PHYTOREMEDIATION OF ARSENIC IN MINE TAILINGS BY *Acacia mangium*

BY

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ABSTRACT

Old mining areas are always associated with metal contamination such as arsenic due to poor waste management and pose a significant threat to human health due to dispersion of toxic metal-containing particulates in air, soil, and water. Therefore, the goals of this project are to investigate the potential of *Acacia mangium* to remediate mine tailings through phytoremediation and explore its ability to survive on contaminated and low nutrient soil. Tailings characterization studies shows that the soil texture is sandy clay, with pH around 4.5 and the highest heavy metal content in the soil is arsenic with concentration 790 mg/kg. The HPLC-ICP-MS results found that there was only arsenate in the tailings and no arsenite was detected. A preliminary test using soil spiked with various arsenic concentrations showed that *Acacia mangium* was able to survive on arsenic-contaminated soil with concentrations up to 500 mg/kg arsenic. *Ex-situ* phytoremediation studies using mine tailings showed no toxicity effect on the *Acacia mangium* throughout the five months of treatment. Bioconcentration and translocation factor indicate that *Acacia mangium* utilize phytostabilization as its main mechanism to uptake arsenic into the plant's tissues. The usage of chemical enhancement, monoammonium phosphate ($NH_4H_2PO_4$) and citric acid ($C_6H_8O_7$), had improved the dry weight biomass (roots, stems and leaves) and arsenic uptake by the roots of *Acacia mangium*. Moreover, 50 mg/kg of phosphate concentration had increased the translocation of arsenic from the roots to the stems by 12-fold increase compared to the un-dosed plants. Further speciation analysis revealed that arsenic in the form of arsenate was the only arsenic species detected in the stems after being amended with monoammonium phosphate; thereby, suggesting a sensible strategy for more efficient targeted arsenic phytoremediation by *Acacia mangium*.

خلاصة البحث

بسبب انتشار الجسيمات السامة المحتوية على معادن في الهواء والتربة والماء، ترتبط مناطق التعدين القديمة دائمًا بالتلوث المعدني مثل الزرنيخ نتيجة لسوء إدارة النفايات وتشكل تهديدًا كبيرًا على صحة الإنسان والبيئة. نتيجة لذلك، تتمثل أهداف هذا المشروع في البحث في إمكانية استخدام أكاسيا مانجيوم *Acacia mangium* لمعالجة مخلفات المناجم والتحقيق في قدرتها على الانتشار في التربة الملوثة. قوام التربة عبارة عن طين رملي، مع درجة حموضة تبلغ حوالي 4.5، وأكبر محتوى من المعادن الثقيلة في التربة هو الزرنيخ، بتركيز 790 مجم / كجم، وفقًا لدراسات خصائص المخلفات. كشفت نتائج HPLC-ICP-MS أن المخلفات تحتوي فقط على الزرنيخ (ارسينات) وليس الزُرنيخيتِ (ارسينيد). تم العثور على أكاسيا مانجيوم لتكون قادرة على الانتشار في التربة الملوثة بالزرنيخ بتركيزات تصل إلى 500 ملغم / كغم في اختبار تجريبي باستخدام تربة مرتفعة بتركيزات متنوعة من الزرنيخ. كشفت اختبارات المعالجة النباتية خارج الموقع باستخدام مخلفات المناجم أن أكاسيا مانجيوم لم يتضرر خلال فترة العلاج التي استمرت خمسة أشهر. تمتص أكاسيا مانجيوم الزرنيخ في أنسجة النبات في الغالب من خلال التثبيت النباتي، وفقًا للتركيز الحيوي وعامل الانتقال. أدى استخدام فوسفات الأمونيوم الأحادي ($NH4H2PO4$) وحمض الستريك ($C6H8O7$) كتعزيزات كيميائية إلى تعزيز الكتلة الحيوية للوزن الجاف (الجذور والسيقان والأوراق) وامتصاص الزرنيخ بواسطة جذور أكاسيا مانجيوم .علاوة على ذلك، أدى تركيز 50 مجم / كجم من تركيز الفوسفات إلى زيادة انتقال الزرنيخ من الجذور إلى السيقان بمقدار 12 ضعفًا مقارنة بالنباتات غير المعالجة. كشف تحليل الانتواع الإضافي أن الزرنيخ في شكل الارسينات كان النوع الوحيد من الزرنيخ المكتشف في السيقان بعد تعديله باستخدام فوسفات الأمونيوم الأحادي، مما يعني ضمناً طريقة معقولة للمعالجة النباتية للزرنيخ المستهدف بشكل أكثر كفاءة بواسطة أكاسيا مانجيوم.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology).

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Shahbudin bin Saad
Dean, Kulliyyah of Sciences

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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SPECIAL DEDICATION,

To my parents and my wife
for their endless loves, supports, and encouragements.

May Allah protect you all always.

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LIST OF SYMBOLS

cm	-	centimetre
g	-	gram
kg	-	kilogram
mg/kg	-	Milligram per kilogram
%	-	percentage
°C	-	degree celcius

LIST OF ABBREVIATIONS

Anova	-	Analysis of variance
SEM	-	Standard error of mean
SD	-	Standard deviation
BF	-	Bioconcentration factor
TF	-	Translocation factor

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The process of mining and extraction of precious metals such as gold from primary ore generates massive quantity of mine wastes or tailings. Cyanidation technology which is a common technique used in the gold mining industry around the world to extract gold from ores caused variable quantities of cyanides, metal-cyano complexes, sulfide, and heavy metals such as arsenic in the liquid effluents. The permissible limit of arsenic in industrial soil is 30 mg/kg and only 5.2E-02 ug/L in the groundwater (Department of Environment, 2015). However, as the demand for gold increased over time, the enormous amounts of mine wastes generated led to elevated levels of arsenic released into the environment which is very harmful to the humans.

Mine wastes produced from the mining process are treated using several methods, such as flotation, bacterial oxidation, and treatment with reagent, and then placed at a storage facility called the tailings storage facility where it often remains non-rehabilitated for years. Phytoremediation is a plant-based remediation technology used to remove contaminants using plants and their associated microorganisms. This approach is found to be effective, sustainable, and economically efficient due to its high removal rate and ability to restore the soil (Behera, 2014; Muthusaravanan et al., 2018). Due to the low bioavailability of metals in soil, chemical enhancement using soil additives such as phosphate and citric acids has been used to boost the uptake of arsenic into plant roots (Agnello et al., 2014; Bolan et al., 2013; Yang et al., 2018).

Acacia mangium is a fast-growing plant species that can be found in many uninhabited areas including old mine wastes areas. Previous studies have shown the potential of Acacia to remediate soil with various contaminants, including heavy metals such as cadmium, chromium, and zinc, demonstrating the plant's ability to survive in soils with high heavy metal concentrations and low nutrient levels (Mahdavi et al., 2014; Majid et al., 2011, 2012). This is a very important trait because mine wastes often contain levels of metals that are phytotoxic to many species. Furthermore, the advantage of having high biomass production also allows Acacia to be widely used for bioenergy production (Gasol et al., 2010), thus adding an economical option for this species to be used post-phytoremediation.

1.2 PROBLEM STATEMENT

Tailing management at the storage facility of mining site had raised environmental concerns as it contains high concentrations of arsenic which is dangerous to the environments. Low pH of the tailings generates acid mine drainage which increase the solubility of arsenic and then leached out together into the surroundings area. This condition increases the arsenic concentration in the nearby soil, stream and groundwater which is very harmful to the humans being. Besides that, tailings also contain relatively low levels of organic matter or macronutrients, and consequentially may not support the growth and survival of many living organisms, including bacteria and these areas often remain un-vegetated. Therefore, the goals of this project are to investigate the potential of *Acacia mangium* to remediate the tailings through phytoremediation technique and to explore their ability to survive on the contaminated soil.

1.3 OBJECTIVES

1. To determine the potential of *Acacia mangium* in phytoremediation of arsenic from mine wastes.
2. To determine the effect of chemical enhancement on arsenic uptake by *Acacia mangium.*
3. To identify arsenic species accumulated in the tailings and plants.

1.4 HYPOTHESIS

1. It is hypothesized that *Acacia mangium* can survive when grown on mine wastes and is able to uptake and accumulate high concentration of arsenic.
2. Chemical enhancement will improve arsenic uptake by the *Acacia mangium.*
3. Arsenate is the most accumulated arsenic species in the stem of *Acacia mangium.*

CHAPTER TWO

LITERATURE REVIEW

2.1 GOLD MINING ACTIVITIES

Mining industry such as for gold, silver and copper are a huge sector in Malaysia and has strengthen the country's economy for many years. Gold is a valuable metal that has high economic value due to their rarity and application in various industries. Malaysia is among one of the important gold producers in Southeast Asia and have widespread gold mining industry throughout the country especially in the Central Belt of Peninsular Malaysia (Ariffin, 2012). Most of the major gold production in Malaysia comes within the Central Gold Belt (Figure 2.1). In 2015, it was estimated that Malaysia produced around 4.7 tonnes of gold (Figure 2.2) worth RM780.8 million with Pahang state being the major gold producer with almost 74 % production compared to other states such as Kelantan and Terengganu (Lim, 2017).

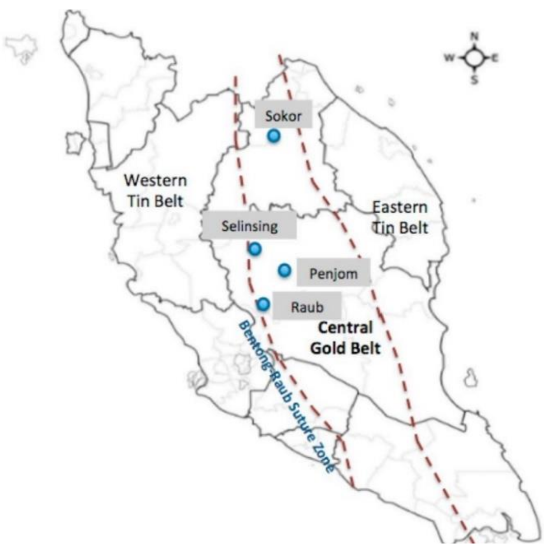

Figure 2.1: Map Showing Mineral Belt of Peninsular Malaysia Modified From Kusin et al., (2019)

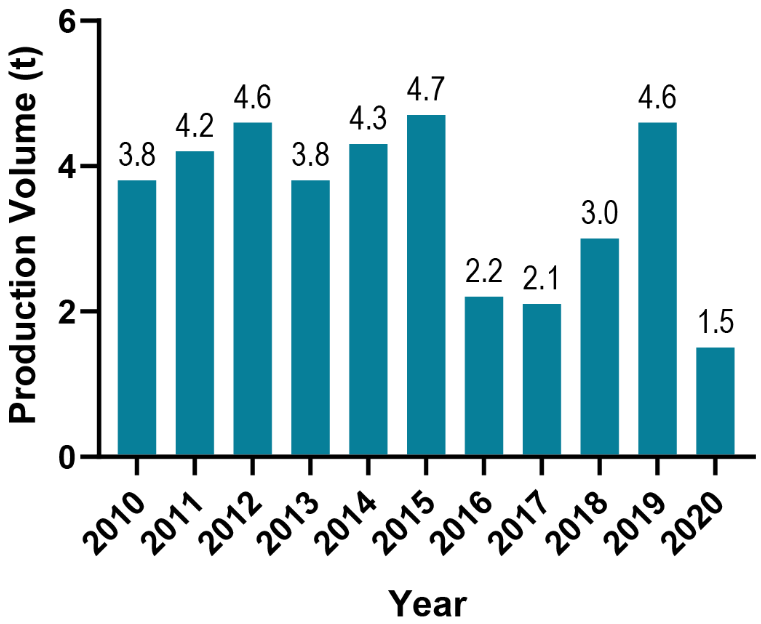

Figure 2.2: Annual Production Volume of Gold Mines in Malaysia from 2010 to 2020 (Joschka Müller, 2021)

Many procedures were involved during the mining activities such as drilling, blasting, crushing, and grinding to pulverize huge quantities of rock and recovered the valuable metals from the ore. The process of mining and extraction of these metals from primary ore consume a vast amount of energy and generate massive quantity of waste. The raw ore taken from the open pit mining site were crushed and transferred into a tank containing leaching solution to dissolves the gold from the ore by using leaching agent such as cyanide, thiosulphate, thiocyanate or halides (Altinkaya et al., 2020; Petersen, 2016). Next, the gold-containing solutions from the leach tank were moved into adsorption tank and a carbon adsorption method using either zinc powder, activated charcoal or ion-exchange resins was used to recover the gold from the solutions (Khosravi et al., 2017). Lastly, the recovered golds from the solutions were refined through few procedures such as desorption, electrowinning, acid wash and smelting in order to remove all impurities and finally obtained gold bar (Abdul-Wahab & Marikar, 2011).

The typical ratio of ore to concentrate is approximately around 30-50:1, which means that 96-98 % of the ore becomes tailings along with the waste material such as ground rock, process effluent, sand, clay and water (Glaister & Mudd, 2010). Waste materials produced from the mining process will be dumped at an established location called the tailings storage facility and will remain non-rehabilitated for years. In line with the requirement from the Department of Environment Malaysia, tailings will be treated using several methods such as flotation, bacterial oxidation and treatment with reagent and nutrients before it can be stored at the tailing storage facility. However, the discharge of huge amount of mining wastes has led to elevated levels of metals such as arsenic into the surrounding soil and water course which could potentially leads to ground water contamination.

2.1.1 Mine Tailings Contamination

There are many environmental concerns for both solid and liquid effluents stored at the tailing storage facility. Various size of particle fractions from the solid effluents such as coarse mine waste, fine clays, chemical precipitates, and slimes were discharged to the tailings which might disturb the stability of the tailing storage facility. The unsteadiness of the dam slope will cause tailings dam failure causing environmental incidents as the mine waste could be released to the surrounding. The database of International Commission of Large Dams (ICOLD, 2001) reported several major spills cases associated to tailings dams failures such as: muddy mass spill at Stava, Italy in 1985 (Luino & De Graff, 2012); sulphide tailings dam spill at Aznalcóllar, Spain in 1998 (Armstrong et al., 2019) and catastrophic alumina spillage at Ajka, Hungary in 2010 (Figure 2.3) (Klebercz et al., 2012).

Figure 2.3: The Crack Dam Corner causing the Alumina Spillage at Ajka, Hungary in 2010 (Turi et al., 2013)

Effluents from the mining or milling operations such as additives to the process, oils, organic and flotation chemical (leaching agents, solvent extractants, reagent, surfactants, oxidants) may also cause an environmental threat within tailings (Ritcey, 2005). Cyanidation technology which is used to extract gold efficiently from the ore will cause variable quantities of cyanides, metal-cyano complexes, sulphide and arsenic discarded together in the liquid effluents which could potentially be leaked out into the environment (Kuyucak & Akcil, 2013; Natarajan & Ting, 2015). Highly toxic sodium cyanide (NaCN) is used as primary reagent by the international mining community to concentrate gold and other precious metals since 1887 until today (Korte et al., 2000; Technology, 2016). Around 90% of gold extracted worldwide use cyanide leaching techniques as it is able to dissolve small flecks of gold and other precious metal even from low-grade ore (Mudder & Botz, 2004).

According to Eisler and Wiemeyer (2004), this cyanidation method involves dissolution of gold from the ore by using dilute cyanide solutions forming gold-cyanide complexes as shown in the following Elsner's reactions [1] and [2]. Gold content in the eluate solutions from the cyanidation process can recovered through reduction method by using zinc powder to produce gold precipitations as shown in reaction [3].

$$2Au + 4NaCN + O_2 + 2H_20 \longrightarrow 2NaAu(CN)_2 + 2NaOH + H_20_2 \quad [1]$$

$$2Au + 4NaCN + H_20_2 \longrightarrow 2NaAu(CN)_2 + 2NaOH \quad [2]$$

$$2NaAu(CN)_2 + Zn \longrightarrow Na_2Zn(CN)_4 + 2Au \quad [3]$$

The gold precipitated then is treated with dilute sulphuric acid to remove zinc residual and finally it was refined through electrowinning and smelting process to produce bars of gold bullion (Abdul-Wahab & Marikar, 2011).

The concentration of cyanide in the effluents had become the prime concern with the gold mill effluents. There are high risk contamination of surface and groundwater in the use, storage, and transport of sodium cyanide. According to the National Institute for Occupational Safety and Health (NIOSH), relatively low dosage exposure of cyanide which is around 4.7 ppm to 10 ppm can causes adverse effects to the humans and animals as it intervenes with oxygen utilisation (National Institute for Occupational Safety and Health, 2014; Technology, 2016). A tailing dam burst at gold mining area in Baia Mare, Romania in 2000 had released cyanide contaminated water into Somes River and killed enormous amount of fish in Hungary and Yugoslavia (Edraki et al., 2014; M. A. Rahman & Hasegawa, 2011a).

Another potential contamination from mine waste are substantial amount of sulphide presence in the tailings (Ritcey, 1989). The depositions of sulphide mineral ore found in the solid effluents can be oxidised to produce sulphuric acid which cause an acidic discharge (Edraki et al., 2014). Pyrite ore (FeS_2) which is a metal sulphide that easily oxidised when exposed to oxygen, water and microorganism, had become one of the main minerals that responsible for generating acid mine drainage (AMD) (Blodau, 2006; Pierre Louis et al., 2015; Plante et al., 2014). Once generated, it is difficult to control the process of AMD and high cost is required for the treatment (Aguiar et al., 2018; Baruah & Khare, 2010; Qureshi et al., 2016). AMD possess a severe pollution problem due to the low pH because it will dissolve high concentration of toxic metal in the tailings and then leach-out together to the environment (Hallberg, 2010; Ritcey, 2005). This condition will increase dissolved metal such as copper and metalloid (arsenic) in the receiving stream which could harming the stream biota and degrading the surrounding soils (Kefeni et al., 2017; MacIngova & Luptakova, 2012).

2.2 ARSENIC ELEMENTS

Arsenic (atomic number 33) is an odourless metalloid located throughout the Earth's crust and widely dispersed in the natural environments (Tangahu et al., 2011). This silver-grey brittle crystalline solid have atomic weight of 74.9, specific gravity 5.73, melting point 817°C (at 28 atm), boiling point 613°C, and vapor pressure 1 mm Hg at 372°C (Mohan & Pittman, 2007). In the earth's crust, arsenic normally can be found associated with sulphur forming sulphide minerals such as realgar (As_2S_2), orpiment (As_2S_3) and arsenopyrite (FeAsS) which are also a gold-bearing ore (Keshavarzi et al., 2012). Arsenic was naturally introduced into the environment through geological sources such as underground deposits or surface watering, erosion of rocks and mineral, volcanic activity and forest fires (Bowell et al., 2014; Vaclavikova et al., 2008; Zhu et al., 2014). However, mining activities and extraction of As-bearing ore deposits had increased the bioavailability of arsenic contaminant in the environment such as in the soil, water, plant and living organisms (Chakraborti et al., 2013; Craw & Bowell, 2014).