UNIVERSITI PUTRA MALAYSIA

LEAD TOXICITY AND PHYTOEXTRACTION POTENTIAL OF *ACACIA AURICULIFORMIS* A. CUNN. EX BENTH. TO REMEDIATE LEAD-POLLUTED SOIL

ZERKOUT ABDERRAHMANE

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By

ZERKOUT ABDERRAHMANE

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the
Degree of Doctor of Philosophy

September 2020

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Muskhazli Mustafa, PhD
Faculty : Science

Phytoremediation takes a longer time to accomplish than other treatment. Phytoextraction with *Acacia auriculiformis*, also known as Auri, Earleaf acacia, Earpod wattle, Northern black wattle, Papuan wattle and Tan wattle, has not been reported previously even though it is a fast-growing species and able to grow well in very poor soil. This study was conducted to determine the impact of lead (Pb) on *A. auriculiformis* seed germination, the influence of Pb on *A. auriculiformis* growth, the capability of *A. auriculiformis* to remove Pb from the soil and the effect of NPK fertilizer on the speed of growth as well as the absorption of Pb from the soil. Seeds were exposed to Pb by soaked it in different Pb concentrations from 0 to 4 g/L (interval of 0.5 g/L). The evaluation of Pb impact on *A. auriculiformis* seed germination and early radicle development was conducted based on germination percentage (GP), germination index (GI), mean germination time (MGT), seedling vigour index (SVI), relative injury rate (RIR) and histological observation on seed embryo development. Biochemical response of *A. auriculiformis* seeds towards Pb stress was assessed by determining the phenolic compounds, malondialdehyde and starch content. Physical growth parameters such as shoot height, leaves number, basal diameter and root length were measured. Several physiological parameters including net photosynthesis, total chlorophylls, internal CO_2 concentration, transpiration rate, the relative water content (RWC) and water use efficiency (WUE) were also measured. The enzymatic respond to Pb toxicity was assessed by measuring the catalase activity. While the concentration of Pb concentration in the soil and plant part was determined using Inductively coupled plasma atomic emission spectroscopy (ICP AES). The bioconcentration factor (BCF) and the translocation factor (TF) were calculated to assess *A. auriculiformis* phytoremediation potential. The effect of the NPK fertilizer with a ratio of 1:1:1on *A. auriculiformis* growth was assesed by measuring the same morphological and physiological parameters as ststed previously. All the collected data were analysed using one way analysis of variance (ANOVA). via IBM SPSS Statistic Version 25 software. The triplicates data were presented as mean ± standard deviation based on the Tukey's HSD test. Study reveals that Pb toxicity has does not affect *A. auriculiformis* seeds germination up to 1.5 g/L. Increasing the concentration of Pb up to 3.5 g/L led to a reduction in GP and GI while an

increment in the MGT caused the germination to be delayed by almost 10 days. The SVI and RIR results indicated that Pb has also affected seeds strength and ability to repair the damage. High starch accumulation in *A. auriculiformis* seeds caused a reduction in nutrient availability which adversely reduces the embryo development. The assessment of growth parameters also showed that Pb did not affect *A. auriculiformis* grown in 1 g/kg Pb-treated soil. The plant grown in 1 g/kg Pb-treated soil showed a 12% decrement in WUE and RWC, but no significant difference was recorded between 1 g/kg, 2 g/kg and 3 g/kg Pb-treated soil. This is coherence with the proline and catalase responses. Catalase findings showed that Pb caused a significant overproduction of the CAT enzyme in *A. auriculiformis*. Thus, proving that *A. auriculiformis* has a good antioxidant defence system to overcome the Pb stress. Analysis of BCF and TF values showed that values for *A. auriculiformis* grown in 1 g/kg Pb-treated soil were 3.5 and 1.28, respectively. The BCF and TF results indicated that *A. auriculiformis* could be used as a phytoextractor at Pb concentration of 1 g/kg and as phytostabilizer at 2 g/kg. The NPK fertilizer application enhanced *A. auriculiformis* morphological parameters by 55% and an increment was observed in net photosynthesis, total chlorophylls, the transpiration rate, the internal CO_2 concentration and RWC. Proline accumulation was significantly reduced ranging from 15% to 50% depend on the Pb concentration in the soil. However, no significant difference between the CAT enzyme production in the presence and the absence of the NPK fertilizer. Data on BCF and the TF for *A. auriculiformis* treated with the NPK fertilizer showed lower value compared to without NPK. Thus, indicated that in the presence of NPK, *A. auriculiformis* is unable to absorb and translocate a significant amount of Pb from the soil to the different parts of the plant. Based on the all data and tolerance index, *A. auriculiformis* has the potential to be used as a phytoremediation agent for Pb. Combination of its ability to grow in unfertile soil and resistance to Pb up to 2 g/kg does give it some advantage as phytoremediation agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KETOKSIKAN PLUMBUM DAN POTENSI FITOEKSTRAK OLEH *ACACIA AURICULIFORMIS* A. CUNN. EX BENTH UNTUK MEMULIHKAN TANAH YANG DICEMARI PLUMBUM

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Fitopemulihan mengambil masa yang lebih lama berbandaing dengan rawatan lain. Fitopemulihan dengan menggunakan Acacia auriculiformis yang juga dikenali sebagai auri, 'Earleaf acacia', 'Earpod wattle', 'Northern black wattle', 'Papuan wattle' dan 'Tan wattle' tidak pernah dilaporkan walaupun ia adalah spesis yang tumbuh dengan pantas dan berupaya tumbuh dengan baik pada tanah yang tidak subur. Kajian ini dijalankan untuk menentukan kesan Pb terhadap percambahan benih *A. auriculiformis*, pengaruh plumbum (Pb) terhadap percambahan biji benih *A. auriculiformis*, pengaruh Pb pada pertumbuhan *A. auriculiformis*, keupayaan *A. auriculiformis* untuk untuk mengeluarkan Pb dari tanah dan kesan baja NPK terhadap kadar tumbesaran serta penyerapan Pb dari tanah. Biji benih didedahkan kepada Pb dengan merendamnya di dalam kepekatan Pb berbeza dari 1 sehingga 4 g/L (selang 0.5 g/L). Penilaian kesan Pb terhadap percambahan biji benih *A. auriculiformis* dan perkembangan awal radikal telah dilaksanakan berdasarkan kepada peratus percambahan (GP), indeks percambahan (GI), purata masa percambahan (MGT), index kekuatan benih (SVI), kadar kecederaan relative (RIR) dan pemerhatian histologi perkembangan embrio biji benih. Tindak balas biokimia biji benih *A. auriculiformis* terhadap tekanan Pb dinilai menerusi penentuan kandungan sebatian prolin, malondaildehid dan kanji. Parameter tumbesaran fizikal seperti tinggi pucuk, bilangan daun, diameter pangkal dan panjang akar telah diukur. Beberapa parameter fisiologi termasuk fotosintesis bersih, jumlah klorofil, kepekatan CO_2 dalaman, kadar transpirasi, kandungan relative air (RWC) dan kecekapan penggunaan air (WUE) juga disukat. Tindak balas enzim terhadap ketoksikan Pb dinilai dengan mengukur aktiviti katalase. Sementara itu, pengukuran kepekatan Pb di dalam tanah dan bahagian tumbuhan ditentukan menggunakan 'Inductively coupled plasma atomic emission spectroscopy' (ICP AES). Faktor biokepekatan (BCF) dan faktor translokasi (TF) telah dikira untuk menilai potensi fitopemulihan *A. auriculiformis*. Kesan baja NPK dengan kadar 1:1:1 keatas pertumbuhan *A. auriculiformis* telah dinilai dengan mengukur parameter morfologi dan fisiologi yang sama seperti yang dinyatakan sebelum ini. Semua data yang dikumpul telah dianalisis mengggunakan analisis varians satu hala (ANOVA) menggunakan perisian IBM SPSS Statistic Version 25. Data tiga replikat ditunjukan sebagai purata ± piawai sisihan berdasrakan kepada Tukey's HSD test. Kajian

mendedahkan bahawa ketoksikan Pb tidak memberikan kesan keatas percambahan biji benih *A. auriculiformis* sehingga 1.5 g/L. Peningkatan kepekatan Pb sehingga 3.5 g/L menyebabkan penurunan dalam GP dan GI manakala peningkatan MGT menyebabkan penagguhan percambahan sehingga hampir 10 hari. Keputusan SVI dan RIR menunjukan bahawa Pb juga memberi kesan terhadap kekuatan dan keupayaan biji benih membaik pulih kerosakan. Pengumpulan kanji yang tinggi di dalam biji benih *A. auriculiformis* menyebabkan penurunan nutrien tersedia yang mengurangkan perkembangan embrio. Penilaian parameter pertumbuhan juga telah menunjukkan bahawa Pb tidak memberikan kesan terhadap *A. auriculiformis* yang tumbuh di dalam tanah yang rawat dengan 1 g/kg Pb. Pokok yang tumbuh di dalam tanah yang rawat dengan 1 g/kg Pb menunjukkan 12 penurunan dalam WUE dan RWC, tetapi tiada perbezaan bererti dicatatkan diantara tanah yang rawat dengan 1 g/kg, 2g/kg dan 3 g/kg Pb. Ini selaras dengan tindak balas prolin dan katalase. Dapatan katalase menunjukkan bahawa Pb telah menyebabkan penghasilan yang melampau enzim CAT di dalam *A. auriculiformis*. Oleh itu, ianya membuktikan bahawa *A. auriculiformis* mempunyai sistem pertahanan antioksida yang baik untuk mengatasi tekanan Pb. Analisis terhadap nilai BCF dan TF menunjukkan bahawa nilai bagi *A. auriculiformis* yang ditanam pada tanah yang rawat dengan 1 g/kg Pb ialah masing-masing pada 3.5 dan 1.28. Keputusan BCF dan TF menunjukkan bahawa berupaya *A. auriculiformis* boleh digunakan sebagai fitopengekstrakan pada kepekatan Pb 1 g/kg dan sebagai fitopenstabilan pada 2 g/kg. Penggunaan baja NPK meningkatkan parameter morfologi *A. auriculiformis* sebanyak 55% dan peningkatan dapat dilihat dalam fotosintesis bersih, jumlah klorofil, kadar transpirasi, kepekatan CO_2 dalaman dan RWC. Pengumpulan prolin dikurangkan dengan berkesan dari julat 15% sehingga 50%, bergantung kepada kepekatan Pb di dalam tanah. Walau bagaianapun, tiada perbezaan bererti diantara penghasilan enzim CAT semasa kehadiran dan tanpa baja NPK. Data BCF dan TF bagi *A. auriculiformis* yang dirawat dengan baja NPK menunjukkan nilai yang lebih rendah berbanding dengan tanpa NPK. Oleh itu, ianya menunjukkan bahawa dengan kehadiran baja NPK, *A. auriculiformis* tidak berupaya menyerap dan menindahkan secara berkesan sejumlah Pb dari tanah ke bahagian berlainan pada pokok. Berdasarkan kepada semua data dan index toleransi, *A. auriculiformis* mempunyai potensi untuk digunakan sebagai agent fitopemulihan bagi Pb. Gabungan kebolehannya untuk tumbuh pada tanah yang tidak subur dan tahan terhadap Pb sehingga 2 g/kg telah memberikan beberapa kelebihan sebagai agen fitopemulihan.

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to **Allah**, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I am extremely grateful to my parents for their love, prayers, caring and sacrifices for educating and preparing me for my future, may **Allah** reward you with Jannatul Firdaus. Special thanks go to my one and only brother Ammar for his support, valuable and courageous advice. I am also very much thankful to my wife Narimen for her love, understanding, prayers and continuing support to complete this research work. I thank her for the moral and emotional support, may **Allah** bless you and keep you always by my side.

I would like to express my deep and sincere gratitude to the chairman of my supervisory committee Associate Professor Dr. Muskhazli Mustafa for giving me the opportunity to do research and providing invaluable guidance throughout this research. He has taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under his guidance. I am extremely grateful for what he has offered me. I would like to thank my supervisor committee Dr. Mohd Hafiz bin Ibrahim and Associate Professor Dr. Hishamuddin bin Omar for helping with this research project.

I would like to say thanks to my friends and research colleagues especially Anna for her dedicated support and for continuously provided encouragement. Finally, many thanks to all participants that took part in the study and enabled this research to be possible directly or indirectly.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 11 March 2021

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ANOVA	Analysis of variance
AAS	Atomic Absorption Spectrophotometer
ASC	Ascorbic acid
BCF	Bioconcentration Factor
Ca	Calcium
CAT	Catalases
Cd	Cadmium
CDF	Cation Diffusion Facilitators
Cu	Copper
CNGC	Cyclic nucleotide gated channel
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DW	Dry weight
EDL	Electron Discharge Lamp
FAA	Formalin-Acetic Acid- Ethanol
FCR	Folin Ciocalteu Reagent
FW	Fresh Weight
g	Gram
GP	Germination Percentage
GI	Germination index
GSH	Glutathione
HMW	High Molecular Weight
H_2O_2	Hydrogen peroxide

h	Hour
ICP-OES	Inductively coupled plasma optical emission spectrophotometer
K	Potassium
kg	Kilogram
LMW	Low Molecular Weight
L	Litre
MTE	Metal Trace Element
mg	Milligram
min	Minute
MGT	Mean Germination Time
MDA	Malondialdehyde
MCW	Methanol:Chloroform:Water
Mg	Magnesium
NSC	Non-Structural Carbohydrate
N	Nitrogen
Ni	Nickel
NRAMP	Natural Resistance-Associated Macrophage Protein
$O_2^{\cdot-}$	Superoxide anion
OH	Hydroxyl radical
OH^-	Hydroxyl anion
Pb	Lead
PCR	Photosynthetic carbon reduction
PCs	Phytochelatins
POD	Peroxidases
Pi	Orthophosphate

P	Phosphorus
ROS	Reactive Oxygen Species
RIR	Relative Pb Injury Rate
RNA	Ribonucleic acid
RO·	Alkoxyl radical
RWC	Relative water content
SOD	Superoxide-dismutase
SVI	Seedling Vigor Index
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TF	Translocation factor
TPC	Total phenolic contents
TI	Tolerance index
TW	Turgescence weight
WUE	Water use efficiency
Zn	Zinc
ZIP	Zinc-regulated transporter, Iron-regulated transporter, Protein

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Heavy metal pollution has developed as a horrible ecological crisis. It is harmful not only to human beings but also to all other living organisms (Raymond *et al.*, 2011). Rapid industrialization, urbanization and human activities have caused serious environmental hazard in the biosphere (Nagajyoti *et al.*, 2008). Heavy metals are accumulated in soils through precipitation, runoff, spillage, dumping of waste or by-product from industrial areas, mining activities, disposal of industrial, agriculture and domestic wastes (Ali *et al.*, 2013a). Heavy metals do not remain in the soil indefinitely but transferred to the biotic food chain through the primary producer, plant (Sherene, 2010); which threaten their survival, affecting their growth and metabolism (Maryam *et al*., 2014; Gangaiah *et al.*, 2013)

Heavy metals are classified into two categories: essential heavy metals and non-essential heavy metals (Raymond *et al.*, 2011). The essential heavy metals are required in plant growth such as Zn, Cu and Fe. The non-essential heavy metals such as Pb, Cd and Ni in high concentration can interfere with other nutrient uptake, physiological functions genetic integrity and others (Mihiri *et al.*, 2016). Furthermore, heavy metal pollution may influence the formation of the embryo sac, the vacuolation of embryonic cells, and degeneration of embryo in the late stage of heart-shaped embryo (Mohsenzadeh *et al.*, 2011).

There are many studies on the effect of heavy metals on plants such as germination study, plant development, the effect on DNA, gene expression, respiration, secondary metabolite, heavy metal distribution and accumulation levels (Anişoara *et al.*, 2010; Dane *et al.*, 2010; Chandra & Kang, 2016; Buscaroli, 2017; Rehman *et al.*, 2018). Different plant species responded differently to different heavy metals (Hu *et al.,* 2012). Some plants can accumulate heavy metals to a varying degree without showing physical defect (Amin *et al.*, 2018) and these attributes make plant a very interesting research subject particularly on heavy metals and its primary role in spreading heavy metal in the food chain.

1.2 Problem statement

Among the non-essential heavy metal, lead (Pb) is the most frequently encountered persistent heavy metal in polluted environment (Pourrut *et al.*, 2011a), and it was classed as the second most harmful substance after Arsenic (Patra *et al.*, 2004). Exposure to Pb was identified as a serious public health problem, particularly for children (Nawab *et al.*, 2015). The exploitation of various types of deposits in the ore leads to the accumulation of Pb on the surface (Lutts & Lefevr, 2015), and due to its non-biodegradability, toxicity, and accumulation, it can enter the human body through intake of food (65%), water (20%) and air (15%), thereby causing hazardous damage to human health, such as kidney damage, disruption of nervous systems, brain damage, high blood pressure, and disruption of the biosynthesis of hemoglobin causing anemia (Zhang *et* al., 2015; Lei *et al.,* 2016).

The main sources of Pb contamination are anthropogenic releases (Salazar & Pignata, 2014). It comes mostly from paints, cable covering, fertilisers and pesticides, and metal production (Arruti *et al.,* 2010). In soil, paint is the major contributor to Pb contamination; it held tightly on the surfaces of very fine clay and organic matter particles (Srinivasan *et al.,* 2014). In Malaysia, Pb was found to be the second highest metal in contaminated soil after Cu in Perlis state, where the pollution index of Cu and Pb noted the highest by 11 and 6% respectively (Siti *et al.*, 2014).

The issue of Pb in contaminated agricultural soil has been a question of great interest in a wide range of fields (Abbasi *et al.*, 2017). Therefore, several remediation techniques have emphasized on the removal of Pb from soil including soil replacement method, thermal desorption, chemical leaching, chemical fixation, electro-kinetic remediation, and vitrify technology (Yao *et al.,* 2012), but none of them seems to performed satisfactorily. The major issues with these techniques are the increase risk of spreading contaminated soil or dust particles during removal, transport of contaminated soil, and the relatively high cost (Bang *et al.*, 2015).

In recent years, it has been reported that plants can help to reduce or remove heavy metals from the soil by their roots, with the aim of restoring area sites to a condition useable for private or public applications (Couselo *et al.,* 2010; Masvodza *et al.,* 2013; Ochonogor & Atagana, 2014). Phytoremediation has become the way out to extract heavy metal properly without affecting the environment and potentially cost-effective (Illié *et al.*, 2015) especially the phytoextraction technique. Phytoextraction is an important aspect of phytoremediation; it consists especially of the extraction of heavy metals from soil and stored them in roots, shoots and leaves (Souza *et al.,* 2013). Disadvantages for using this technique is that it takes longer time than other treatments, due to plant limitation, where in most cases high contaminant concentration can reduce the speed of plant growth (Laghlimi *et al.*, 2015; Ali *et al.*, 2013b; Moosavi & Mohamd, 2013). However, these drawbacks can be reduced or eliminated through intensive research on identification of fast growing and high heavy metals tolerance plants. The choice of phytoremediation candidates is important. Some plants can be short-lived, too small to be significant as pool to contain heavy metals or serves as food for herbivores. Ideal phytoremediation agents should be a hardy plant, long-lived, fast growing, big and inedible.

1.3 Significance of the study

Acacia plants fit the above description perfectly, they are adapted to a wide range of environments, both tropical and temperate, and this adaptability has made them popular for planting on degraded lands in Asia and elsewhere (Turnbull *et al.,* 1997). Acacias have the potential to rehabilitate the soil through absorption and storage of heavy metals in its leaves, shoots and roots which make them the best phytoremediation candidates (Yang *et al.,* 2005). The phytoremediation potential of acacias has been previously reported in numerous studies. Majid *et al.,* (2012) showed that *A. mangium* can absorb up to 36.25 ppm of Pb with total plant biomass of 115.39 g in 3 months. Another study by Mohd *et al.,* (2013) showed that the aboveground biomass of a single *A. mangium* tree is projected to accumulate 5.74 g of Zn, 1.15 g of Cu and 0.06 g of Cd in 12 months. It is clearly observed that heavy metals toxicity affects the biomass yield and the speed of growth of *A. mangium* thus reduce its absorption potential and phytoremediation efficiency. Therefore, it is necessary to find other *Acacia* plants that can resist higher concentrations and absorb more toxic metals in their parts.

Acacia auriculiformis could be a perfect candidate in this matter. It has a high biomass yield and adapts well to degraded soil conditions, poor soil quality, even in saline and seasonally waterlogged soils and dry season (Lokman *et al.,* 2009). It is a fast-growing multipurpose tree species in the Leguminosae family that can reach 30 m of height and 30cm of diameter (Gilman & Watson 2013). Its economic impact is predominantly positive, and it is one of the most favoured trees in degraded sites (Ishiguri *et al.*, 2004). However, phytoremediation using *Acacia auriculiformis* species on Pb contaminated soils has never been reported. Based on the attributes mentioned, theoretically, *A. auriculiformis* should be able to tolerate, absorb and accumulate a large amount of Pb from the soil providing a perfect remediation technique to clean up Pb from the soil. If this is the case, the timber from this tree can be used as a building material for the house which can last for 50 years or more (Chakraborty *et al.,* 2019). *Acacia auriculiformis* is an exotic species mostly planted as roadside avenue tree by the side of highways, railway tracks and parks. It is only recently its invasive potential has been noted (Olatunja *et al.,* 2012).

1.4 Objective of the study

Based on the positive attributes of *A. auriculiformis*, the main aim of the study is to assess the potential of using *A. auriculiformis* that grows fast as a phytoremediator agent to absorbs a considerable amount of Pb from the soil. The outline of the research approach is shown in Figure 1.1 and the design of the study was based on the following specific objectives;

i. To determine the impact of Pb on *A. auriculiformis* seed germination pattern and early seedling development.
ii. To evaluate the ability of *A. auriculiformis* plant to grow on Pb contaminated soil.
iii. To determine the distribution of Pb in different parts of *A. auriculiformis* plant.
iv. To assess the effect of NPK fertiliser on *A. auriculiformis* growth and heavy metal absorption capability.

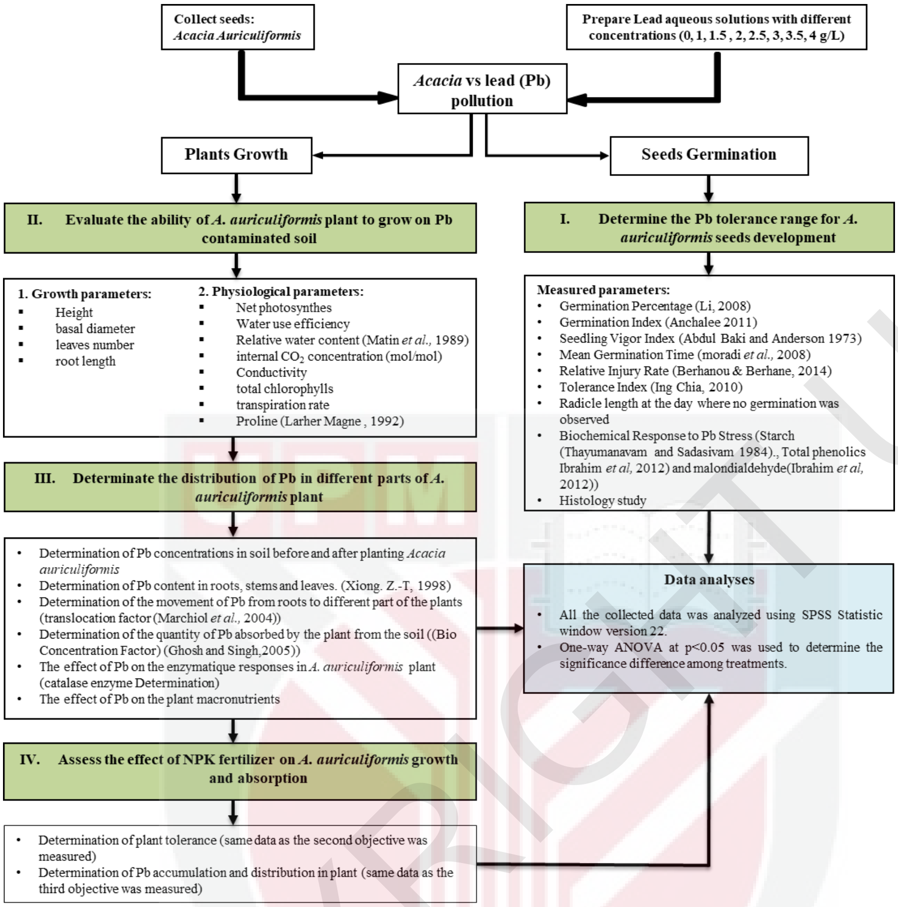

Figure 1.1: Summary of research design and attainment of objectives

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LIST OF PUBLICATIONS

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