BIODIVERSITAS
Volume 20, Number 10, October 2019
Pages: 2949-2957

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d201025

The phytoremediation potential of non-edible oil-producing plants for gold mine tailings

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Manuscript received: 11 August 2019. Revision accepted: 16 September 2019.

Abstract. *Andriya NN, Hamim H, Sulistijorini, Triadiati. 2019. The phytoremediation potential of non-edible oil-producing plants for gold mine tailings. Biodiversitas 20: 2949-2957.* Plants can be used as phytoremediation agents to reduce environmental pollutants including heavy metal contaminants produced due to industrial activities. The objective of this study was to analyze the morphological, anatomical, and physiological responses of four non-edible oil-producing plants namely *Jatropha curcas*, *Ricinus communis*, *Reutealis trisperma*, and *Melia azedarach* and their ability to absorb and accumulate lead (Pb) when grown in different concentrations of gold mine tailings. The study was conducted using a completely randomized design involving two factors, four different species of plants and three different concentrations of tailings (0%, 50%, and 100%). Gold mine tailings caused a decrease in the growth of all species indicated by a significant reduction in plant height, leaf number, leaf area, shoot as well as root dry weight, while it significantly increased green RGB values of leaves. Pb accumulation was detected in the root as well as leaf tissues of the plant-based on histochemical analysis. The treatment with tailings also caused an increase in lipid peroxidation levels as indicated by increased malondialdehyde content in the roots and leaves. On the other hand, chlorophyll and carotenoid content decreased due to tailings treatment, along with the relative water content. Among the four species investigated, *R. trisperma* was found to be the most resistant species to gold mine tailings based on its ability to maintain growth even during gold tailing stress, which is also supported by principal component analysis.

Keywords: Gold mine tailings, malondialdehyde, non-edible oil species, phytoremediation, *Reutealis trisperma*

INTRODUCTION

Among the many major environmental problems is the increase in industrial area driven by economic activities. Since 1900, industries began to increase rapidly, which is followed by the increase of environmental pollution (Nriagu 1979). This is because various industries produced waste materials that were not degraded and became harmful to the environment. One of the sources of environmental contamination which needs to be handled is the waste of heavy metals from gold mining activities. The main activity of gold mining is the process of separation of gold ores from the rock which will produce an outcast of gold mine waste. Tailing is the main waste of gold mining activities and is known to contain heavy metals (Fashola et al. 2016). Tailings are generally solids containing fine sand and dust, and at the end, these materials will be deposited in open land and they may pollute the surrounding environment due to heavy metal content. The contaminating and toxic heavy metals produced by gold mining are lead, arsenic, and mercury which are the dominant contents of the tailings (Ogola et al. 2002). Therefore, these materials should be handled and managed properly to reduce negative impact on environment.

Phytoremediation is one of the potential alternative methods to remove contaminants from a land (Sarwar et al. 2017), sediment (Cho-Ruk et al. 2006), or water (Hanks et al. 2015) by utilizing plants that are tolerant to heavy metal contaminants. This method has been recognized as an efficient and effective method to reduce contaminants from many sites, including heavy metal contaminated areas (Edao 2017). The effectiveness of this method to reduce heavy metal in soil depends on the capacity of accumulator plants to absorb and accumulate certain heavy metals in their aerial parts (Yargholi 2008), while the plants are still able to grow well with higher biomass. Therefore, identifying plants with higher capacity to absorb heavy metals, which are known as accumulator plants, is very important.

There are several plants that can be used as phytoremediation agents of heavy metals, ranging from weeds and shrubs to trees. According to Capuana (2011), some types of trees are suitable for phytoremediation because they have rapid growth, produce high biomass, have deep rooting system, and are easy to harvest. *Jatropha curcas* (jatropha), for example, is a potential plant for phytoremediation of land or soil polluted with various heavy metals (Edao 2017). *Ricinus communis* (castor bean) has also been known to have the ability to stabilize residues and lower metal levels (Olivares et al. 2013), as well as to grow well in the presence of lead (Zhang et al. 2015). *Reutealis trisperma* (candlenut), a tree species, has good adaptability to grow in marginal lands such as post-tin mining soil, very dry land, and acidic soils (Pranowo et al. 2015), and was able to adapt to the gold mine's liquid waste (Hamim et al. 2017). *Melia azedarach* (melia) was effective to eliminate arsenic contamination (Sarwar et al. 2013). These four species produce non-edible oil which can be used as biodiesel feedstock, and therefore utilization of these species for phytoremediation will have double benefits, reducing contamination and producing non-edible oil.

With this background, the current study was aimed at analyzing morphological, anatomical and physiological responses of *J. curcas*, *R. communis*, *R. trisperma*, and *M. azedarach* in response to gold mine tailings, in general, and observing their ability to absorb and accumulate lead (Pb) from tailing media, in particular.

MATERIALS AND METHODS

The experiment was carried out from April to November 2018 in the Field Laboratory of Department of Biology, Bogor Agricultural University (IPB University). The anatomical and physiological responses were conducted in the Laboratory of Ecology and Plant Resources, and Laboratory of Plant Physiology, Genetics and Molecular Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University). The analysis of soil and tailing was carried out at Laboratory of Integrated Chemistry, Bogor Agricultural University (IPB University), Bogor, Indonesia.

Materials

Plant materials used in the experiments were 3-month-old seedlings of *J. curcas*, *R. communis*, *R. trisperma* and *M. azedarach* obtained from Research Institute for Industrial and Refreshment Crops, Ministry of Agriculture, Republic of Indonesia, Pakuwon, Sukabumi. Gold mine solid tailings were obtained from tailing dam of Indonesian gold-mine industry Aneka Tambang Inc. (PT. ANTAM) UPBE Pongkor, Bogor, Indonesia.

Procedures

Growing plants under different tailing treatments

Growth media was prepared using a mixture of soil and tailings with different concentrations (based on the treatment) into 6 kg capacity polybags, and then every polybag was fertilized using 500 grams of compost. One plant was grown in each polybag. The study was conducted using a completely random design with two factors and three replications. The first factor was 4 types of plants, namely *J. curcas* (JC), *R. communis* (RC), *R. trisperma* (RT), and *M. azedarach* (MA). The second factor was 3 tailing concentrations in the growth media, i.e. 0% (control), 50% and 100% of tailing. The growth of the plants was observed for 8 weeks. The samples of soil and tailings were analyzed to determine nutrient content and heavy metal component using atomic absorption spectrometry (AAS).

Plants growth parameters observed

Observations included different plant growth parameters, such as the shoot height, leaf number, leaf area, and green RGB (red green blue) measured using the ImageJ software from the scanned images using the HP 1050 scanner. Shoot and root dry weight of the plants were measured after 5 days of drying in the oven at 70ºC.

Histochemical analysis of Pb in the roots and leaf tissues

The accumulation of lead (Pb) in the root and leaf tissues was studied by histochemical analysis. The root and leaf tissue sections were prepared using microtome and were stained using dithizone reagent, before microscopic observation. The existence of Pb metal in the tissues was indicated by red color of the tissues (Seregin and Kozhevnikova 2011).

Lipid Peroxidation Analysis (MDA content)

The level of lipid peroxidation was analyzed by measuring the levels of malondialdehyde (MDA), based on the method proposed by Wang et al. (2013), with some modifications. 0.5 grams of leaves were ground in 10 mL of 5% trichloroacetic acid (TCA), in a mortar. The samples were then centrifuged at a speed of 3000 rpm for 25 min, at a temperature of 4°C. Two milliliters of supernatant was taken and mixed with 3 mL of 0.5% thiobarbituric acid (TBA) in 5% TCA which was then heated using a water bath for 30 min at 80°C. The sample was cooled and centrifuged at 3000 rpm for 25 minutes at a temperature of 4°C. The measurement of absorbance was carried out spectrophotometrically at 450, 532, and 600 nm. The concentration of MDA was calculated using the equation given below:

$$C_{MDA}\ (\mu mol\ mL^{-1}) = 6.45 \times (D_{532} - D_{600}) - 0.56 \times D_{450}$$

Where:
C_{MDA} : malondialdehyde concentration
D_{450} : absorbance at wavelengths 450 nm
D_{532} : absorbance at wavelengths 532 nm
D_{600} : absorbance at wavelengths 600 nm

Chlorophyll and carotenoid analysis

Photosynthetic pigments, such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid was estimated according to the method of Quinet et al. (2012). 0.1 gram of fresh leaves were crushed with mortar in 10 mL of 80% acetone. The samples were centrifuged at a speed of 3000 rpm/min for 10 min at a temperature of 4°C. Measurement of absorbance was carried out using a spectrophotometer with wavelengths of 470, 646, and 663 nm. The calculations were based on the Lichtenthaler equation (1987) as follow:

C_a : $12.25\ A_{663} - 2.79\ A_{646}$
C_b : $21.50\ A_{646} - 5.10\ A_{663}$
C_t : $7.15\ A_{663} + 18.71\ A_{646}$

$$\text{Carotene} = \frac{1000\ A470 - 1.82\ Ca - 85.02\ Cb}{198}$$

Where:
Ca : chlorophyll a
Cb : chlorophyll b
Ct : total chlorophyll
A663 : absorbance at 663 nm
A646 : absorbance at 646 nm
A470 : absorbance at 470 nm

Relative water content analysis

The determination of relative water content was carried out using the method of Patade et al. (2011), with some modifications. The leaves were cut and weighed to get the fresh weight. They were then soaked in containers containing aquadest for 24 hours in dark conditions. The samples were then weighed to obtain turgid weight. They were further dried in the oven at 80ºC for 48 hours and weighed to determine the dry weight. The relative water content was determined using the following formula:

$$\text{Relative Water Content (\%)} = \frac{\text{(fresh weight} - \text{dry weight)}}{\text{(turgid weight dry weight)}} \times 100$$

Lead content Analysis

Pb content in shoot and root tissues was measured using atomic absorption spectrometry (AAS). The value of Pb obtained was then used as the basis for calculating the bioconcentration factor (Zhuang et al. 2007) and the translocation factor (Padmavathiamma and Li 2007), as shown below:

$$\text{Bioconcentration Factor} = \frac{\text{Concentration of Pb (shoot and root)}}{\text{Concentration of Pb in media}}$$

$$\text{Translocation Factor} = \frac{\text{Concentration of Pb in shoot}}{\text{Concentration of Pb in root}}$$

Data analysis

All data obtained were analyzed using Analysis of Variance, followed by Duncan Multi Range Test (DMRT) with α = 5%, using SPSS 16.0 software. Morphological and physiological data were used for principal components analysis (PCA) by PAST 3 software (Hammer 2015). Before PCA analysis, the data were standardized with Microsoft Exel 2016 (Jolliffe and Cadima 2016).

RESULTS AND DISCUSSION

Pb and Hg in gold mine tailings and soil

The analysis of Pb and Hg in planting media was carried out before the treatment, results of which are given in Table 1. Pb content was far higher than Hg in both tailing and soil. Pb content in gold mine tailing was considerably high which influenced plant growth. The data also showed that the soil used as media in this study also slightly contaminated by Pb.

Plant growth

The plant growth showed significant reduction in response to tailing treatments, especially at concentration of 100%. Gold mine tailing at 50% did not notably affect the growth of all the species, even though some of the studied parameters decreased (Figure 1).

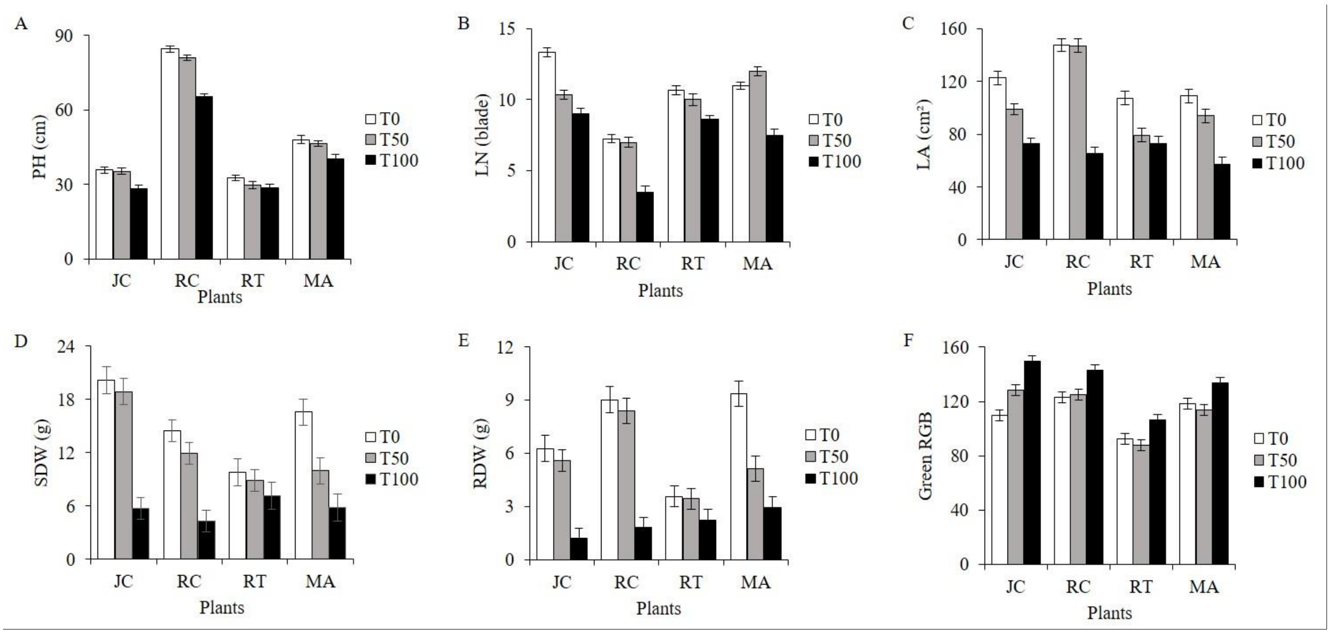

Figure 1. Growth responses of four study species *J. curcas* (JC), *R. communis* (RC), *R. trisperma* (RT) and *M. azedarach* (MA) to different concentrations of tailing treatments: T0, (without tailing); T50, (50%) and T100 (100%) after a growth period of 8 weeks. Note: Plant height (PH) (A), leaf number (LN) (B), leaf area (LA) (C), shoot dry weight (SDW) (D), root dry weight (RDW) (E), Green RGB (F). Bar lines indicate standard error of DMRT at α of 5%.

The result showed that there was a decrease in plant height with increasing concentration of tailings in the medium. The plant height reduction occurred in all species, in the range of 15.9% to 22.8%, except in *R. trisperma* in which the decrease was only 12.3% (Figure 1A). The leaf number and leaf area also showed a sharp decline in all species in the range of 31.8% to 51.7% for leaf number and 40.8%-55.8% for leaf area, except in *R. trisperma* in which decrease was 19.4% and 31.9% for both parameters, respectively (Figures 1B and 1C). The dry weight of shoot and roots also decreased in the range of 65.0% to 71.5% and shoot dry weight decreased in the range of 68.4% to 81.0%, except in *R. trisperma* which showed a decrease of 27.0% and 37.4%, respectively (Figures 1D and 1E). Tailing treatment also changed leaf color from green to yellowish as indicated by 15.3% to 36.1% increase of green RGB values of all plants, except in *M. azedarach* which recorded an increase by about 12.6% (Figure 1F).

Plant growth is highly influenced by environmental changes and conditions. Gold mine tailing at 100% significantly reduced growth of all the species (Figure 1), even though all the species grew well till the end of the 8 weeks study period suggesting the adaptability of all the species. Among the species studied, *R. trisperma* appears to be least affected by gold mine tailing based on growth performance. A previous study also showed that contamination resulting from gold mining activities in the form of tailings contained some heavy metals and other essential elements (Mg, Fe, Mn, and Zn) as well as non-essential element such as Pb and Ag at significant concentration (Hilmi et al. 2018). In this experiment heavy metal, Pb was detected at relatively high concentration of 63.31 ppm while Hg content was considerably low (Table 1). The existence of heavy metals, especially non-essential heavy metals even in low quantities, can affect the growth and development of a plant (Page and Feller 2015). Heavy metals such as lead (Pb) may affect plant growth processes, damage to leaves, and reduce biomass from plants (Page and Feller 2015; Kiran and Prasad 2017).

Pb accumulation in root and leaf tissue

According to Seregin and Kozhevnikova (2011), histochemical method can help to investigate the distribution and accumulation of heavy metals in plant tissues. Histochemical observation showed the presence of Pb in endodermis and vascular bundle of the roots of all species, which was indicated by the red color (Figure 2). In addition, Pb was also detected in the leaf tissues. In leaf tissues, the existence of Pb metal was found in the vascular bundle, precisely in the xylem (Figure 3). This data showed that all the species absorbed and transported Pb to leaves even in the absence of tailing in the growth medium (T0), because Pb was also found in the soil media (Table 1). The graphs also indicated that accumulation of Pb was more prominent in roots than in shoots. Some earlier studies have confirmed that accumulation of Pb in the root was higher than in the shoot, in plants such as *Symphytum officinale* (Chin 2007), *Pharagmites australis* (Al-Akeel 2016), and *Anthocephalus cadamba* (Setyaningsih et al. 2017). The absence of any toxicity symptoms in all the plants suggests that all the species were able to tolerate heavy metal (Pb) present in the tailings, even though growth responses of the plants varied depending on the species.

MDA content of leaf and roots

Malondialdehyde (MDA) content was analyzed to detect lipid peroxidation in response to gold mine tailing treatment. MDA levels increased significantly ($P<0.5$) with increasing concentration of tailings up to 100% (Figure 4), except in *R. trisperma* which did not show any considerable increase of MDA content in the leaf and roots which was only up to 6.8% and 13.5%, respectively as compared to control plant (Figures 4A and 4B). The MDA content in the leaves was higher than that of the roots, even though the increase in MDA content in response to gold mine tailings was almost similar in both organs (Figure 4). *R. trisperma* was the only species in which the MDA content was not influenced by tailing treatment, while *M. azedarach* was the most affected species as MDA increase due to gold mine tailing treatment was maximum in this plant.

Heavy metal content, especially Pb, in the tailings (Table 1) may be the cause of the increased reactive oxygen species (ROS) in the tissues as indicated by the increased MDA content in the leaf and roots (Figure 4). Heavy metals induce oxidative stress in plants due to the formation of free radicals (Aransiola 2013) leading to increase in lipid peroxidation (Rascio and Navarie-Izzo 2011). Some investigators have proven that heavy metal caused significant increase of ROS in many species (Hilmi et al. 2018; Page and Feller 2015). Kiran and Prasad (2017) have also reported that the treatment of tailings containing heavy metals such as lead caused the increase of MDA levels.

Chlorophyll and carotenoid content

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were also measured to investigate the physiological responses of gold mine tailing based on the development of plant pigments. After 8 weeks of treatment, the levels of chlorophyll and carotenoids decreased with increasing concentration of gold mine tailings (Figure 5), suggesting that gold mine tailings treatment caused the decline of chlorophyll as well as carotenoids. The decrease of all pigments tended to have similar pattern among the species.

Table 1. Tailing and soil content as media

Parameter	Tailing	Soil
Pb	63.31	13.44
Hg	0.03	0.03

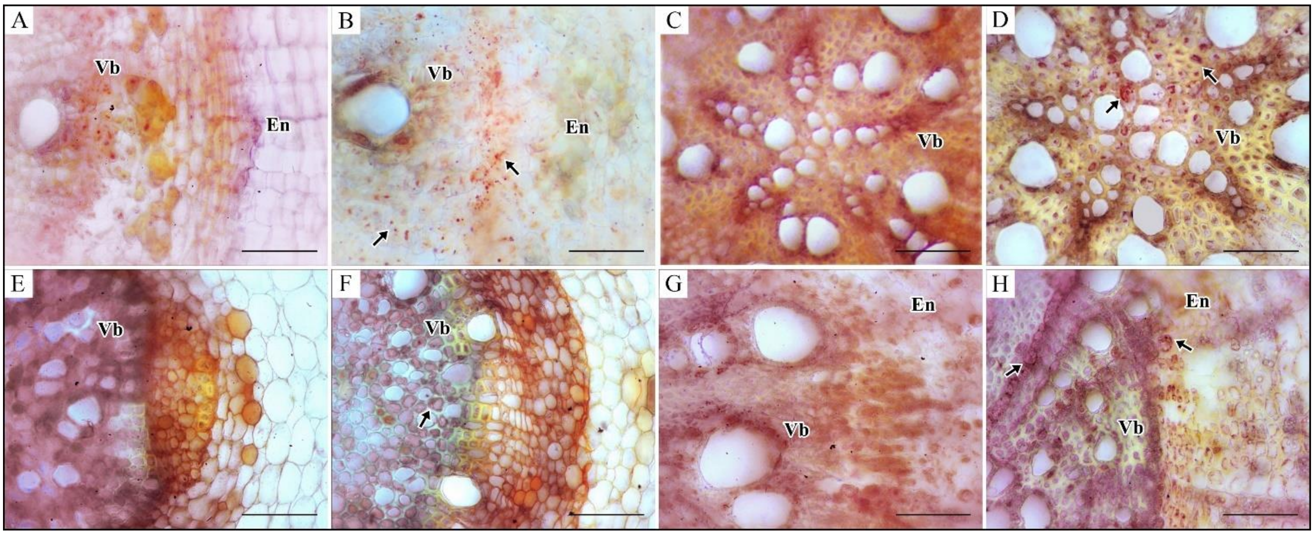

Figure 2. Histochemical analysis to detect Pb metal in the root tissues of four species grown without (T0) and with 100% of gold mine tailings (T100). Note: *J. curcas* T0 (A), *J. curcas* T100 (B), *R. communis* T0 (C), *R. communis* T100 (D), *R. trisperma* T0 (E), *R. trisperma* T100 (F), *M. azedarach* T0 (G), *M. azedarach* T100 (H). The red color with an arrow (→) indicated the presence of Pb metal. En: endodermis, Vb: vascular bundle. Bar length: 50 µm.

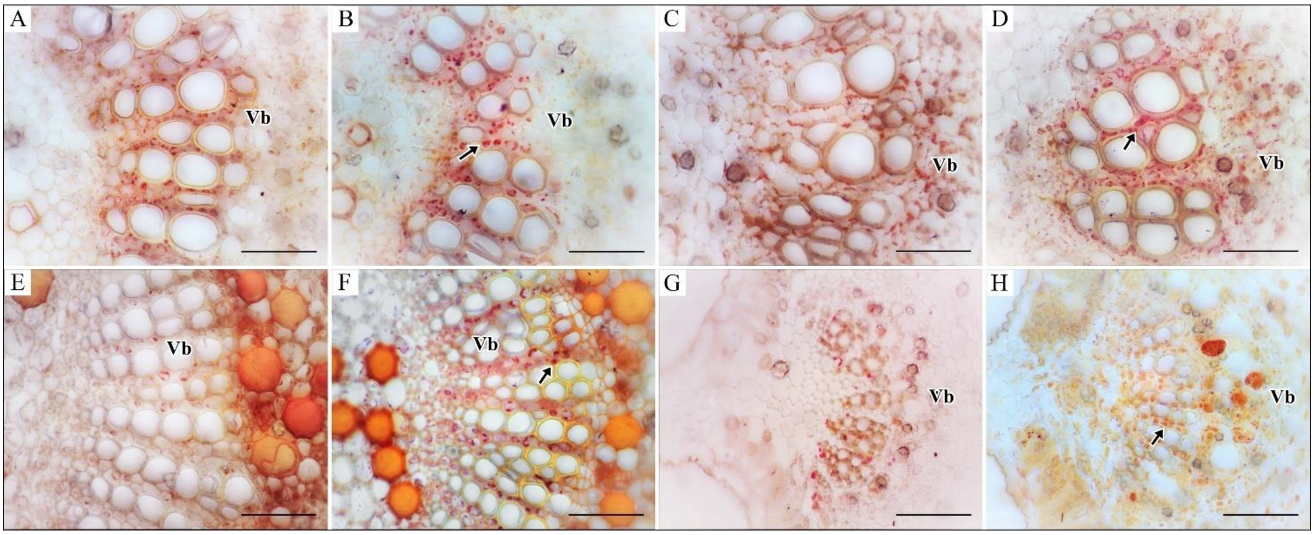

Figure 3. Histochemical analysis to detect Pb metal in the roots tissues of four species grown without (T0) and with 100% of gold mine tailings (T100). Note: *J. curcas* T0 (A), *J. curcas* T100 (B), *R. communis* T0 (C), *R. communis* T100 (D), *R. trisperma* T0 (E), *R. trisperma* T100 (F), *M. azedarach* T0 (G), *M. azedarach* T100 (H). The red color indicated the presence of Pb metal. Vb: vascular bundle. The red color with row (→) indicated the presence of Pb metal. En: endodermis, Vb: vascular bundle. Bar length: 50 µm.

Table 2. Pb content in the shoot and root tissues of *Jatropha curcas*, *Ricinus communis*, *Reutealis trisperma* and *Melia azedarach* grew in tailing concentrations of 0%, 50%, and 100% for 8 weeks.

Plants	Pb Content (ppm)					
	Shoot			Root		
	T0	T50	T100	T0	T50	T100
Jatropha curcas	<0.50b	<0.50b	3.72a	<0.50c	<0.50c	<0.50c
Ricinus communis	<0.50b	<0.50b	5.10a	<0.50c	<0.50c	7.95a
Reutealis trisperma	<0.50b	<0.50b	0.75b	<0.50c	2.42bc	4.40b
Melia azedarach	<0.50b	<0.50b	<0.50b	<0.50c	<0.50c	<0.50c

Note: The number followed by the same letter shows no distinct results based on the DMRT test

Table 3. Bioconcentration factor (BCF) and translocation factor (TF) of Pb for *Jatropha curcas*, *Ricinus communis*, *Reutealis trisperma* and *Melia azedarach* in response to gold mine tailings of 0%, 50%, and 100% for 8 weeks

Plants	Bioconcentration Factor (BCF)			Translocation Factor (TF)		
	T0	**T50**	**T100**	**T0**	**T50**	**T100**
Jatropha curcas	0.07b	0.03c	0.07b	1.00b	1.00b	7.44a
Ricinus communis	0.07b	0.04c	0.21a	1.00b	0.59b	0.77b
Reutealis trisperma	0.07b	0.08b	0.08b	1.00b	0.22b	0.18b
Melia azedarach	0.07b	0.03c	0.02c	1.00b	1.00b	1.00b

Note: The number followed by the same letter shows no distinct results based on the DMRT test

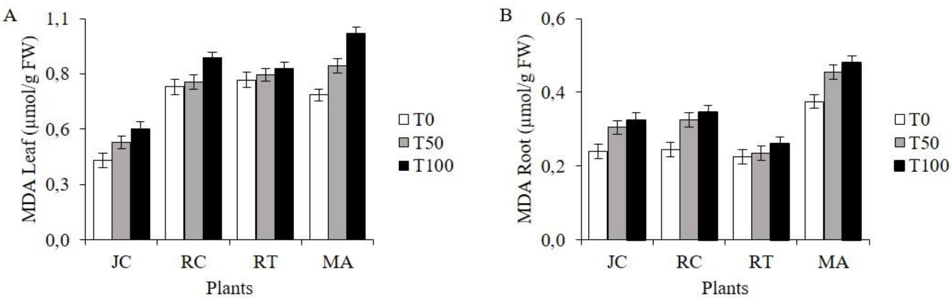

Figure 4. MDA changes in leaf (A) and root (B) of *J. curcas* (JC), *R. communis* (RC), *R. trisperma* (RT)and *M. azedarach* (MA) in response to tailing content of 0% (T0), 50% (T50) and 100% for 8 weeks. Bar lines indicate standard error

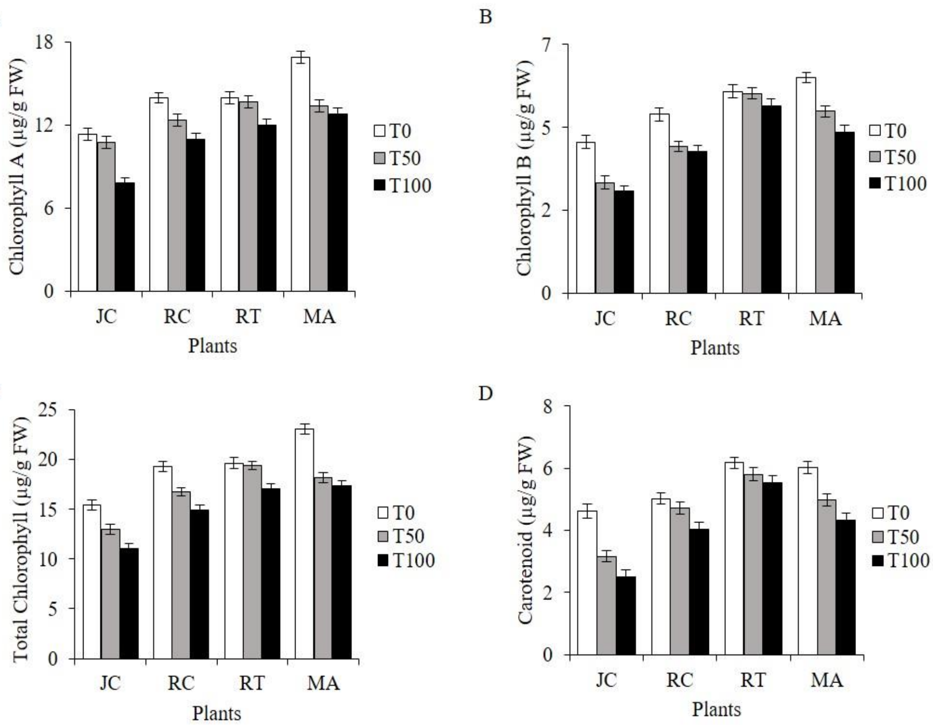

Figure 5. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) content of *J. curcas* (JC), *R. communis* (RC), *R. trisperma* (RT), *M. azedarach* (MA) in response to 0% (T0), 50% (T50), and 100% (T100) tailings treatment for 8 weeks. Bar lines indicate standard error

The level of chlorophyll a decreased in all plants in the range of 21.6% to 31.4%, except in *R. trisperma* in which the decrease was just 14.4% (Figure 5.A). Chlorophyll b decreased in the range of 20.9% to 32.6% except in *R. trisperma* which only decreased by 7.0% (Figure 5.B). Consequently, gold mine tailings caused the decrease of total chlorophyll of all plants in the range of 22.7% to 28.8%, except for *R. trisperma* which decreased only by about 13.2% (Figure 5.C). Carotenoids content also decreased up to 45.6% in similar pattern for all plants with *R. trisperma* showing the lowest reduction of 10.5% (Figure 5.D).

The decrease of plant pigments suggested that the plants were under stress which disturbed synthesis of important pigments including chlorophyll and carotenoid (Figure 5). The extent of chlorophyll reduction may be an important indicator of plant response to gold mine tailings. Kiran and Prasad (2017) also suggested that chlorophyll and carotenoids can be used as indicators of sensitivity of plants to heavy metals such as Pb. Pb has been reported to cause the formation of radical oxygen species (ROS) which damage chlorophyll pigments and the photosynthesis process (Najeeb et al. 2017). According to another study, the damage of pigments was caused by heavy metals that replaced the essential metals in the structure of pigments which eventually interferes with the functioning of the pigment (Aransiola 2013). Rascio and Navarie-Izzo (2011) showed that heavy metal poisoning also caused disruption of electron transport in the chloroplast membrane.

Relative water content (RWC)

The relative water content was measured after 8 weeks of tailings treatment, to find out the effect of heavy metal on plant water balance. The results showed that relative water content varied across the species. RWC values decreased significantly by gold mine tailings, except in *R. trisperma* (Figure 6). The decrease of RWC was significant for 100% gold mine tailings with the values ranging from 6.4% to 19.9%, except for *R. trisperma* in which just 3.3% decrease was observed (Figure 6). RWC is an important parameter indicative of plant water status (Hamim et al. 2016). The decrease of RWC in response to gold mine tailings may be associated with the negative impact of heavy metals on water balance due to root dieback or the decrease of hairy roots in response to heavy metals (Rucinska-Sobkowiak 2016). The dramatic decrease in dry weight of roots in response to goldmine tailings as shown in Figure 1, supports the suspicion that hairy roots were most affected.

Analysis of Pb in shoot and root tissues

The concentration of Pb in the plant tissues was analyzed because it was the only heavy metal present in higher concentration in the tailings (Table 1). The analysis was carried out in the shoot and root tissues of the plants after 8 weeks of gold mine tailing treatment. Pb content in the root was higher than that in the shoot, even though there was a wide variation across the species in their response to 100% tailings, while at 50% tailings did not induce Pb accumulation except in *R. trisperma* roots (Table 2). *R. communis* showed the highest Pb concentration in both shoot and roots, while the lowest was found in *M. azedarach* (Table 2). The higher Pb accumulation in the roots indicates that the plant resists transferring Pb to the shoot. Yoon (2006) reported that roots have higher ability to accumulate heavy metals than the shoot, and this is also found in the tolerant plants.

The Pb content in the tissue was used to calculate the bioconcentration factor (BCF) and translocation factor (TF). The bioconcentration factor (BCF) is important to determine shoot and root capabilities to absorb Pb, while translocation factor (TF) is important component to determine its translocation from the root to the shoot. In general, BCF in all plants is low, less than one (Table 3). Zhuang (2007) explained that plants have difficulty in mobilizing metals in the rooting area. *R. communis* showed the highest BCF value, while *J. curcas* had the highest TF value in 100% tailings treatment (Table 3). In contrast, *R. trisperma* had the lowest TF value with a moderate BCF value which was almost similar to that of *J. curcas* (Table 3).

A combination of BCF < 1 and TF ≥ 1 or BCF and TF < 1 is indicative of plant's potential as phytostabilizer and refers to its metal tolerance strategy, that is exclusion (Gajić et al. 2018). Exclusion is one of the plant strategies to maintain lower rate of heavy metal absorption and accumulation (Seregin and Kozhevnikova 2011; Gajić et al. 2018). The low value of BCF and FT shows that the plant has limitations in absorbing and accumulating metals in its biomass (Yoon 2006). In the current study, *R. trisperma* exhibited best growth performance with lowest BCF and FT. (Table 3). Considering the Pb content (Table 2) together with BCF and FT values, it may be suggested that this species can be categorized as a phytoremediator plant with phytostabilization mechanism, indicated by the low heavy metal translocation from the root to the shoot. Padmavathiamma and Li (2007) explained that phytostabilizers are those plants which reduce the mobility of heavy metals from the soil to the shoot by accumulating them in the root tissues.

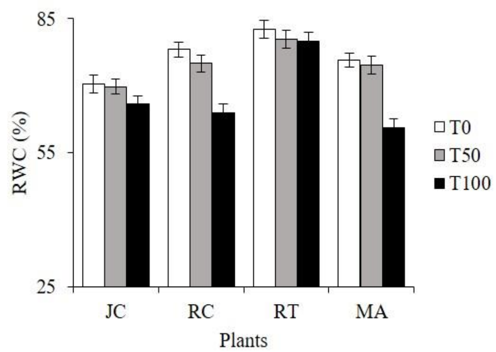

Figure 6. Relative water content (RWC) of *J. curcas* (JC), *R. communis* (RC), *R. trisperma* (RT), *M. azedarach* (MA) in response to gold mine tailing treatment at the concentration of 0% (T0), 50% (T50) and 100% (T100) for 8 weeks. Bar lines indicate standard error.

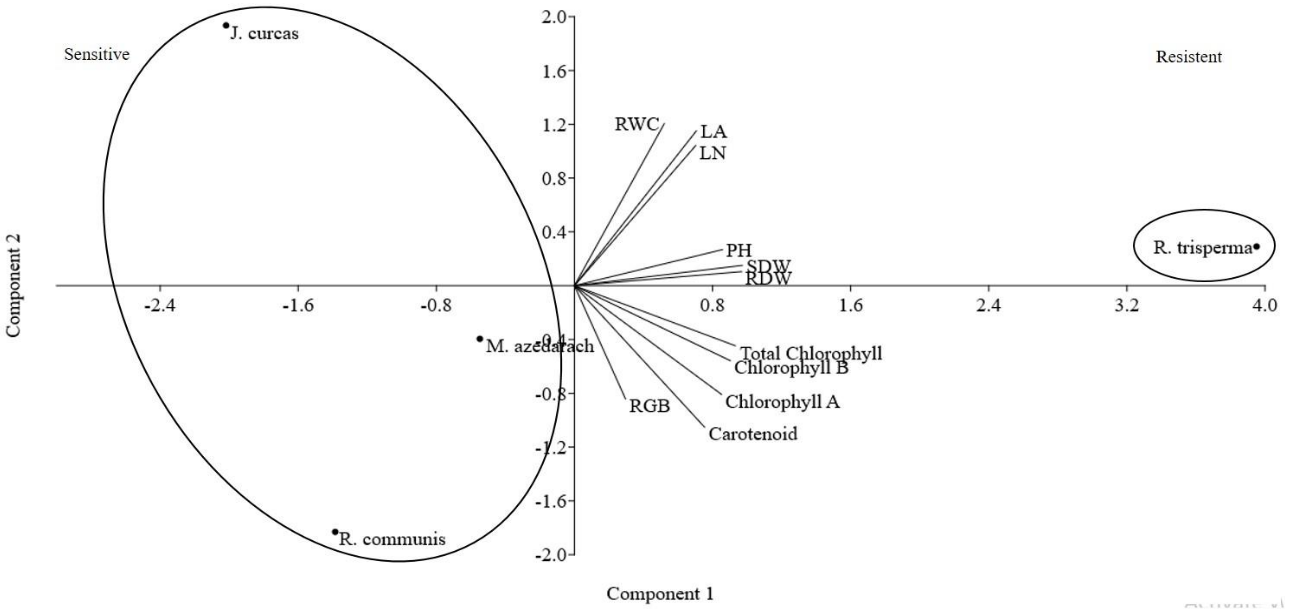

Figure 7. Principle component analysis of morphological and physiological responses of four species to gold mine tailing treatment parameter

Principal Component Analysis

All the morphological and physiological parameters studied were subjected to Principal Component Analysis. The analysis showed that component 1 represented 73.58% of the variations and component 2 was 18.94%. Using *k-mean* clustering, the analysis categorized the plants to 2 groups as most resistant and less resistant species (Figure 7). *J. curcas*, *R. communis*, and *M. azedarach* were included in the category of less resistant plants while *R. trisperma* was in the group of plants most resistant to gold mine tailings (Figure 7). In almost all parameters, *R. trisperma* showed superior response to gold mine tailing treatments, when compared to the other 3 species. These data are in agreement with the findings of Hamim et al. (2017) and Hilmi et al. (2018) that *R. trisperma* is a potential plant for phytoremediation program in gold mining area. With its capacity to produce non-edible oil (Pranowo et al. 2015), utilization of *R. trisperma* for phytoremediation program will have additional benefits, as the plant can reduce heavy metal pollutant while producing non-edible oil for biodiesel production.

ACKNOWLEDGEMENTS

The authors would like to thank PT Aneka Tambang UPBE Pongkor, Bogor, Indonesia for the opportunity to explore and use gold mine tailing material for this study.

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