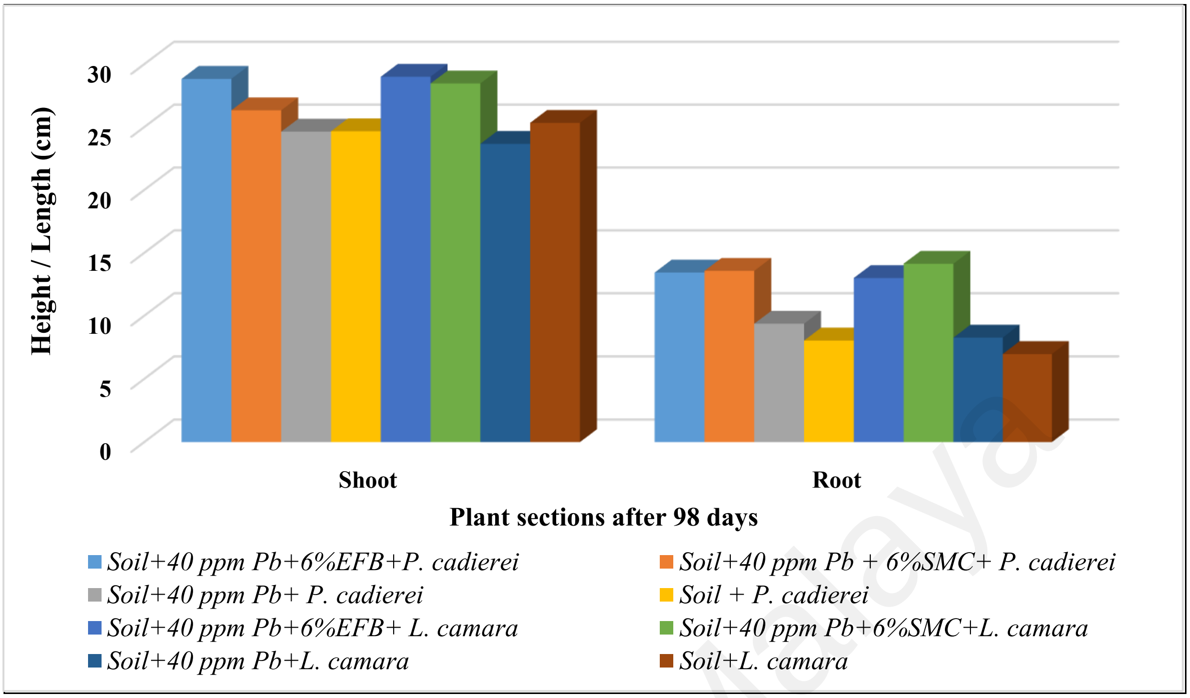


Figure 4.82: Postharvest comparison of plant shoots height and roots length exposed to 40 ppm Pb contaminated soil during phytoremediation with controls.

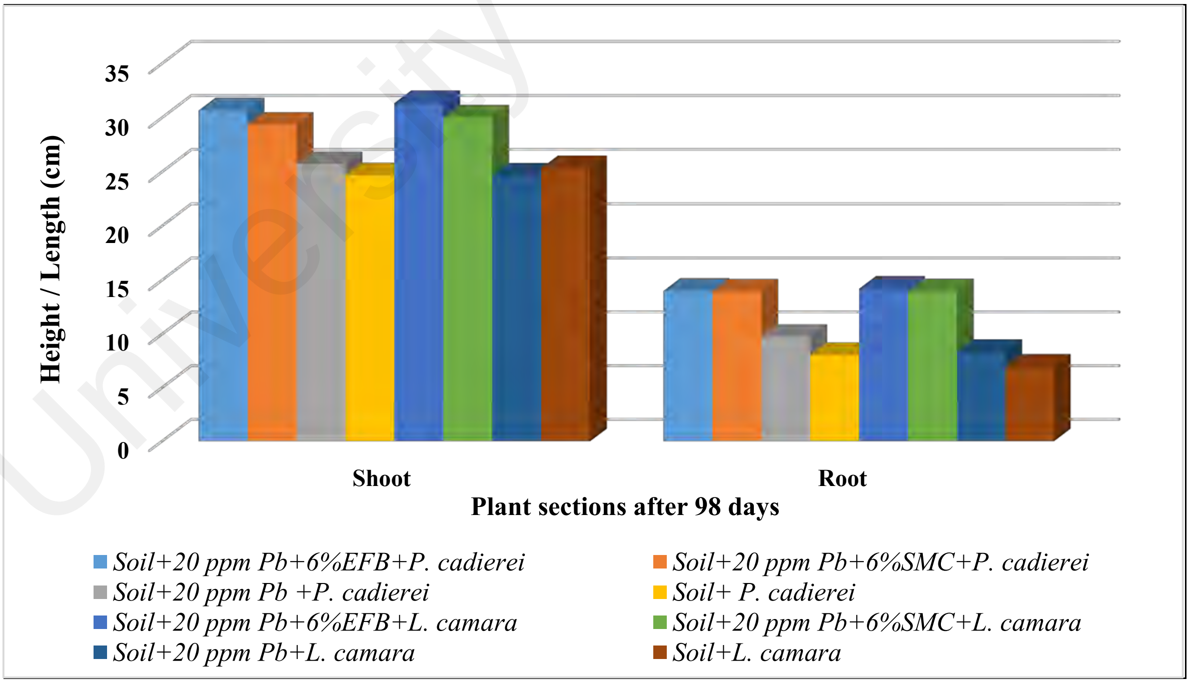


Figure 4.83: Postharvest comparison of plant shoots height and roots length exposed to 20 ppm Pb contaminated soil during phytoremediation with controls.

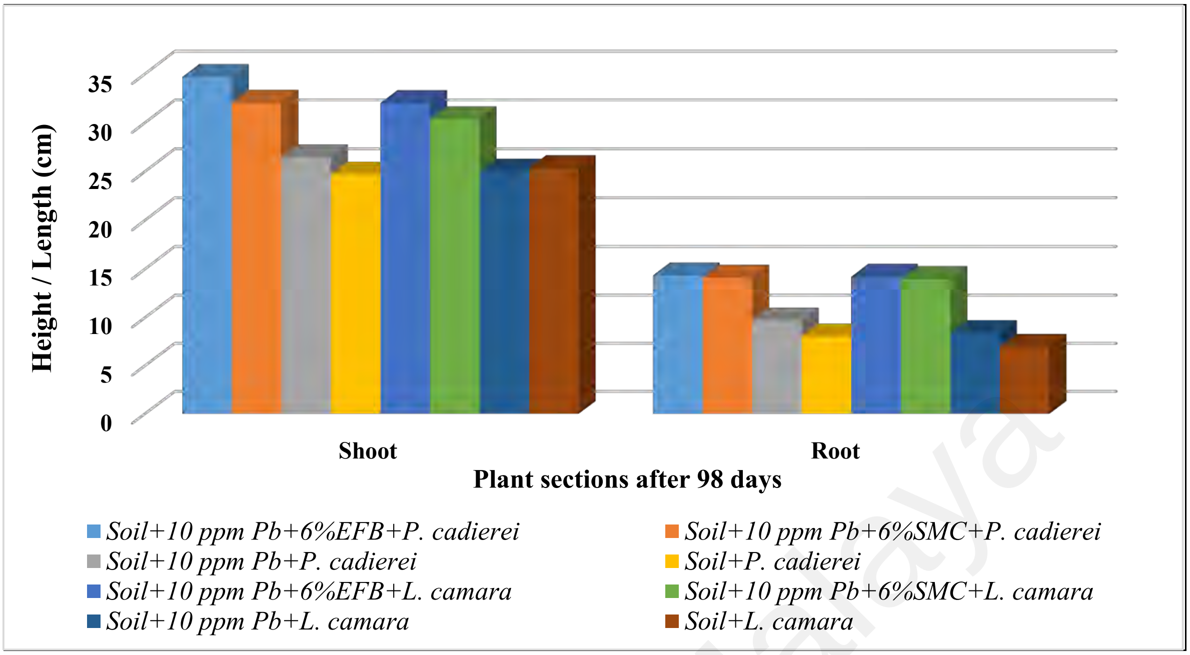


Figure 4.84: Postharvest comparison of plant shoots height and roots length exposed to 10 ppm Pb contaminated soil during phytoremediation.

Furthermore, the effects of Cr (40 to 10 ppm) on root length and shoot height of *Pilea sp.* and *Lantana sp.* are presented in **Figures 4.85** to **4.87**, after 98 days soil phytoremediation. The root and shoot lengths of *Pilea sp.* in EFB, SMC amended, and unamended soils, recorded between 13.4 to 12.87 cm and 34.3 to 28.03 cm, 13.19 to 12.64 cm and 30.64 to 27.33 cm, and also 8.56 to 8.30 cm, respectively. Significant differences were seen between the root and shoot height/length of amended, and the unamended treatments [F: (p<0.05) =301.51]. This also reflected on Cr stabilization in soils, as rightly elaborated earlier on. However, correspondingly, *Lantana sp.* recorded root length and shoot height of about 14.13 to 13.77 cm and 35.0 to 27.33 cm for EFB supplemented Cr-soils, 13.29 to 12.96 cm and 32.37 to 27.33 cm for SMC amended Cr-soils, and in addition 8.77 to 8.33 cm and 26.70 to 23.83 cm, for unamended soil. Hence, significant difference F: (p<0.05) =344.11 in favour of roots and shoots from organic amended treatments were observed.

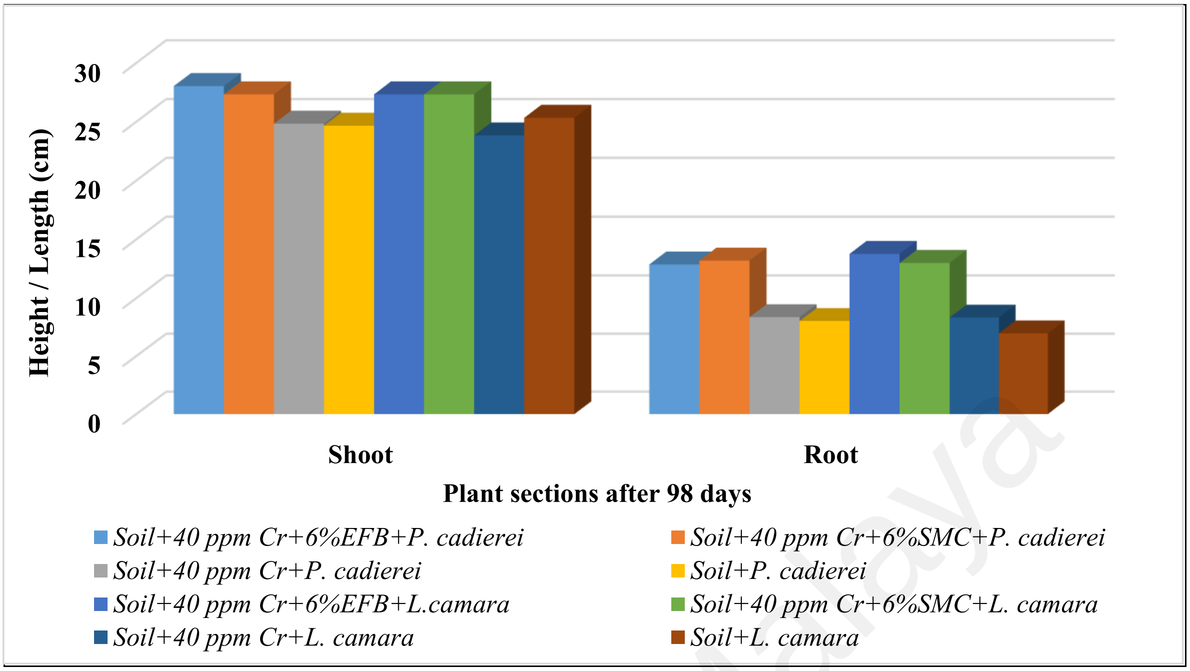


Figure 4.85: Postharvest comparison of plant shoots height and roots length exposed to 40 ppm Cr contaminated soil during phytoremediation.

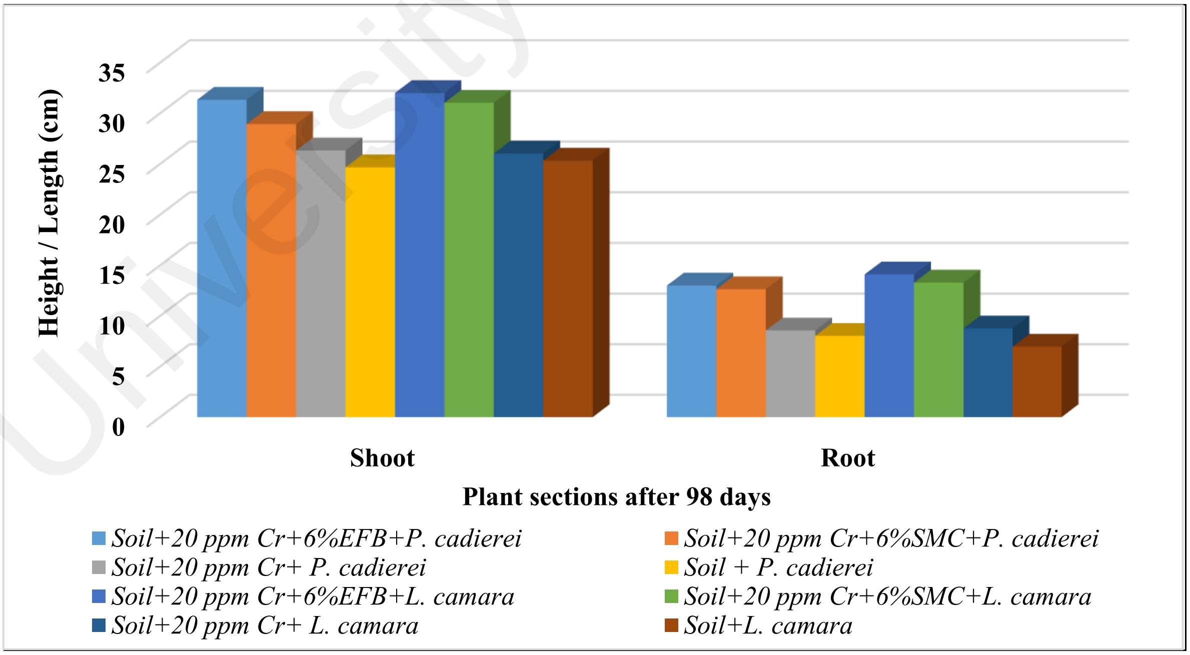


Figure 4.86: Postharvest comparison of plant shoots height and roots length exposed to 20 ppm Cr contaminated soil during phytoremediation.

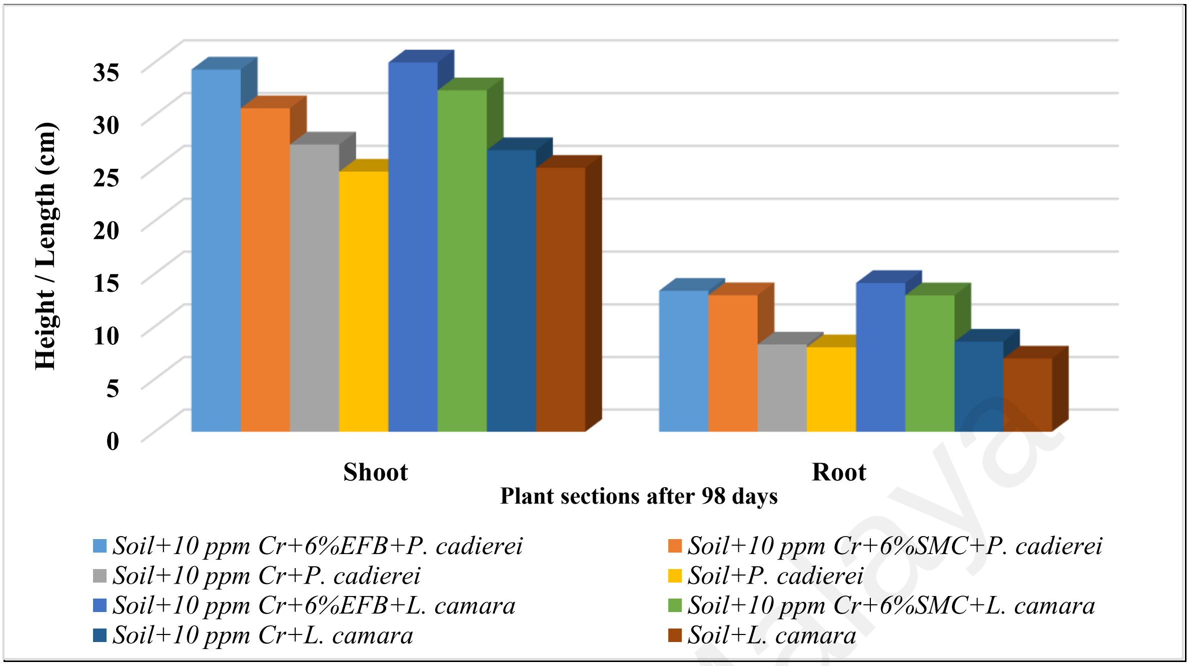


Figure 4.87: Postharvest comparison of plant shoots height and roots length exposed to 10 ppm Cr contaminated soil during phytoremediation.

Although, no significant differences were recorded among the supplemented plants, but their shoot heights, nominally appeared lower at this order 10 ppm Cr > 20 ppm Cr > 40 ppm Cr, respectively. This trend was also implicated by John et al., (2009) on the effects of Cd toxicity on *Brassica juncea* L. However, this current study, indicted Cr toxicity to trigger stunted growth on *Pilea sp.* and *Lantana sp.* unamended bioindicator plants, respectively.

Graphical analysis of root length and shoot heights at Pb and Cr co-contaminated soil under *Pilea sp.* and *Lantana sp.* are shown in **Figure 4.88**. There was a clear case of both root and shoot reductions of about 8.63% and 9.83%, and also 9.02% and 9.83% for *Pilea sp.* and *Lantana sp.* lengths, when compared to values gotten from single-Cr soil plants (**Figure 4.86**). Further to this, about 13.5% and 14.1%, together with 7.62% and 7.98% root and shoot declines were noticed at *Pilea sp.* and *Lantana sp.* harvest, when correlated to lone-Pb soil-plants (**Figure 4.83**), at significant level F: ($p<0.05$) =401.02 for root lengths, and F: ($p<0.05$) =511.04 for shoot heights, respectively.

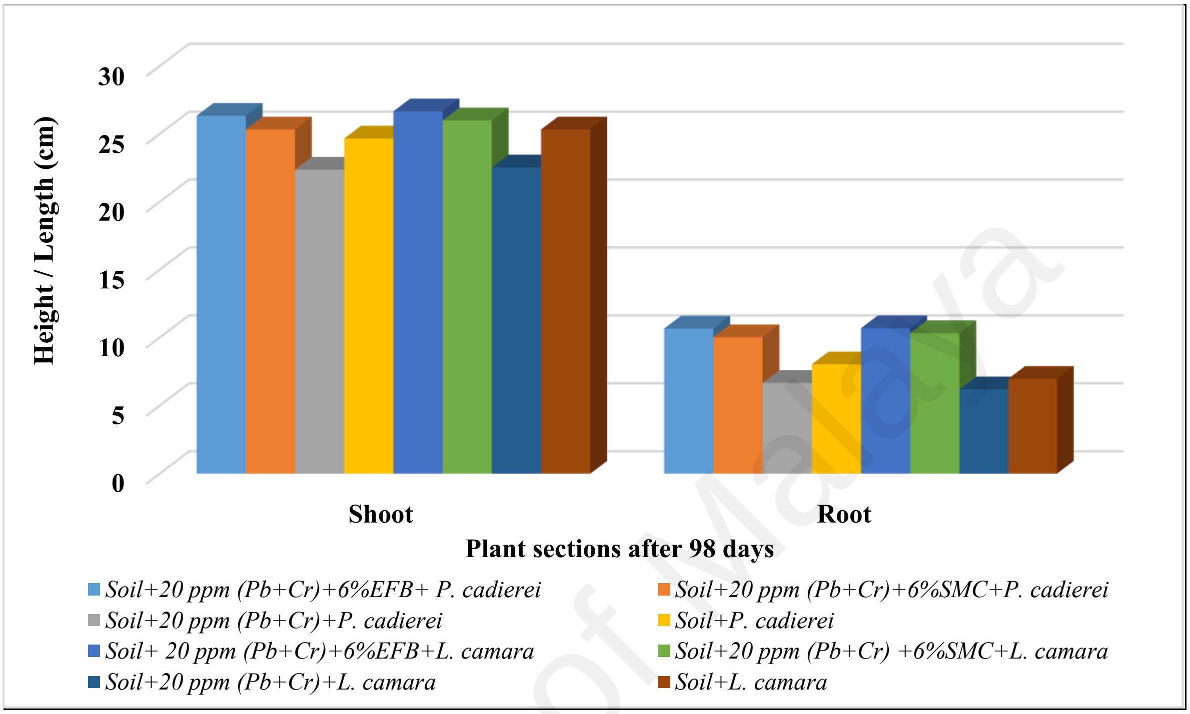


Figure 4.88: Postharvest comparison of plant shoots height and roots length exposed to 20 ppm (Pb+Cr) contaminated soil during phytoremediation.

This means, multiple metal contaminated soil, can technically impede plant developmental process, which thereafter could affect its phytoremediation ability. Meanwhile, combined stress of five-heavy metals, which reduced the root length and shoot heights of *alfalfa* plant, had been reported by Peralta-videa et al., (2002) during phytoremediation.

4.5.4 Plant uptake rate, isotherms and thermodynamics of heavy metal

Tables 4.5 and **4.6** show the rates of uptake and/or phytoreduction at different Pb treatments, while **Tables 4.7** and **4.8** equally represent Cr counterparts within the periods of 98 days by *Lantana sp.* and *Pilea sp.* with their accompanying half-life.

Table 4.5: Phytoreduction/uptake rates and half-life of *Lantana sp.* in Pb contaminated soil

Conc. and treatments	Pb Uptake rate (*k*) day^{-1}	Half-life (ln2/k) days	R^2
Soil +80 mg/kg Pb + EFB+ *Lantana sp.*	0.1256 ^{a}	5.52	0.9658
Soil +80 mg/kg Pb + SMC + *Lantana sp.*	0.1023 ^{a}	6.78	0.9534
Soil +80 mg/kg Pb + *Lantana sp.*	0.0377 ^{b}	18.39	0.9745
Soil +80 mg/kg Pb only.	0.0056 ^{a}	123.75	0.9103
Autoclaved soil +80 mg/kg Pb + 0.5%NaN_3	0.0019 ^{a}	364.74	0.8374
Soil +40 mg/kg Pb + EFB+ *Lantana sp.*	0.1358 ^{a}	5.10	0.9540
Soil +40 mg/kg Pb + SMC + *Lantana sp.*	0.1075 ^{a}	6.45	0.9460
Soil +40 mg/kg Pb + *Lantana sp.*	0.0398 ^{b}	17.42	0.9402
Soil +40 mg/kg Pb only.	0.0090 ^{a}	77.02	0.9113
Autoclaved soil +40 mg/kg Pb + 0.5%NaN_3	0.0033 ^{a}	210.01	0.8898
Soil +20 mg/kg Pb + EFB+ *Lantana sp.*	0.1395 ^{a}	4.97	0.9553
Soil +20 mg/kg Pb + SMC + *Lantana sp.*	0.1118 ^{a}	6.20	0.9471
Soil +20 mg/kg Pb+ *Lantana sp.*	0.0586 ^{b}	11.83	0.8440
Soil +20 mg/kg Pb only.	0.0084 ^{a}	82.52	0.8749
Autoclaved soil +20 mg/kg Pb + 0.5%NaN_3	0.0043 ^{b}	161.20	0.6518
Soil +10 mg/kg Pb + EFB+ *Lantana sp.*	0.3069 ^{a}	2.26	0.8700
Soil +10 mg/kg Pb + SMC + *Lantana sp.*	0.2580 ^{b}	2.68	0.8499
Soil +10 mg/kg Pb+ *Lantana sp.*	0.1161 ^{b}	5.97	0.7818
Soil +10 mg/kg Pb only.	0.0179 ^{a}	38.72	0.9395
Autoclaved soil +10 mg/kg Pb + 0.5%NaN_3	0.0080 ^{a}	86.63	0.9005

Values followed by letter **b** indicate sig. difference ($p<0.05$) level, while "**a**" indicates not sig. ($p<0.05$) level.

Therefore, under *Lantana sp.*, soils polluted with 80 to 10 mg/kg Pb, but supplemented with EFB recorded uptake rates 0.1256 to 0.3069 day^{-1} (R^2= 0.9658- 0.8700), while with SMC recorded about 0.1023 to 0.2580 day^{-1} (R^2= 0.9534- 0.8499). In similar order, the unamended soil with *Lantana sp.* recorded 0.0377 to 0.1161 day^{-1} (R^2= 0.9745-0.8440). This result implicated EFB in facilitating Pb reduction rates, and best Pb uptake rate was at 10 mg/kg soil contamination ($p<0.005$). Hence, the optimum, and probably due to contamination level and biota associations, however this was elaborated further in Alaribe & Agamuthu, (2015). Therefore, this result observed that low Pb density in soil correspond to enhanced half-life, thus amended treatments recorded half-life ranged between 5.52 to 2.26 days (EFB), and 6.78 to 2.68 days (SMC). However, 18.39 to 5.97 days for (unamended), 123.75 to 38.72 days (control soil), as well as 364.74 to 86 days for (autoclaved soil) were also observed. This agreed with Bolan et al., (2003b) who reported that when biowaste were added to metal polluted soil, it quickens the soil remediation time, especially when metals are present at low concentration.

Table 4.6: Phytoreduction/uptake rates and half-life of *Pilea sp.* in Pb contaminated soil

Conc. and treatments	Pb Uptake rate (*k*) day^{-1}	Half-life (ln2/k) days	R^2
Soil +80 mg/kg Pb + EFB+ *Pilea sp.*	0.1040 ^{a}	6.66	0.9538
Soil +80 mg/kg Pb + SMC *Pilea sp.*	0.0957 ^{b}	7.24	0.9424
Soil +80 mg/kg Pb + *Pilea sp.*	0.0414 ^{b}	16.74	0.9759
Soil +80 mg/kg Pb only.	0.0056 ^{b}	123.75	0.9103
Autoclaved soil +80 mg/kg Pb + 0.5%NaN_3	0.0019 ^{a}	364.74	0.8374
Soil +40 mg/kg Pb + EFB+ *Pilea sp.*	0.1118 ^{a}	6.20	0.9493
Soil +40 mg/kg Pb + SMC + *Pilea sp.*	0.0996 ^{b}	6.96	0.9290
Soil +40 mg/kg Pb + *Pilea sp.*	0.0414 ^{b}	16.74	0.9774
Soil +40 mg/kg Pb only.	0.0090 ^{b}	77.02	0.9113
Autoclaved soil +40 mg/kg Pb + 0.5%NaN_3	0.0033 ^{a}	210.01	0.8898
Soil +20 mg/kg Pb + EFB+ *Pilea sp.*	0.1136 ^{a}	6.10	0.9477
Soil +20 mg/kg Pb + SMC + *Pilea sp.*	0.0994 ^{b}	6.97	0.9302
Soil +20 mg/kg Pb+ *Pilea sp.*	0.0544 ^{b}	12.84	0.8894
Soil +20 mg/kg Pb only.	0.0084 ^{b}	82.52	0.8749
Autoclaved soil +20 mg/kg Pb + 0.5%NaN_3	0.0043 ^{b}	161.20	0.6518
Soil +10 mg/kg Pb + EFB+ *Pilea sp.*	0.2629 ^{a}	2.64	0.8678
Soil +10 mg/kg Pb + SMC + *Pilea sp.*	0.2332 ^{a}	2.97	0.8078
Soil +10 mg/kg Pb+ *Pilea sp.*	0.0923 ^{b}	7.51	0.7740
Soil +10 mg/kg Pb only.	0.0179 ^{b}	38.72	0.9395
Autoclaved soil +10 mg/kg Pb + 0.5%NaN_3	0.0080 ^{b}	86.63	0.9005

Values followed by letter **b** indicate sig. difference ($p<0.05$) level, while "**a**" indicates not sig. ($p<0.05$) level.

Therefore, under *Pilea sp.* (Table 4.6), the half-life and rates of Pb reduction ranged from 6.66 to 2.64 days and 0.1118 to 0.262 day^{-1}; R^2= 0.9538-0.8678 for EFB amended soil, 7.24 to 2.97 days and 0.0994 to 0.2332 day^{-1}; R^2 = 0.9424-0.8078 for SMC amended soil, 123.75 to 7.51 days and 0.0056 to 0.0923 day^{-1}; R^2= 0.9759-0.7740 for *Pilea sp.* (unamended) soil. Half-life implicated 16.74 to 7.52 days, for rates of 0.0923 and 0.041 day^{-1} of Pb, to be reduced from soil, if not amended. It would be much longer if the Pb soil is left alone for natural attenuation. Agamuthu et al., (2010) observed similar trend, in soil contaminated with lubricating oil. This collectively means that, if contaminated soils are left un-facilitated, it can last forever to regain cleanliness.

Table 4.7: Phytoreduction/uptake rates and half-life of *Lantana sp.* in Cr contaminated soil

Conc. and treatments	Cr Uptake rate (*k*) day^{-1}	Half-life (ln2/k) days	R^2
Soil +40 mg/kg Cr + EFB+ *Lantana sp.*	0.1154 $^{\mathbf{a}}$	6.01	0.9736
Soil +40 mg/kg Cr + SMC + *Lantana sp.*	0.0965 $^{\mathbf{b}}$	7.18	0.9760
Soil +40 mg/kg Cr + *Lantana sp.*	0.0529 $^{\mathbf{b}}$	13.10	0.9918
Soil +40 mg/kg Cr only.	0.0081 $^{\mathbf{b}}$	85.57	0.9672
Autoclaved soil +40 mg/kg Cr + 0.5%NaN_3	0.0024 $^{\mathbf{a}}$	288.81	0.8650
Soil +20 mg/kg Cr + EFB+ *Lantana sp.*	0.1181 $^{\mathbf{a}}$	5.87	0.9446
Soil +20 mg/kg Cr + SMC + *Lantana sp.*	0.1024 $^{\mathbf{a}}$	6.77	0.9433
Soil +20 mg/kg Cr+ *Lantana sp.*	0.0497 $^{\mathbf{b}}$	13.95	0.8991
Soil +20 mg/kg Cr only.	0.0091 $^{\mathbf{b}}$	76.17	0.9774
Autoclaved soil +20 mg/kg Cr + 0.5%NaN_3	0.0027 $^{\mathbf{b}}$	256.7	0.7677
Soil +10 mg/kg Cr + EFB+ *Lantana sp.*	0.2236 $^{\mathbf{a}}$	3.10	0.9570
Soil +10 mg/kg Cr + SMC + *Lantana sp.*	0.2073 $^{\mathbf{a}}$	3.34	0.9620
Soil +10 mg/kg Cr+ *Lantana sp.*	0.1009 $^{\mathbf{b}}$	6.87	0.9204
Soil +10 mg/kg Cr only.	0.0168 $^{\mathbf{b}}$	41.26	0.9135
Autoclaved soil +10 mg/kg Cr + 0.5%NaN_3	0.0047 $^{\mathbf{b}}$	147.49	0.9401

Values followed by letter **b** indicate sig. difference ($p<0.05$) level, while "**a**" indicates not sig. ($p<0.05$) level.

Table 4.8: Phytoreduction/uptake rates and half-life of *Pilea sp.* in Cr contaminated soil

Conc. and treatments	Cr Uptake rate (*k*) day^{-1}	Half-life (ln2/k) days	R^2
Soil +40 mg/kg Cr + EFB+ *Pilea sp.*	0.1025 ^{a}	6.76	0.9663
Soil +40 mg/kg Cr + SMC + *Pilea sp.*	0.0725 ^{b}	9.56	0.9528
Soil +40 mg/kg Cr + *Pilea sp.*	0.0422 ^{b}	16.43	0.9818
Soil +40 mg/kg Cr only.	0.0081 ^{b}	85.57	0.9672
Autoclaved soil +40 mg/kg Cr + 0.5%NaN_3	0.0024 ^{a}	288.81	0.8650
Soil +20 mg/kg Cr + EFB+ *Pilea sp.*	0.1078 a	6.43	0.9088
Soil +20 mg/kg Cr + SMC + *Pilea sp.*	0.0750 ^{b}	9.24	0.8828
Soil +20 mg/kg Pb+ *Pilea sp.*	0.0451 ^{b}	15.37	0.7935
Soil +20 mg/kg Cr only.	0.0091 ^{b}	76.17	0.9774
Autoclaved soil +20 mg/kg Cr + 0.5%NaN_3	0.0027 ^{b}	256.7	0.7677
Soil +10 mg/kg Cr + EFB+ *Pilea sp.*	0.2130 ^{a}	3.25	0.9720
Soil +10 mg/kg Cr + SMC + *Pilea sp.*	0.1834 ^{b}	3.78	0.9720
Soil +10 mg/kg Cr+ *Pilea sp.*	0.0902 ^{b}	7.68	0.9159
Soil +10 mg/kg Cr only.	0.0168 ^{b}	41.26	0.9135
Autoclaved soil +10 mg/kg Cr + 0.5%NaN_3	0.0047 ^{b}	147.49	0.9401

Values followed by letter **b** indicate sig. difference ($p<0.05$) level, while "**a**" indicates not sig. ($p<0.05$) level.

Meanwhile, under 40-10 ppm Cr remediated soil, in both *Lantana sp.* and *Pilea* application, half-life reduced with respect to lower Cr concentration at specific treatments. This was also observed in Pb. However, soil amended with organic waste recorded higher rates of Cr uptake in all treatments, when compared to unamended soil. But higher metal uptake were seen in soil contaminated with 10 ppm Cr. Logically because *Lantana sp.* and *Pilea sp.* in soil polluted with 10 ppm Cr did not experience much stress due to low Cr contamination, compared to those in 40 and 20 ppm Cr marred soils. Hence, they are able to grow better, and uptake the metal at higher rate than those in 40 and 20 ppm Cr. These results in Table 4.7 and 4.8, factually points to soil amended with EFB to have recorded higher uptake rates of Cr.

Also, at co-contaminated soil in **Table 4.9**, results showed that at concurrent exposure of Pb and Cr, higher uptake rate of Pb was observed, and this necessitated to enhance half-life of Pb than Cr. Adediran et al., (2009) implicated Cr to have low mobility in absorption substrates when merged with Pb. Therefore, Adediran et al., (2009) arguments could be the facilitating factor behind this result, where higher uptake rate of Pb was recorded, in the presence of Cr, and this directly reduced Pb half-life than Cr.

Table 4.9: Phytoreduction/uptake rates and half-life of *Lantana sp.* and *Pilea sp.* in (Pb + Cr) co-contaminated soil.

Conc. and treatments	Uptake rate (*k*) day^{-1}		Half-life (ln2/k) days		R^{2}	
	Pb	Cr	**Pb**	Cr	**Pb**	Cr
Soil +20 ppm (Pb+Cr) + EFB+ *Lantana sp.*	**0.0744** ^{a}	0.0639^{a}	**9.32**	10.85	**0.9299**	0.9236
Soil +20 ppm (Pb+Cr) +EFB+ *Pilea sp.*	**0.0611** ^{a}	0.0491^{a}	**11.35**	22.38	**0.9402**	0.9477
Soil +20 ppm (Pb+Cr) + SMC *Lantana sp.*	**0.0510** ^{a}	0.0389^{a}	**13.59**	17.82	**0.9308**	0.9322
Soil +20 ppm (Pb+Cr) +SMC+ *Pilea sp.*	**0.0386** ^{b}	0.0289^{a}	**17.96**	23.98	**0.8813**	0.9168
Soil +20 ppm (Pb+Cr) + *Lantana sp.*	**0.0325** ^{a}	0.0290^{a}	**21.32**	23.90	**0.8642**	0.8377
Soil +20 ppm (Pb+Cr) + *Pilea sp.*	**0.0299** ^{a}	**0.2380**^{a}	**23.18**	29.12	**0.8130**	0.8010
Soil +20 ppm (Pb+Cr) only.	**0.0077** ^{b}	0.0062^{b}	**90.02**	111.8	**0.6287**	0.4942
Autoclaved soil +20 ppm (Pb+Cr) + 0.5%NaN_3	**0.0016** ^{a}	0.0009^{a}	**433.22**	770.2	**0.8270**	0.9311

Values followed by letter **b** indicate sig. difference ($p<0.05$) level, while “**a**” indicates not sig. ($p<0.05$) level.

Generally, visual assessment of both *Pilea sp.* and *Lantana sp.* in response to environmental stimuli at various doses of Pb (80 to 10 ppm), and Cr (40 to 10 ppm) monitored after 98 days showed no signs of absolute necrosis (**Plates 4.1 to 4.8)**. However, plants exposed to 80 ppm Pb and 40 ppm Cr contaminated soils, expressed some sings of induced phytotoxicity, especially the yellowing of leaves. This was due to oxidative stress, and may be the displacement of essential cations (Van Oosten & Maggio, 2015; Shanker et al., 2005). Unamended plants also recorded some signs of chlorosis. Such signs concurred with the findings of Doumette et al., (2008), which implied a strong resistance potentials of *Pilea sp.* and *Lantana sp.* to Pb and Cr polluted soil.

A: Sample of *Pilea sp.* plant under 80 ppm Pb soil amended with organic waste.

B: Sample of *Pilea sp.* plant under 40 ppm Cr soil amended with organic waste.

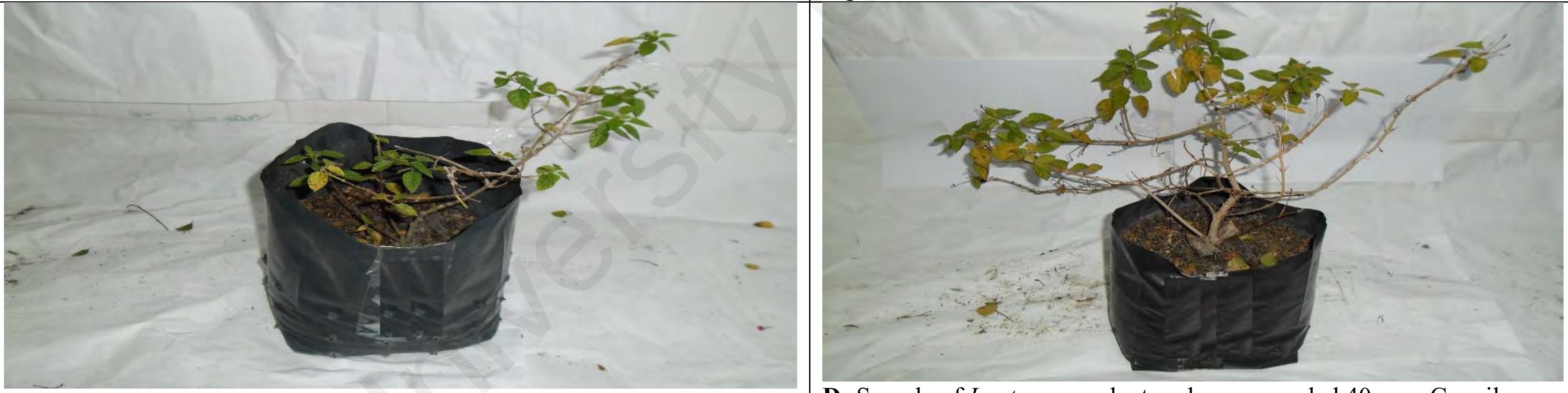
C: Sample of *Pilea sp.* plant under unamended 80 ppm Pb soil.

D: Sample of *Pilea sp.* plant under unamended 40 ppm Cr soil.

Plate 4.1A-D: Sampled appearances of *Pilea sp.* under 80 ppm Pb and 40 ppm Cr contaminated soils.

A: Sample of *Lantana sp.* plant under 80 ppm Pb soil amended with organic waste

B: Sample of *Lantana sp.* plant under 40 ppm Cr soil amended with organic waste.



C: Sample of *Lantana sp.* plant under unamended 80 ppm Pb soil.

D: Sample of *Lantana sp.* plant under unamended 40 ppm Cr soil.

Plate 4.2A-D: Sampled appearances of *Lantana sp.* under 80 ppm Pb and 40 ppm Cr contaminated soils.

Besides plant uptake rates, phytoremediation isotherms and other thermodynamic parameters, revealed insightful details about this study (**Table 4.10)**. Freundlich *n* and *k* represents adsorption intensity and capacity were used as indicators. Therefore, ***n***-values of 2.34, 2.38, and 3.39 were obtained at EFB, SMC amended, and Pb-unamended *Pilea sp.* soils; R^2 = 0.9303-0.9920, respectively. Furthermore, ***n***-magnitudes of 2.44, 2.38, and 2.97 were correspondingly witnessed at EFB-amended, SMC-aided, and Pb-unamended *Lantana sp.* soils; R^2 = 0.9012-0.9840. However, high ***n***-values, ranged from 5.99 to 6.59 were observed in Pb-soil, and at Pb-autoclaved soil controls. Meanwhile, the introduction of organic wastes, and plants lowered the ***n***-values (as seen above). Favourable adsorption is conferred with Freundlich constant ***n***, ranged from 1 to 10 (Senthil Kumar et al., 2010; Adediran et al., 2009).

This result indicated opposite movements of Pb from the soil towards the plants, and away from the bulk contaminated Pb soil. Due to Pb-root phytoextraction activities, through complexation by organic wastes. Hence, Pb adsorption and/or affinity to soil, decreased to about 47.14% and 42.2% when EFB and SMC was amended to plants in soils, with respect to Pb-soil adsorption intensity.

In continuation, the free energy ΔG, of phytoremediation obtained in **Table 4.10**, further substantiated Freundlich ***n***-values emphasized above, because, ΔG ranged from -4.130 to 0.075 $KJmol^{-1}K^{-1}$; R^2 = 0.9303, and -3.445 to 0.504 $KJmol^{-1}K^{-1}$; R^2 = 0.9477, were seen in EFB and SMC amended *Pilea sp*. soils, respectively. -5.01 to -0.25 $KJmol^{-1}K^{-1}$; R^2 = 0.9012, and -3.93 to -0.49 $KJmol^{-1}K^{-1}$; R^2 = 0.9327, were seen in EFB and SMC amended *Lantana sp.* soils, respectively. Similar ΔG trend of -7.5 to -7.02 $KJmol^{-1}K^{-1}$ were observed by Cantu et al., (2014) on Cr sorption to nano-materials, and was referred to as more spontaneous process.

Table 4.10: Phytoremediation modelling of 10 to 80 ppm Pb remediated soil under different treatment conditions.

			Phytoremediation Freudlich isotherm parameters		
Conc. and treatments	**ΔG** = (K J $mol^{-1}K^{-1}$)	**K_c**	***n***	***K_f*** (mg/kg)	R^2
Soil+ (10- 80) ppm Pb + EFB+ *Pilea sp.*	- 4.130 to 0.075	5.10- 0.81	2.38	0.02	0.9303
Soil + (10- 80) ppm Pb + SMC+ *Pilea sp*.	- 3.445 to 0.504	3.95- 0.74	2.32	0.01	0.9477
Soil + (10- 80) ppm Pb+ *Pilea sp.*	0.18 to 3.37	0.93-0.26	3.39	0.56	0.9920
Soil+ (10- 80) ppm Pb + EFB+ *Lantana sp.*	- 5.01 to -0.25	7.35- 1.10	2.51	0.03	0.9012
Soil+ (10- 80) ppm Pb + SMC+ *Lantana sp*.	- 3.93 to -0.49	4.79- 0.82	2.44	0.05	0.9327
Soil + (10- 80) ppm Pb+ *Lantana sp*.	- 0.79 to 2.94	1.37- 0.31	2.97	0.50	0.9840
Soil + (10- 80) ppm Pb	4.96 to 8.16	0.14- 0.04	5.59	0.62	0.9505
Autoclaved soil + (10- 80) ppm Pb + 0.5%NaN_3	6.91 to 10.73	0.06- 0.01	6.59	0.67	0.9980

Note: **ΔG** = Free energy; **K_c** = Equilibrium constant; ***n***= Freudlich intensity of adsorption; ***K_f*** = Freudlich capacity of metal adsorption to soil; R^2 = Coefficient of determination.

This implicated ΔG, have fluctuated between negative (i.e., spontaneity), and to values closer to zero (i.e., near to equilibrium) in this study. Hence, this phytoremediation process is considered to be exothermic, where Pb was inferred to be leaving the soil surrounding, at high equilibrium constant; $\mathbf{K}_c$ = 7.35 to 5.10. The unamended treatments had ΔG ranged from -0.79 to 3.37; R^2 = 0.9505-0.9980, compared to the more negative values recorded in amended treatments. This shows that amended treatments were more thermodynamically favoured (Cantu et al., 2014). However, the ΔG results from (soil-Pb only), and the (autoclaved-soil) controls, indicated endothermic when further correlated to the amended plant treatments. Meaning, little or no Pb left the system. Adediran et al., (2009) reported ΔG of -14 $KJmol^{-1}K^{-1}$ in dynamic adsorption of Pb^{2+} with *Lumbricina* cast soil. This contrasted with this study, may be due to plant and substrate differences.

Similarly, **Table 4.11** shows the modelling results for 10 to 40 ppm Cr soil, phytoremediation process. Under *Pilea sp.* application, Freundlich ***n***-values ranged 2.23 to 2.47; R^2 = 0.9327-0.9599, and ΔG; -2.63 to 0.28 $KJmol^{-1}K^{-1}$ and -1.83 to 1.23 $KJmol^{-1}K^{-1}$ were identified at EFB and SMC amended spots, respectively. Equally, ***n***-values of 2.46 to 2.25; R^2 = 0.9327-0.9599, ΔG; -2.84 to 0.32 $KJmol^{-1}K^{-1}$ and -2.39 to -0.49 $KJmol^{-1}K^{-1}$, and also equilibrium constant ($\mathbf{K}_c$) ranged from 3.10 to 1.13 and 2.57 to 0.87 were obtained at EFB and SMC amended soils of *Lantana sp.*, respectively. This indicates, reduction in Cr-binding affinity to soil, and its subsequent elimination with plant, enhanced by organic additives. Thus, induced favourable thermodynamic for phytoremediation (Chen et al., 2009; Cantu et al., 2014), when compared to other controls devoid of organic wastes and/or *Pilea sp. /Lantana sp*. However, Senthil-Kumar et al., (2010) agreed with this current study, where 1.504 and 0.789 of adsorption intensity (***n***) and capacity (K_f) were obtained, and was inferred to have showed favourable separation of Pb^{2+} ions from their respective substrates.

Table 4.11: Phytoremediation modelling of 10 to 40 ppm Cr remediated soil under different treatment conditions.

			Phytoremediation Freudlich isotherm parameters		
Conc. and treatments	**ΔG** = (KJmol^{-1}K^{-1})	**K$_c$**	***n***	***K$_f$*** (mg/kg)	R^2
Soil+ (10- 40) ppm Cr + EFB+ *Pilea sp.*	- 2.63 to 0.28	2.63- 0.89	2.23	0.03	0.9701
Soil + (10- 40) ppm Cr + SMC+ *Pilea sp*.	- 1.83 to 1.23	2.07- 0.62	2.47	0.20	0.9599
Soil + (10- 40) ppm Cr + *Pilea sp*.	0.50 to 3.03	0.81-0.30	3.09	0.50	0.9734
Soil+ (10- 40) ppm Cr + EFB+ *Lantana sp.*	- 2.84 to 0.32	3.10- 1.13	2.46	0.07	0.9654
Soil+ (10- 40) ppm Cr + SMC+ *Lantana sp*.	- 2.39 to -0.49	2.59- 0.87	2.25	0.04	0.9757
Soil + (10- 40) ppm Cr+ *Lantana sp.*	0.08 to 2.41	0.97- 0.38	3.15	0.49	0.9633
Soil + (10- 40) ppm Cr	5.49 to 7.52	0.11- 0.05	7.20	0.55	0.9370
Autoclaved soil + (10- 40) ppm Cr + 0.5%NaN$_3$	9.77 to 8.95	0.02- 0.03	3.59	0.68	0.9980

Note: **ΔG** = Free energy; **K$_c$** = Equilibrium constant; ***n***= Freudlich intensity of adsorption; ***K$_f$*** = Freudlich capacity of metal adsorption to soil; R^2 = Coefficient of determination.

Further modelling of the co-contaminated (Pb+Cr) soil, shown in **Table 4.12**, was more complicating but anticipated, due to uncommon metal speciation. Surprisingly, ***n***-values obtained were absolutely negative across all phytoremediation treatments and controls, from -9.10 to -0.66; R^2=1, and -0.35 to -0.22; R^2 =1, respectively. Correspondingly, ΔG ranged from 1.99 to 4.44 $KJmol^{-1}K^{-1}$; R^2=1, and -2.66 to 4.32 $KJmol^{-1}K^{-1}$; R^2=1 for all *Pilea sp.* and *Lantana sp.* treatments, respectively. Implicating more of endothermic effects (Chen et al., 2009). In addition to 7.26 to 12.44 $KJmol^{-1}K^{-1}$ for compared controls devoid of plant and/or organic wastes. This unfolding trend, obviously reveals the problematic nature of co-contaminated metal substrates, because, Freundlich indicators ***n*** and $\boldsymbol{K_f}$, suggested this to be unfavourable (Senthil-Kumar et al., 2010), for adsorption of Pb and Cr to soil.

However, data from ΔG (**Table 4.12**), implied that metal elimination was more or less inactive in the system via phytoextraction. Therefore, the accompanying equilibrium constants were also observed to be poor. This could cause metal leaching from soil, especially when the pH is acidic. Therefore, phytostabilization may be implicated as the mechanism, through which *Pilea sp.* and *Lantana sp.* dealt with this situation. Sun et al., (2011) could not investigate this contrasting thermodynamic effect in their study on phytoremediation of co-contamination metal soil. This was considered important in this current study, and has provided more insight into the precarious dynamics of soil-multiple metal contamination.

Table 4.12: Phytoremediation modelling of 20 ppm (Pb+Cr) remediated soil under different treatment conditions.

			Phytoremediation Freudlich isotherm parameters		
Conc. and treatments	**ΔG** = (KJmol^{-1}K^{-1})	**K$_c$**	***n***	***K$_f$*** (mg/kg)	R^2
Soil+ 20 ppm (**Pb**+Cr) + EFB+ *Pilea sp.*	**1.99** to 2.83	0.45- 0.33	-9.10	0.91	1.0
Soil + 20 ppm (Pb+Cr) + SMC+ *Pilea sp.*	3.18 to 3.99	0.28- 0.20	-4.20	1.93	1.0
Soil + 20 ppm (Pb+Cr) + *Pilea sp.*	3.81 to 4.44	0.22-0.17	-0.66	4.30	1.0
Soil+ 20 ppm (Pb+Cr) + EFB+ *Lantana sp.*	- 2.66 to 2.22	1.06- 0.41	-1.20	3.49	1.0
Soil+ 20 ppm (Pb+Cr) + SMC+ *Lantana sp*.	2.50 to 3.24	0.37- 0.27	-4.20	1.93	1.0
Soil + 20 ppm (Pb+Cr) + *Lantana sp*.	3.48 to 4.32	0.25- 0.18	-0.73	4.15	1.0
Soil + 20 ppm (Pb+Cr)	7.26 to 7.92	0.06- 0.04	-0.35	5.83	1.0
Autoclaved soil + 20 ppm (Pb+Cr) + 0.5%NaN$_3$	11.30 to 12.44	0.011- 0.001	-0.22	7.47	1.0

Note: **ΔG** = Free energy; **K$_c$** = Equilibrium constant; ***n***= Freudlich intensity of adsorption; ***K$_f$*** = Freudlich capacity of metal adsorption to soil; R^2 = Coefficient of determination.

The experimental data fitted into the intra-particle diffusion model for 80 to 10 ppm Pb phytoremediated soil, are shown in **Tables 4.13** to **4.14**. However, the intra-particle diffusion rate constant (**K_{int})** of Pb (80 to 10 ppm) in *Lantana sp.* ranged from 7.064-1.354 mg/kg/day$^{0.5}$; R^2= 0.9758-0.981 for EFB amended soil, 6.364 -1.306 mg/kg/day$^{0.5}$; R^2=0.9758-0.981 for SMC amended soil, 3.014- 0.869 mg/kg/day$^{0.5}$; R^2=0.9740-0.98696 for *Lantana sp.* (unamended) control, 0.436-0.166 mg/kg/day$^{0.5}$; R^2=0.9089-0.9441 for Pb control, and 0.146-0.018 mg/kg/day$^{0.5}$; R^2=0.9078-0.8236 for autoclaved control soil. Significant among these values is the 80 ppm EFB amended soil, with kinetic relationship y= **K_{int}** (7.064 mg/kg/day$^{0.5}$) x **-C_i** (2.003 mg/kg). This proved that concentration gradient was an important diffusion factor (Cantu et al., 2014), and EFB enhanced multi-linearity absorption of Pb at the contaminated soil medium, during phytoremediation (**Appendix Figure 1**). This results agreed with Bhalara et al., (2014) isotherm model on uranium ion removal from contaminated substrate.

Similarly, the corresponding **K_{int}**-values under *Pilea sp*. at 80-10 ppm Pb soil recorded 6.259-1.201 mg/kg/day$^{0.5}$; R^2= 0.9482-0.9317(EFB), 5.983-1.201 mg/kg/day$^{0.5}$; R^2= 0.9482-0.9317 (SMC), and 2.966-0.069 for *Pilea sp.* (unamended) control. Meanwhile, *Pilea sp.* amended with EFB at 80 ppm dosage also recorded highest rate constant, hence Pb multi-linearity absorption is expressed as thus; y= **K_{int}** (6.259 mg/kg/day$^{0.5}$) x **-C_i** (6.523 mg/kg), see **Appendix Figure 2**. Graphs of q_t vs. $t^{0.5}$ from the respective relationships, indicated more than one point interaction of Pb during phytoremediation. This agreed with the reports of Kumar et al., (2009) on Cr adsorption, but contrasted because of the amendments applied.

Table 4.13: Intraparticle diffusion in 80 and 40 ppm Pb phytoremediated soil.

Conc. and treatments	Kinetic relationship (y=)	K*int* (mg/kg$^{-0.5}$)	C*i* (mg/kg)	Coefficient = (R^2)
<u>80 ppm Pb</u>				
Soil +80 mg/kg Pb + EFB+ *Pilea sp.*	6.259 x -6.523	6.259	-6.523	0.9592
Soil +80 mg/kg Pb + SMC *Pilea sp.*	5.983 x -8.857	5.983	-8.857	0.9557
Soil +80 mg/kg Pb + *Pilea sp*.	2.966 x -3.93	2.966	-3.930	0.9802
Soil +80 mg/kg Pb + EFB+ *Lantana sp.*	7.064 x -6.858	7.064	-6.858	0.9758
Soil +80 mg/kg Pb + SMC + *Lantana sp.*	6.364 x -7.403	6.364	-7.403	0.9233
Soil +80 mg/kg Pb + *Lantana sp.*	3.014 x -2.003	3.014	-2.003	0.9740
Soil +80 mg/kg Pb only.	0.436 x +0.336	0.436	0.336	0.9100
Autoclaved soil +80 mg/kg Pb + 0.5%NaN_3	0.146 x -2.003	0.146	-2.003	0.8236
<u>40 ppm Pb</u>				
Soil +40 mg/kg Pb + EFB+ *Pilea sp.*	3.304 x - 3.870	3.304	-3.870	0.9598
Soil +40 mg/kg Pb + SMC *Pilea sp.*	3.063 x -4.526	3.063	-4.526	0.9482
Soil +40 mg/kg Pb + *Pilea sp.*	1.479 x -1.931	1.479	-1.931	0.9808
Soil +40 mg/kg Pb + EFB+ *Lantana sp.*	3.708 x -3.927	3.708	-3.708	0.9751
Soil +40 mg/kg Pb + SMC + *Lantana sp.*	3.225 x -4.136	3.225	-4.136	0.9566
Soil +40 mg/kg Pb + *Lantana sp.*	1.407 x -1.097	1.407	-1.097	0.9413
Soil +40 mg/kg Pb only.	0.348 x -0.157	0.344	-0.157	0.9089
Autoclaved soil +40 mg/kg Pb + 0.5%NaN_3	0.156 x -0.10	0.156	-0.10	0.9058

Table 4.14: Intraparticle diffusion of 20 and 10 ppm Pb phytoremediated soil.

Conc. and treatments	Kinetic relationship (y=)	K*int* (mg/kg$^{-0.5}$)	C*i* (mg/kg)	Coefficient = (R^2)
20 ppm Pb				
Soil +20 mg/kg Pb + EFB+ *Pilea*	1.669 x -1.953	1.669	-1.953	0.9589
Soil +20 mg/kg Pb + SMC *Pilea*	1.528 x -2.253	1.528	-2.253	0.9500
Soil +20 mg/kg Pb + Pilea	0.979 x -1.873	0.979	-1.873	0.8891
Soil +20 mg/kg Pb + EFB+ *Lantana*	1.913 x -2.141	1.913	-2.141	0.9741
Soil +20 mg/kg Pb + SMC + *Lantana*	1.659 x -2.219	1.659	-2.219	0.9619
Soil +20 mg/kg Pb + *Lantana*	0.978 x -1.284	0.978	-1.284	0.9145
Soil +20 mg/kg Pb only.	0.164 x -0.067	0.164	-0.067	0.8820
Autoclaved soil +20 mg/kg Pb + 0.5%NaN_3	0.022 x 0.023	0.022	0.023	0.8575
10 ppm Pb				
Soil +10 mg/kg Pb + EFB+ *Pilea*	1.275 x -1.016	1.275	-1.016	0.9703
Soil +10 mg/kg Pb + SMC *Pilea*	1.264 x -1.891	1.264	-1.891	0.9317
Soil +10 mg/kg Pb + Pilea	0.069 x +0.051	0.069	0.051	0.8705
Soil +10 mg/kg Pb + EFB+ *Lantana*	1.354 x -0.996	1.354	-0.996	0.9817
Soil +10 mg/kg Pb + SMC + *Lantana*	1.306 x -1.553	1.306	-1.553	0.9704
Soil +10 mg/kg Pb + *Lantana*	0.869 x -1.477	0.869	-1.477	0.8696
Soil +10 mg/kg Pb only.	0.166 x -0.054	0.166	-0.054	0.9441
Autoclaved soil +10 mg/kg Pb + 0.5%NaN_3	0.078 x -0.06	0.0178	0.006	0.9078

However, at 40 to 10 ppm Cr phytoremediated soil, the $\mathbf{K}_{int}$ relationship obtained in both *Lantana sp.* and *Pilea sp.* supplemented with EFB at 40 ppm Cr recorded highest values, thus; y= $\mathbf{K}_{int}$ (3.260 mg/kg/day$^{0.5}$) x -$\mathbf{C}_i$ (2.117 mg/kg); R^2= 0.9840-0.9345, and y= $\mathbf{K}_{int}$ (3.067 mg/kg/day$^{0.5}$) x -$\mathbf{C}_i$ (3.393 mg/kg); R^2= 0.9819-0.9532, respectively (**Tables 4.15** to **4.16)**. **Appendix Figures 3** and **4** show the typical graph illustration. However, this does not imply that multi-linearity were not prevalent at lower Cr-concentrated soil, but rather, EFB and higher concentration factor, enhanced the instantaneous and gradual absorption stages of Cr, and up to its final equilibrium points. Demirbas et al., (2004) argued that the solute uptake rate controls the resident time of adsorbate uptake at the solid-solution interface, and that the adsorption rate constant can be used to compare the performance of each system.

Furthermore, the co-contaminated soil (**Table 4.17**), revealed the diffusion rate constant ($\mathbf{K}_{int}$) ranged from 1.210 to 1.069 mg/kg; R^2= 0.9384-0.9306, and 0.734 to 0.860 mg/kg; R^2= 0.9608-0.8224 for *Lantana sp.* amended EFB and SMC soils, respectively. Differently, 1.033 to 0.859 mg/kg; R^2= 0.9557-0.9529, and 0.691 to 0.535 mg/kg; R^2= 0.9044-0.9314 for *Pilea sp*. were recorded for EFB and SMC treated soils, respectively. Hence, this is lower than the $\mathbf{K}_{int}$ recorded in single metal contaminated soils, and this can influence the metals pattern of multi-linear absorption (Bhalara et al., (2014) (**Appendix Figures 5** and **6)**. However, the reasons for this might be the effects of Pb and Cr combination, cation antagonisms, and unspecific speciation that slowed the individual metals rates of mobility and/or diffusion. If diffusion to strategic metals removal points are impeded during phytoremediation, invariably metal elimination will be poor.

Table 4.15: Intraparticle diffusion of 40 and 20 ppm Cr phytoremediated soil.

Conc. and treatments	Kinetic relationship (y=)	K*int* (mg/kg$^{-0.5}$)	C*i* (mg/kg)	Coefficient = (R^2)
40 ppm Cr				
Soil +40 mg/kg Cr + EFB+ *Pilea sp.*	3.067 x - 3.393	3.067	-3.393	0.9800
Soil +40 mg/kg Cr + SMC *Pilea sp.*	2.418 x -2.761	2.418	-2.761	0.9719
Soil +40 mg/kg Cr + *Pilea sp.*	1.495 x -1.539	1.495	-1.539	0.9832
Soil +40 mg/kg Cr + EFB+ *Lantana sp.*	3.260 x -2.117	3.260	-2.117	0.9840
Soil +40 mg/kg Cr + SMC + *Lantana sp.*	2.881 x -2.321	2.881	-2.881	0.9916
Soil +40 mg/kg Cr + *Lantana sp.*	1.781 x -1.063	1.781	-1.063	0.9880
Soil +40 mg/kg Cr only.	0.313 x -0.033	0.313	-0.033	0.9639
Autoclaved soil +40 mg/kg Cr + 0.5%NaN_3	0.186 x 0.01	0.186	0.01	0.8634
20 ppm Cr				
Soil +20 mg/kg Cr + EFB+ *Pilea sp.*	1.553 x - 1.733	1.553	-1.733	0.9819
Soil +20 mg/kg Cr + SMC *Pilea sp.*	1.171 x -1.419	1.171	-1.419	0.9205
Soil +20 mg/kg Cr + *Pilea sp.*	0.778 x -1.163	0.778	-1.163	0.8313
Soil +20 mg/kg Cr + EFB+ *Lantana sp.*	1.65 x -1.183	1.65	-1.183	0.9697
Soil +20 mg/kg Cr + SMC + *Lantana sp.*	1.526 x -1.649	1.526	-1.649	0.9743
Soil +20 mg/kg Cr + *Lantana sp.*	0.811 x -0.834	0.811	-0.834	0.9232
Soil +20 mg/kg Cr only.	0.176 x -0.066	0.176	-0.066	0.9766
Autoclaved soil +20 mg/kg Cr + 0.5%NaN_3	0.096 x +0.049	0.096	0.049	0.7841

Table 4.16: Intraparticle diffusion of 10 ppm Cr phytoremediated soil.

Conc. and treatments	Kinetic relationship (y=)	K*int* (mg/kg$^{-0.5}$)	C*i* (mg/kg)	Coefficient = (R^2)
Soil +10 mg/kg Cr + EFB+ *Pilea sp.*	1.176x -1.04	1.176	-1.04	0.9532
Soil +10 mg/kg Cr + SMC *Pilea sp.*	1.201x -1.284	1.201	-1.284	0.9730
Soil +10 mg/kg Cr + *Pilea sp.*	0.701x -0.954	0.701	-0.954	0.954
Soil +10 mg/kg Cr + EFB+ *Lantana sp.*	1.228x -0.716	1.228	-0.716	0.9345
Soil +10 mg/kg Cr + SMC + *Lantana sp.*	1.256x -1.666	1.256	-1.666	0.9509
Soil +10 mg/kg Cr + *Lantana sp.*	0.755 x -0.755	0.755	-0.755	0.9421
Soil +10 mg/kg Cr only.	0.158x -0.161	0.158	-0.161	0.9212
Autoclaved soil +10 mg/kg Cr + 0.5%NaN_3	0.0271x +0.027	0.0271	0.027	0.9400

Table 4.17: Intraparticle diffusion of 20 ppm (Pb+ Cr) phytoremediated soil

Conc. and treatments	K*int* (mg/kg$^{-0.5}$)	C*i* (mg/kg)	Coefficient = (R^2)
Soil+ 20 ppm (**Pb**+Cr) + EFB+ *Pilea sp.*	1.033 to 0.859	-1.489 to -1.114	0.9557-0.9529
Soil + 20 ppm (Pb+Cr) + SMC+ *Pilea sp.*	0.691 to 0.535	-1.134 to -0.857	0.9044-0.9314
Soil + 20 ppm (Pb+Cr) + *Pilea sp.*	0.539 to 0.445	- 0.981 to -0.842	0.7969-0.8108
Soil+ 20 ppm (Pb+Cr) + EFB+ *Lantana sp.*	1.210 to 1.069	-1.503 to -1.269	0.9384-0.9306
Soil+ 20 ppm (Pb+Cr) + SMC+ *Lantana sp.*	0.734 to 0.860	-0.964 to -1.371	0.9608-0.8224
Soil + 20 ppm (Pb+Cr) + *Lantana sp.*	0.588 to 0.533	-0.893 to -0.866	0.0884-0.8525
Soil + 20 ppm (Pb+Cr)	0.149 to 0.120	-0.214 to 0.057	0.625- 0.4875
Autoclaved soil + 20 ppm (Pb+Cr) + 0.5%NaN_3	0.032 to 0.020	0.033 to 0.020	0.8136-0.9311

4.5.5 SEM phytoremediation observation on soil and plant tissues

Scanning electron microscopy (SEM), shown in **Plates 4.3A-C**, represent the impacts of reduction of metals during phytoremediation. The shiny areas in SEM, B-image, indicates Pb-deposits in un-remediated soil. However, after phytoremediation, the intensity of shining reduced at SEM section C, after 98 days. This was also observed with Cr in **Plates 4.4A-C**, where its sparkling intensity dropped in polluted soil, implicating reduction, after 98 days under amended conditions. Meanwhile, these are representative images, chosen from 40 ppm Pb or Cr remediated soils, due to visual clarity, and equal doses of Pb and Cr monitored, in addition to resource limitation. However, this result agreed with Ahmad et al., (2012) who reported decrease in Pb-intensity of brightness, in a polluted soil imaged with SEM, after immobilization with eggshell waste.

Plate 4.3A: **SEM** image of the **control soil.**

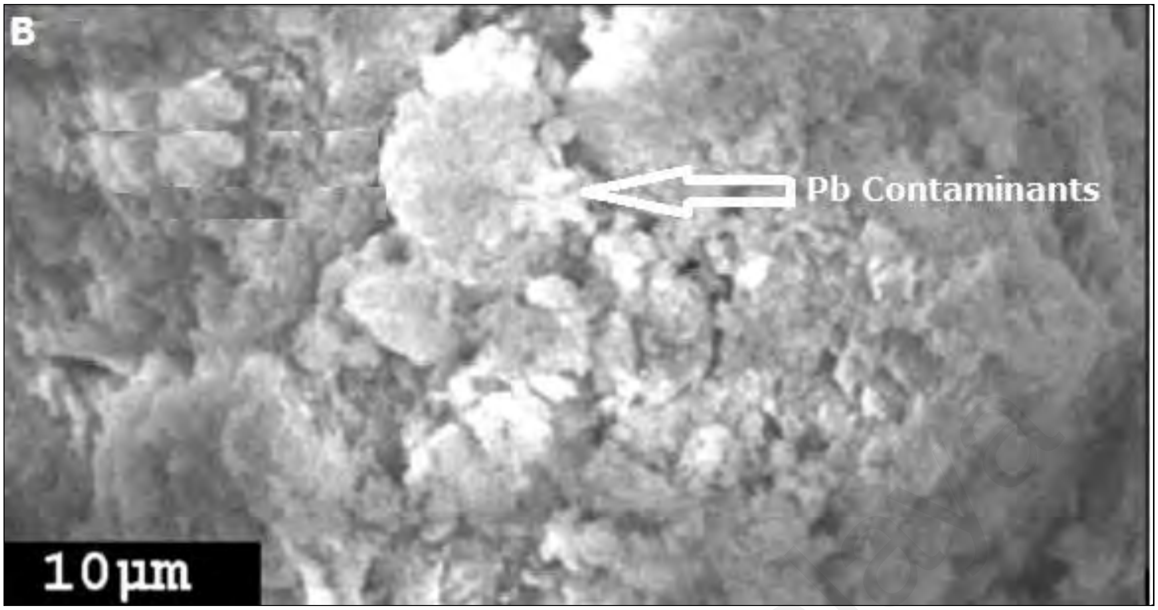


Plate 4.3B: SEM of 40 ppm Pb-contaminated soil with amendments

Note: The **shiny areas** in (**B**) shows high Pb concentration and/or contaminant in sediments.

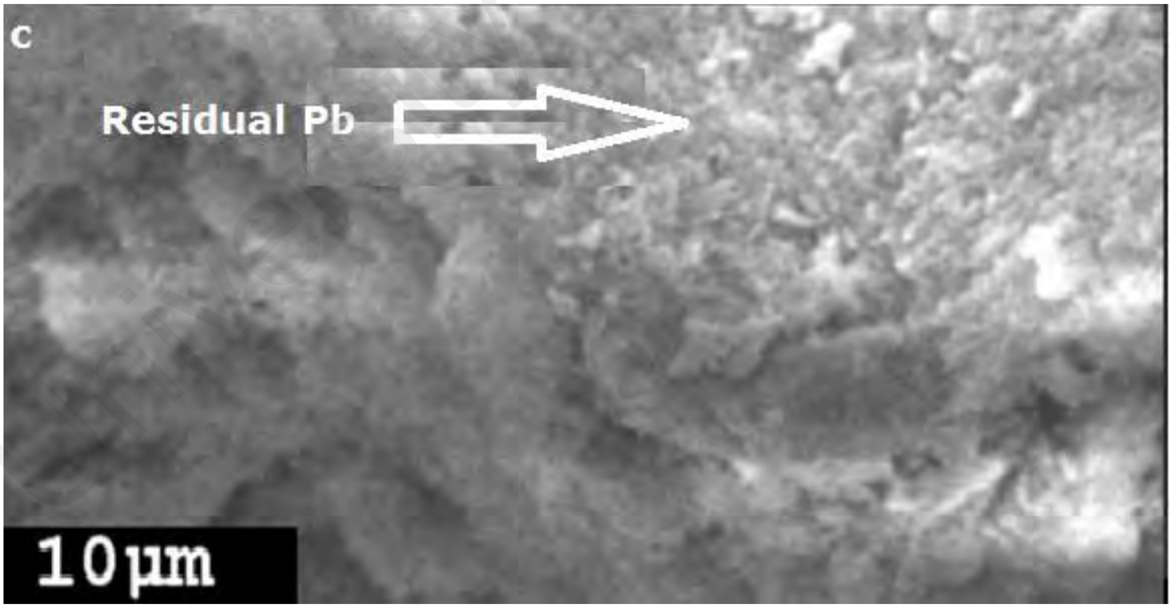


Plate 4.3C: SEM of 40 ppm Pb-post phytoremediated soil after 98days.

Note: The less **shiny areas** shows drop at Pb concentration and/or contaminant in sediments.

Plate 4.4A: SEM of the control soil.

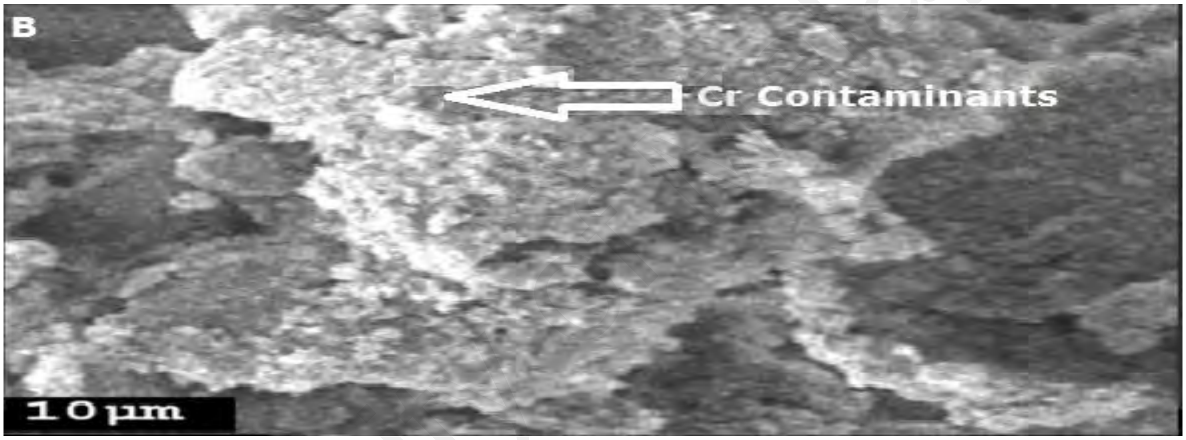


Plate 4.4B: SEM of 40 ppm Cr-contaminated soil with amendments.

Note: The **shiny areas** in (**B**) reflects high Cr concentration and/or contaminant in soil.

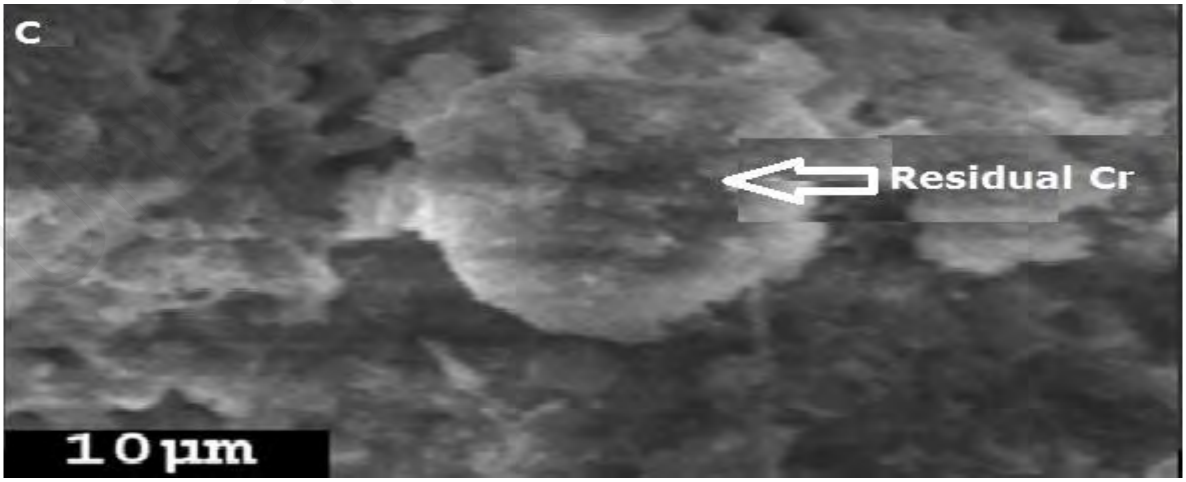


Plate 4.4C: SEM of 40 ppm Cr-post phytoremediated soil after 98days.

Note: The less **shiny areas** indicates drop in Cr concentration and/or contaminant in soil.

Similarly, light micrograph analysis shown in **Plates 4.5A-C**, implicated evidence of Pb and Cr translocated are deposited as black spots, along the xylem and phloem walls of *Pilea sp.*, when compared to the control image in A. Meanwhile, this recent evidence suggest moderate metal accumulation by plant. Kumar et al., (1995) also reported such dark spots in transports tissues of *Brassica juncea* during phytoextraction of Zn and Cd contaminated soil. Furthermore, cortex breakdown was also observed in *Lantana sp.* under Pb and Cr influence (**Plates 4.6A-C**). Indicating metal translocation to the root and shoot area when compared to the control plant. This structural changes implies adaptive significance due to metal presence (Aravind & Prasad, 2005).

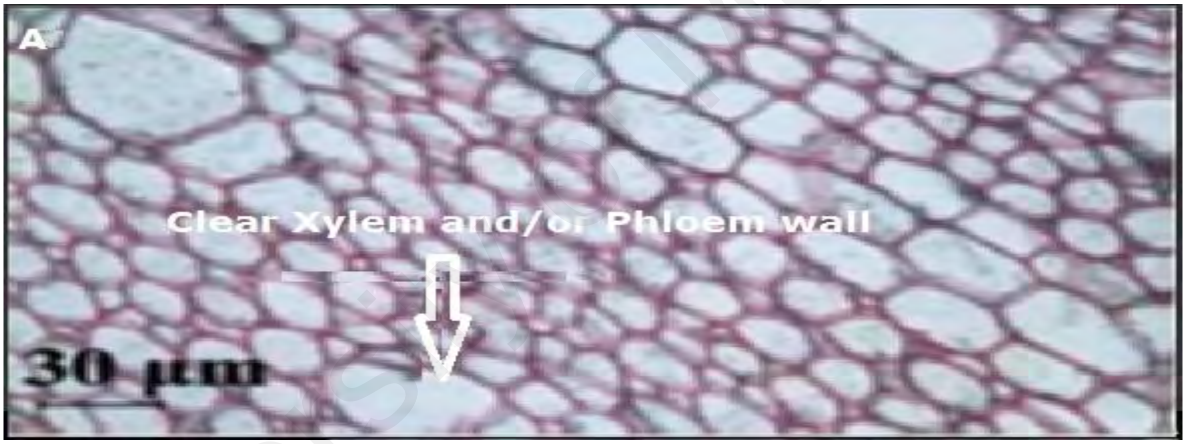


Plate 4.5A: Light micrographs showing the transverse section of *Pilea sp.* stems of control.

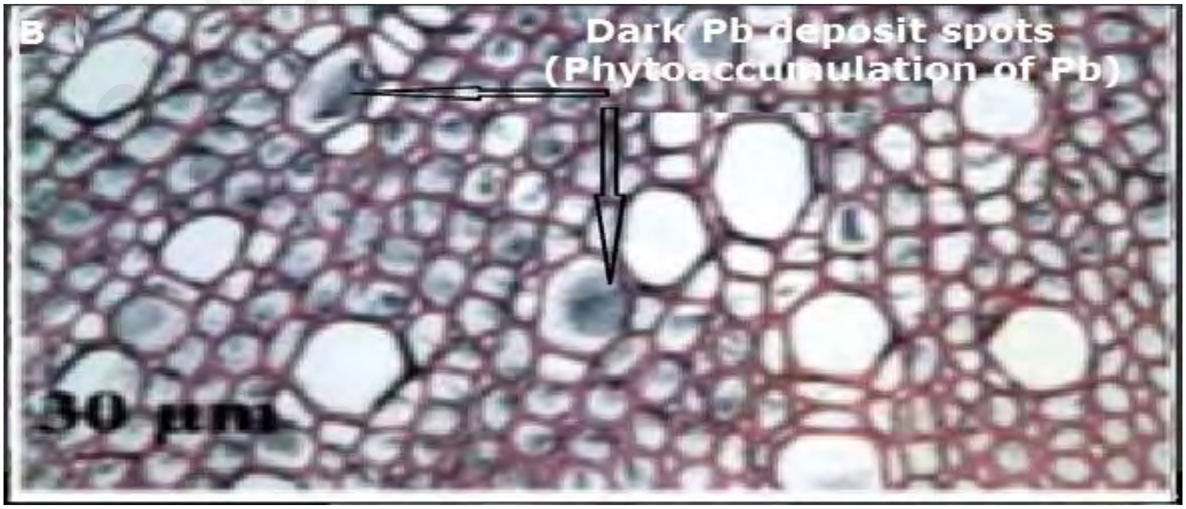


Plate 4.5B: *Pilea sp.* stem from 40 ppm **Pb** organic waste amended soil after 98 days.

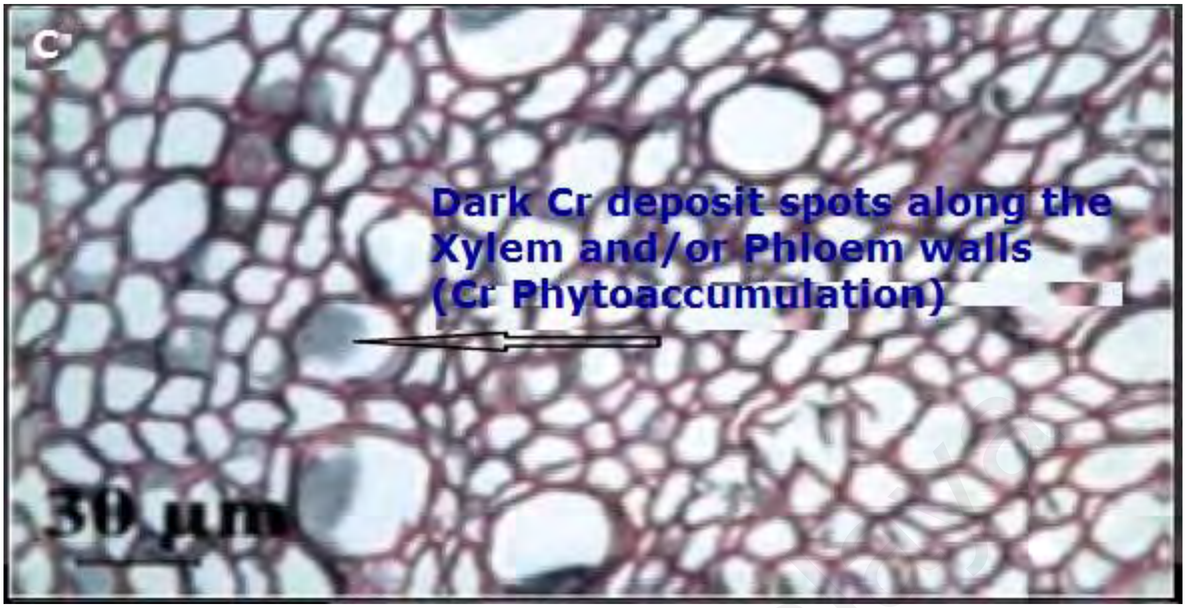
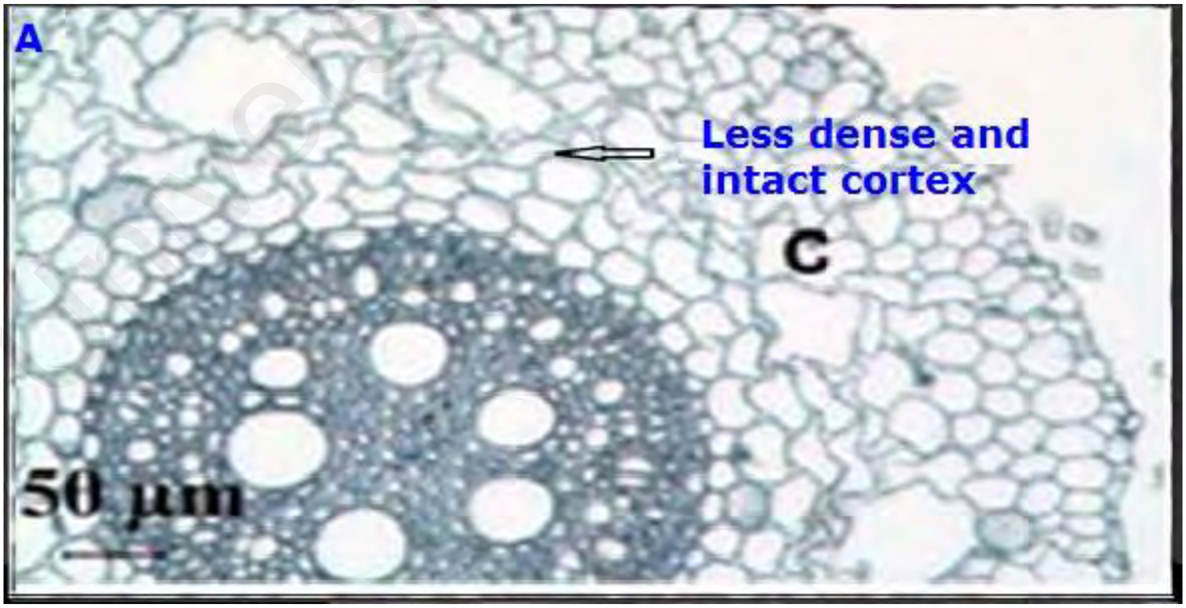


Plate 4.5C: Light micrographs of *Pilea sp.* stem in 40 ppm **Cr**- amended with organic wastes after 98 days. Stained with sulfide-silver for localization of metals.

Note: The Pb (**B**), and Cr (**C**) precipitates were seen as black deposits along the walls of xylem and phloem vessels compared to clean control (**A**) stems.



Plates 4.6A: Light micrographs showing the transverse section of *Lantana sp.* stems in control treatment.

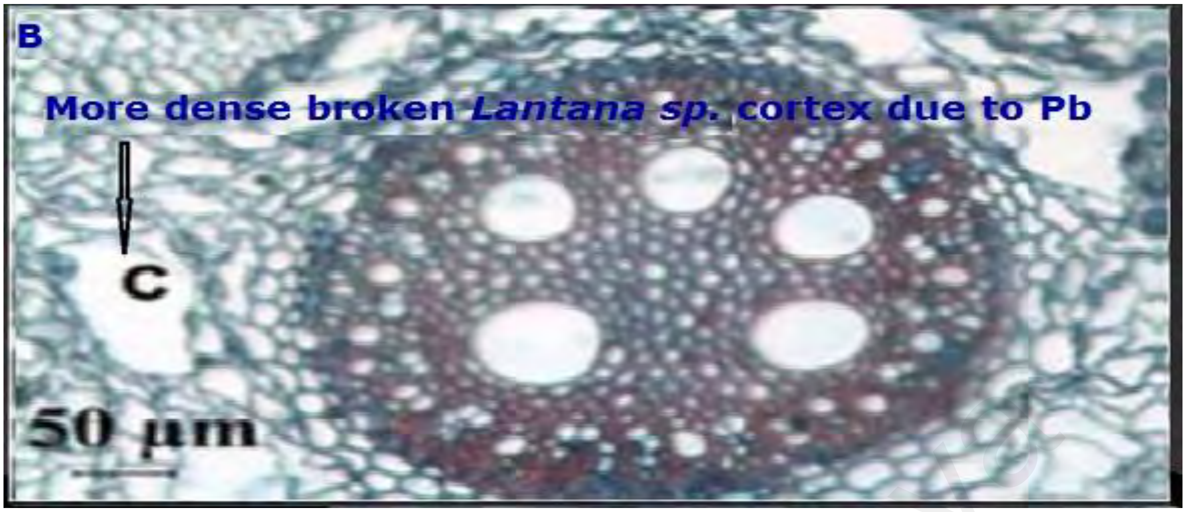


Plate 4.6B: Transverse *Lantana sp.* stem from 40 ppm **Pb** soil amended with organic waste after 98 days.

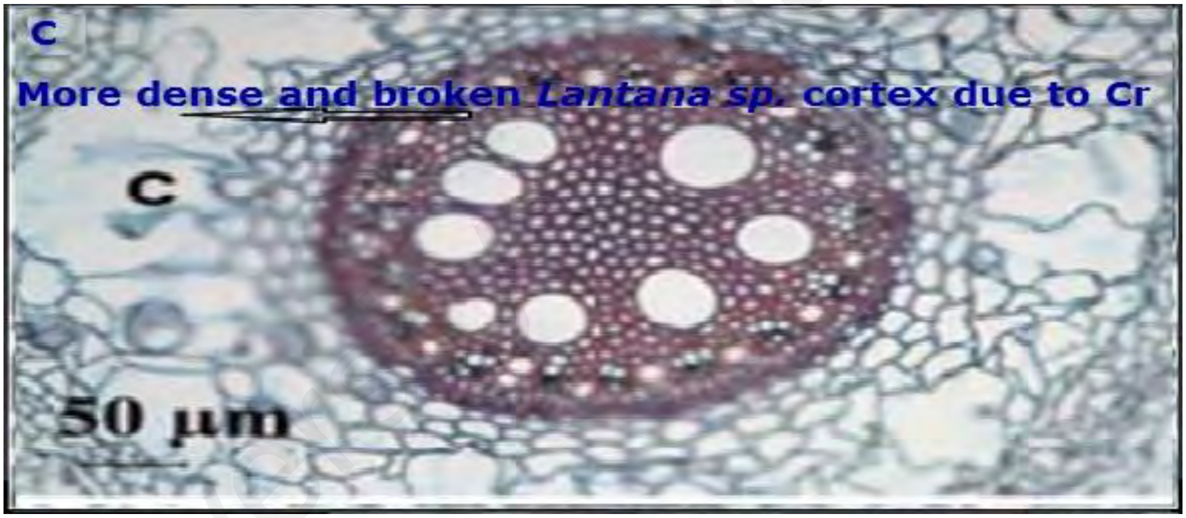


Plate 4.6C: Light micrographs of transverse *Lantana sp.* stem from 40 ppm **Cr**-soil amended with organic waste after 98 days. Stained with sulfide-silver for localization of metals.

(**Note: inserted "C"= cortex;** means breakdown of the cortex cells in **B** and **C** due to translocation).

4.5.6 Phytoremediation schematic flow of metal mass balance (STAN)

The STAN software generated a schematic balance of metal losses and/or changes in the sinks (i.e plant and soil), during Pb and/or Cr phytoremediation periods. These values were predetermined and imputed into the STAN (subSTance flow ANalysis), to express the respective metal sinks and possible losses. Therefore, **Figures 4.89a-4.89f**, show comprehensive schematics on 80 ppm Pb phytoremediation system boundaries. However, Pb sinks ranged from +44.14 to +64.41 mg/kg and +28.74 to +12.78 mg/kg were observed in soil and *Pilea sp.* treatments. While change in Pb-positive stocks between +38.03 to +61.41 mg/kg and +32.08 to +12.78 mg/kg were seen under soil and *Lantana sp.* sinks, respectively, after 98 days phytoremediation.

The export (E), suggests the amount of Pb that escaped the entire system, through biotic and abiotic losses, and via phytovolatilization. Hence, (I) is the injected soil pollutant. Hongyeng & Agamuthu, (2014) reported similar STAN-schematic analysis of carbon flow, in organic and convention farms. However, their study agreed with this current study, in terms of material mass balance analysis (law of conservation of mass), but contrasted because Hongyeng & Agamuthu, (2014) investigated nutrient flow in farms, while this current study dealt with metal polluted soil phytoremediation.

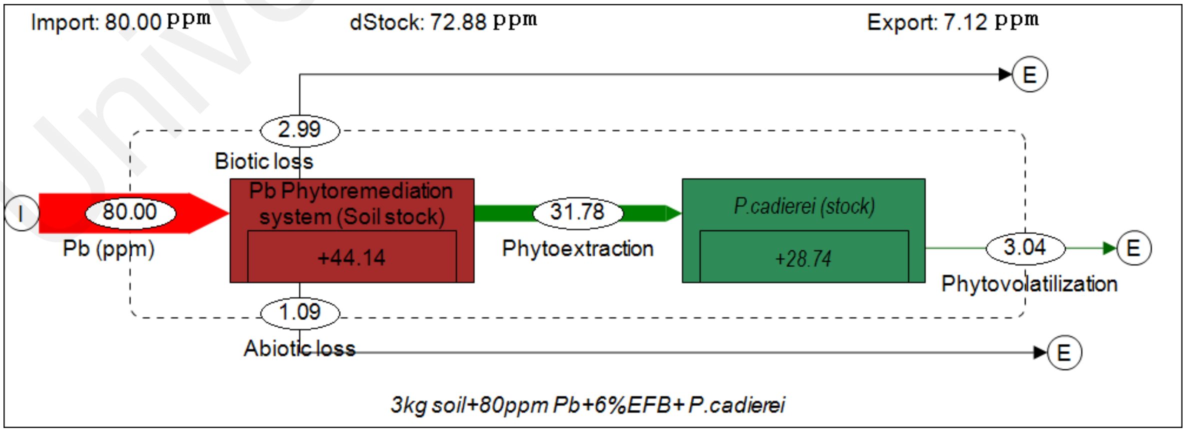


Figure 4.89a: STAN phytoremediation schematic mass flow of 80 ppm Pb soil with EFB under *Pilea sp.*

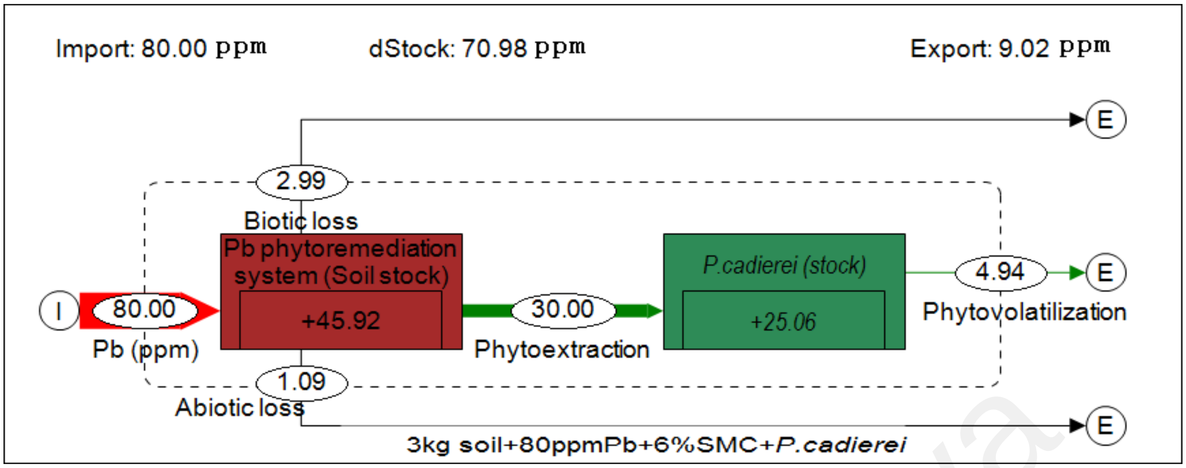


Figure 4.89b: STAN phytoremediation schematic mass flow of 80 ppm Pb soil with SMC under *Pilea sp*.

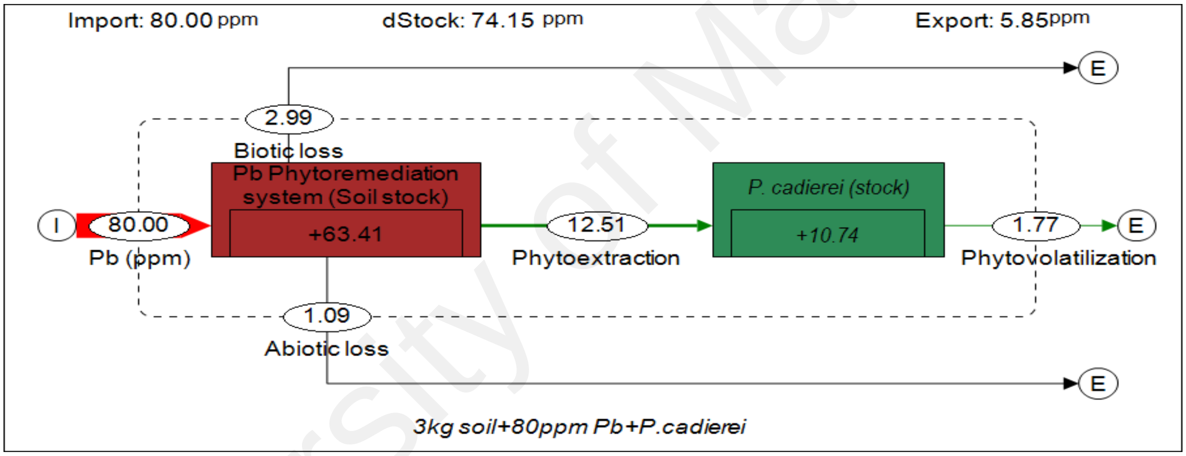


Figure 4.89c: STAN phytoremediation schematic mass flow of 80 ppm Pb soil under *Pilea sp.* alone.

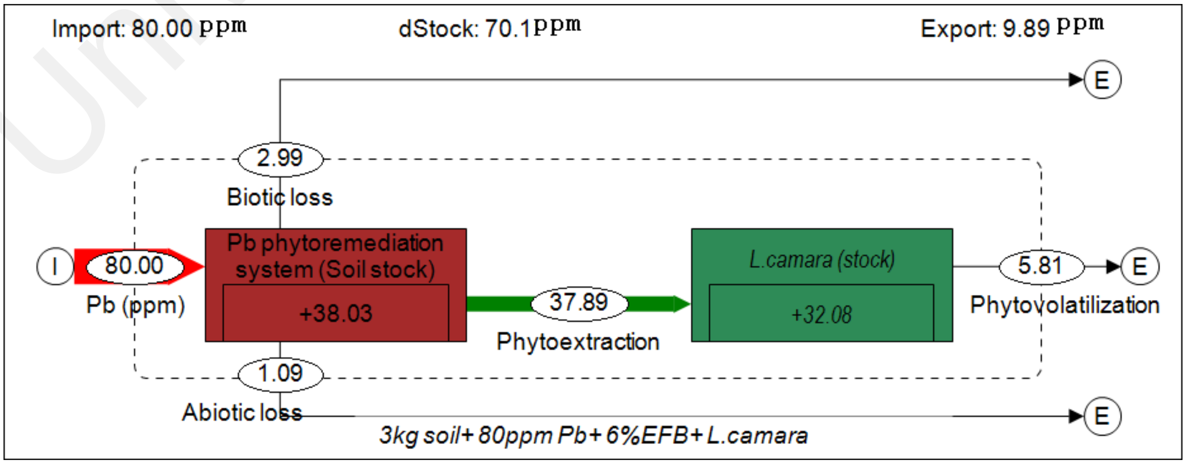


Figure 4.89d: STAN phytoremediation schematic mass flow of 80 ppm Pb soil with EFB under *Lantana sp*.

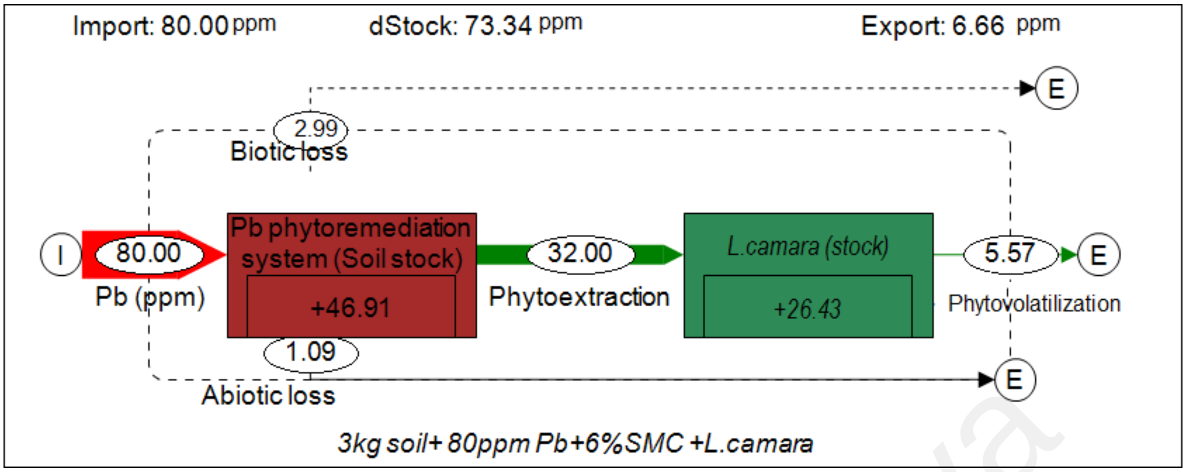


Figure 4.89e: STAN phytoremediation schematic mass flow of 80 ppm Pb soil with SMC under *Lantana sp*.

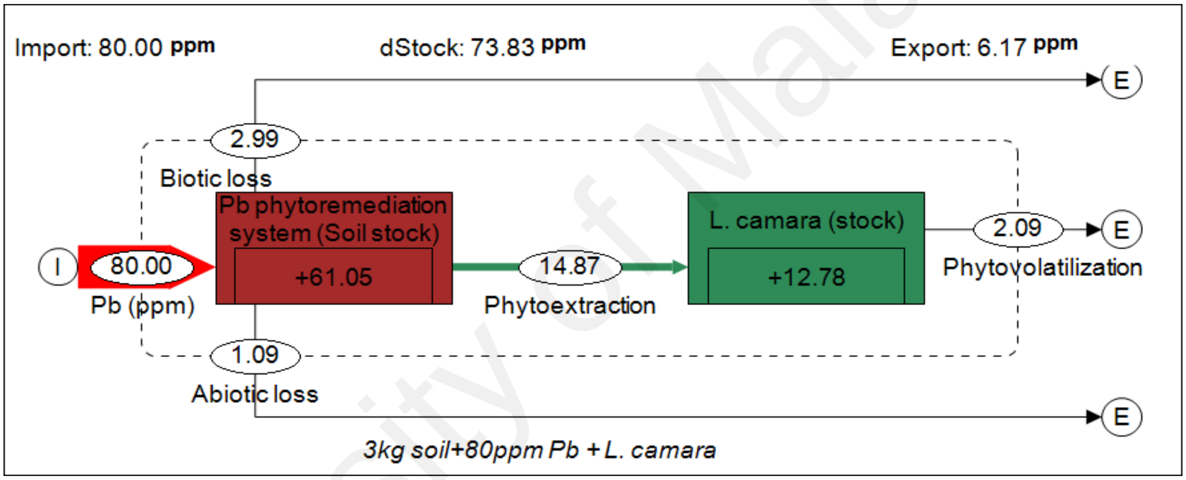


Figure 4.89f: STAN phytoremediation schematic mass flow of 80 ppm Pb soil under *Lantana sp.* alone.

However, Reisinger et al., (2009) conducted heavy metal (Pb, Cd, and Hg) mass flow analysis study, to support the Austrian environmental policy on metal pollution minimization. To this effect, Reisinger et al., (2009) partially agreed to this current study, but contrasted because this study focused mainly on soil-heavy metal phytoremediation, but theirs were directed towards environmental policy formulations.

It is evident that at post-phytoremediation, some metal loads may still be present in the soil (i.e., soil residual-stocks), depending on the metal concentration and/or plant phytoextraction capacity. Based on this premise, STAN-model could immediately inform the decision of the environmental scientist, to propose repetitive phytoremediation approach, towards mitigating the residual soil metal load if possible. Similarly, it may proffer appropriate insight for disposing and/or handling of the metal polluted floras after harvest, as may be suggested at the 80 ppm Pb remediated soil.

At 40 ppm Pb phytoremediated mass flow system, phytovolatilization ranged from 3.91 to 0.47 mg/kg, hence relatively lower to values obtained in 80 ppm Pb. Therefore, low concentration may have contributed to this effect, according to Yoshida et al., (2015). However, this and other details of 40 ppm Pb schematic mass flow balance, are presented in **Figures 4.90a** to **4.90f**.

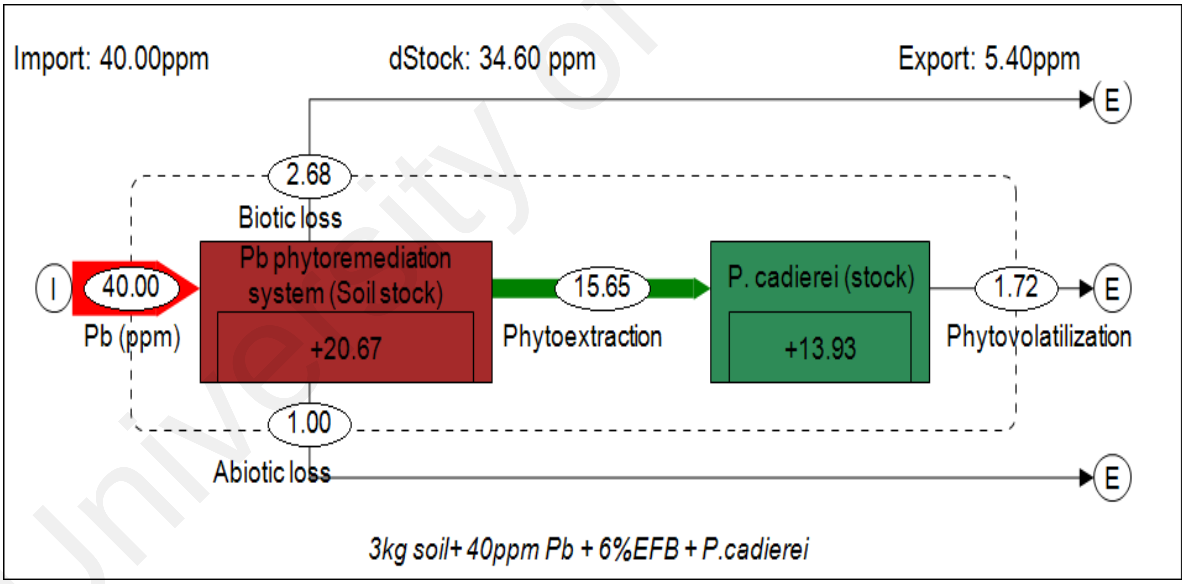


Figure 4.90a: STAN phytoremediation schematic mass flow of 40 ppm Pb soil with EFB under *Pilea sp*.

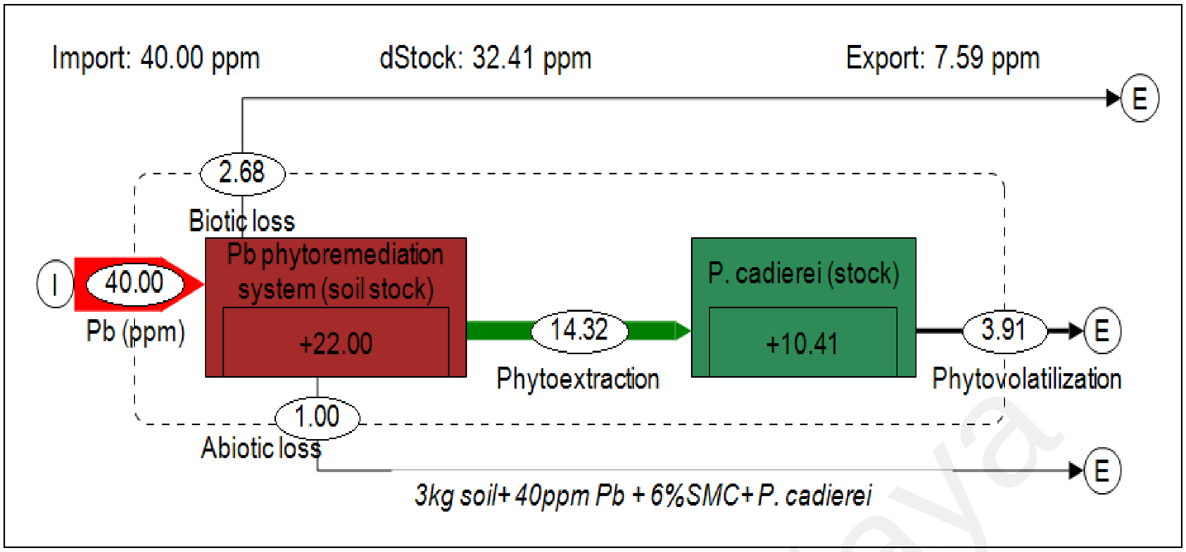


Figure 4.90b: STAN phytoremediation schematic mass flow of 40 ppm Pb soil with SMC under *Pilea sp*.

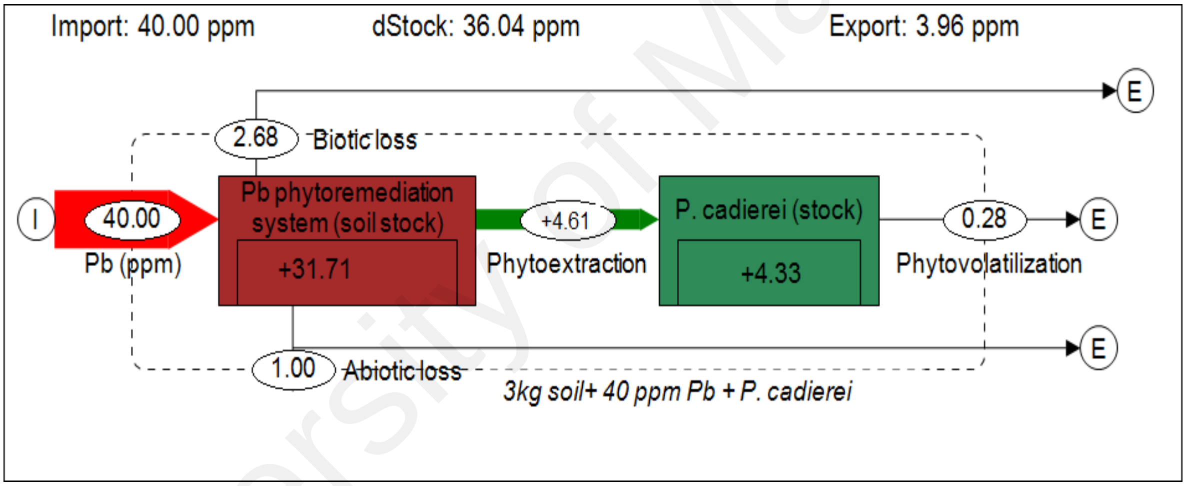


Figure 4.90c: STAN phytoremediation schematic mass flow of 40 ppm Pb soil under *Pilea sp.* alone.

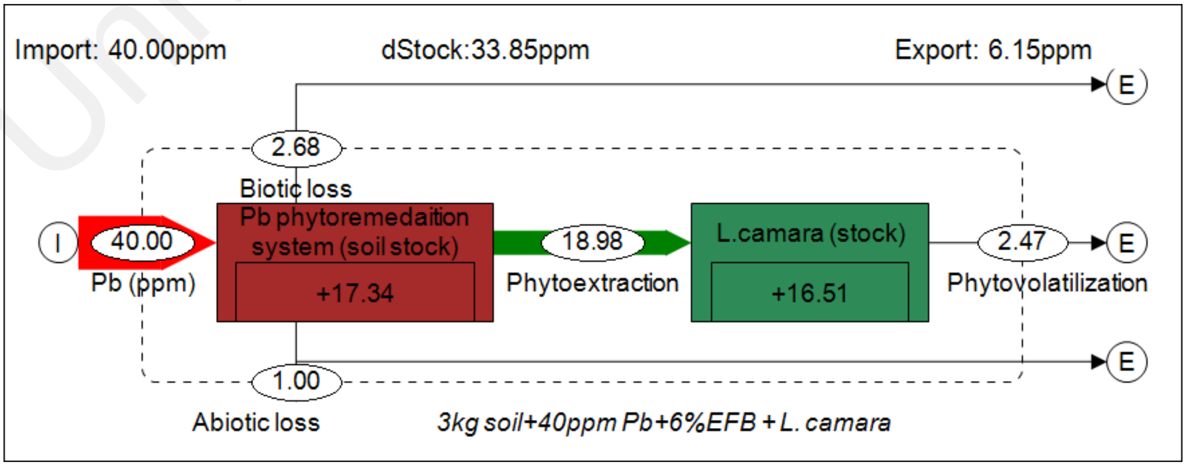


Figure 4.90d: STAN phytoremediation schematic mass flow of 40 ppm Pb soil with EFB under *Lantana sp*.

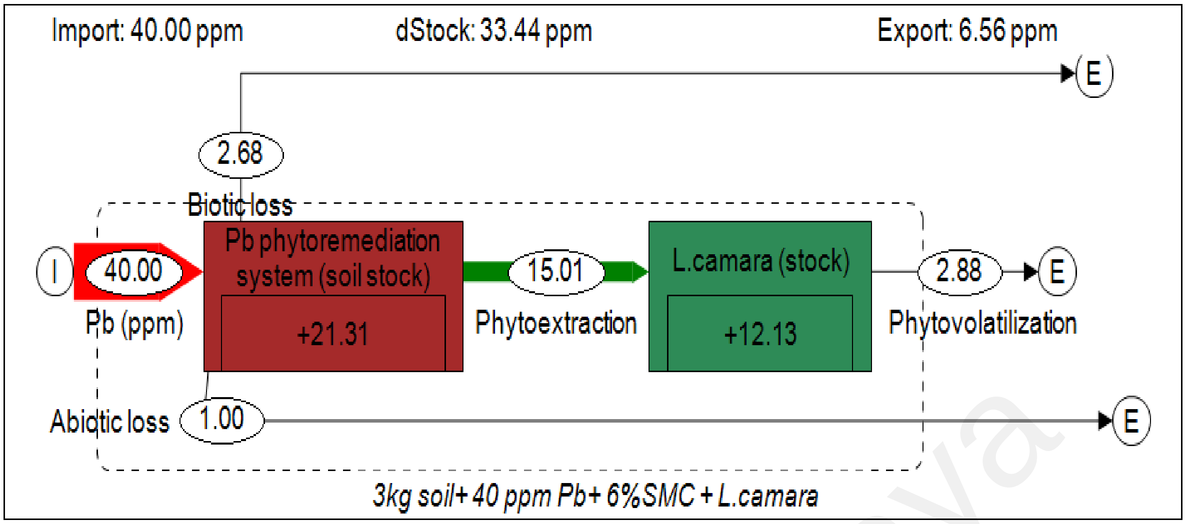


Figure 4.90e: STAN phytoremediation schematic mass flow of 40 ppm Pb soil with SMC under *Lantana sp*.

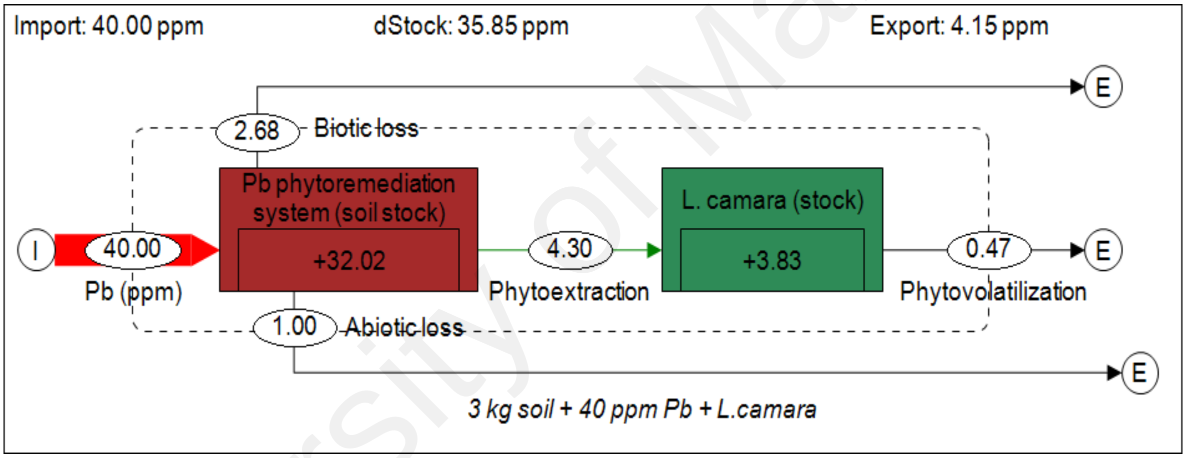


Figure 4.90f: STAN phytoremediation schematic mass flow of 40 ppm Pb soil under *Lantana sp.* alone.

Furthermore, the recorded drift in the amount of Pb-metal stocks in *Lantana sp./Pilea sp.* and/or soils, reflected to Rechberger et al., (2014) claims that uncertainties exist in material flow analysis.

In continuation, the sink dispersion of Pb at 20 ppm Pb phytoremediated system, is presented in **Figures 4.91a** to **4.91f**. Generally, soil-sink retained more Pb, than plant counterparts. This may be due to bioavailability and affinity factor. However, mass balance suggest that repeated phytoremediation can decline the soil Pb sink level (Brunner & Kral, 2014).

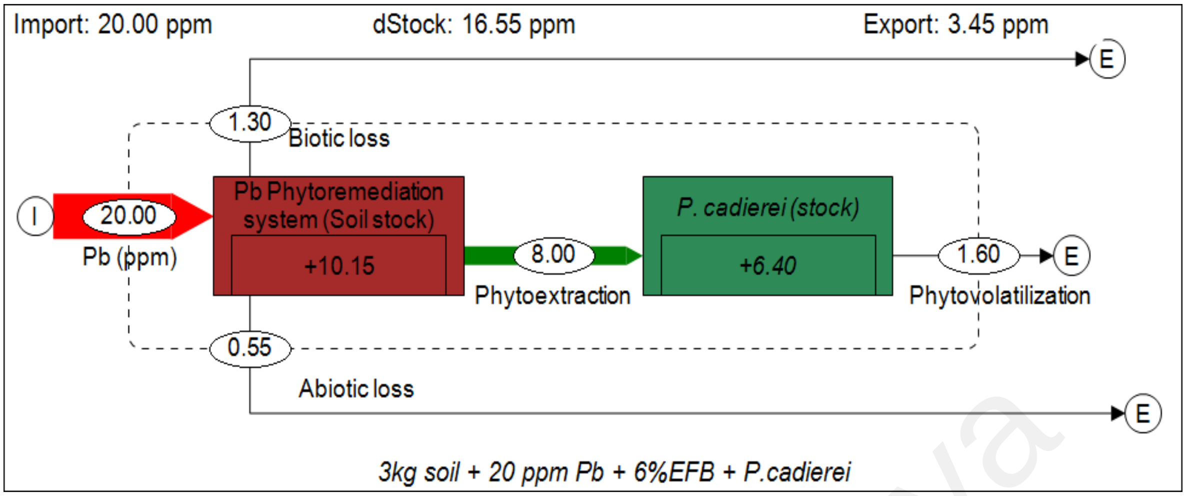


Figure 4.91a: STAN phytoremediation schematic mass flow of 20 ppm Pb soil with EFB under *Pilea sp*.

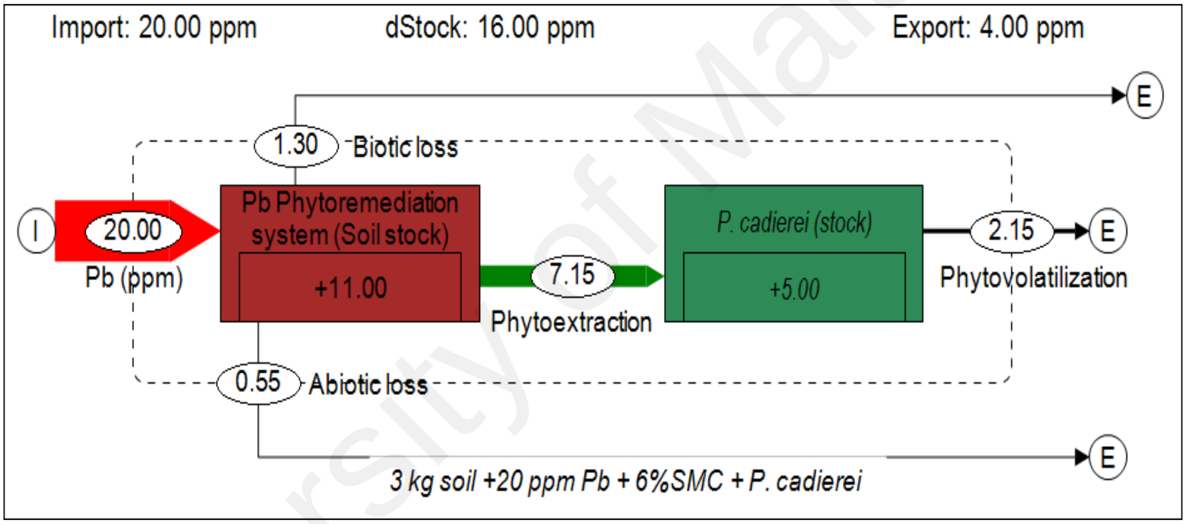


Figure 4.91b: STAN phytoremediation schematic mass flow of 20 ppm Pb soil with SMC under *Pilea sp*.

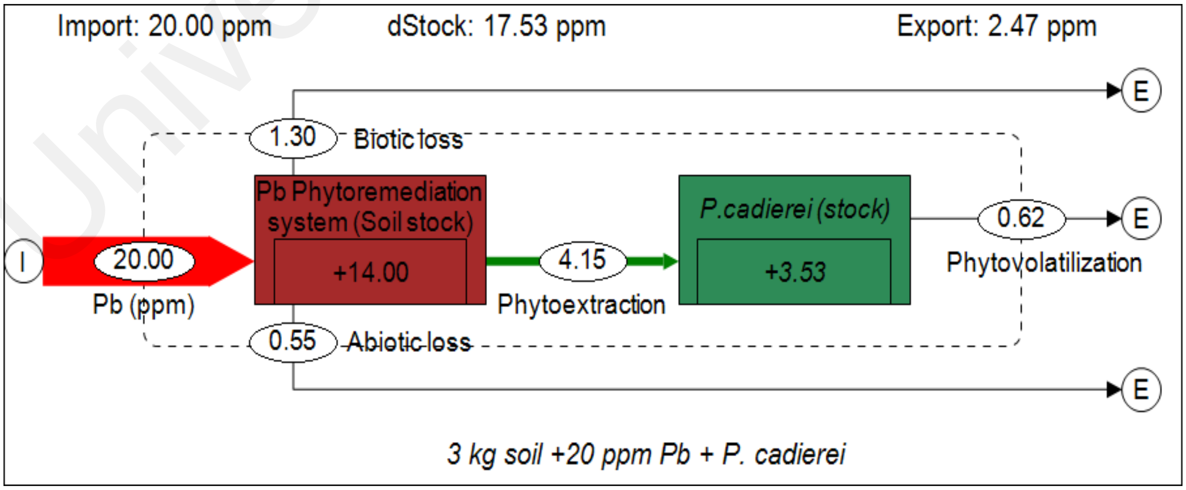


Figure 4.91c: STAN phytoremediation schematic mass flow of 20 ppm Pb soil under *Pilea sp.* alone.

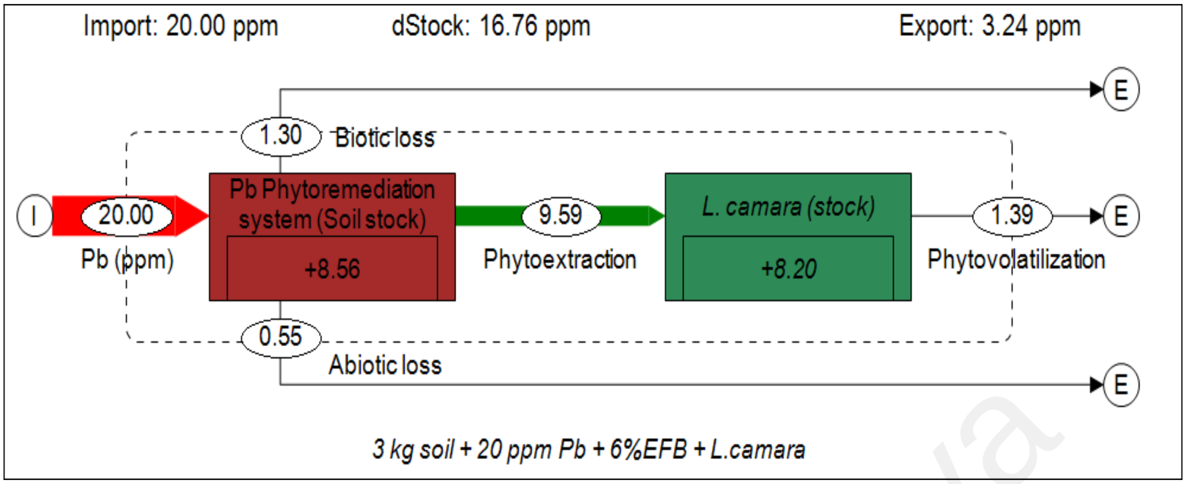


Figure 4.91d: STAN phytoremediation schematic mass flow of 20 ppm Pb soil with EFB under *Lantana sp*.

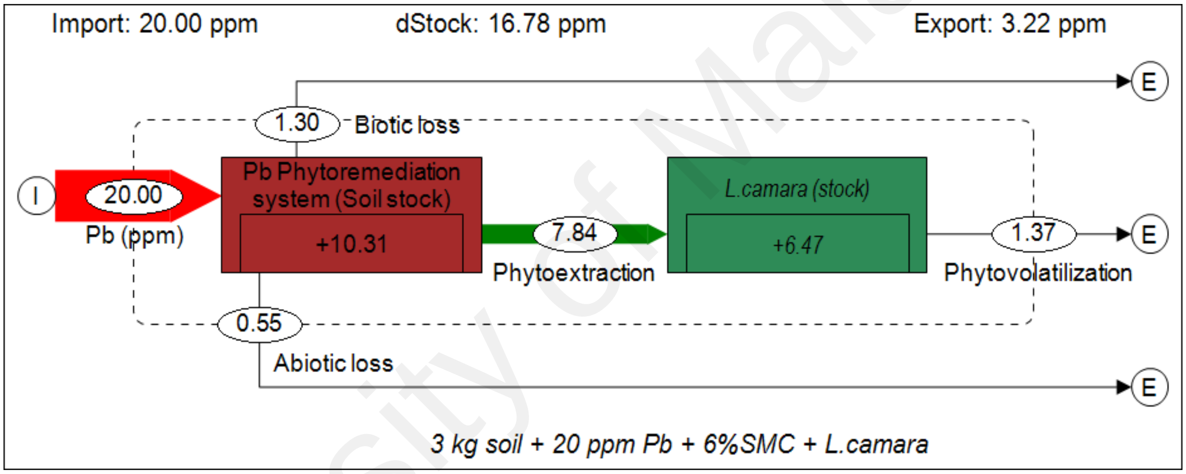


Figure 4.91e: STAN phytoremediation schematic mass flow of 20 ppm Pb soil with SMC under *Lantana sp*.

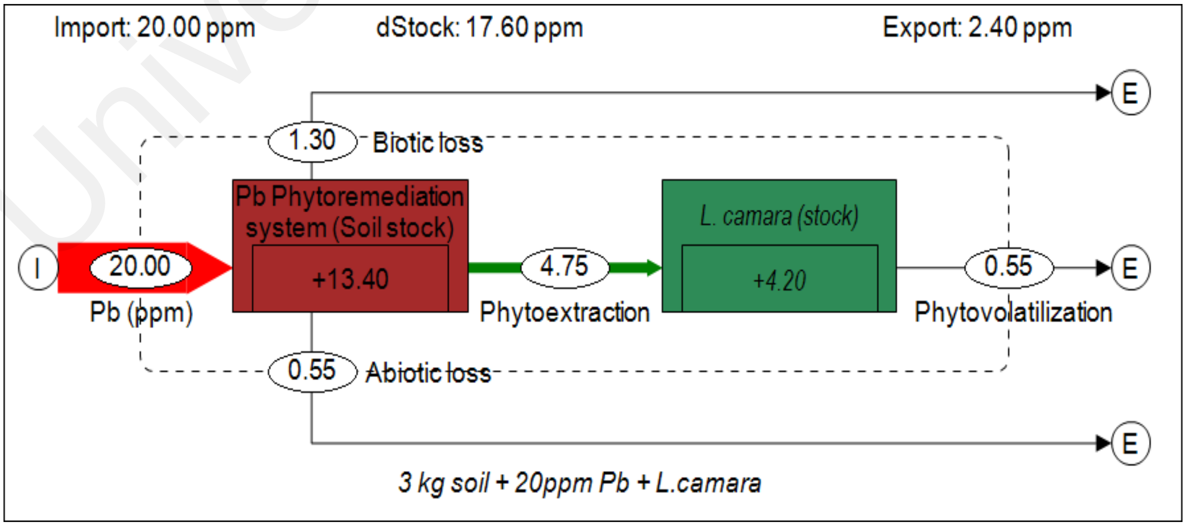


Figure 4.91f: STAN phytoremediation schematic mass flow of 20 ppm Pb soil under *Lantana sp.* alone.

Finally, in 10 ppm Pb phytoremediation STAN. The schematic mass balance constructs are presented in **Figures 4.92a** to **4.92f**. However, changes in stock at system boundaries recorded more Pb sink to plant, under amended scenario. As opposed to the soil stock of Pb in the mass flow after phytoremediation. Baertschi et al., (2013) referred to this type of mass balancing as relative, and not absolute, while Brunner & Rechberger, (2004) substantiated this by arguing that the model completely relies on predetermined input of data for accuracy and completeness.

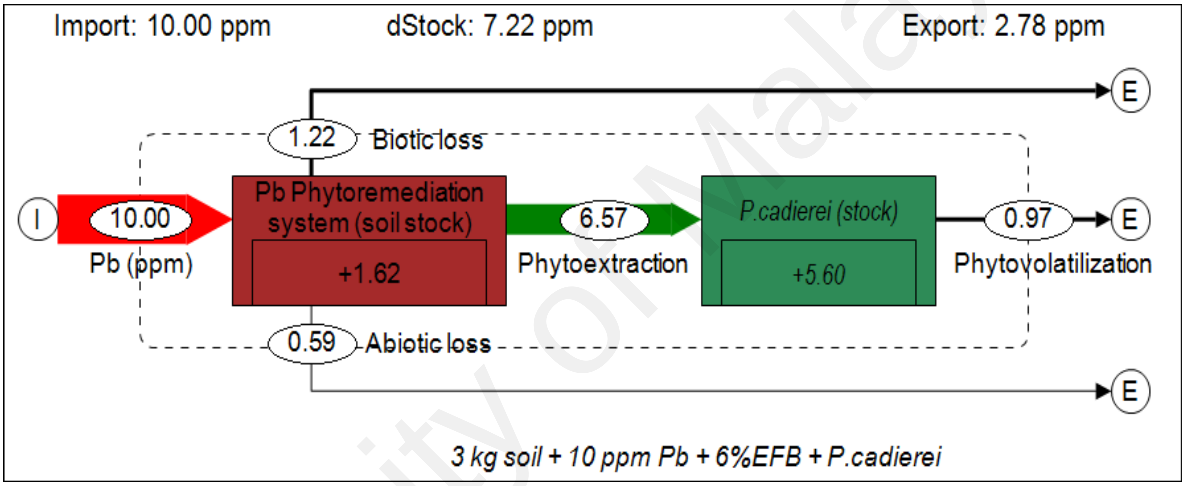


Figure 4.92a: STAN phytoremediation schematic mass flow of 10 ppm Pb soil with EFB under *Pilea sp*.

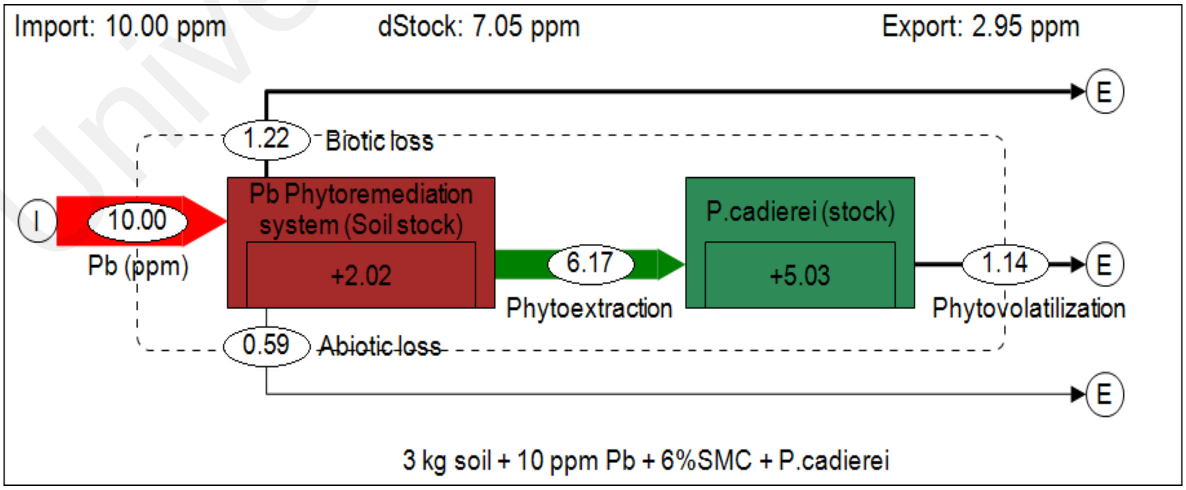


Figure 4.92b: STAN phytoremediation schematic mass flow of 10 ppm Pb soil with SMC under *Pilea sp*.

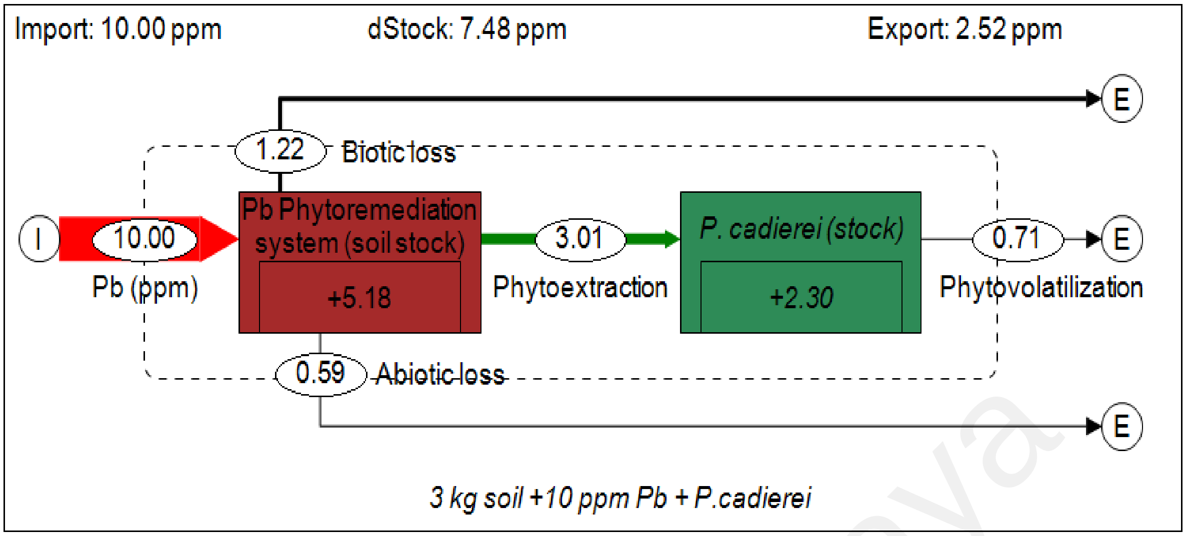


Figure 4.92c: STAN phytoremediation schematic mass flow of 10 ppm Pb soil under *Pilea sp.* alone.

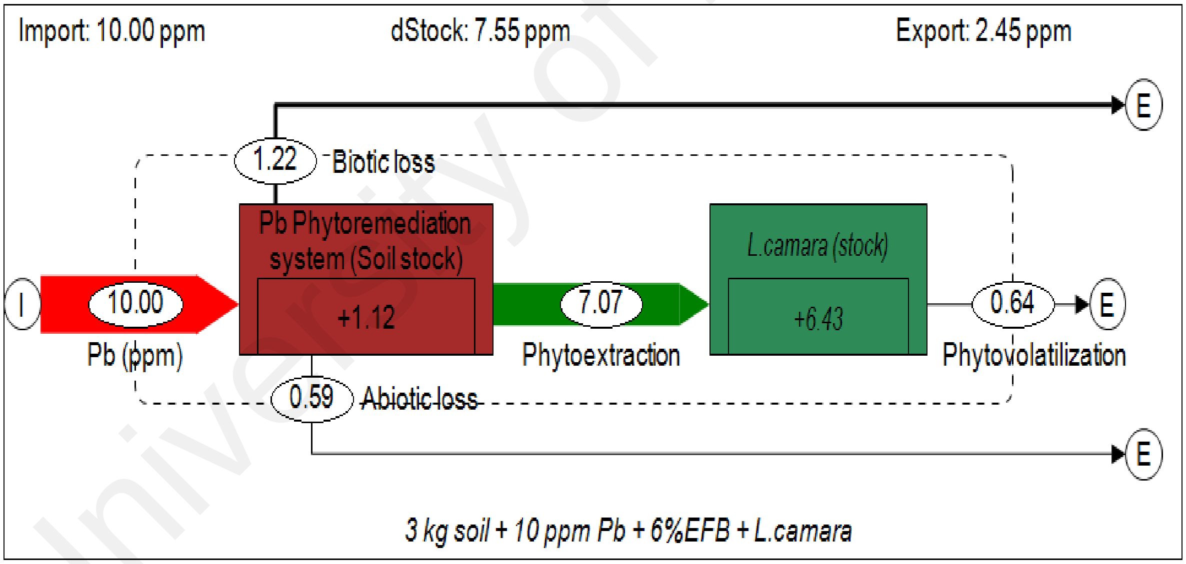


Figure 4.92d: STAN phytoremediation schematic mass flow of 10 ppm Pb soil with EFB under *Lantana sp*.

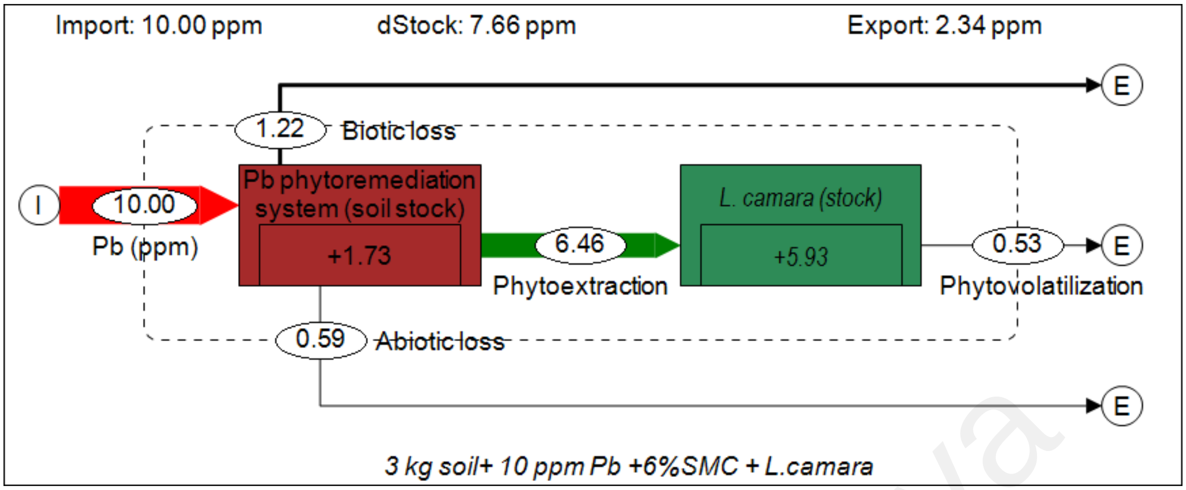


Figure 4.92e: STAN phytoremediation schematic mass flow of 10 ppm Pb soil with SMC under *Lantana sp*.

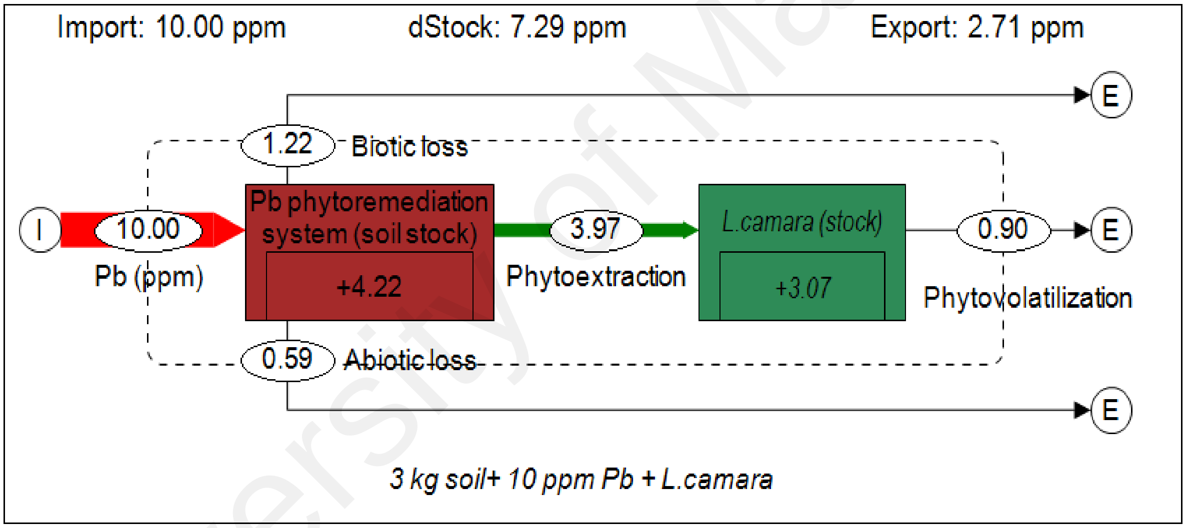


Figure 4.92f: STAN phytoremediation schematic mass flow of 10 ppm Pb soil under *Lantana sp.* alone.

Differently, STAN analysis under 40 ppm Cr phytoremediation system, are shown in **Figures 4.93a** to **4.93f**. These implicated +21.13 to +30.88 mg/kg of Cr and +13.96 to 5.01 mg/kg Cr, as the stock changes for soil and *Pilea sp.*, respectively. Correspondingly, +18.74 to +28.93 mg/kg Cr and +16.62 to +7.15 mg/kg Cr were stock changes (i.e, sinks) in soil, and *Lantana sp.* at post-phytoremediated system boundaries. Arena & Di Gregorio, (2014) argued that in material flow analysis, system boundaries are important in determining loads. This concurred with this study on Cr mass flow. However, positive stock changes were recorded in both sediment and indicator plants; this indicates Cr surplus. Possibly, due to Cr absorption and/or adsorption in the system, as well as export impacts (E). Therefore, this is what this model tends to schematically elaborate, in a nutshell.

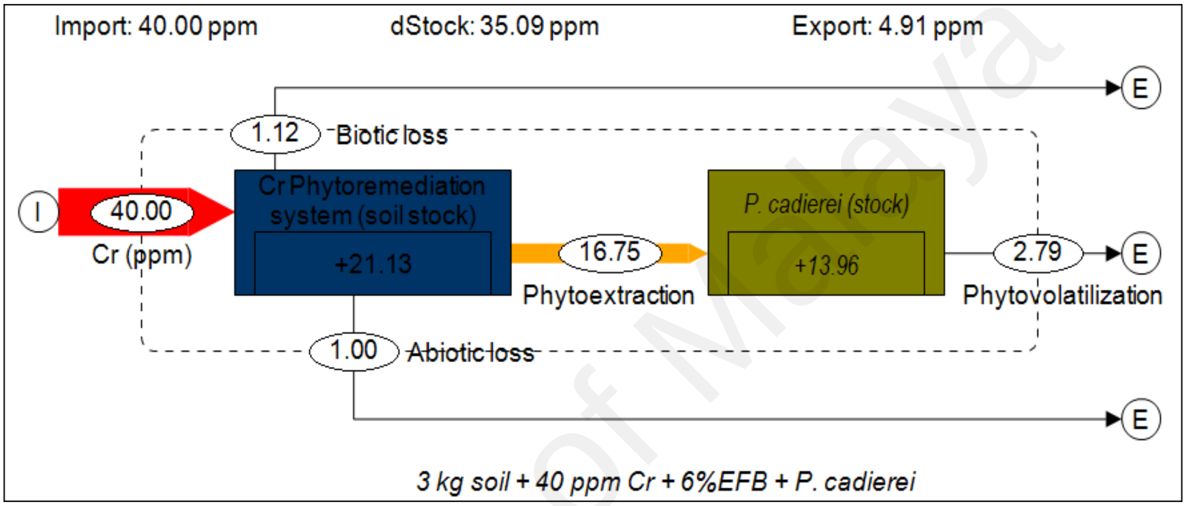


Figure 4.93a: STAN phytoremediation schematic mass flow of 40 ppm Cr soil with EFB under *Pilea sp*.

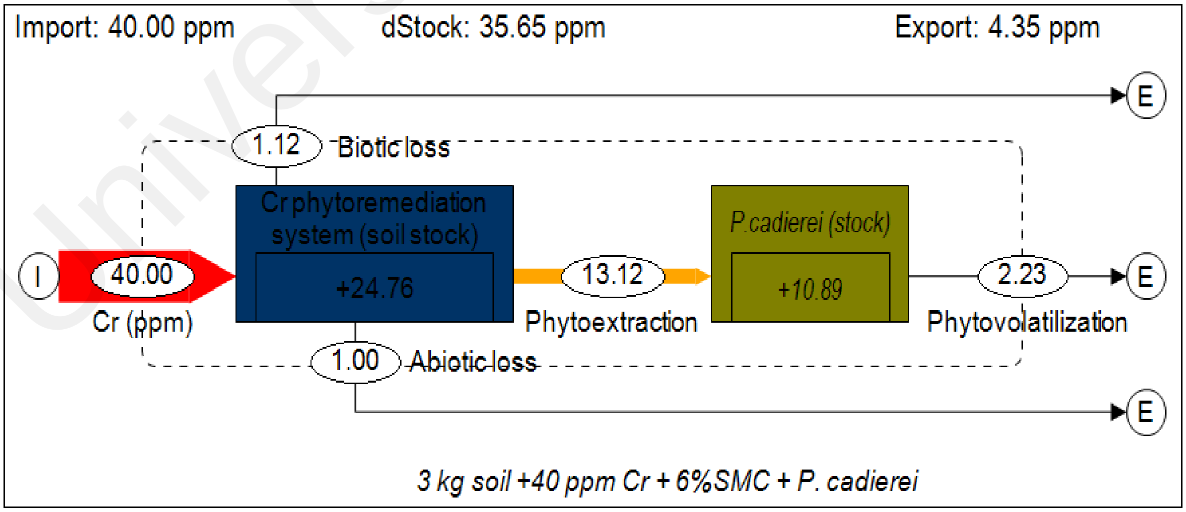


Figure 4.93b: STAN phytoremediation schematic mass flow of 40 ppm Cr soil with SMC under *Pilea sp*.

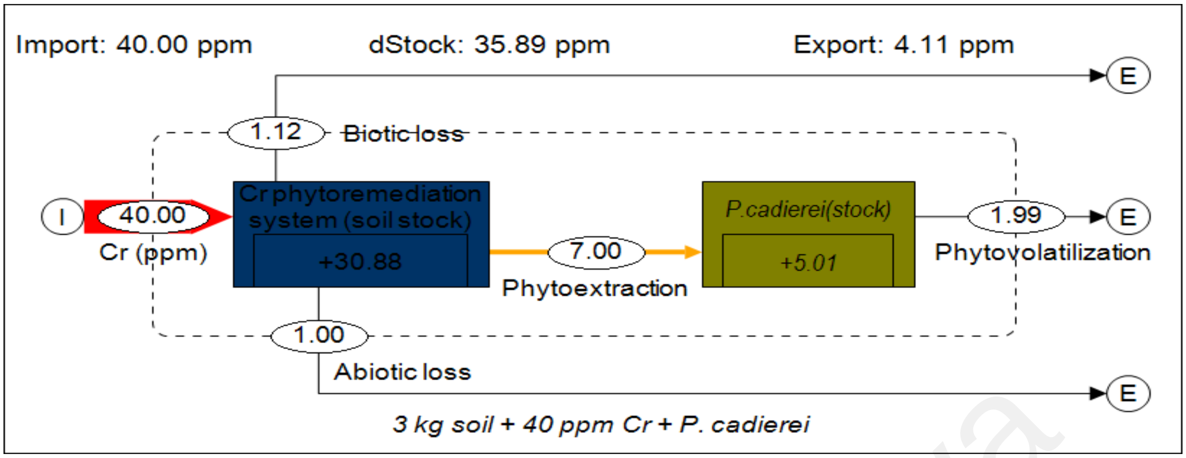


Figure 4.93c: STAN phytoremediation schematic mass flow of 40 ppm Cr soil under *Pilea sp.* alone.

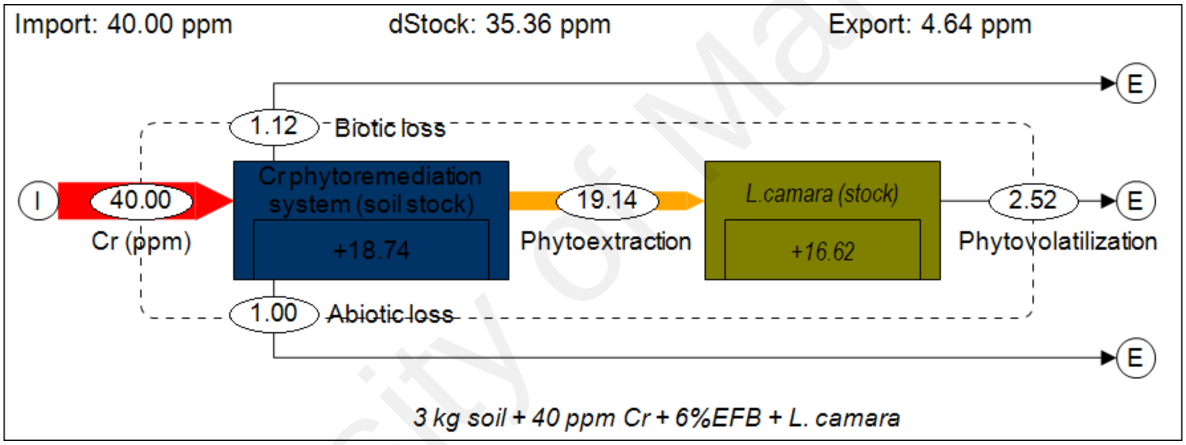


Figure 4.93d: STAN phytoremediation schematic mass flow of 40 ppm Cr soil with EFB under *Lantana sp*.

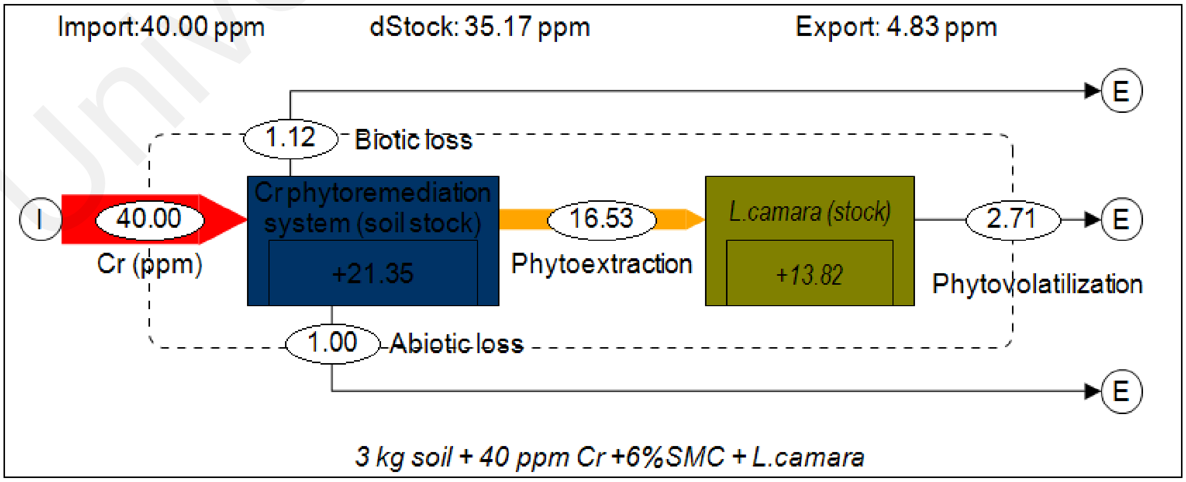


Figure 4.93e: STAN phytoremediation schematic mass flow of 40 ppm Cr soil with SMC under *Lantana sp*.

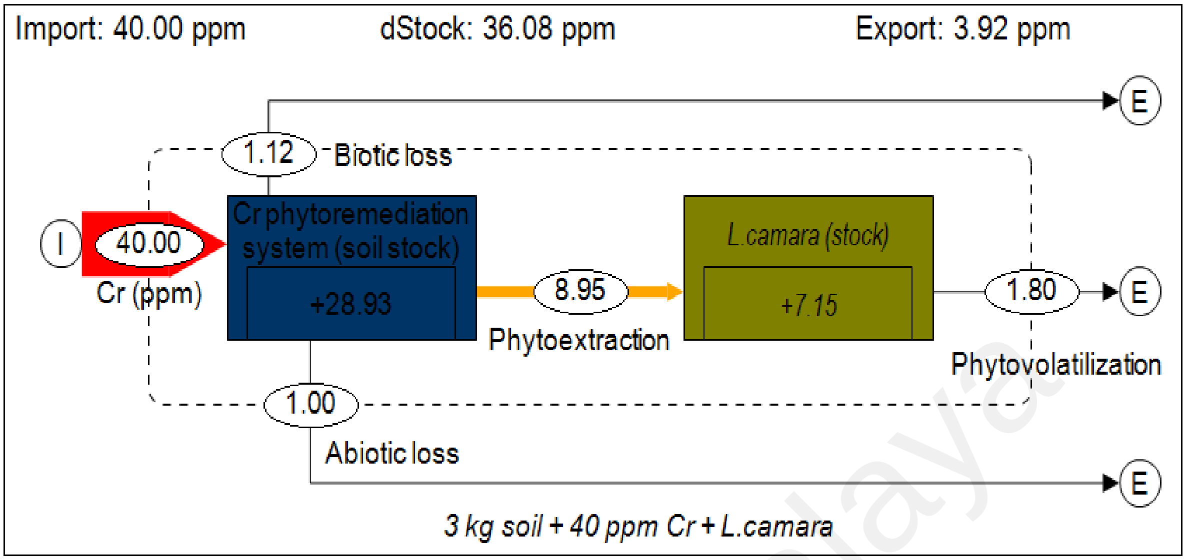


Figure 4.93f: STAN phytoremediation schematic mass flow of 40 ppm Cr soil under *Lantana sp.* alone.

Equally, in 20 ppm Cr STAN phytoremediation complex presented in **Figures 4.94a** to **4.94f**. However, indicated positive Cr stock in soil and also in *Pilea sp.*, which ranged from +10.41 to +14.70 mg/kg and +5.27 to 2.01 mg/kg respectively, at post-remediation. *Lantana sp.* had a change in stock of about +7.60 to +2.80 mg/kg. These flows implied higher Cr accumulation in *Lantana sp.* over *Pilea sp.* at the independent system boundaries. Bai et al., (2015) recorded this type of trend in a Pb mass flow -smelting process. However, phytovolatilization was evident in the model as Cr export route, from the plants stocks.

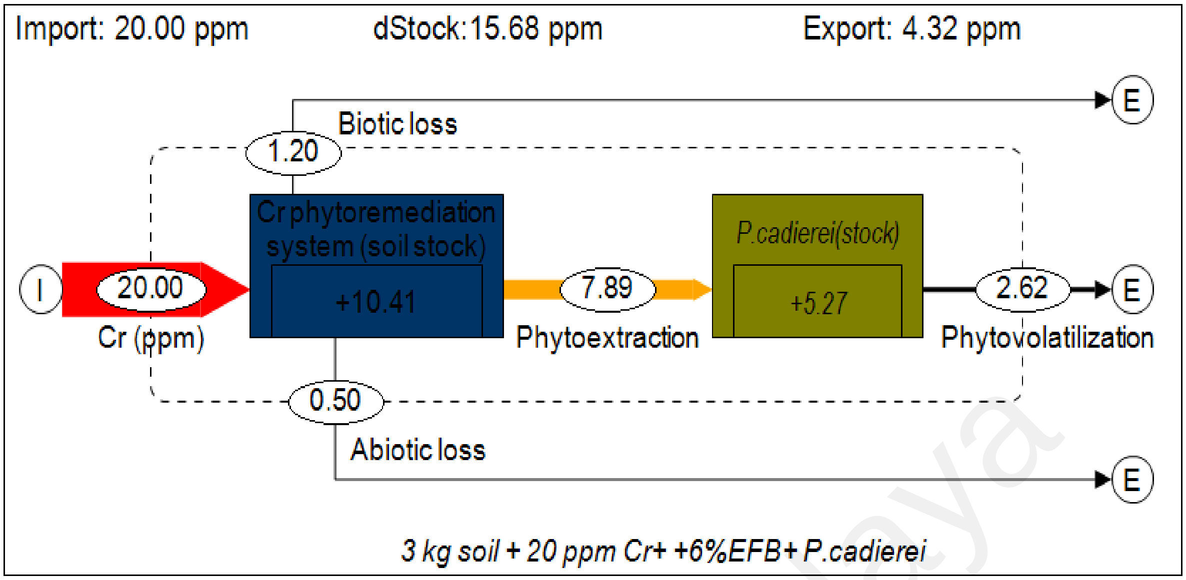


Figure 4.94a: STAN Phytoremediation schematic mass flow of 20 ppm Cr soil with EFB under *Pilea sp*.

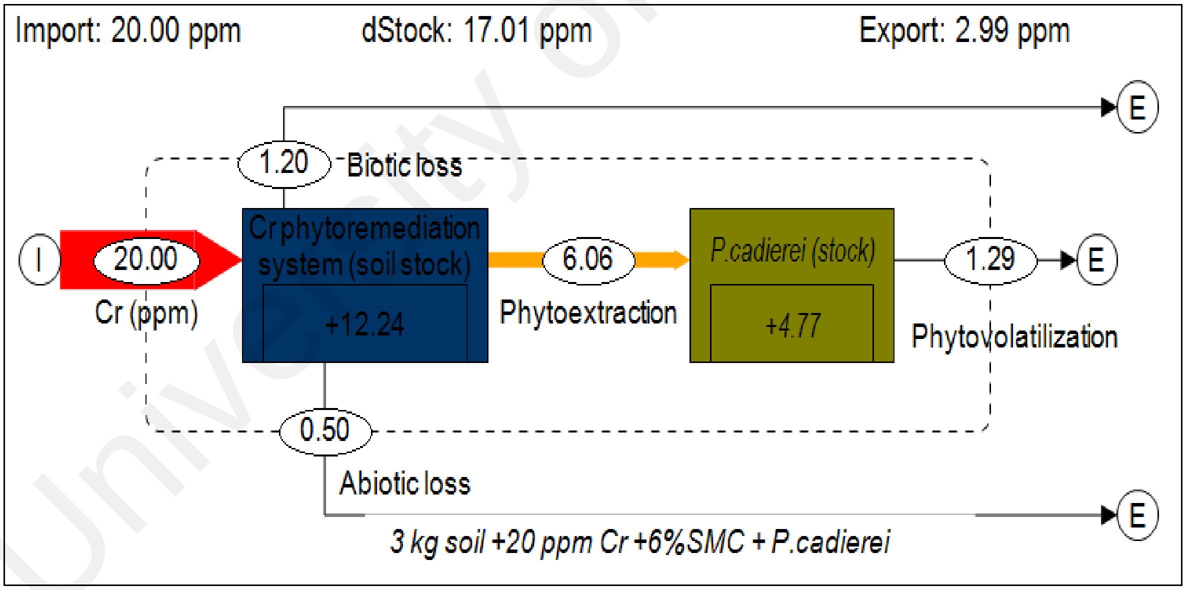


Figure 4.94b: STAN Phytoremediation schematic mass flow of 20 ppm Cr soil with SMC under *Pilea sp*.

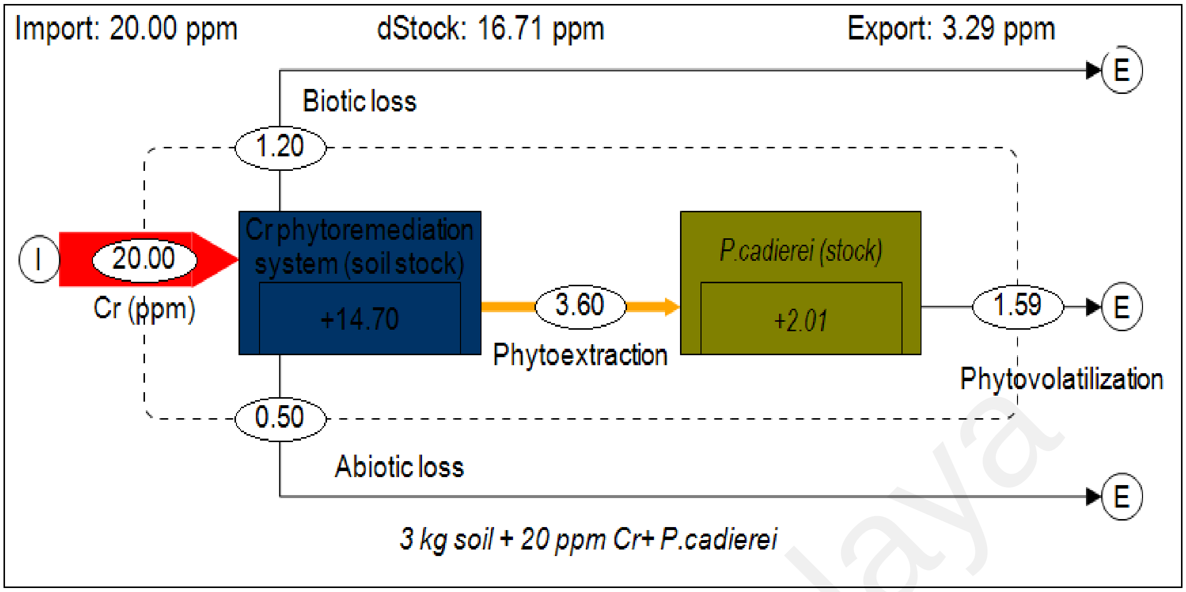


Figure 4.94c: STAN Phytoremediation schematic mass flow of 20 ppm Cr soil under *Pilea sp.* alone.

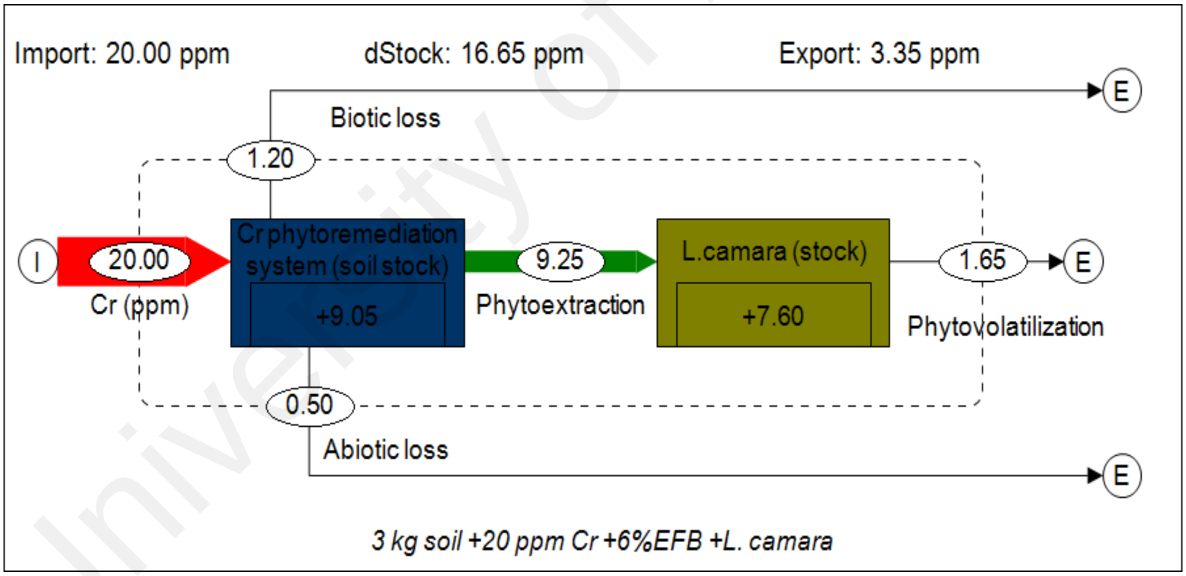


Figure 4.94d: STAN phytoremediation schematic mass flow of 20 ppm Cr soil with EFB under *Lantana sp*.

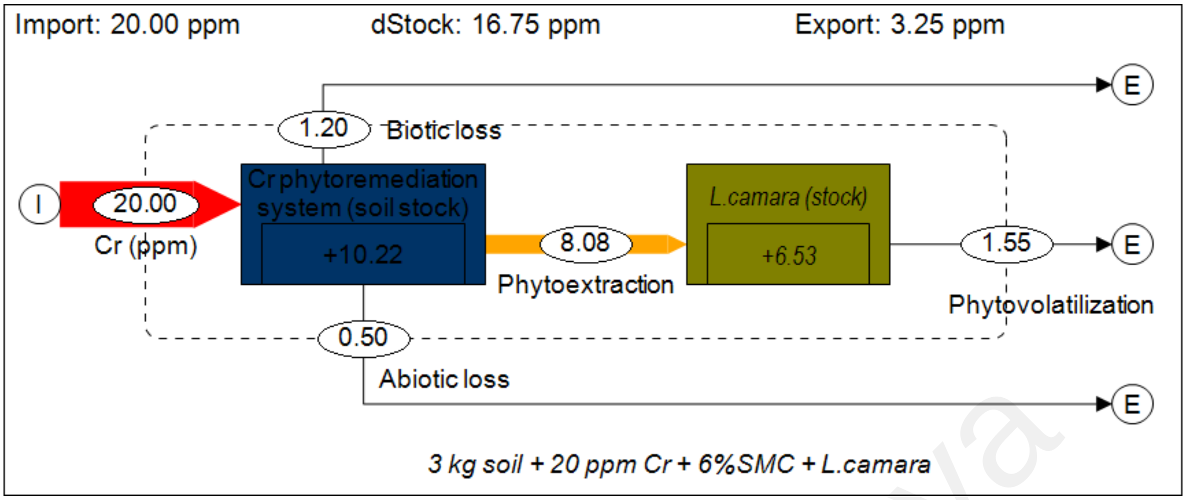


Figure 4.94e: STAN phytoremediation schematic mass flow of 20 ppm Cr soil with SMC under *Lantana sp*.

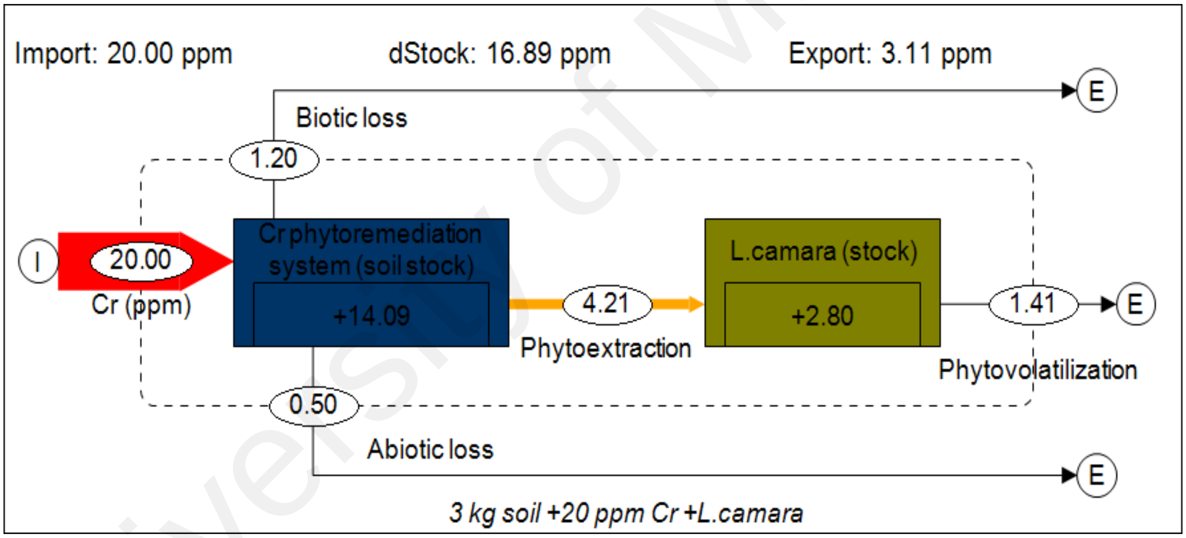


Figure 4.94f: STAN Phytoremediation schematic mass flow of 20 ppm Cr soil under *Lantana sp.* alone.

Also, higher Cr stocks were evident in *Pilea sp.* and *Lantana sp.*, as opposed to soil, under amended 10 ppm Cr STAN phytoremediation complex, seen in **Figures 4.95a** to **4.95f**. Thus, indicating boundary movement of metals, and the effects of concentration implicated as well.

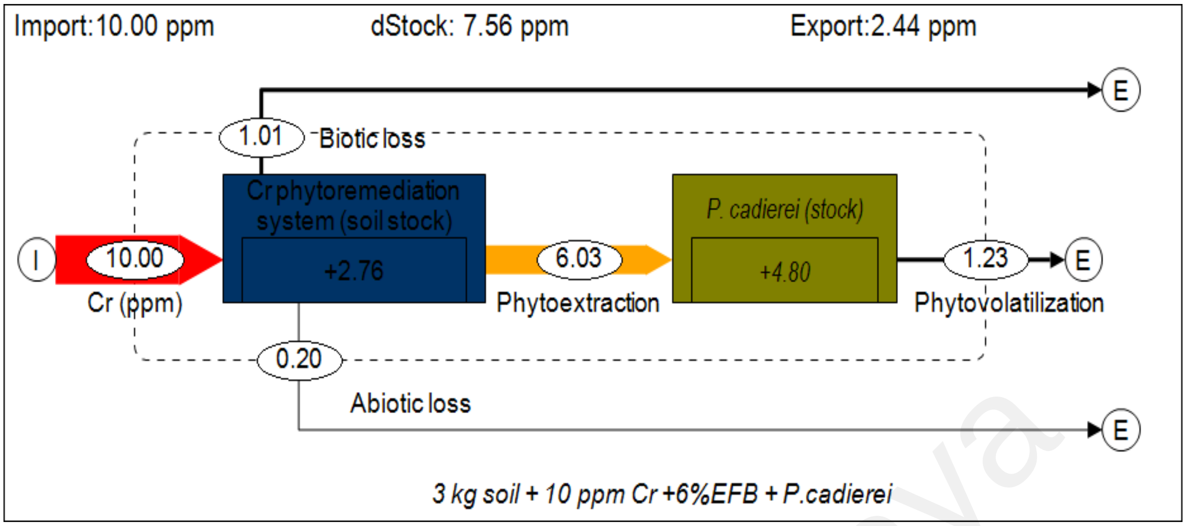


Figure 4.95a: STAN Phytoremediation schematic mass flow of 10 ppm Cr soil with EFB under *Pilea sp*.

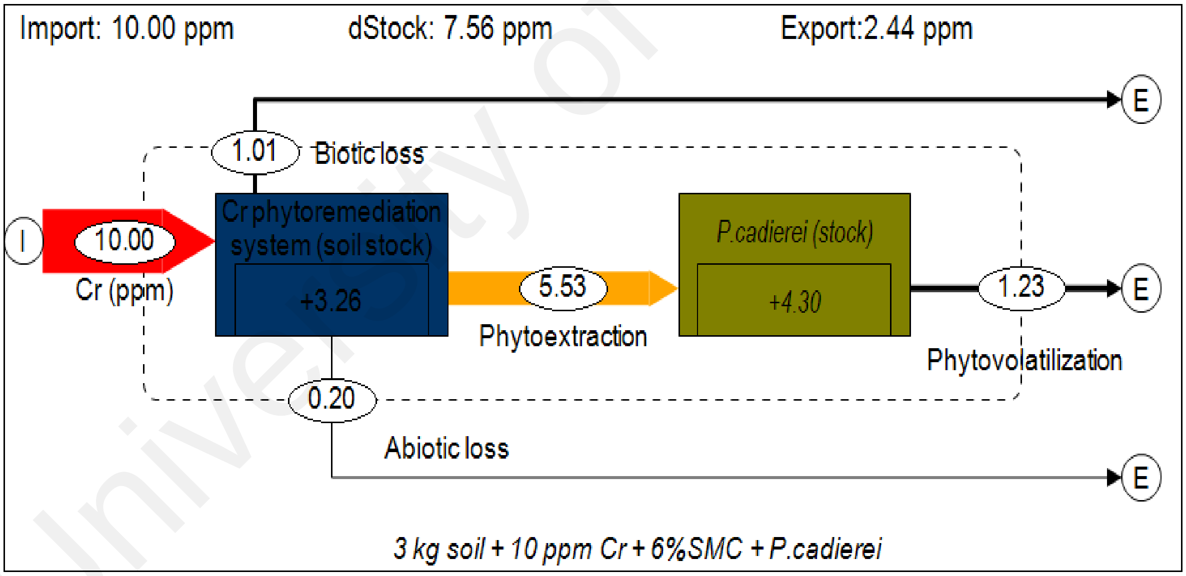


Figure 4.95b: STAN Phytoremediation schematic mass flow of 10 ppm Cr soil with SMC under *Pilea sp*.

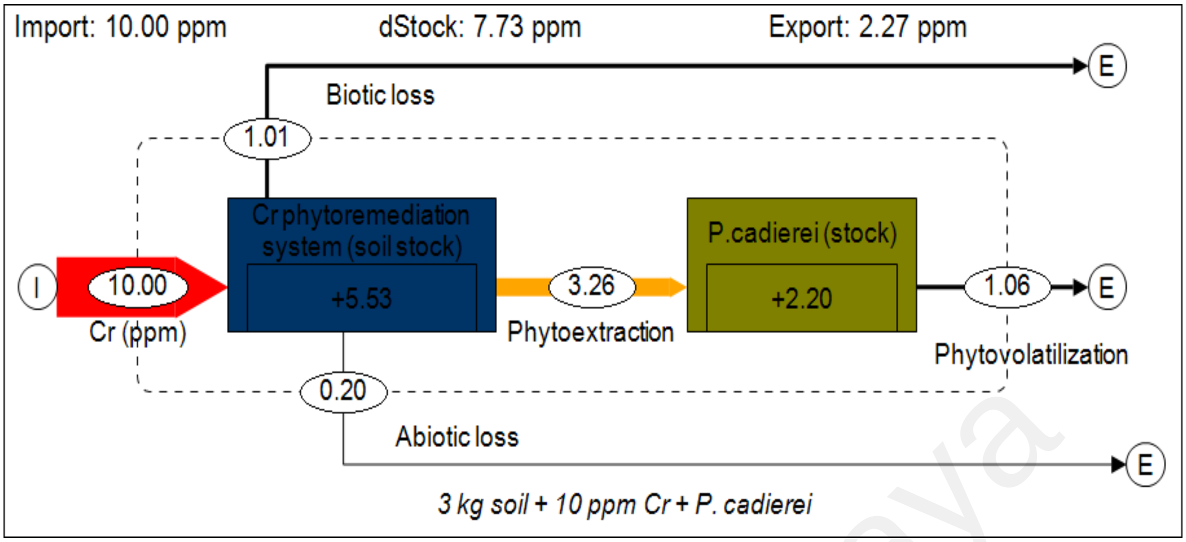


Figure 4.95c: STAN Phytoremediation schematic mass flow of 10 ppm Cr soil under *Pilea sp.* alone.

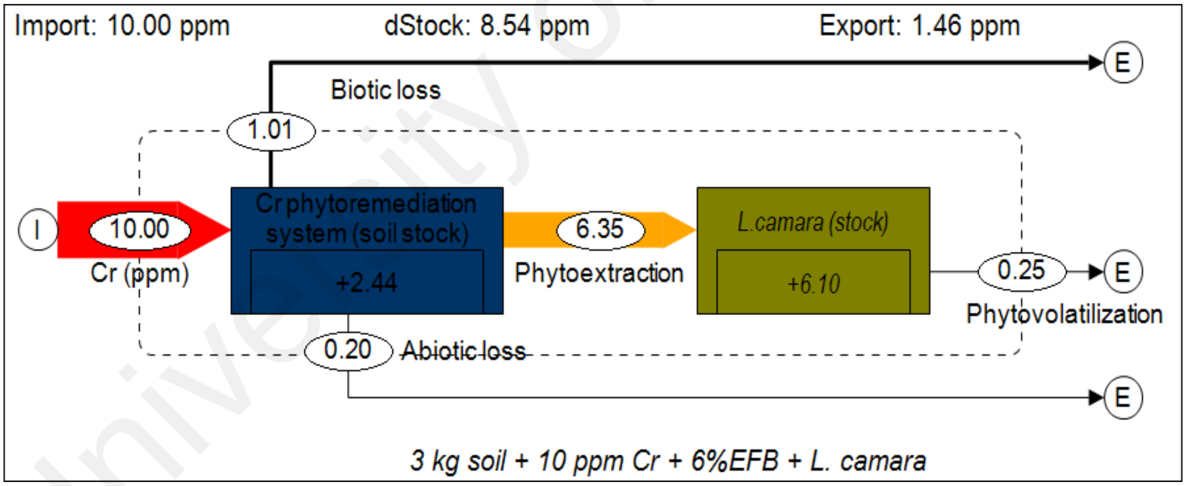


Figure 4.95d: STAN phytoremediation schematic mass flow of 10 ppm Cr soil with EFB under *Lantana sp*.

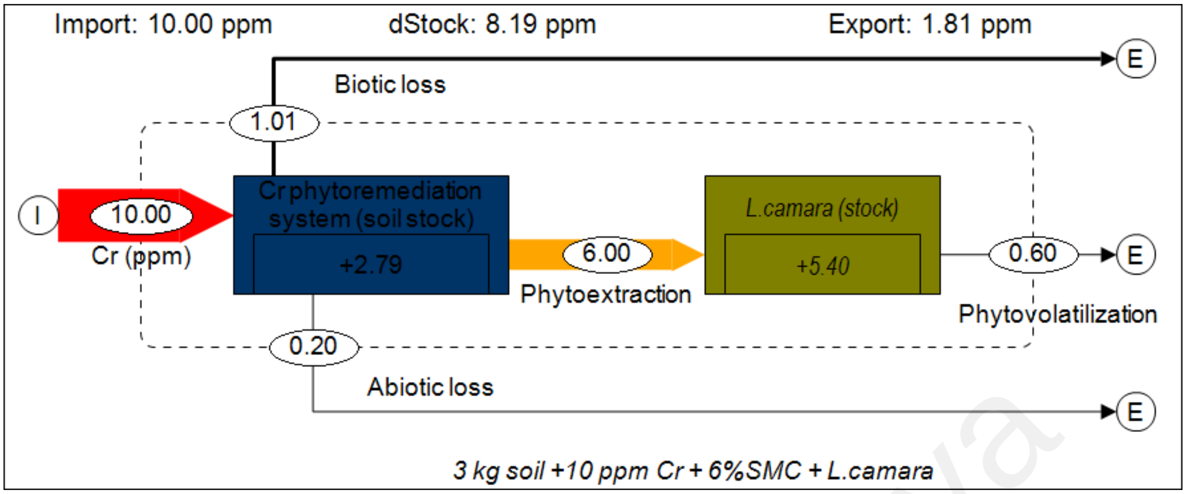


Figure 4.95e: STAN phytoremediation schematic mass flow of 10 ppm Cr soil with SMC under *Lantana sp*.

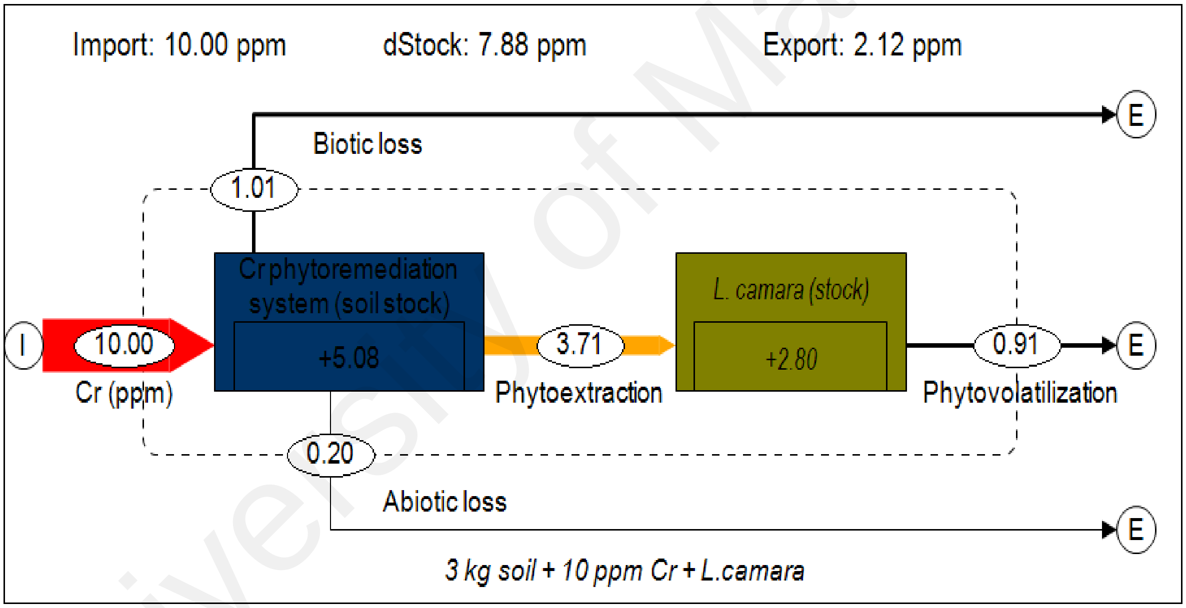


Figure 4.95f: STAN Phytoremediation schematic mass flow of 10 ppm Cr soil under *Lantana sp.* alone.

In continuation, the dissemination of elemental mass balance at co-contaminated soil (**Figures 4.96a** to **4.96f**), implicated complexity. Obviously, due to merged boundaries (Cascarosa et al., 2013). Hence, more positive stocks of Pb and Cr were seen on soil, than in plants. However, phytovolatilization and other Pb and Cr exports routes were considered low, due to unspecific conditions.

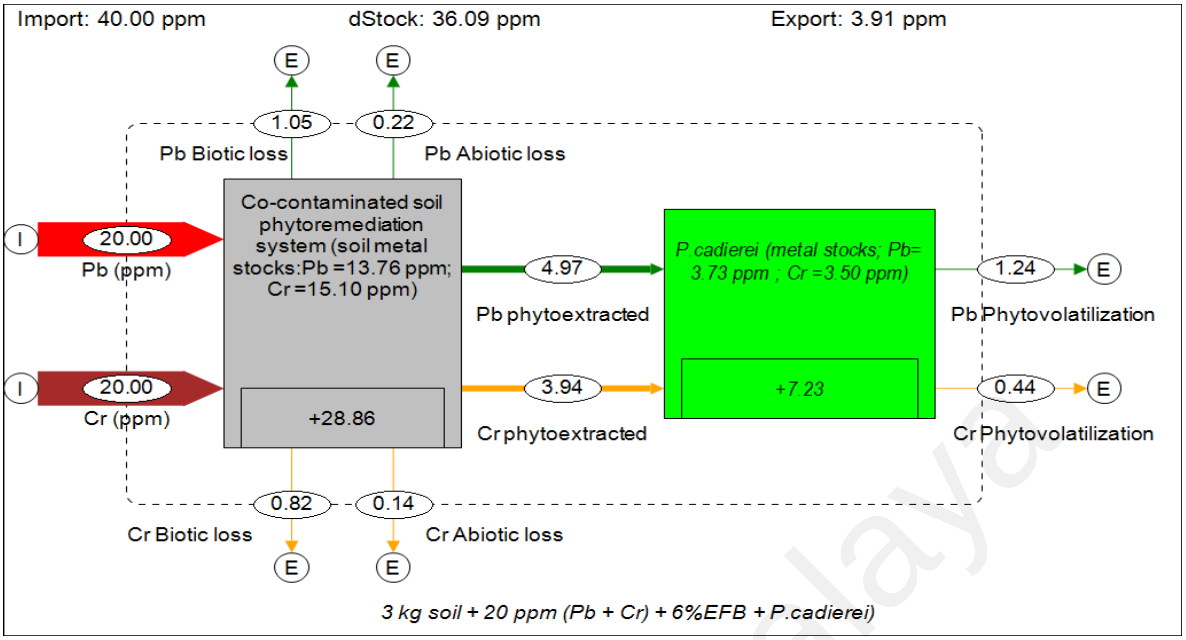


Figure 4.96a: STAN Phytoremediation schematic mass flow of co-contaminated soil with EFB under *Pilea sp.*

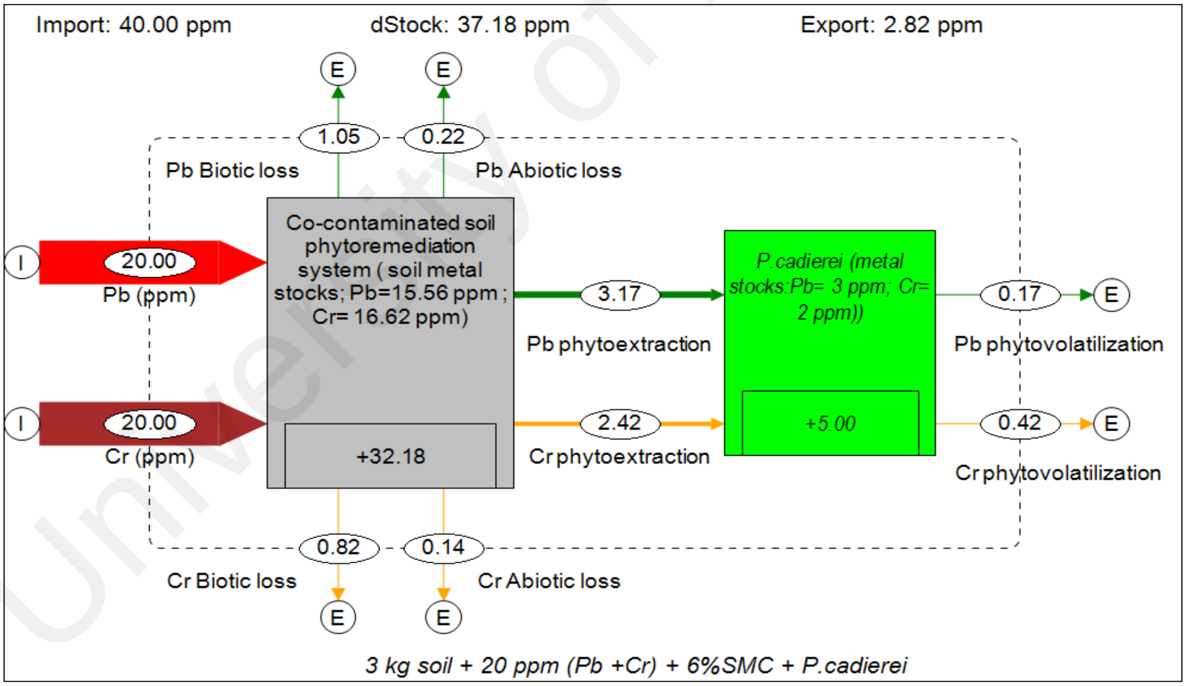


Figure 4.96b: STAN Phytoremediation schematic mass flow of co-contaminated soil with SMC under *Pilea sp.*

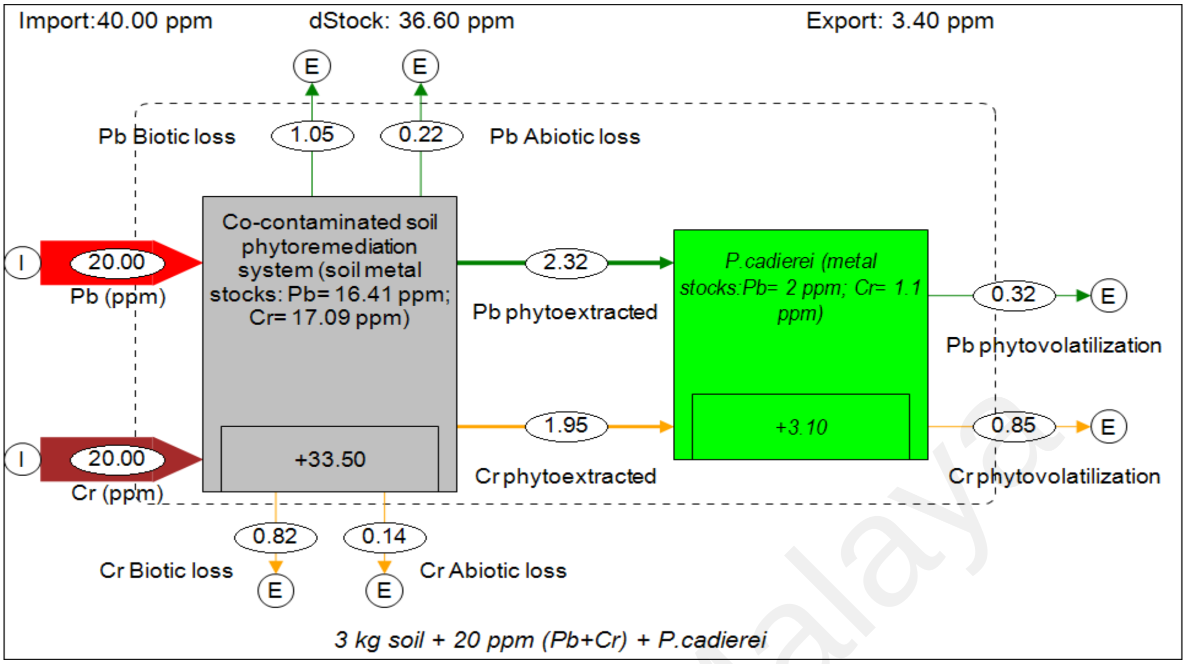


Figure 4.96c: STAN Phytoremediation schematic mass flow of co-contaminated soil under *Pilea sp.* alone.

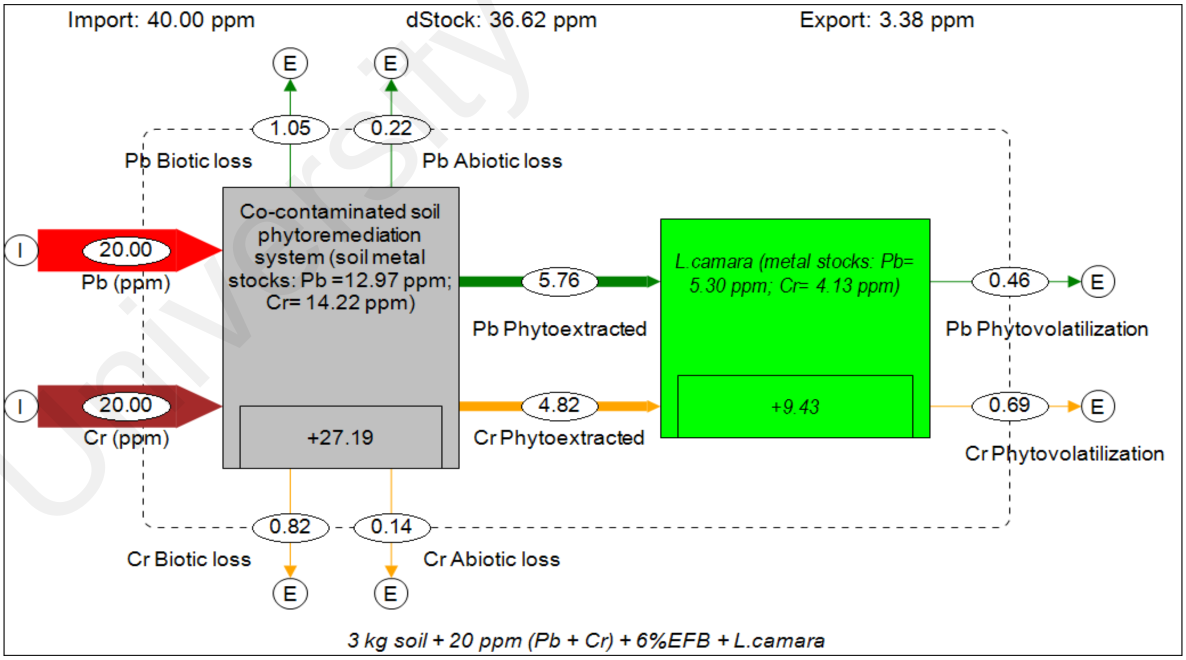


Figure 4.96d: STAN Phytoremediation schematic mass flow of co-contaminated soil with EFB under *Lantana sp*.

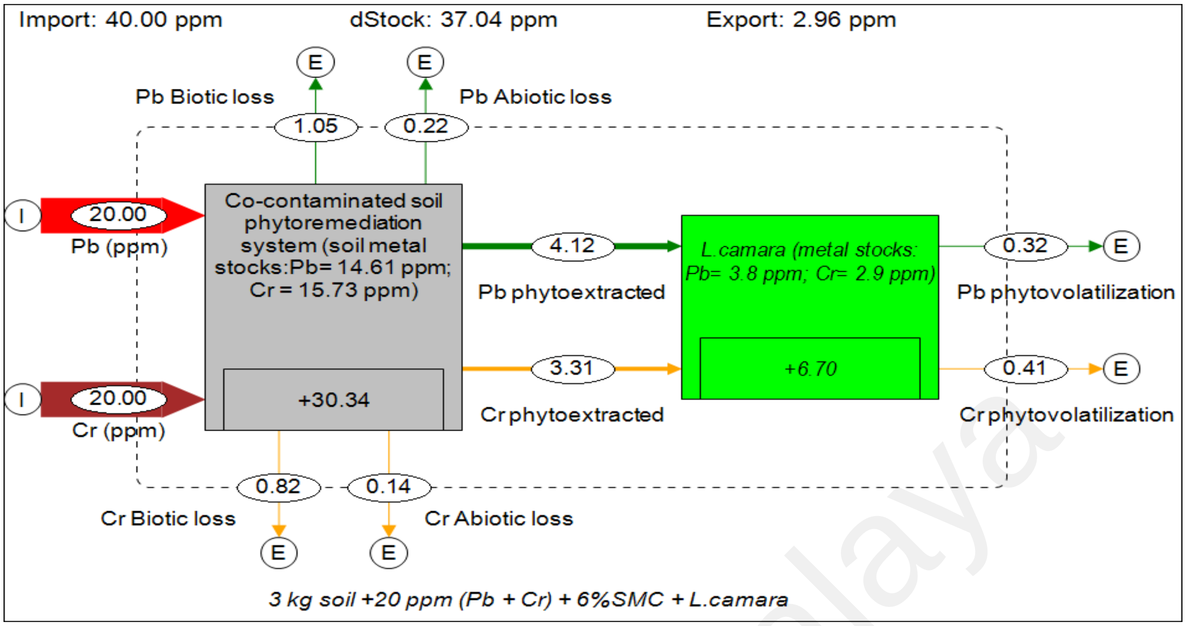


Figure 4.96e: STAN Phytoremediation schematic mass flow of co-contaminated soil with SMC under *Lantana sp*.

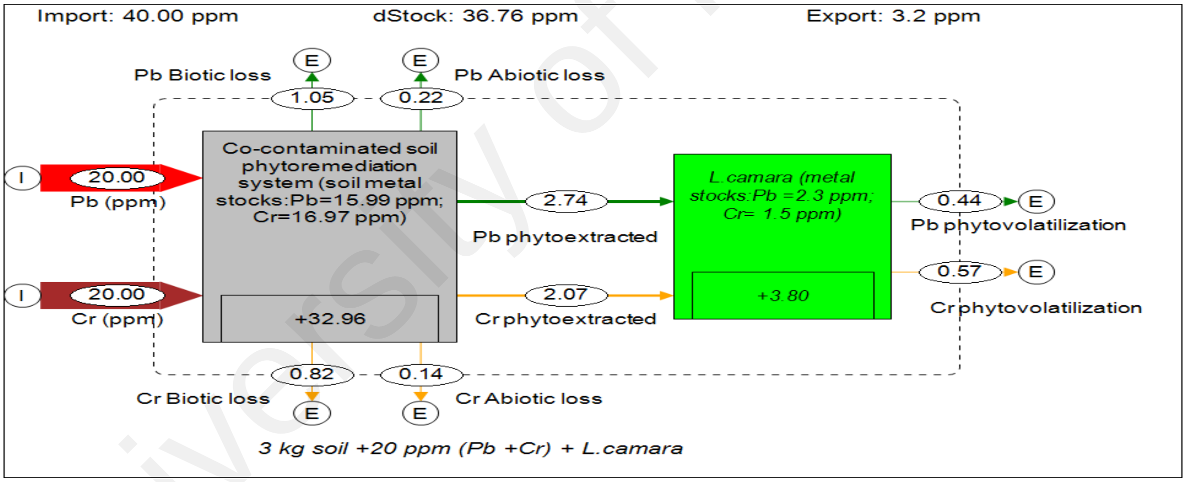


Figure 4.96f: STAN Phytoremediation schematic mass flow of co-contaminated soil under *Lantana sp.* alone.

In conclusion, STAN Pb and Cr fluxes examined, may not be absolutely definite. Hence, there may be some drawbacks, either in underestimating or overestimating abiotic and/or biotic variables. Rather, this mass balance model, basically offers an alternative methods, for evaluating where the pollutants are stocked, at a quick glance in a phytoremediation system.

4.6 Metal reductions by HMRB-isolates in bioaugmentation validation study

Figure 4.97 presented a bioaugmentation study result after 90 days monitoring, where 20 ppm Pb contaminated soil, inoculated with *Enterobacter cloacea* (single isolate), and *Enterobacter cloacea* + *Lysinibaccillus sphaericus* (consortia) recorded 26.03% and 30.33% reductions of Pb, when compared to 1.5% recorded in zero inocula control ($p<0.05$). Differently, *Bacillus pumilus* (single inocula), and *Bacillus pumilus* + *Lysinibaccillus sphaericus* (consortia) recorded 27.04% and 24.33% Cr reductions, when compared to 1.1% decline obtained from zero isolate Cr control ($p<0.05$). This variation was due to supplemented consortiums, and recorded pH values ranged from pH 6.11 to 6.35. However, He et al., (2010) have also isolated thirteen Cu-resistant bacteria, from Cu polluted soil; including *Bacillus* sp., and was identified to possess 99% database similarity with known 16S rRNA bacteria gene.

When this bioaugmentation results obtained from these consortiums were compared to phytoremediation of 20 ppm Cr or Pb polluted soils, under *Pilea sp.* and *Lantana sp.* that recorded 47.87 to 28.15% and 57.2 to 32.99% reductions of Cr and Pb, respectively. Therefore, provided revalidating and/or supporting evidence on microbial impacts to soil-metal reduction, hence the HMRB-isolated (*Enterobacter cloacea*; *Lysinibaccillus sphaericus*; *Bacillus pumilus*) had contributed in metal elimination during phytoremediation.

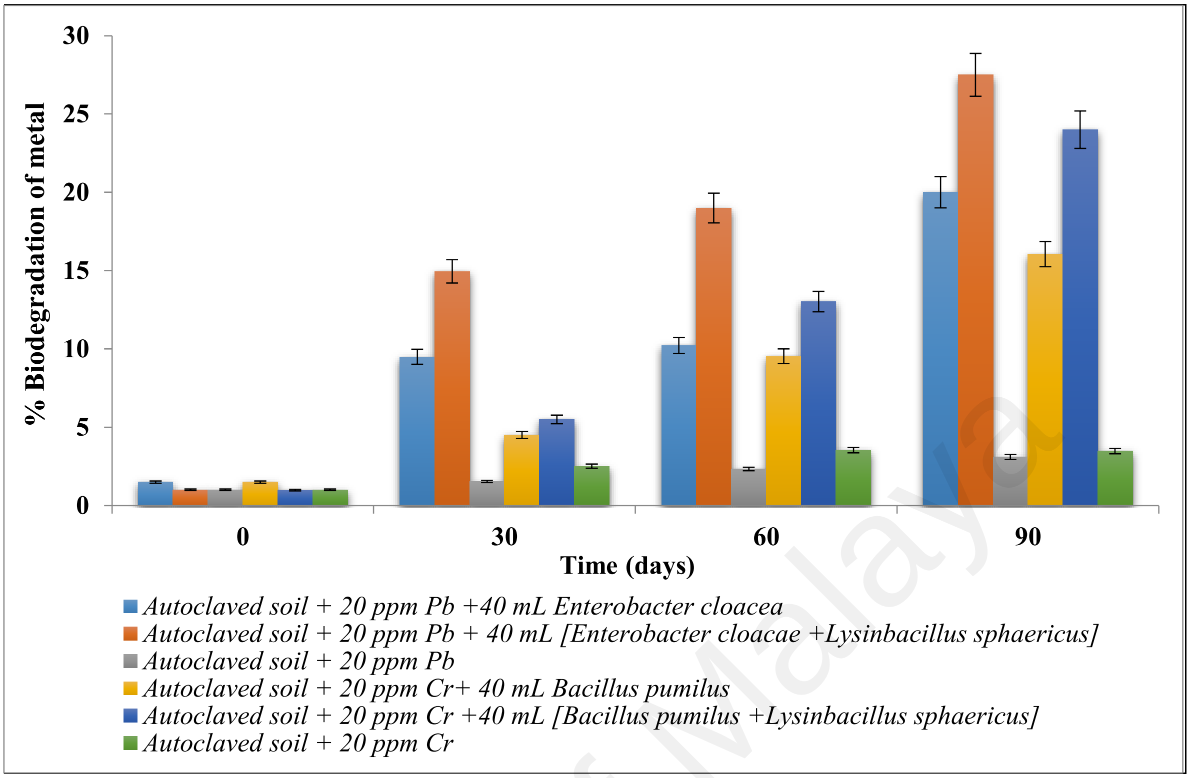


Figure 4.97: Strain validation under consortium bioaugmentation.

Note: readings were taken at n=3.

Jacque et al., (2008) further reported six microbial consortium; *Bacillus cereus* included, that degraded 90% PAHs. Implying that autochthonous microbes, have the potential to degrade pollutants in their primary environment when optimized.

4.7 General discussion

The entire rational behind this study, was to apply ecologically safe method in decontaminating heavy metal (Pb and Cr) contaminated soil. By applying *Pilea sp.* and/or *Lantana sp.*, incorporated with non-toxic but nutrient viable (biodegradable biowaste; EFB and SMC to the soil. These organic wastes utilized, contained appreciable amounts of N and P, reported to be limiting nutrients for both plant and/or microorganisms, which can effectively support their metabolisms, and hence propel the elimination of pollutants from soil and/or sediments.

The phytoremediation studies, which was also tailored to screen *Pilea sp.* and *Lantana sp.* as emerging metallophytes, involved the contamination of soil with various doses of (Pb; 80, 40, 20 and 10 ppm, Cr; 40, 20 and 10 ppm, and 20 ppm Pb+Cr) pollutants, under natural condition for 98 days. However, the organic wastes used demonstrated great potentials in enhancing system complexation, and hence metal elimination, especially EFB. To this development, *Pilea sp.* and *Lantana sp.* expressed high tolerance in combating metal stress, but *Lantana sp.* was a more resilient bioindicator. Sadly, both plants experienced greater challenges under co-contaminated soil condition; hence, a more problematic scenario, due to speciation uncertainties. Empty fruit bunch and SMC under *Lantana sp.* enhanced the elimination of Pb by 52.47%, 55.66%, 57.2%, and 88.03%, and 45.1%, 46.73%, 48.47%, and 82.73% for 80, 40, 20, and 10 ppm Pb doses, respectively. Correspondingly, EFB and SMC under *Pilea sp.* reduced Pb by 44.83%, 48.32%, 49.26%, and 83.83%, and also 42.6%, 44.99%, 45.0%, and 79.77%, respectively.

Similarly, under *Lantana sp.* 53.18 to 75.63%, and 46.62 to 72.13% of Cr were reduced at EFB and SMC amended Cr- soils. 47.18 to 72.43%, and 38.09 to 67.45% elimination of Cr were evident at EFB and SMC supplemented soils, affixed with *Pilea sp.* respectively, at 40 to 10 ppm Cr-phytoremediated soil. Finally, under co-contaminated condition of about 31.22 to 24.5%, and 22.18 to 16.9% of Pb and Cr were extinguished under *Pilea*, when amended with EFB and SMC, respectively. Parallelly, 35.13 to 28.8%, and 26.95 to 21.37% of Pb and

Cr were absented by *Lantana sp.*, under EFB and SMC amended co-polluted soil, respectively. The potentials of EFB boosting phytoremediation than SMC might not be unconnected to its N content, when correlated to SMC used in this study.

Also, the diverse capabilities of *Lantana sp.* and *Pilea sp.* towards phytoremediation and/or phytostabilization metal polluted soil, may be linked to their exudates, and plant adaptive features to heavy metal stress. However, *Lantana sp.* appeared harder in nature than *Pilea*, and that could foster better survival of the former, under harsh environmental condition. Meanwhile, with the exclusion of Alaribe & Agamuthu, (2015) and this current study, no other studies have attempted to review the metallophyte potentials of *Lantana sp.* and/or *Pilea sp.*, thus far, demonstrated to be resourceful. Therefore, phytoremediation studies, focused towards screening for indigenous plants efficiency on target heavy metals, should be inexhaustive.

In terms of BAF and TF, both *Lantana sp.* and *Pilea sp.* recorded appreciable accumulation of Pb and Cr, at their root zones. This may be as a result of root to shoot metal-translocation inertia. Generally, both plants have BAF and TF < 1, indicating an accumulator plant, and not a hyperaccumulator. But, it is still feasible for soil-metal phytoremediation. Rosselli et al., (2003) have also established similar arguments on *A. incana*. However, root and shoot lengths relatively varied between treatments.

Meanwhile, Pb and Cr uptake rates of *Lantana sp.*, under EFB and SMC amended soils exceeded *Pilea*. This might not only be the impacts of nutrients alone, but also, the organic acid exudates seen in *Lantana sp.* rhizosphere region. Which potentially produced more H^+, and hence displace Pb and/or Cr at their soil binding sites, thus enhanced phytoextraction. In extension, Ali et al., (2013) clearly stated that uptake of heavy metal ion from soil solution, is mediated by differentiated transporters (i.e., channel proteins) or H^+ fused carrier proteins present in the plasma membrane of roots. Therefore, non-essential heavy metals, suchlike Pb and Cr, can effectively compete for, and enter the roots through the same transmembrane transporters, used by essential heavy metals having similar oxidation states. This basically explains some of the fundamental mechanisms of phytoextraction.

The isotherms and thermodynamics applied to correlate the data's from this study, sustainably proved favourable when polluted-soils were amended with organic waste, upon phytoremediation. This agreed with numerous authors (Bolan et al., 2014; Ali et al., 2013; Wu et al., 2010), who emphasized on similar phenomena. Another reason, might be that organic wastes amendments tends to improve soil aeration, and texture. Thus, fostering the diffusion of complexed metals, towards plant rhizosphere roots, as well as desorbing metals away from soil matrices.

However, the limitations in spiking soil with Pb or Cr may involve diverse and/or induced speciation, which may differ from the speciation of a naturally contaminated soil, due to inherent soil ecological factors. This was further elucidated by Hu et al., (2014), who observed relative differences in Cu and Se speciations in both spiked and naturally contaminated soils, during remediation of Cu and/or Se contaminated soils. However, indepth speciations of Pb and/or Cr in the contaminated soil was outside the context of this study, but would be recommended for future study.

Finally, another important aspect of phytoremediation, especially for soil contaminated with heavy metals, is the handling and/or disposal of post-remediation harvested plants, believed to be highly contaminated with metals. However, if the plant biomass are not properly managed, it can re-introduce metals into the environment when discarded. Therefore, safe disposal via proper incineration and/or hazardous waste landfill is very imperative.

For example, under Malaysia regulation, such contaminated plants are directed by the Department of Environment to be conveyed to *Kualiti-Alam* incineration facility (*http://www.kualitialam.com.my/capabilities-services-hazardous-waste-management.php*): assessed on May 12th, 2016. Thereafter, the plant-ash is converted to solidified cement, and further disposed into a hazardous waste landfill. Furthermore, Robinson et al., (2015) alternatively reported the recovery of heavy metals from plant ash (i.e., bio ore), through phytomining techniques as another option. This concurred with Zhang et al., (2014) who reported 6-20% Ni recovery from hyperaccumulators. Meanwhile, Robinson et al., (2015) also argued that phytomining would require significant capital outlay, and thus, may not be feasible without government assistance.

Eventually, this study recommends that research on phytoremediation of soil contaminated with heavy metals is obviously interdisciplinary in nature, and may require background knowledge in environmental engineering, plant biology, biotechnology, soil microbiology, ecology, and also soil chemistry. Therefore, the integration and/or holistic scientific knowledge, may help in surmounting the challenging questions about phytoremediation, and its commercialization in the future.

CHAPTER 5: CONCLUSION

5.1 Conclusion

Phytoremediation study of heavy metal (Pb and Cr) contaminated soil, with indigenous *Lantana sp.* and *Pilea sp.* plants was carried out. The established Pb bioaccumulation and translocation phenomenon ranged between 0.16 to 0.41 and 0.59 to 0.82 for *Lantana sp.* uptake, and 0.11 to 0.36 and 0.39 to 0.75 for *Pilea*, respectively. While, the corresponding Cr treatments recorded BAF and TL of 0.14 to 0.61 and 0.41 to 0.56 for *Lantana sp.*, in extension to 0.10 to 0.48 and 0.22 to 0.83 obtained for *Pilea sp.* uptakes, respectively. This indicated that both native floras, are indicators and/or accumulator plants for phytoremediation of metal polluted soil, but not hyperaccumulators, since their BAF and TF values are < 1, as opposed to >1 designated for hyperaccumulators. However, they possess metallophyte traits. *A. incana* recorded BAF value < 1, for Cd soil phytoremediation, hence was conferred Cd accumulator. Therefore, considering the BAF and TF values obtained for both *Lantana sp.* and *Pilea sp.*, the former can be implicated to possess higher metal phytoextraction potentials.

Heavy metal in soil amended with organic wastes decreased significantly, when compared to the unamended treatments. However, treatments with EFB, gave about 44.8% to 88.8% reduction of Pb in soil. More so, 42.6% to 82.7% Cr-elimination from soil were evidenced when amended with EFB, for both *Pilea sp.* and *Lantana sp.* spots. Correspondingly, SMC-amended soil recorded Pb and Cr disappearance of 42.6% to 82.7%, and 38 % to 72.1% from soil, respectively. Correlation with unamended Pb and Cr control soil, revealed 23% to 49% and 20.7% to 57.8% elimination of metals. Therefore, biostimulation materials applied, enhanced heavy metal decontamination from soil, while still supporting plant nutrient needs. But comparatively, EFB have more biostimulating impacts than SMC, based on this study.

Clearly, higher number of HMRB were isolated from amended soils. However, during the 90 days autochthonous bioaugmentation revalidated study, isolates actually reduced the concentrations of Pb and/or Cr from soil. This therefore, substantiated the associated role bacteria played in actual phytoremediation. Secondly, the impacts of rhizosphere root exudates recorded in this study implicated numerous metabolites, suchlike organic acids, and peptides, presumed to be phytosiderophores, phytochelatins and/or methionine molecules. These enhanced solubilizing Pb and/or Cr ions, and invariably improved their bioavailability for plant uptake.

Interestingly, first-order kinetic rates of Pb and Cr uptake under amended treatments ranged 0.2629 to 0.1040 day^{-1} and 0.223 to 0.1154 day^{-1}, when compared to 0.0923 to 0.1009 day^{-1} recorded for unamended *Pilea sp.* and *Lantana sp.* spots, respectively. However, half-life implicated 15 to 22 times faster elimination of Pb and/or Cr contents in soil, when amended with organic wastes. Meanwhile, intra-particle diffusion model, indicated high multi-linear absorption of Pb and Cr when amended, as opposed to un-supplemented soil during phytoremediation. Hence, SEM analysis showed less shiny and/or bright images of soils at post- phytoremediation, inferring the disappearance of heavy metal loads.

Furthermore, under co-metal polluted soil, both *Pilea sp.* and *Lantana sp.* recorded less BAF and TF-values, including less kinetic uptake rates. This led to poor Pb and/or Cr disappearance from soil, when compared to the phytoremediated single metal soil-pollutant, after 98 days. Revealing its problematic nature, therefore not all metallophytes that combated single-metal polluted soil, can suppress multiple contamination. Hence, this study recommends that more studies may be needed to decomplex these unforeseen factors.

STAN modelling made apparent, the inherent components where metals were channelled in the phytoremediation system. This offers prompt and holistic assessments of the phytoremediation system. Perhaps, may inform the decision of the environmental scientist and/or engineer about possible phytoremediation lapses and/or success, as to enhance future study.

Therefore, the proposed null hypothesis can be rejected based on these statistical views:

The null hypothesis that states that *Pilea sp.* and *Lantana sp.* plants are not indicator plants for phytoremediation of soil contaminated with heavy metal; could be rejected. Because, the ANOVA computed F-statistics ($p<0.05$), for phytoextraction and/or reductions of Pb and/or Cr from plants and/or soil, were substantially larger than 1, in many treatments as detailed in chapter 4. Therefore, if the F-statistics computed were equal or less than 1, in all the determining parameters investigated, then the null hypothesis would have been considered acceptable (Howell, 2003). However, this was relatively not the case. Secondly, microbial proliferation enhanced the solubilization of metals, because the computed F-statistics ($p<0.05$), indicated that bacterial growths at amended soils, that recorded higher metal elimination during monitoring were much higher than 1, when compared to the bacterial density observed at metal control soils, that proffered less metal removals.

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APPENDIX: Tables

Appendix Table 1: Percentage phytoreduction of 80 ppm Pb in soil under *Pilea sp.* and *Lantana sp.* after 98 days.

Treatments **Statistical sig.**	**Time (days) and (%) Pb reduction**							
Pilea sp.	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
A1= ***a***	2.72±0.01	4.86±0.01	12.37±0.02	21.13±0.01	37.35±0.01	38.74±0.01	44.83±0.01	***b c f g h***
A2= ***b***	1.21±0.01	2.37±0.01	7.36±0.01	14.74±0.01	29.88±0.01	33.74±0.02	42.60±1.25	***c f g h***
A3= ***c***	0.34±0.03	1.13±0.01	6.12±0.01	9.27±0.648	13.60±0.01	18.73±0.01	20.73±0.01	***g h***
Lantana sp.								
A1.1= ***d***	3.74±0.01	7.49±0.14	13.74±0.03	24.99±0.11	39.83±0.13	44.83±0.03	52.46±0.02	***abcefgh***
A2.1= ***e***	2.48±0.02	4.98±0.02	9.74±0.22	18.46±0.01	33.47±0.09	37.45±0.07	45.10±1.25	***b c f g h***
A3.1= ***f***	2.32±0.01	4.85±0.01	7.49±0.12	10.99±0.13	18.63±0.01	19.99±0.01	23.69±1.25	***c g h***
Controls								
A4= ***g***	0.63±0.02	1.72±0.58	2.44±0.02	2.63±0.01	3.69±0.02	3.71±0.01	3.73±0.07	***h***
A5= ***h***	0.17±0.07	0.56±0.02	0.63±0.02	0.75±0.013	1.13±0.01	1.61±0.59	1.37±0.01	**z**

Indications

A1 & **A1.1 =** 3 kg soil + 80 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*

A2 & **A2.1** = 3 kg soil + 80 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*

A3 & **A3.1** = 3 kg soil + 80 ppm Pb +*Pilea cadierei /Lantana camara*

A4; A5 = 3 kg soil + 80 ppm Pb only; 3 kg autoclaved soil + 80 ppm Pb + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 2291.688 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **A1, A2, A3, A1.1, A2.1, A3.1, A4, and A5.**

z = no significant difference**.**

df = degree of freedom; **F =** critical value.

Appendix Table 2: **ANOVA** for 80 ppm Pb in soil Pb reduction (***p*< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig (p<0.05)**
Between Groups	8215.148	7	1173.593	**2291.688**
Within Groups	8.194	16	.512	
Total	8223.342	23		

Appendix Table 3: Percentage phytoreduction of 40 ppm Pb in soil under *Pilea sp.* and *Lantana sp.* after 98 days.

Treatments				**Time (days) and (%) Pb reduction**				**Statistical sig.**
Pilea sp.	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
B1= ***a***	2.72±0.01	4.86±0.11	10.56±0.04	21.10±0.04	37.35±0.01	38.73±0.01	48.32±1.49	***bce fgh***
B2= ***b***	1.90±0.11	2.48±0.03	7.38±0.04	14.72±0.04	29.88±0.01	33.75±0.01	44.99±0.03	***c f g h***
B3= ***c***	0.36±0.03	1.24±0.02	6.23±0.03	8.74±0.03	13.65±0.03	18.74±0.01	20.74±0.03	***g h***
Lantana sp.								
B1.1= **d**	3.80±0.03	7.12±0.04	13.73±0.03	24.98±0.01	40.04±0.03	44.95±0.03	56.66±0.03	***bcdefgh***
B2.1=***e***	2.49±0.001	4.78±0.03	7.93±0.01	19.20±1.45	33.98±0.02	38.27±1.28	46.73±0.01	***b c f g h***
B3.1= ***f***	2.42±0.12	2.73±0.02	5.91±2.74	10.97±0.02	17.37±0.03	19.96±0.03	19.95±0.03	***g h***
Controls								
B4= ***g***	0.68±0.03	1.43±0.03	2.44±0.01	2.63±0.01	2.69±0.03	3.93±0.01	6.70±0.03	***h***
B5= ***h***	0.23±0.03	0.86±1.43	0.38±0.03	0.75±0.013	0.95±0.73	2.12±1.05	2.49±0.01	***z***

Indications

B1 & **B1.1 =** 3 kg soil + 40 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*

B2 & **B2.1** = 3 kg soil + 40 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*

B3 & **B3.1** = 3 kg soil + 40 ppm Pb +*Pilea cadierei /Lantana camara*

B4; B5 = 3 kg soil + 40 ppm Pb only; 3 kg autoclaved soil + 40 ppm Pb + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 4677.750 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **B1, B2, B3, B1.1, B2.1, B3.1, B4, and B5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

Appendix Table 4: **ANOVA** for 40 ppm Pb in soil Pb reduction (**p< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig. (p<0.05)**
Between Groups	9095.005	7	1299.286	**4677.750**
Within Groups	4.444	16	.278	
Total	9099.450	23		

Appendix Table 5: Percentage phytoreduction of 20 ppm Pb in soil under *Pilea* and *Lantana* after 98 days.

Treatments				**Time (days) and (%) Pb reduction**				**Statistical sig.**
Pilea sp.	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(p<0.05)**
E1=***a***	3.00±0.05	4.90±0.10	10.57±0.02	21.15±0.05	37.38±0.03	38.95±0.05	49.25±1.49	***b c f g h***
E2= *b*	2.00±0.05	2.37±0.03	7.45±0.05	14.95±0.04	29.50±0.01	33.75±0.05	45.00±0.05	***c f g h***
E3= ***c***	0.50±0.05	1.27±0.03	5.09±0.14	8.85±0.05	13.55±0.05	18.03±0.03	30.00±0.05	***g h***
Lantana sp.								
E1.1= ***d***	3.75±0.05	7.13±0.03	13.78±0.03	24.99±0.05	40.75±0.05	45.75±3.36	57.20±0.11	***abcefg h***
E2.1= ***e***	2.10±0.05	4.50±0.03	7.95±0.05	19.82±0.49	33.50±0.05	38.35±1.17	48.47±0.70	***bcfg h***
E3.1= ***f***	1.92±0.02	3.73±0.08	6.03±0.85	10.05±0.01	17.32±0.03	19.95±0.05	32.99±0.01	***c g h***
Controls								
E4= ***g***	0.63±0.03	1.40±0.05	2.31±0.03	2.69±0.02	3.47±0.03	3.55±0.05	6.50±0.04	***g***
E5= ***h***	0.25±0.05	0.32±0.06	0.35±0.05	0.70±0.05	0.83±0.03	0.90±0.05	2.75±0.05	**z**

E1 & **E1.1** = 3 kg soil + 20 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*

E2 & **E2.1** = 3 kg soil + 20 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*

E3 & **E3.1** = 3 kg soil + 20 ppm Pb +*Pilea cadierei /Lantana camara*

E4; **E5** = 3 kg soil + 20 ppm Pb only; 3 kg autoclaved soil + 20 ppm Pb + 0.5%NaN_3

F (df = 7, 16, p< 0.05) = 11692.259 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **E1, E2, E3, E1.1, E2.1, E3.1, E4, and E5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

Appendix Table 6: **ANOVA** for 20 ppm Pb in soil Pb reduction (***p*****< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig. (*p*<0.05)**
Between Groups	8560.817	7	1222.974	**11692.259**
Within Groups	1.674	16	.105	
Total	8562.490	23		

Appendix Table 7: Percentage phytoreduction of 10 ppm Pb in soil under *Pilea* and *Lantana* after 98 days

Treatments				**Time (days) and (%) Pb reduction**				**Statistical sig.**
Pilea sp.	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
G1= *a*	8.00±0.10	9.60±0.10	28.80±0.01	38.80±0.10	57.00±1.01	60.10±0.01	83.83±0.15	***b c f g h***
G2= *b*	1.800±0.10	5.93±0.06	18.80±0.10	20.13±0.06	39.63±0.25	55.43±0.01	79.77±0.49	***c f g h***
G3= *c*	1.60±0.01	1.93±0.10	11.20±0.10	13.0±1.01	13.05±0.03	29.73±0.06	48.23±0.10	***g h***
Lantana sp.								
G1.1= *d*	8.20±0.27	10.97±0.06	30.36±0.55	46.40±2.78	40.04±0.03	66.675±1.49	88.83±0.06	***b cdefg h***
G2.1= *e*	3.60±2.02	9.99±0.02	20.97±1.05	30.00±0.17	33.98±0.02	59.70±0.61	82.73±1.42	***b c f g h***
G3.1= *f*	1.33±0.61	4.93±0.15	16.30±1.04	17.73±0.25	17.37±0.03	30.00±0.10	57.80±0.35	***c g h***
Controls								
G4= *g*	1.50±0.10	2.90±0.10	4.70±0.01	6.0±1.01	6.02±1.00	9.53±0.06	12.20±0.10	***h***
G5= *h*	0.80±0.02	1.93±0.06	2.80±0.10	2.90±0.10	2.90±0.73	5.00±0.01	5.77±0.12	***z***

Indications

G1 & G1.1 = 3 kg soil + 10 ppm Pb + 6%EFB+ *Pilea cadierei /Lantana camara*

G2 & G2.1 = 3 kg soil + 10 ppm Pb + 6%SMC+ *Pilea cadierei /Lantana camara*

G3 & G3.1 = 3 kg soil + 10 ppm Pb +*Pilea cadierei /Lantana camara*

G4; G5 = 3 kg soil + 10 ppm Pb only; 3 kg autoclaved soil + 10 ppm Pb + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 12048.408 defined the statistical impacts of this phytoremediation treatments.

Detail of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **G1, G2, G3, G1.1, G2.1, G3.1, G4, and G5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

Appendix Table 8: **ANOVA** for 10 ppm Pb in soil Pb reduction (***p*** **< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig. (*p*<0.05)**
Between Groups	22682.936	7	3240.419	**12048.408**
Within Groups	4.303	16	.269	
Total	22687.239	23		

Appendix Table 9: Percentage phytoreduction of 40 ppm Cr in soil under *Pilea* and *Lantana* after 98 days.

Treatments				**Time (days) and (%) Cr reduction**				**Statistical sig.**
Pilea	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
C1= ***a***	1.58±0.80	4.58±1.41	12.46±0.01	24.95±0.03	29.72±0.04	34.90±0.13	47.81±0.03	***b c f g h***
C2= ***b***	0.99±0.01	4.27±0.88	9.68±0.10	17.40±0.03	24.79±0.16	25.71±1.61	38.09±0.10	***c f g h***
C3= ***c***	1.60±0.01	2.23±0.03	7.18±0.10	12.47±0.01	14.82±0.12	17.28±0.17	22.98±1.76	***g h***
Lantana								
C1.1= ***d***	1.68±0.15	9.73±0.03	22.13±0.03	29.70±0.03	34.22±0.04	40.58±1.42	53.18±0.06	***b c d e f g h***
C2.1= ***e***	1.25±0.03	8.88±0.97	15.95±0.02	23.78±0.04	29.93±0.04	34.68±0.04	46.62±0.10	***b c f g h***
C3.1= ***f***	1.03±0.03	5.48±0.03	12.46±0.04	14.97±0.04	14.97±0.04	23.63±0.04	27.57±0.17	***c g h***
Controls								
C4= ***g***	0.63±0.10	1.46±0.68	2.55±0.03	3.38±0.01	4.01±0.03	4.80±0.18	4.95±0.03	***h***
C5= ***h***	0.50±0.03	0.98±0.01	1.49±0.01	2.23±0.03	2.94±0.70	2.76±0.01	2.75±0.001	**z**

C1 & **C1.1** = 3 kg soil + 40 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*

C2 & **C2.1** = 3 kg soil + 40 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*

C3 & **C3.1** = 3 kg soil + 40 ppm Cr +*Pilea cadierei /Lantana camara*

C4; **C5** = 3 kg soil + 40 ppm Cr only; 3 kg autoclaved soil + 40 ppm Cr + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 283.841 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **C1, C2, C3, C1.1, C2.1, C3.1, C4, and C5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

Appendix Table 10: **ANOVA** for 40 ppm Cr in soil Cr reduction (***p*< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig. (*p*<0.05)**
Between Groups	7792.295	7	1113.185	**283.841**
Within Groups	6.272	16	.392	
Total	7798.567	23		

Appendix Table 11: Percentage phytoreduction of 20 ppm Cr in soil under *Pilea* and *Lantana* after 98.

Treatments				**Time (days) and (%) Cr reduction**				**Statistical sig.**
Pilea	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
D1=***a***	1.07±0.80	5.37±0.08	12.50±0.05	24.99±0.03	29.88±0.03	34.98±0.01	47.87±0.13	***b c f g h***
D2=***b***	0.95±0.05	4.11±1.42	9.68±0.01	12.48±0.01	24.97±0.03	22.22±0.01	38.83±0.01	***c f g h***
D3=***c***	0.63±0.02	2.23±0.01	6.10±0.15	7.44±2.03	10.27±0.21	13.43±2.84	28.15±0.35	***g h***
Lantana								
D1.1=***d***	1.78±0.04	9.35±0.05	22.17±0.03	29.90±0.03	30.30±0.05	41.33±0.03	54.77±0.05	***a b c e f g h***
D2.1=***e***	0.95±0.03	5.38±0.12	15.85±0.05	23.60±0.04	25.98±1.14	35.43±2.84	48.90±0.82	***b c f g h***
D3.1=***f***	0.83±0.03	4.98±0.03	9.30±0.82	10.07±0.03	16.67±2.89	17.27±0.45	29.52±0.03	***c g h***
Controls								
D4= ***g***	0.63±0.02	1.03±0.03	2.53±0.03	3.38±0.02	4.32±0.59	4.50±0.05	6.0±0.50	***h***
D5= ***h***	0.45±0.05	1.25±0.05	1.45±0.05	1.50±0.05	1.50±0.05	1.62±0.02	2.50±0.05	***z***

D1 & **D1.1** = 3 kg soil + 20 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*

D2 & **D2.1** = 3 kg soil + 20 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*

D3 & **D3.1** = 3 kg soil + 20 ppm Cr +*Pilea cadierei /Lantana camara*

D4; D5 = 3 kg soil + 20 ppm Cr only; 3 kg autoclaved soil + 20 ppm Cr + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 987 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **D1, D2, D3, D1.1, D2.1, D3.1, D4, and D5.**

z = no significant difference**;** **df** = degree of freedom; **F** = critical value.

Appendix Table 12: **ANOVA** for 20 ppm Cr in soil Cr reduction (***p*****< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F -Sig. (*p*<0.05)**
Between Groups	22658.432	7	323.919	**987.163**
Within Groups	5.247	16	.328	
Total	22663.678	23		

Appendix Table 13: Percentage phytoreduction of 10 ppm Cr in soil under *Pilea* and *Lantana* after 98 days.

Treatments				**Time (days) and (%) Cr reduction**				**Statistical sig.**
Pilea sp.	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**(*p*<0.05)**
F1=**a**	2.00±0.01	5.90±0.10	30.13±0.06	45.00±0.06	57.80±0.10	58.90±0.20	72.43±0.61	***c f g h***
F2=***b***	1.17±0.06	4.10±0.10	27.50±0.10	36.90±0.01	50.20±0.10	54.07±0.06	67.45±5.54	***c f g h***
F3=***c***	1.09±0.01	2.10±0.01	10.99±0.01	18.80±2.03	22.77±0.12	28.87±0.06	44.69±0.35	***g h***
Lantana sp.								
F1.1=***d***	3.40±0.95	7.15±1.91	37.50±0.10	49.60±0.52	59.60±0.36	60.20±0.27	75.63±3.99	***c f g h***
F2.1= ***e***	2.57±2.11	5.50±1.23	28.80±0.26	38.20±2.77	55.00±0.95	27.20±0.10	72.13±0.10	***c f g h***
F3.1=***f***	1.17±0.12	3.13±0.06	19.99±0.10	25.13±3.86	28.37±0.15	32.20±2.52	49.16±0.06	***g h***
Controls								
F4= ***g***	1.09±0.01	1.70±0.01	2.20±0.10	3.90±0.10	5.00±0.10	8.93±0.15	10.13±0.06	***h***
F5= ***h***	0.30±0.10	0.90±0.01	1.20±0.01	1.43±0.06	1.63±0.06	1.87±0.05	1.97±0.06	**z**

F1 & **F1.1** = 3 kg soil + 10 ppm Cr + 6%EFB+ *Pilea cadierei /Lantana camara*

F2 & **F2.1** = 3 kg soil + 10 ppm Cr + 6%SMC+ *Pilea cadierei /Lantana camara*

F3 & **F3.1** = 3 kg soil + 10 ppm Cr +*Pilea cadierei /Lantana camara*

F4; **F5** = 3 kg soil + 10 ppm Cr only; 3 kg autoclaved soil + 10 ppm Cr + 0.5%NaN_3

F (df = 7, 16, *p*< 0.05) = 294.755 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates above

a, b, c , d, e, f, g, and ***h*** denotes significant levels at treatments **F1, F2, F3, F1.1, F2.1, F3.1, F4, and F5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

Appendix Table 14: **ANOVA** for 10 ppm Cr in soil Cr reduction (***p*** **< 0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F-Sig (*p*<0.05)**
Between Groups	17523.356	7	323.919	**294.755**
Within Groups	136.663	16	.328	
Total	17760.019	23		

Appendix Table 15: Percentage phytoreduction of 20 ppm (Pb + Cr) under *Pilea sp.* and *Lantana sp.* after 98 days in soil.

Treatments	**Time (days) and (%) Pb and Cr reduction in co-contaminated soil**							**Statistical significance**
Pilea	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**F (df = 7, 16, $p< 0.05$) = 719.482**
H1 = a →Pb	0.50±0.05	2.32±0.03	6.92±0.05	9.50±0.05	20.08±0.03	22.00±0.05	31.20±0.06	***b c d e h f j i k l m n o p***
b→ Cr	0.45±0.04	2.20±0.05	2.20±0.04	8.95±0.04	19.50±0.05	19.57±0.02	24.50±2.60	***c d e f j k l m o n p***
H2 = ***c*** →Pb	0.45±0.01	1.45±0.05	3.95±0.04	5.22±0.33	9.50±0.04	14.45±0.05	22.18±0.010	***d e f k l m o n p***
d→ Cr	0.22±0.03	0.80±0.04	2.95±0.04	4.92±0.03	9.00±0.03	10.18±0.06	16.90±2.50	***f m n o p***
H3 = ***e***→ Pb	0.20±0.01	0.60±0.01	2.55±1.60	3.55±0.05	9.00±0.05	9.68±0.28	17.97±0.78	***f l m n o p***
f→ Cr	0.17±0.03	0.68±0.03	1.88±1.03	4.00±0.04	5.07±0.03	9.00±0.05	14.57±0.03	***m n o p***
Lantana								
H1.1 = ***g***→ Pb	1.22±1.95	4.10±0.05	9.55±0.05	10.50±0.05	10.50±0.05	29.05±0.05	35.13±0.03	***a b c d e f h i j k l m n o p***
h→ Cr	0.50±0.001	4.00±0.04	4.25±0.43	9.85±0.05	9.85±0.05	28.45±0.04	28.80±0.30	***b c d e f j k l m n o p***
H2.1 = *i*→ Pb	0.60±0.01	1.92±0.03	4.25±0.43	9.50±0.04	9.50±0.04	19.85±0.30	26.95±0.05	***b c d e f j k m n o p***
j→ Cr	0.45±0.01	2.13±0.03	3.97±0.02	5.50±0.04	5.50±0.05	15.50±0.43	21.37±0.10	***d e f l m n o p***
H3.1= *k*→ Pb	0.50±0.01	1.80±0.06	3.77±0.36	4.83±0.36	4.83±0.29	11.00±0.05	20.05±0.07	***d l m n o p***
l→ Cr	0.36±0.03	0.85±0.05	3.12±0.08	4.85±0.30	4.85±0.30	10.98±0.03	15.17±0.01	***m n o p***
Controls								
H4 = ***m***→ Pb	0.05±0.01	0.25±0.01	0.75±0.01	0.92±0.02	0.92±0.02	30.00±0.05	5.25±0.22	***o p***
n→ Cr	0.13±0.03	0.45±0.02	0.75±0.05	0.95±0.03	0.95±0.03	1.25±0.04	4.10±0.03	***o p***
H5 = ***o***→ Pb	0.05±0.01	0.25±0.01	0.30±0.001	0.40±0.001	0.40±0.001	1.85±2.29	1.12±0.03	***z***
p→ Cr	0.10±0.00	0.35±0.02	0.42±0.06	0.50±0.01	0.50±0.02	0.65±0.001	0.70±0.35	***z***

Indications (Continuation of Appendix Table A1.8)

H1 & H1.1 = 3 kg soil + 20 ppm (Pb +Cr) + 6%EFB+ *Pilea cadierei /Lantana camara*:

H2 & H2.1 = 3 kg soil + 20 ppm (Pb + Cr) + 6%SMC+ *Pilea cadierei /Lantana camara*

H3 & H3.1 = 3 kg soil + 20 ppm (Pb+ Cr) +*Pilea cadierei /Lantana camara*

H4; H5 = 3 kg soil + 20 ppm (Pb + Cr) only; 3 kg autoclaved soil 20 ppm (Pb + Cr) + 0.5% NaN_3

F (df = 7, 16, p< 0.05) = 719.482 defined the statistical impacts of this phytoremediation treatments.

Details of statistical designates in Appendix Table A1. 8 above

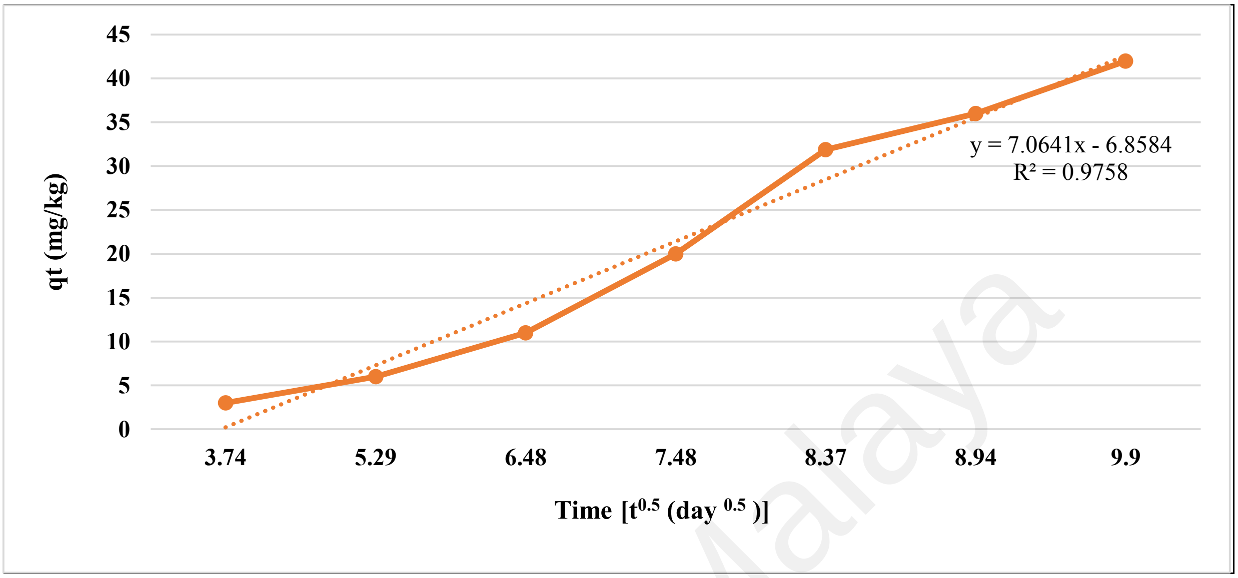
a, b; c , d; e, f; g, h; i, j; k, l; m, n; and o, p; denotes significant levels at treatments **H1; H2; H3; H1.1; H2.1; H3.1; H4; and F5.**

z = no significant difference**; df =** degree of freedom; **F =** critical value.

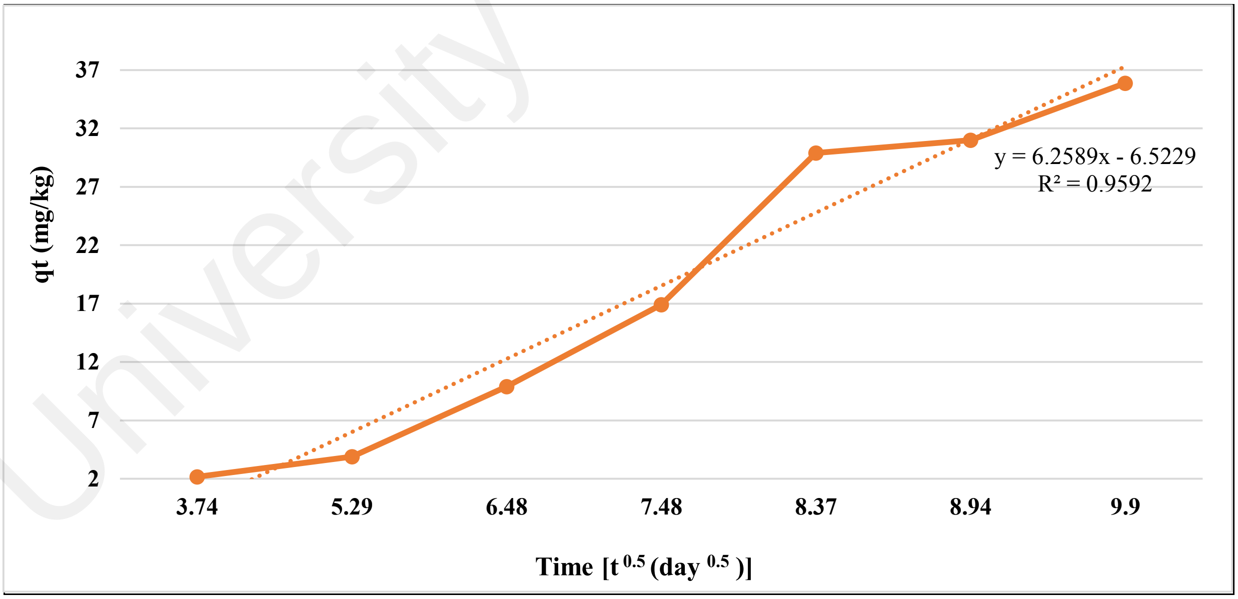
Appendix Table 16: **ANOVA** for 20 ppm (Pb + Cr) in co-contaminated soil metal reduction (***p***< **0.05**).

Treatments	**Sum of square**	**df**	**mean square**	**F**	**Sig.**
Between Groups	5093.141	7	339.543	**719.482**	.000
Within Groups	15.102	16	.472		
Total	5108.243	23			

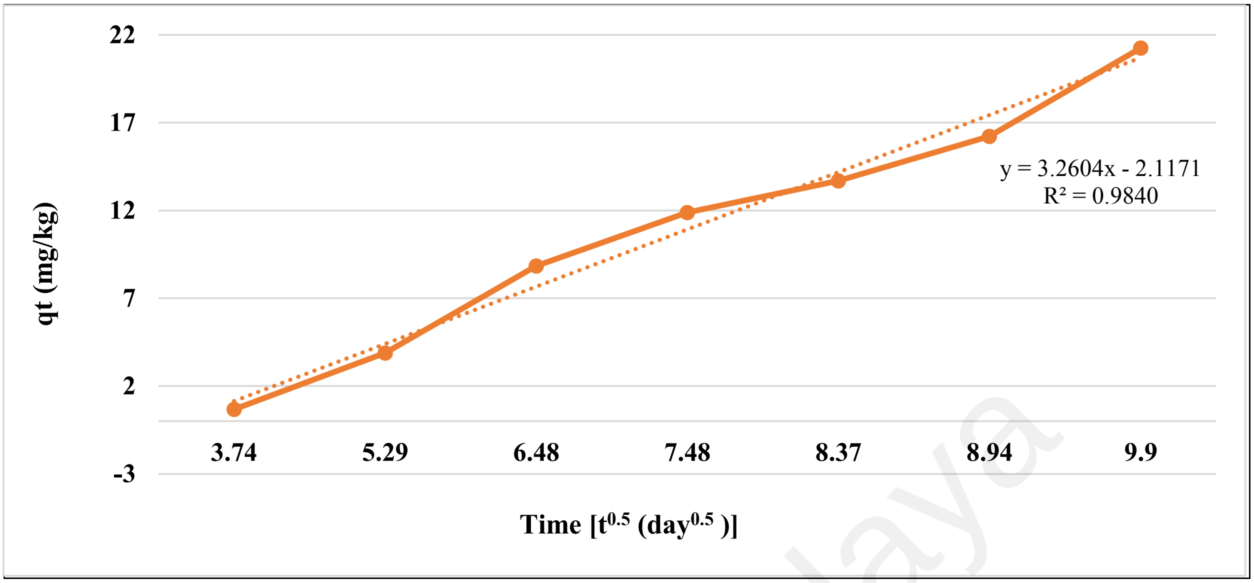
APPENDIX: Figures



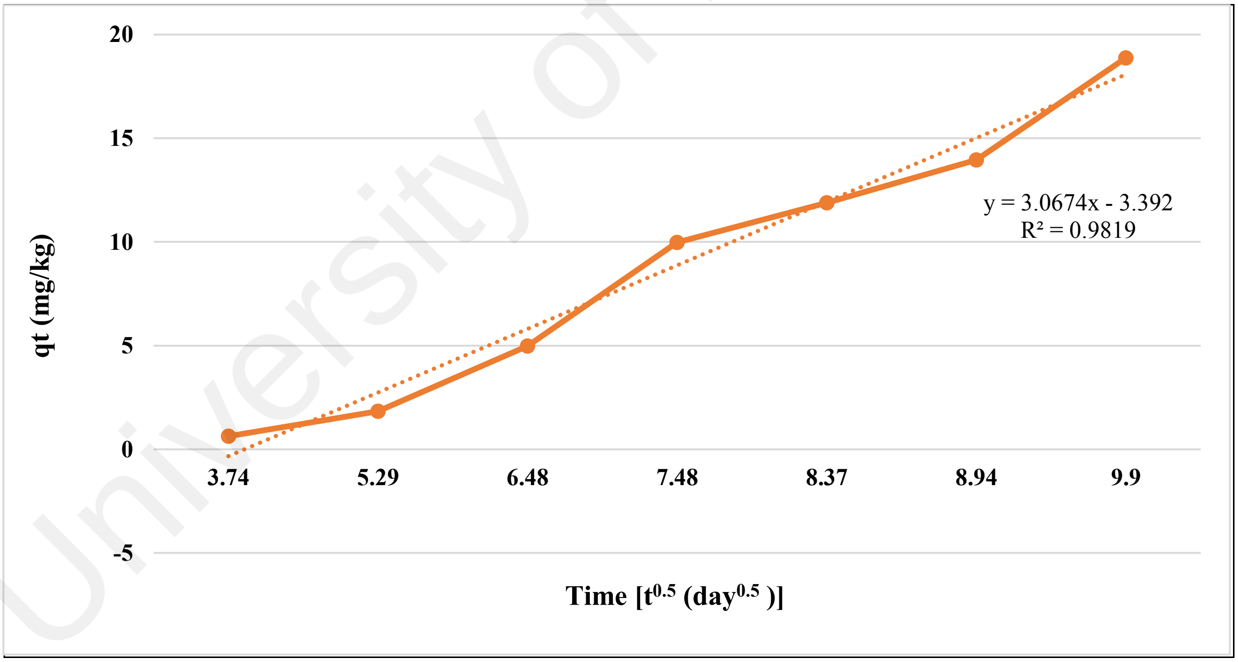
Appendix Figure 1: Intra-particle diffusion in soil of 80 ppm Pb with EFB under *Lantana sp.* after 98 days.



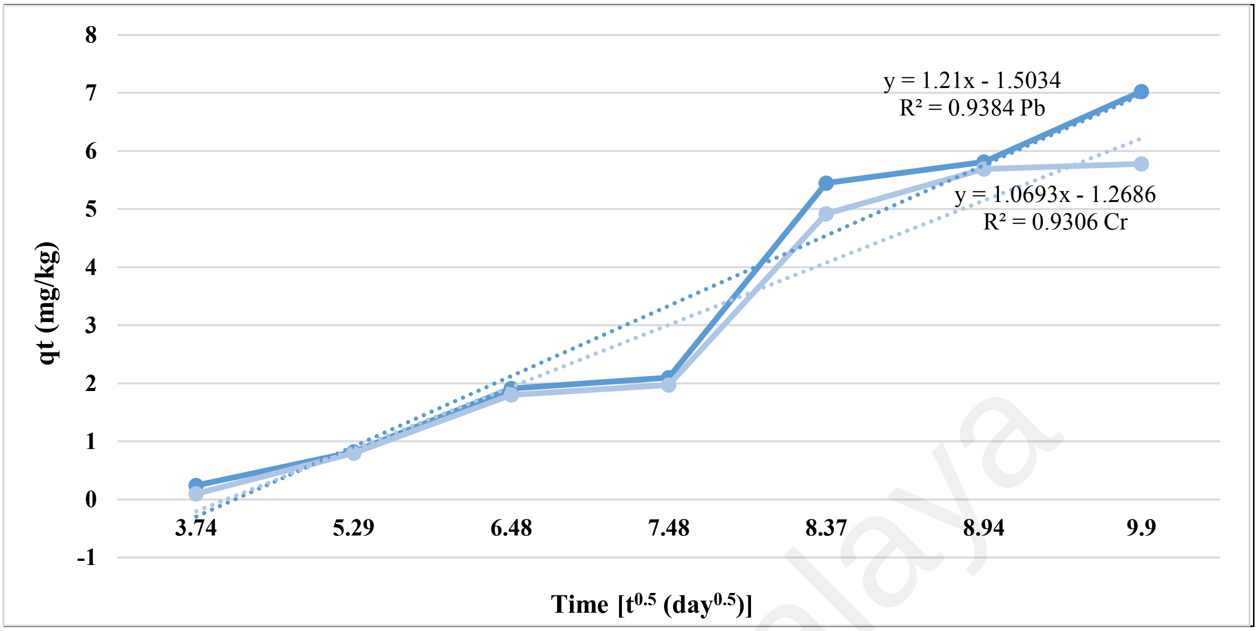
Appendix Figure 2: Intra-particle diffusion in soil of 80 ppm Pb with EFB under *Pilea sp.* after 98 days.



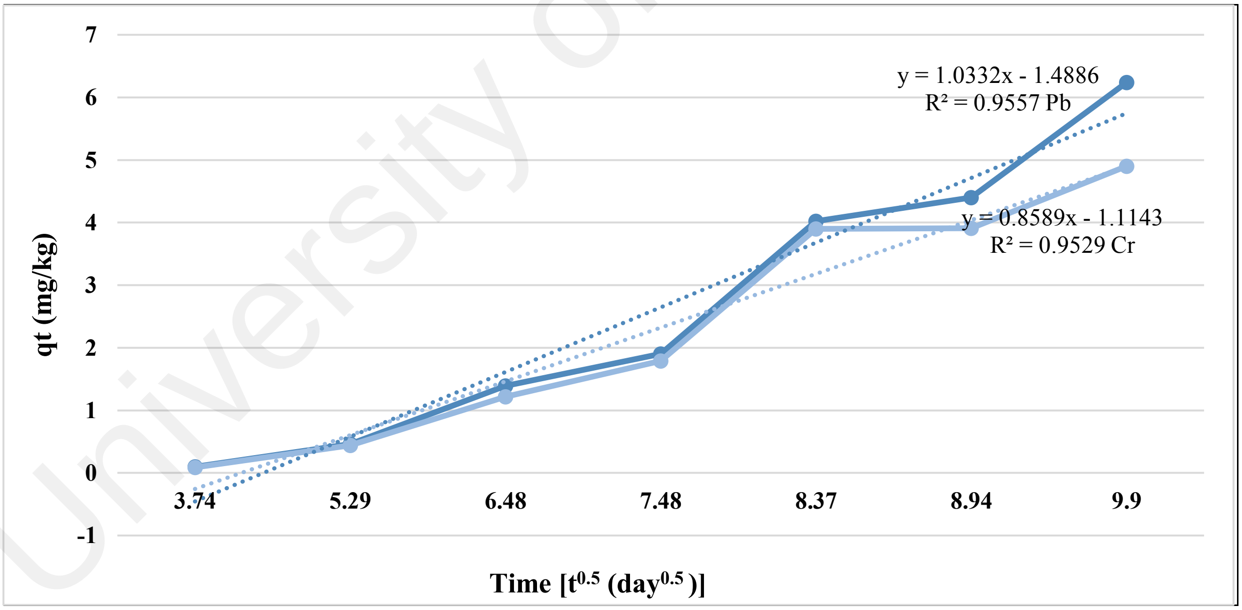
Appendix Figure 3: Intra-particle diffusion in soil of 40 ppm Cr with EFB under *Lantana sp.* after 98 days.



Appendix Figure 4: Intra-particle diffusion in soil of 40 ppm Cr with EFB under *Pilea sp.* after 98 days.



Appendix Figure 5: Intra-particle diffusion in soil of 20 ppm (Pb+ Cr) with EFB under *Lantana sp.* after 98 days.



Appendix Figure 6: Intra-particle diffusion in soil of 20 ppm (Pb+ Cr) with EFB under *Pilea sp.* after 98 days.

APPENDIX: Publication and Conference

Publication:

- Alaribe, F.O., Agamuthu, P. (2015). Assessment of phytoremediation potentials of *Lantana camara* in Pb impacted soil with organic waste additives. *Ecological Engineering* 83: 513-520.
- Alaribe, F.O., Agamuthu, P. (2016). Fertigation of *Brassica rapa* L. using treated landfill leachate as a nutrient recycling option. *South Afr J. Sci*. 112 (3/4):1-8.

Accepted manuscript for publication:

- Alaribe, F.O., Agamuthu, P. (2015). *Lantana camara*- A predictive ecological bioindicator plant for decontamination of Pb impaired soil under supplemented scenario. *Pedosphere* (Manuscript ID pedos201502067.R3).

Conference proceedings:

- Agamuthu, P. and Alaribe, F.O. (2015). Removal of Cr using *Pilea cadierei* in Chromium polluted soil under amended condition: Screening for emerging phytoremediation plants. *International Conference on Waste Management and Environment* (ICWME): "Paradigm Transformation in Waste Management towards a Greener Environment" University of Malaya, Kuala Lumpur 20-22 August (*Oral presentation*).

Accepted conference abstract for oral presentation

- Agamuthu, P., and Alaribe, F.O. An approach to decontaminating chromium induced soil with *Pilea cadierei* under amended monitoring. 8th *International Conference on Environmental Science and Technology*, Houston, Texas, USA 6-10 June, 2016.

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Assessment of phytoremediation potentials of *Lantana camara* in Pb impacted soil with organic waste additives

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A B S T R A C T

Anthropogenic metal activities have led to soil contamination, posing a severe risk to each trophic level. Therefore, ecosustainable remediation techniques and/or strategy which is the focus of this study must be continuously explored, since metal processing and accidental leakages and depositions into the environment are both inevitable in the global drive for industrialization. Different concentrations at 80–10 mg/kg of Lead (Pb), were used to contaminate a characterized (Mollisol) soil with empty fruit bunch (EFB) and spent mushroom compost (SMC) as amendments with *Lantana camara* alongside controls. Metal reductions ranged 52.46 to 88.03% and 45.10 to 82.73% were recorded under EFB and SMC, respectively. Heavy metal resistant bacteria (HMRB) counts of 79.67×10^6 to 56.1×10^6 CFU/g soil and 63.33×10^6 to 39.0×10^6 CFU/g soil were recorded under EFB and SMC conditions, respectively, at "Freundlich model" absorption intensity (n) of 2.51 ($R^2 = 0.9840$) and 2.44 ($R^2 = 0.9012$) in same order as above. Results validation with free energy change, kinetics and diffusion studies revealed that *L. camara* is a potential indicator plant for phytoremediation.

1. Introduction

The presence of heavy metals in soils may adversely affect soil ecology, agricultural production/ product and water quality (Wang et al., 2001). Extreme anthropogenic activities such as metal mining, smelting and refining, atmospheric depositions, manufacturing and distribution of painting materials, are the major sources of metal enrichment in soils which eventually cause biogeochemical and ecosystem imbalance (Kotas and Stasicka, 2000; Bolan et al., 2003a,b; Galloway et al., 1982; Gray et al., 2003; Adriano, 2001; Gabriela et al., 2013). Studies have shown that certain heavy metals damage human DNA and induce cancer and fertility degenerations (Selena et al., 2008; Dabonne et al., 2010; Peng et al., 2007).

In order to mitigate such problems, efforts had been made to develop remediation techniques for metal polluted soils (Nishanta et al., 2006; Ensley, 2000). Despite the assumed suitable technologies already in practice, metal contamination has continued to pose consistent ecological problems (Abramovitch et al., 2003), hence the continuous search for environmentally, dynamic and sustainable remediation strategy remains inconclusive. The use of plants to remove toxic contaminants like metals or hydrocarbon from soils is known as phytoremediation (Agamuthu et al., 2010). Therefore, according to Cobbett (2003), there are about 420 species belonging to about 45 plants families recorded as hyperaccumulators of heavy metals. However, only few species have been tested in the laboratory to confirm their metal accumulation behaviours despite large field collection of hyperaccumulators (Krämer, 2003). Also, intrigues exist in plants pattern of metal accumulation (Ernst, 2000; Galeas et al., 2006; Klein et al., 2008). For example, two-thirds of the 320 species of Ni hyperaccumulators so far discovered are found exclusively in the tropical regions (Proctor, 2003) and the prevalence of greater weathering of naturally occurring ore-bodies in the tropical regions could be responsible for imposing stronger selective pressures on local floras. Plants species are divided into two main groups with respect to metal adaptations in soil; the pseudometallophytes that grow on both contaminated and non-contaminated soils, and the absolute metallophytes that grow only on metal-contaminated and naturally metal rich soils (Bothe, 2011). Whilst *Thlaspi caerulescens* is the most studied plant species with regards to phytoextraction (Wang et al., 2006), *Cardaminopsis halleri* and *Armeria maritima* are absolute metallophytes and *Agrostis tenuis* a pseudometallophytes have also been studied for their metal-tolerance strategies. The main purpose of this research was to assess the phytoremediation capabilities of *Lantana camara* as an emerging metallophytes for remediation of Pb contaminated soil, while adopting kinetics to test its relevance.

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Fertigation of *Brassica rapa* L. using treated landfill leachate as a nutrient recycling option

Optimising nutrient availability and minimising plant metal contamination are vital in sustainable agriculture. This paper reports experiments in which treated leachate was used at different concentrations with predetermined N content for fertigation of *Brassica rapa* L. (leafy vegetable). An inorganic fertiliser, with N content equivalent to the leachate amount, was also prepared, as well as a control. Growth (leaf length, leaf width and stem height), harvest parameters (total number of leaves, root length and root dry weight) and specific growth rates (mm/day) were determined for three consecutive seasons. The dry weights of leaves, roots and stems in the leachate treatments were within the ranges of 1.95–3.60 g, 1.18–3.60 g and 0.33–1.37 g, with biomasses of 1.75 g, 1.14 g and 0.2 g, respectively, which were higher than those of the control. *B. rapa* L. fertigated with 25% diluted treated leachate recorded high specific growth rate and a leaf length of 0.53 mm/day and 0.23.17±0.58 cm, respectively (%N=0.023; $p<0.05$). The maximum permissible mineral concentration set by the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) was compared with that of the grown plants. Treated leachate can increase plant nutrient content.

Introduction

Fertigation is the technique of supplying dissolved fertiliser to crops through irrigation systems. This approach can improve application efficiency and reduce the need to export fertiliser off-field.[1,2] Current nutrient management practices using chemical fertilisers exhibit adverse environmental effects. Continuous production of crops under intensified agriculture over years, has resulted in large-scale removal of nutrients from soil, thereby causing a negative nutrient balance and declining soil fertility.[3] Hence, an adequate proportion of nutrient supply, especially in the form of bio-fertilisers, to soil and/or crops is essential for enhancing global food security.[4] Long-term irrigation with wastewater may result in the accumulation of heavy metals in soils and plants, consequently decreasing food safety and increasing health risk, which are environmental concerns.[5,6] Health risks caused by heavy metal contamination of soil have been widely reported.[7–12] Crops and vegetables grown in soils contaminated with heavy metals may have a larger accumulation of metals than those grown in uncontaminated soils. Risk assessment of heavy metal accumulation in wastewater irrigated leafy vegetables, such as palak (*Beta vulgaris* L.), amaranthus (*Amaranthus caudatus* L.), and cabbage (*Brassica oleracea* L.) was reported by Anita et al.[6] Intake of metal-contaminated vegetables is a pathway for heavy metal toxicity to humans.[13,14] Intawongse and Dean[15] determined the bioavailability of Cd, Cu, Zn and Mn in the human gastrointestinal tract from the edible part of the vegetables by using an in-vitro gastrointestinal extraction technique. They found that the edible portions of 'lettuce and radish' are more responsible than other vegetables for heavy metal accumulation in humans.

Malaysia has approximately 260 landfills, of which 10% are engineered (safe collection and leachate treatment facilities) and 90% are un-engineered.[16] Agamuthu[16] indicated that the volume of leachate generated is about 3 million litres per day. Leachate is produced by precipitation and percolation of 'biodegradable and non-biodegradable' wastes deposited in landfills. Landfill leachate mainly consists of large amounts of organic matter, including dissolved organic matter, phenols, ammoniacal nitrogen, phosphates, heavy metals, sulfides, solids, inorganic salts, and other toxicants, and is characterised by hardness, acidity, alkalinity, and salinity.[16,17] The complex pollution and toxicity impacts of leachates have imposed the need to standardise pre-treatments prior to discharge for quality improvement.[18] Treated leachates contain nutrients and minerals that can possibly be transformed for fertigation. Scholars have reported N transport and distribution characteristics in soils for basin irrigation using fertigation and conventional fertilisation.[19-21,5] Cheng and Chu[22] reported the positive and detrimental effects of landfill leachate on plant growth, depending on plant species leachate concentration. Menser et al.[23] explained that irrigation with leachate can lead to yield reduction, leaf damage, premature senescence and poor plant survival. By contrast, Liang et al.[24] suggested that the use of landfill leachate as irrigation water in dry seasons can enhance the growth, survival, and stomatal conductance of *Acacia confusa*, *Leucaena leuocephala* and *Eucalyptus torelliana*. Cureton et al.[25] reported significantly high growth rates of *Phalaris arundinacea*, *Salix babylonica* and *Populus nigra* subjected to leachate application; however, phytotoxicity symptoms, such as brown leaves and necrotic spots, were observed in poplar leaves. Mapanda et al.[26] reported that more than 100 ha of land under horticultural production in Harare utilises wastewater for irrigation of crops such as maize (*Zea mays*) and leafy vegetables (*Brassica* spp.). Growth rate and biomass production are common indicators of imposed stress. Although leaf length, leaf width and shoot length exhibit fluctuating asymmetry, non-directional deviation from anticipated symmetry is proposed as an environmental stress indicator.[27,28] Nominal consumers consider undamaged, dark green and large leaves as characteristics of good-quality leafy vegetables; however, the external morphology of vegetables cannot guarantee safety from contamination. In particular, heavy metals rank the highest among the chief contaminants of leafy vegetables.[26,29,30]

Leafy vegetables grow in places with adequate water supply and well-drained, fertile and preferably alkaline soils. These plants prefer a pH range of 5.5–8.2 although they can tolerate a pH within the range of 4.3–7.5.[31] Leafy vegetables prefer cool, moist and reasonably fertile soils. Shallow rooted plants are intolerant of drought and thus need to be grown in moist, fertile soil to produce high-quality leaves.[32] *Brassica* and mustards need adequate nitrogen and sulfur[33] and an N:S ratio ranging from 4:1 to 8:1 is appropriate for the *Brassica* species. Treated landfill leachate can be utilised as a source of water or plant nutrients, and as a soil conditioner for crop production.

Lantana camara- A Predictive Ecological Bioindicator Plant for Decontamination of Pb Impaired Soil under Supplemented Scenario

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ABSTRACT

Heavy-metal extraction and processing from ores release elements into the environment. Soil, being "unfortunate" sink, its bionomics are impaired and affected by metal pollution. Metals sneak into the food chain and pose risk to humans and other edaphic-dependent organisms. For decontamination, the use of ecosystem-friendly approach involving plants is known as phytoremediation. In this study, different Pb concentrations (80, 40, 20, and 10 mg/kg) were used to contaminate a characterized soil, supplemented with empty fruit bunch (EFB) or spent mushroom compost (SMC), with non-edible plant – *Lantana camara*. Pb removal ranged from 45.51% to 88.03% for supplemented soil and 23.7% to 57.8% for unsupplemented soil ($p < 0.05$). EFB-supplemented and *L. camara* remediated soil showed the highest counts of heavy-metal-resistant bacteria "HMRB" (79.67×10^6 CFU/g soil to 56.0×10^6 CFU/g soil), followed by SMC (63.33×10^6 CFU/g soil to 39.0×10^6 CFU/g soil). Aerial metal uptake was 32.08 ± 0.8 to 5.03 ± 0.08 mg/kg dry wt., and bioaccumulation factor was 0.401 to 0.643 ($p < 0.05$). Half-life's ($t_{1/2}$) of 7.24–2.26 days (supplemented), 18.39–11.83 days (unsupplemented), and 123.75–38.72 days (soil controls) were respectively recorded. Freundlich isotherms showed that the intensity (***n***) of metal absorption ranged from 2.44 to 2.51 for supplemented soil with regression coefficients (R^2) between 0.9012 and 0.9840. The computed free-energy change (ΔG) for Pb absorption ranged from −5.01 kJ mol^{-1} K^{-1} to 0.49 kJ mol^{-1} K^{-1} for EFB and −3.93 kJ mol^{-1} K^{-1} to 0.49 kJ mol^{-1} K^{-1} for SMC.