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Profile of Secondary Metabolites and Metal in *Jatropha curcas* L. and *Reutealis trisperma* Plants Grown in the Media Contaminated by Gold Mining Tailings using LC-MS/MS

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Abstract: The increase in industrial development hurts the environment. One of the efforts to reduce heavy metal contaminants from the environment is through a phytoremediation program. This study aimed to analyze the morphological response, metal content, and changes in metabolites of the compounds based on LC-MS/MS analysis of *Jatropha curcas* and *Reutealis trisperma* treated with gold mine tailings stress. The study was conducted using a completely randomized design with two factors consisting of two plant species, Jatropha curcas and Reutealis trisperma, and three gold mine tailings concentrations (0, 50, and 100 %). The work procedures carried out are planting preparation and tailings treatment, growth observation and sampling for analysis, analysis of secondary metabolites in plant tissues, identification of metal components in leaves with XRF, and identification of metabolite profiles with LC-MS/MS. Gold mine tailings caused a significant decline in plant growth, represented by plant height and leaf number. The higher the concentration of tailings, the more disturbed plant growth. We find about 33 metabolites that are thought to be composed of amino acids, terpenoids, phenols, flavonoids, and other organic compounds.

Keywords: phytoremediation; LC-MS/MS; XRF spectrum; *R. trisperma*; *J. curcas.*

1. Introduction

Gold mining is one of the human activities that are at significant risk of heavy metal pollution due to produced tailings. Tailings are waste residues of mining activities that are dominated by sand texture, are poor in nutrients, and often contain heavy metal elements [1]. Bayu [2] found that Pb is one of the heavy metals found in tailings in high concentrations.

Heavy metals in the environment are very dangerous for living things, and since heavy metals cannot be biologically degraded, they will accumulate in the tissues. The higher concentration of metals can be a source of toxic and carcinogenic materials for living beings [3]. The impact of heavy metal contamination on humans and animals includes the presence of heavy metal content in the blood, which ends in the onset of various diseases.

Countermeasures of heavy metal waste contained in our environments, including tailings, can be carried out with the help of plants (known as phytoremediation). Phytoremediation is using plants to remove pollutants from contaminated soil or water. This method has been recognized as an efficient and effective method to reduce contaminants from many sites, including areas contaminated with heavy metals [4]. Plants that can survive in polluted environments and can accumulate heavy metals are called hyperaccumulator plants [5,6]. The effectiveness of phytoremediation in reducing heavy metals depends on the capacity of the plant to absorb and accumulate certain heavy metals in the canopy part [7] while plants are still able to grow well and have higher biomass. Therefore, it is necessary to constantly develop efforts to obtain plants with a high ability to absorb heavy metals as accumulator plants.

There are several criteria for plants that are used as phytoremediation agents of heavy metals, such as having fast growth, producing high biomass, a deep root system [8], having tolerant properties, and having the ability to absorb heavy metals. Plants such as Jatropha curcas and Reutealis trisperma can be used as phytoremediators on land polluted with gold mine waste. The two species can, in addition, be used as phytoremediators. J. curcas is a potential plant for phytoremediation of soil or polluted soil with a wide variety of heavy metals [9]. R. trisperma has good adaptability to grow on marginal lands such as very dry soils and acidic soils [10] and can adapt to the liquid waste of gold mines [11]. Until now, no publications have been related to aspects of J. curcas metabolites and R. trisperma as a response to heavy metal deposits. Therefore, this study aimed to observe the compound's morphological response, nutrient content response, and metabolite changes response based on LC-MS/MS analysis of J. curcas and R. trisperma that get treated with gold mine tailings.

2. Materials and Methods

2.*1. Experimental methods.*

Pretreatment, castor bean plants Jatropha curcas and Reutealis trisperma are germinated in the beds. After germination, the plant is planted in small pots measuring 7 cm to 1 month old. After 1 month, the plant is transferred into polybags that have been prepared for treatment. The growth medium was prepared using a mixture of soil and compost in a ratio of 1:3 (v/v), and the plants were treated with gold mine tailings with different concentrations. Each polybag is filled with the mixture until it reaches a weight of 6 kg per polybag. To help the initial growth, each polybag is given 500 grams of compost. One plant is planted in each polybag. The study was conducted using a complete randomized design with two factors and three tests. The first factor is 2 types of plants, namely Jatropha curcas and Reutealis trisperma. The second factor is 3 concentrations of tailings in the growth medium, namely a mixture of ordinary media without the addition of tailings (as a control), gold mine tailings with a concentration of 50%, and gold mine tailings with a concentration of 100%.

2.2. Growth observation and sampling for analysis.

Plant growth is observed for 3 months with a note, height, and number of leaves. After observing the growth of plants *Jatropha curcas* and *Reutealis trisperma* is ready to be harvested at the age of 3 months for testing on the metabolite profile of the plant.

2.3. Identification of metal element components on leaves with XRF.

The research method uses micro-XRF, which is a tool to see the distribution of elements in a sample. The first step is a sample of *Jatropha curcas* leaves, and intact *Reutealis trisperma* is inserted into the chamber. Next, turn on the vacuum to 2 mBar. The goal is to increase the accuracy of the readings on the *Light Element*. Furthermore, the sample analyzed is the lower leaf part because the whole leaf is quite large. Next set of power x-rays, step size, and dwell time for analysis of Jatropha curcas and *Reutealis trisperma* samples; the analysis time takes about 30 minutes. The results came out as images of the distribution of elements in the *Jatropha curcas* and *Reutealis trisperma* [12].

2.4. Analysis of secondary metabolites of plant tissues.

The sample is cleaned and then dried; after drying, it is mashed then in the extraction of 10 grams of *Jatropha curcas* leaf powder and *Reutealis trisperma* by dissolving on ethanol PA in a beaker in a ratio of 1:10 and extraction temperature (30 and 40 °C). Ultrasonication was performed for 30 min by using 42 Hz ultrasonic waves, after which a supernatant filtered extract was collected, and the solvent was evaporated with a rotary evaporator in vacuum at a temperature of 45 °C to obtain an extract, then collected in a glass bottle and stored at a temperature of 30 °C [13].

2.5. Identification of metabolites with LC-MS/MS.

Identification of chemical components by treating as much as 10 mg of concentrated extract of the sample, which is weighed and then dissolved in 2 ml of ethanol. The dissolving of the extracts used an ultrasonicator for 30 min. The sonication results are put in a 5 ml measuring flask, then ethanol until the brick mark. Furthermore, the solution was filtered with a 0.2 µm PTFE filter membrane, and as much as 2.50 µl filtrate was injected into UHPLC-Q-Orbitrap-MS/MS. Jatropha curcas and Reutealis trisperma metabolites were separated using Vanquish Flex UHPLCQ-Orbitrap HRMS with accuser column C18 (100×2.1 mm, 1.5 m). A gradient elution system with a flow rate of 0.2 mL/min for 50 min was used to separate metabolites. The composition of the phase of motion used, namely 0.1 % formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a gradient elution system of 0-3.45 minutes (8-25% B), 3.45-6.9 minutes (25-54% B), 6.9-7 minutes (54-100% B), 7-9 minutes (100% B), 9-15 minutes (8% B), with positive and negative ion modes [15].

3. Results and Discussion

3.1. The heavy metal content of gold mine tailings and soil.

Plant growth is a morphological response that can be directly observed and is highly influenced by environmental conditions. The measurement of heavy metal levels carried out in this study was the metal lead (Pb). The determination of heavy metals tested in this study was based on previous research that Pb had relatively high and very low mercury levels [16].

Based on the results of the analysis in this study, the pH of the *tailings* tends to be alkaline (pH 7.23), while the soil is acidic (pH 4.90). Pb levels in *tailings* and soil were 63.35 ppm and 13.43 ppm, respectively (Table 1). The Pb levels obtained hampered the growth processes of the two plants during the study. The characteristics of the gold-mine *tailings* are sand, which is dominant at 83.45%, dust at 15.77%, and clay at 0.90%, while the soil tends to be clay textured at 74.70%, low porosity with a composition of sand at 11.65%, and dust at 13.70%.

Sabelli *et al*. [17] explained that the presence of heavy metals, especially nonessential heavy metals, in low amounts can affect plant growth and development. In addition, the Pb content obtained has also passed the quality standard for potential sources of toxicity to the environment. According to PP No. 85 1999 concerning the TCLP quality standard for contaminants in waste to determine toxic characteristics, the quality standard for lead is 5 ppm.

Table 1. Parameters of heavy metals *tailings* and soil as a growing medium.

Media			**Heavy metals**		
	pH	**Pb (ppm)**	**Hg (ppm)**	**Ag (ppm)**	**As (ppm)**
Tailing	7.22	63.31	0.03	0.04	0.06
Soil	4.91	13.44	0.03	0.04	0.06

3.2. Effect of tailings soil concentration on plant growth.

Observations on the morphology of the two plants showed that, compared to control plants (0% *tailings*), the 50% *tailings* treatment tended to show growth inhibition, while the 100% *tailings* treatment showed significant growth inhibition in crowns and roots (Figure 1).

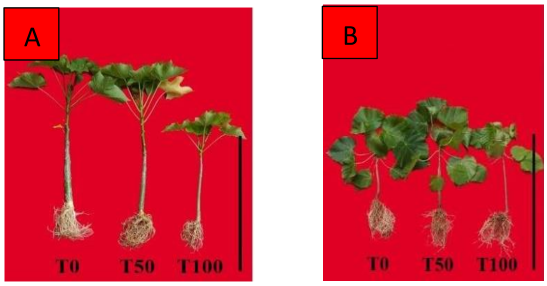

Figure 1. Morphology of species (**A**) *J. curcas*, (**B**) *R. trisperm* after *tailings treatment* 0 % (T0), 50 % (T50), and 100 % (T100) for 8 weeks. 30 cm**.**

The results of measurements of the morphological characters of the two plants showed a significant decrease in response to the 100% *tailings* treatment compared to the control plants for 8 weeks (Figure 2). The morphological characteristics that experience growth inhibition are plant height and several leaves. Observations on the increase in height of *jatropha curcas* and *Reutealis trisperma* plants treated with gold-mine *tailings* during the study within 8 weeks are presented in (Figure 2).

The results showed that growth inhibition occurred in plant height in line with the increasing concentration of tailings waste used as a planting medium. This shows that the tailings treatment affects plant height. The results of the analysis showed that treatment of gold-mine tailings caused a significant reduction in plant height and number of leaves in *J. curcas* but not statistically significant in *R. trisperma* (Figure 2).

Treatment of 50% and 100% *tailings* caused a decrease in plant height in *J. curcas* by 9.51% and 28.80%, respectively, while in *R.trisperma* it tended to decrease by 15.60% and 25.78%. In addition, 50% and 100% tailings treatment also caused a decrease in the number of leaves in *J. curcas* by 26.70% and 47.90%, respectively, while in *R. trisperma* it tended to decrease by 14.70% and 27.80% respectively. The greater the concentration of tailings in the soil medium, the lower the average increase in plant height.

This occurs because of the uptake of heavy metals in plants and results in the inhibition of plant growth. According to [18], heavy metals such as Pb, Al, Mn, Cr, Hg, and other heavy metals can inhibit the growth of the height and number of plant leaves. Apart from the toxic effect of the heavy metals contained in the tailings, this decline is related to the availability of organic matter and nutrients in the planting medium, which was only given 500 grams of compost at the beginning of the treatment.

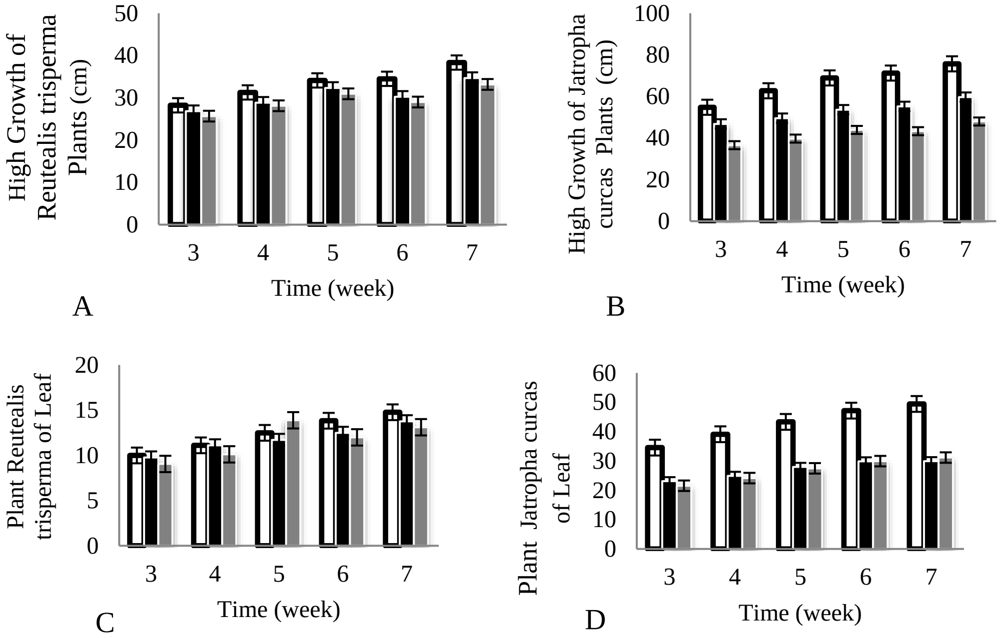

Figure 2. Height gain of (**A**) *Reutealis trisperma* plant, (**B**) *Jatropha curcas*, and (**C**) Number of *Reutealis trisperma* leaves, (**D**) *Jatropha curcas* leaves on the influence of concentration *tailing* T0 (blue), T50 (red), T100 (green).

Many studies have also found that low concentrations of heavy metals in growth media reduce plant growth. According to [19], the height of privet plants decreased with increasing given concentrations of heavy metals. [20], heavy metals are toxic to legumes because they can cause chlorosis, reduced plant growth, decreased productivity, and limited nutrient uptake. [21] also found that growth in height and the number of leaves decreased significantly in the four plants used, including *J. curcas* and *R. trisperma*, after treatment with 100% *tailings*.

Growth inhibition is the main common response when plants are under abiotic stress, including heavy metal stress [22]. High amounts of nonessential heavy metals affect plant growth and development, damage leaves, and reduce plant dry weight [23]. Heavy metal toxicity also reduces enzyme activity due to metal binding to the site of enzyme activation, which ultimately causes growth inhibition [24]. Nonetheless, both of these plants survived until the end of the observation, which shows that these plants have good adaptability to gold-mine *tailings*.

3.3. Pb accumulation in leaves.

Heavy metals can be detected using histochemical methods with specific reagents. The reagent used to detect Pb is dithizone, with a positive red color on the tissue. Histochemical observations of Pb accumulation were also detected in leaf tissue. In the leaf tissue, to be precise, in the leaf veins, the presence of Pb metal is found in the vascular tissue, especially in the xylem.

These results indicate that *J. curcas* and *R. trisperma* were able to absorb and distribute Pb metal from the leaf media. No toxic symptoms were found in *J. curcas* and *R. trisperma*, this indicates that *J. curcas* and *R. trisperma* can tolerate heavy metals contained in *tailings*, although there is a different growth response between *J. curcas* and *R. trisperma*.

Lead accumulation, especially in leaves, was also reported by [16] on *Reutalis trisperma* treated with 100% gold-mine *tailings* where the *tailings* contained a lot of lead. Lead was detected in all leaf tissues, such as the upper and lower epidermis, parenchyma, and stomata. Furthermore, [20] also reported that lead was detected in leaf tissue, especially the xylem in *Jatropha curcas*, *Ricinus communis*, *Reutealis trisperma*, and *Melia azedarach* plants that were treated with gold-mine *tailings* with a concentration of 100%.

3.4. Pb heavy metal levels in shoots and roots.

In this experiment, the heavy metal content analyzed from plant tissue was Pb because Pb has a very high concentration in tailings (Table 2). Analysis was carried out on the crowns and roots of both species after being treated with gold-mine tailings stress for 8 weeks. The results showed that the Pb content in the roots was not too different compared to the canopy except for *J. curcas*, in the 100% tailings treatment which accumulated Pb in the canopy up to more than 9 times. The 50% tailings treatment did not induce Pb accumulation in the shoots and roots except for the roots of *R. trisperma* (Table 2). The data showed a different typology of the two plants, *tailings* treatment caused *J. curcas* to accumulate Pb in the canopy while *R. trisperma* accumulated Pb in the roots (Table 2).

Table 2. Levels of Pb metal in leaf and root tissues of *J. curcas*, *R. trisperma*, which were treated with 0%, 50%, and 100% tailings stress for 8 weeks.

Plant	**Pb Content (ppm)**					
	Shoots			**Roots**		
	T0	T50	T100	T0	T50	T100
J. curcas	0.40b	0.40b	3.75a	0.40c	0.40c	0.40c
R. trisperma	0.40b	0.40b	0.76b	0.40c	2.45bc	4.50b

Note: Numbers followed by the same letter show results that are not significantly different based on the DMRT (Duncan Multiple Range Test) tests

The results of the analysis of this study showed that the 100% tailings treatment did not cause a significant increase in the BCF value in both *J. curcas* and *R. trisperma*, while the highest TF value was in *J. curcas*, which was 7.45 (Table 3). In contrast, in *R. trisperma* the 100% *tailings* treatment caused the TF value to decrease (Table 3).

Plants with low TF values can be used as phytostabilizers; these plants keep metals outside their tissues and are still able to live without introducing metals into plant cells [25].

The combination of BCF and TF values < 1 has the potential as a phytostabilizer, indicating that *R. trisperma* has a higher ratio of Pb levels in roots than in shoots. This indicates that the species is included in the plant Phyto stabilization, which is indicated by the low translocation process from roots to shoots. [17] Explained that phyto-stabilizing plants are plants that reduce the mobility of metals in the soil towards the canopy so that they tend to accumulate in their roots.

Table 3. Bioconcentration factor (BCF) and translocation factor (TF) values of Pb metal from *J. curcas*, *R. trisperma* treated with 0%, 50%, and 100% *tailings* stress for 8 weeks

Plant	**BCF**			**TF**		
	T0	T50	T100	T0	T50	T100
J. curcas	0.06b	0.04c	0.06b	1.00b	1.00b	7.45a
R. trisperma	0.06b	0.09b	0.09b	1.00b	0.20b	0.25b

Note: Numbers followed by the same letter show results that are not significantly different based on the DMRT (Duncan Multiple Range Test) tests

3.5. Metal components on Jatropha curcas and Reutealis leaves castor.

Observation of the metal components of pecan leaves and fence spacing using the XRF micro instrument obtained data on the results of the *Reutealis trisperma* spectrum on the control treatment and 100% tailings treatment with observable metal elements, namely Ca, K, Mg, P, Fe, Ti, Mn and Si, with the value of metal element levels can be presented on Table 4. There is a difference in the comparison between the control treatment and the tailings treatment 100% judging from the existing metal element content, where the Ca in the *tailings* treatment is 100% greater than the Ca in the control treatment, the K in the control treatment is greater than the K in the tailings treatment 100% and Fe in the control treatment is greater than Fe in the *tailings* treatment 100%.

The data also show that the higher concentration of tailings in the soil affects the metal elements present in plants.

As for spectrum *Jatropha curcas* data on control treatment and 100% *tailings* treatment at fence distance, metal data were obtained Ca, Mg, Si, P, S, K, Fe, Mn, and Ti with metal element content values presented in Table 4. There is a comparative difference between the control treatment and the tailings treatment in 100% fencing distance, judging from the existing metal element content data, where the Si in the control treatment is greater than the 100% t*ailings* treatment Si, the S in the control treatment is greater than the 100% tailings treatment S and the K in the control treatment is greater than the 100% *tailings* treatment K.

A very prominent difference between the two types of plants is that in *Reutealis trisperma* no sulfur (S) element in the leaves is measured because the element sulfur (S) is so small that it is covered with other metal elements so that it is not detected in the XRF spectral data, whereas in *Jatropha curcas* there is elemental sulfur (S) to appear in the XRF spectrum data. The reason why sulfur (S) in *Reutealis trisperma* cannot be detected is that the group's LOD (Limit of detection) is below 50 ppm. The LOD (Limit of detection) of XRF (X-ray fluorescence) is around 50 ppm, so the possibility of sulfur in *Reutealis trisperma* below 50 ppm can be even smaller. This shows that the higher concentration of *tailings* in the soil affects the metal elements present in plants.

Table 4. Womb metal elements of *Jatropha* and *Reutealis* leaves

Mineral	**Metal Content (mg/100 g)**			
	Jatropha		***Reutealis***	
	T0	T100	T0	T100
Ca	20.96	26.80	45.82	34.65
K	57.56	55.37	32.60	52.73
P	1.65	1.88	1.88	2.27
Mn	0.25	0.42	0.49	0.75
Fe	0.38	0.28	0.32	0.73
Mg	9.39	8.30	15.77	5.70
Si	7.73	4.87	2.10	1.67
S	1.63	1.22	-	-
Ti	0.73	1.05	1.03	1.68

3.6. Secondary metabolite profile with LC-MS/MS.

Based on the results obtained from chromatogram data of *jatropha* extract *J. curcas* and *R. trisperma* of the two types planted in each *tailings* treatment, different separation patterns are similar to each other but only differ in the area of each detected peak (Figure 3). This suggests that the distribution of metabolites in all samples is relatively almost the same, only at the level of their concentration. This suggests that the distribution of metabolites in all samples is relatively almost the same, only at the level of their concentration.

Based on the identification results using the Xcalibur application, 33 compounds were obtained. The identified compounds consist of 6 compounds from the amino acid group, 9 compounds from the flavonoid group, 4 compounds from the phenolic acid group, 7 compounds from the triterpenoid group, 6 compounds from the steroid group, and 1 compound from the alkaloid group. The data can be presented in Table 5.

The absorption of Pb metal that occurred in the leaf metabolites of *J. curcas* and *R. trisperma* occurred in the leaf cells. It can be seen that there were not many changes due to the 100% tailings treatment. The changes were only seen in the shape and size of the chloroplasts. Chloroplasts are part of the leaf cells that are affected the most due to heavy metal stress; the most frequent change is a decrease in the number and swelling of the chloroplasts, changes in the shape of the membrane thylakoids reduced the number of grant, increased starch grains, and plastoglobuli [26]. In leaf cells that were treated with 100% treatments, there was also a slight accumulation of heavy metals in the cell wall. This was because the leaf cell walls also had a strong affinity for binding to heavy metals, causing accumulation in the cell wall area and preventing heavy metal ions from entering. In leaf cells [27].

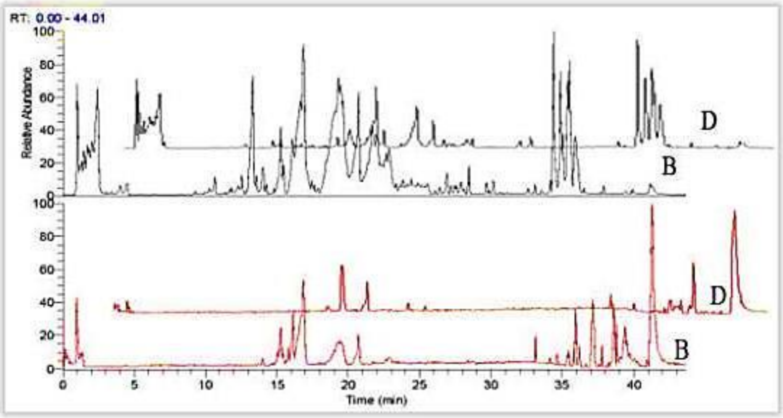

Figure 3. Sample chromatograms on positive ionization (bottom) and negative ionization (top) modes of D (*Jatropha*) and B (*Reutealis*).

3.6.1. Amino acid groups.

Metabolites of the amino acid group identified in *Jatropha* and *Reutealis* extracts are Valine (1), Tryptophan (5), Leucine (2), Phenylalanine (4), 5-Aminopentanoate (3), Ethyl 3,5-bis[(4-nitrobenzol) amino] benzoate (6). In *Jatropha*, on average, there is a decrease in each tailing's concentration of 50 and 100%, while *Reutealis* increases in *tailings* by 100%, but in *tailings* concentrations, 50% decrease data can be seen in Table 3. This shows that the higher the *tailings* concentration in the soil, the more it affects the plant's growth.

Leucine (3) and phenylalanine (4) release HCOOH with m/z values of 86 and 120, respectively, followed by NH_3 release with m/z values of 69 and 103. The valine compound (1) is fragmented by releasing CH_2O_2 molecules at m/z 72 $[M+H-46]^+$ and molecule NH_2 at m/z 56 $[M+H-16]^+$, while tryptophan (2) was identified in the positive ionization mode with $[M+H]^+$ at m/z 188, 170 and 143 (Table 4). This group of compounds is indicated by the presence of M-17 and M-18 fragments, which indicate the release of H_2O and NH_3 [28]. 5-Aminopentanoate (5) serves as a branching point for the biosynthesis of plant-derived alkaloids [29], positive ion mode, undergoes fragmentation at m/z 118$[M+H]^+$, 101$[M+H-OH]^+$, 70$[M+H-OH-CH_2O]^+$, Ethyl 3,5-bis[(4-nitrobenzol) amino] benzoate (6) identified in the negative ion mode, fragmented at m/z 477$[M-H]^-$, 205$[M-H-C_{12}H_6N_3O_5]^-$ and 163 $[M-H-C_{12}H_6N_3O_5-C_2H_2O]^-$.

3.6.2. Flavonoid groups.

Several flavonoid group compounds that have been successfully identified using positive ion mode in *Jatropha* and *Reutealis* extracts Apigenin (13), Kaempferol (12), Ruthin (10), (+)-dihydrokaempferol (9), Palomi'd 529 (7), Daidzein (11), Epicatechin gallate (15), 3-Methylplumbagin (14), Ethyl 1,3-dihydroxy-2-naphthoate (8). In *Jatropha*, the average has decreased, but there is also an increase in tailings concentration of 50% of routine compounds, while in *Reutealis*, the average has a dramatic increase in tailings concentration of 100%, but there is also a decrease in tailings concentration of 50%, data can be seen in Table 3. This shows that the higher the tailings concentration in the soil, the more it affects the plant's growth.

Flavonoids play a role in giving a yellow color to higher plants, so it is suspected that this group of compounds plays an important role in forming the yellow color of *Jatropha curcas* and *Reutealis*. In addition, flavonoids also act as natural antioxidants and anti-cancer. The benefits of flavonoids for plants are that they can influence plant defense responses against micro-organisms so that plants remain durable due to the production of nematodes in plant roots.

Rutin (9) is fragmented in the positive ion mode m/z 611$[M+H]^+$, 423$[M+H-C_{11}H_8O_3]^+$, 329$[M+H-C_{11}H_8O_3-C_6H_6O]^+$ and $[M+H-C_{11}H_8O_3-C_6H_6O-C_9H_6O_3]^+$. Kaempferol (8) is fragmented in the positive ion mode, m/z 273$[M+H]^+$, 193$[M+H-C_2H_4O]^+$, 177$[M+H-C_2H_4O-O]^+$, and 147$[M+H-C_2H_4O-OCH_2O]^+$ and Epicatechin gallate (13) undergo fragmentation in a negative ion mode, m/z 441 $[M-H]^-$, 289$[M-H-C_7H_4O_4]^-$, 271$[M-H-C_7H_4O_4-H_2O]^-$ and 169$[M-H-C_7H_4O_4-H_2O-C_8H_6]^-$. Apigenin (7) was identified as fragmented in the positive ion mode, m/z 271$[M+H]^+$, 253$[M+H-H_2O]^+$, 225$[M+H-H_2O-CO]^+$, and 211$[M+H-H_2O-COCH_2]^+$. (+)-dihydrokaempferol (10) was identified to undergo fragmentation in the negative ion mode m/z 288[M-H]-, 259$[M-H-CO]^-$, 243$[M-H-CO-O]^-$ and 215$[M-H-CO_2-CO]^-$. Palomi'd 529 (11) was identified as fragmented in the negative ion mode, m/z 405$[M-H]^-$, 390$[M-H-CH_3]^-$ and 358$[M-H-CH_3-CO-2H_2]^-$ Daidzein (12) was identified as fragmented in the positive ion mode m/z 255$[M+H]^+$, 214$[M+H-OH-C_2]^+$, 185$[M+H-OH-C_2-COH]^+$ and 172$[M+H-OH-C_2-COH-CH]^+$. 3-Methylplumbagin (14) was identified as experiencing fragmentation in the positive ion mode, m/z 203$[M+H]^+$, 188$[M+H-CH_3]^+$, 160$[M+H-CH_3-CO]$. Ethyl 1,3-dihydroxy-2-naphthoate (15) was identified as experiencing fragmentation in a positive ion mode, m/z 233$[M+H]^+$, 218$[M+H-CH_3]^+$, 190$[M+H-CH_3-CO-COH]^+$.

3.6.3. Phenolic acid groups.

Phenolic acid compounds identified in *Jatropha* and *Reutealis* extracts are Benzaldehyde (19), Gallic acid (16), Ellagic acid (17), and Methyl gallate (18). In

Jatropha, on average, there is an increase in band area in *tailings* of 50 and 100%, while in Reutealis, there is a decrease in tape area in *tailings* of 100%, but in *tailings* of 50%, there is an increase in band area in benzaldehyde compounds, data can be seen in table 3. This shows that the higher the *tailings* concentration in the soil, the more it affects the plant's growth.

The phenolic acid detected in the leaf extract is Benzaldehyde (16), detected in the positive ion mode, fragmented at m/z 107$[M+H]^+$, 79$[M+H-CO]^+$, 77$[M+H-CO-H_2]^+$. Gallic acid (17) has excellent pharmacological activity as an active anti-inflammatory ingredient, without toxic effects, treats inflammation, is very easily absorbed orally identified by negative ion mode, undergoes fragmentation at m/ z 169$[M-H]^-$, 125$[M-H-CO_2]^-$, 97$[M-H-CO_2-CO]^-$ and 81$[M-H-CO_2-CO-O]^-$. Ellagic acid (18) is identified with the positive ion mode. Experiencing fragmentation at m/z 300$[M+H]^+$, 283$[M+H-HO]^+$, 230$[M+H-HO-3(HO)-H_2]^+$. Methyl gallate (19) identified with negative ion mode, fragmented at m/z 183$[M-H]^-$168$[M-H-CH_3]^-$, 139$[M-H-CH_3-CHO]^-$ and 123$[M-H-CH_3-CHO-O]^-$.

3.6.4. Alkaloid groups.

The alkaloids identified on the leaves are 2-phenylethylamine (20). In *Jatropha* there is a decrease in band area per *tailings* concentration of 50 and 100%, but in *Reutealis* it has increased the band area at a *tailing* concentration of 100%. The data can be seen in Table 3. This shows that the higher the *tailings* concentration in the soil, the more it affects the plant's growth.

The alkaloid identified in the leaves is 2-phenylethylamine (20), which serves to analyze the ability to form tyramine by bacteria to evaluate the potential risk of tyramine biosynthesis in food products (Marcobal *et al*. 2022), helps to overcome the dopamine deficit that causes Parkinson's disease, identified with the positive ion mode, experiencing fragmentation at m/z 122$[M+H]^+$, 105$[M+H-NH_2]^+$ and 80$[M+H-NH_2-C_2]^+$.

3.6.5. Triterpenoid groups.

Terpenoid compounds identified in *Jatropha* and *Reutealis* extracts are Hemigossypolone (26), Betulinaldehyde (21), Betulinic acid (22), Marsformosanone (23), Pomolic acid (26), Lupeol (25) and Myristyl sulfate (24). In *Jatropha*, on average, there is a decrease in band area with every tailing's concentration of 50-100%; there is also an increase in the band area. While the average *Reutealis* experienced a decrease in band area with every *tailings* concentration of 50 and 100%, some also experienced an increase. The data can be seen in Table 3. This shows that the higher the *tailings* concentration in the soil, the more it affects the growth of the plant Tabel 5.

Terpenoids are formed from a combination of isoprene-isoprene consisting of IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl pyrophosphate). Terpenoid synthesis occurs through the fusion between the head and tail or the tail with the isoprene tail. Two IPPs combine to form monoterpenoids, IPP combines with DMAPP to form sesquiterpenoids, and two DMAPPs combine to form diterpenoids. Retro processes or terpenoid bond breaking can occur at the head and tail of isoprene, such as the synthesis process. The fragmentation or bond-breaking pattern tends to be the same as that of flavonoids, namely through the retro Aldol process, which is accompanied by condensation or termination of the H_2O group.

Hemigossypolone (21), identified as experiencing fragmentation in the negative ion mode, m/z 272$[M\text{-}H]^-$, 219$[M\text{-}H\text{-}CH\text{-}CH_2]^-$, Betulinic acid (23), identified as experiencing fragmentation with negative ion mode, m/z 455 $[M\text{-}H]^-$, 439$[M\text{-}H\text{-}O]^-$ and 421$[M\text{-}H\text{-}O\text{-}H_2O]^-$. Betulinaldehyde (22) was identified as experiencing fragmentation in a positive ion mode, m/z 441$[M+H]^+$, 315$[M+H\text{-}C_8H_{14}O]^+$ and $[M+H\text{-}C_8H_{14}O\text{-}COH]^+$, Marsformosanone (24) was identified as experiencing fragmentation in positive ion mode, m/z 423$[M+H]^+$, 405$[M+H\text{-}H_2O]^+$, 269$[M+H\text{-}H_2O\text{-}C_{10}H_{16}]^+$, 243$[M+H\text{-}H_2O\text{-}C_{10}H_{16}\text{-}C_2H_2]^+$, 229$[M+H\text{-}H_2O\text{-}C_{10}H_{16}\text{-}C_2H_2\text{-}CH_2]^+$. Pomolic acid (25) was identified as experiencing fragmentation in the positive ion mode, m/z 473$[M+H]^+$, 437$[M+H\text{-}O_2\text{-}2H_2]^+$ and 409$[M+H\text{-}O_2\text{-}2H_2\text{-}CO]^+$. Lupeol (26) was identified as experiencing fragmentation in the positive ion mode m/z 427$[M+H]^+$, 409$[M+H\text{-}H_2O]^+$, 229$[M+H\text{-}H_2O\text{-}C_{14}H_{12}]^+$ and 217$[M+H\text{-} H_2O\text{-}C_{14}H_{12}\text{-}C]^+$.

Table 5. Changes in the area to control area (%) leaf *Jatropha* and *Reutealis.*

Golongan Senyawa	***Jatropha***				***Reutealis***			
	T50		T100		T50		T100	
	up	down	up	down	up	down	up	down
Amino acids								
- *Leucine*	x	-48,98	X	-13,78	x	-94,56	96,54	X
- *Tryptophan*	x	-7,87	X	-90,82	x	-84,61	58,94	X
- *Phenylalanine*	x	-0,77	X	-6,66	x	-29,05	x	-30,16
Flavonoid								
- *Rutin*	30,44	x	32,52	x	x	-11,27	x	-4,22
- *(+)-dihydrokaempferol*	x	-99,95	X	-39,81	22,51	X	32,89	X
- *Ethyl 1,3-dihydroxy-2-naphthoate*	x	-99,19	X	-18,95	x	-5,69	94,67	X
Phenolics acids								
- *Gallic acid*	4,34	x	8,63	x	x	-17,37	x	-18,02
- *Ellagic acid*	16,18	x	7,73	x	x	-12,60	x	-4,54
- *Methyl gallate*	21,09	x	21,19	x	3,13	X	x	2,22
- *Benzaldehyde*	7,70	x	4,23	x	11,91	X	x	2,65
Alkaloid								
- *2-Phenylethylamine*	x	-38,47	X	-42,44	4,75	X	6,67	X
Triterpenoid								
- *Betulinaldehyde*	20,77	x	21,08	x	x	-10,90	x	-10,07
- *Lupeol*	28,41	x	X	-24,38		-25,75	x	-21,76
- *Marsformosanone*	x	-20,47	11,23	x	17,05	X	18,58	X
- *Myristyl sulfate*	x	-13,56	X	-91,21	10,99	X	16,17	X
Steroid								
- *Kaempferitrin*	70,33	x	86,97	x	16,45	X	x	-29,34
- *Rhodalin*	38,33	x	39,31	x	x	-19,52	x	-19,84
- *4,4-Dimethylzymosterol*	x	-86,92	47,01	x	15,12	X	14,51	X
- *3-Dehydroteasterone*	13,27	x	18,44	x	8,88	X	21,62	X

Ket: x = not up/down.

Table 6. Results of identification of secondary metabolite compounds of *Jatropha* and *Reutealis* leaves.

No	**Metabolite**	**Class chemical**	**RT (min)**	**Formula**	**MW**	**Error (ppm)**	**MS fragment ion (m/z)**	**Change in band area to control area (%)**			
								Jatropha		*Reutealis*	
								T50	T100	T50	T100
1	Valine	Asam amino	1.02	$C_5H_{11}NO_2$	117	-1,9	72, 56	-6,52	-4,44	-28,92	-28,35
2	Leucine	Asam amino	1.10	$C_6H_{13}NO_2$	131	-1,22	86, 120, 69, 103	-46,98	-13,78	-94,56	96,54
3	5-Aminopentanoate	Asam amino	1.23	$C_{15}H_{11}N0_2$	117	-0,85	118, 101, 70	-6,15	-2,61	-2,23	35,06
4	Phenylalanine	Asam amino	1.56	$C_9H_{11}NO_2$	165	-2,48	86, 120, 69, 103	-0,77	-6,66	-29,05	-30,16
5	Tryptophan	Asam amino	3.04	$C_{11}H_{12}N_2O_2$	204	-2,56	86, 120, 69, 103	-7,87	-90,82	-84,61	58,94
6	Ethyl 3,5-bis[(4-nitrobenzoyl) amino] benzoate	Asam amino	14.3	$C_{23}H_{18}N_4O_8$	478	-3,76	477, 205, 163	-9,05	-4,68	-85,83	48,11
7	Palomid 529	Flavonoid	1.13	$C_{24}H_{22}O_6$	406	-0,74	405, 390, 358, 357	-92,39	-41,12	-5,34	-4,31
8	Ethyl 1,3-dihydroxy-2-naphthoate	Flavonoid	4.54	$C_{13}H_{12}O_4$	232	-0,86	233, 218, 190,161	-99,19	-18,95	-5,69	94,67
9	(+)-dihydrokaempferol	Flavonoid	4.75	$C_{15}H_{12}O_6$	288	-1,39	288, 259, 243	-99,95	-39,81	22,51	32,89
10	Rutin	Flavonoid	7.22	$C_{27}H_{30}O_{16}$	610	-1,98	611, 423, 329, 167	30,44	32,52	-11,27	-4,22
11	Daidzein	Flavonoid	7.23	$C_{15}H_{10}O_4$	254	-2,76	255, 214, 185, 172	-91,18	-16,93	-10,38	-5,26
12	kaempferol	Flavonoid	7.62	$C_{15}H_{10}O_6$	286	-1,59	287, 253, 201	19,47	-19,37	-11,12	17,61
13	Apigenin	Flavonoid	8.25	$C_{15}H_{10}O_5$	270	-1,22	271, 253, 225, 211	9,39	13,00	10,46	32,94
14	3-Methylplumbagin	Flavonoid	9.74	$C_{12}H_{10}O_2$	202	-1,48	203, 188, 160	-8,04	-11,49	16,99	36,89
15	Epicatechin gallate	Flavonoid	14.9	$C_{22}H_{18}O_{10}$	442	-2,04	441, 289, 271, 169	4,51	34,91	-6,93	-35,30
16	Gallic acid	Asam fenolat	1.51	$C_7H_6O_5$	170	-5,52	169, 125, 97, 81	4,34	8,63	-17,37	-18,02
17	Ellagic acid	Asam fenolat	5.82	$C_{14}H_6O_8$	302	-0,6	300, 283, 230	16,18	7,73	-12,60	-4,54
18	Methyl gallate	Asam phenolate	8.03	$C_8H_8O_5$	184	-5,43	168, 139, 123	21,09	21,19	3,13	2,22
19	Benzaldehyde	Asam phenolate	16.9	C_7H_6O	106	0	107, 79, 77	7,70	4,23	11,91	2,65
20	2-Phenylethylamine	Alkaloid	5.36	$C_8H_{11}N$	121	0	122, 105, 80	-38,47	-42,44	4,75	6,67
21	Betulinaldehyde	Triterpenoid	5.71	$C_{30}H_{48}O_2$	440	-2,27	441, 315, 286	20,77	21,08	-10,90	-10,07
22	Betulinic acid	Triterpenoid	7.91	$C_{30}H_{48}O_3$	456	3,07	455, 439, 421	-25,26	-25,39	-9,79	-10,27
23	Marsformosanone	Triterpenoid	8.43	$C_{30}H_{46}O$	422	-3,32	423, 269, 243, 229	-20,47	11,23	17,05	18,58

No	**Metabolite**	**Class chemical**	**RT (min)**	**Formula**	**MW**	**Error (ppm)**	**MS fragment ion (m/z)**	**Change in band area to control area (%)**			
								Jatropha		*Reutealis*	
								T50	T100	T50	T100
24	Myristyl sulfate	Triterpenoid	9.64	$C_{14}H_{30}O_4S$	294	-1,3	-	-13,56	-91,21	10,99	16,17
No	Metabolite	Class chemical	RT (min)	Formula	MW	Error (ppm)	MS fragment ion (m/z)	Change in band area to control area (%)			
								Jatropha		*Reutealis*	
								T50	T100	T50	T100
25	Lupeol	Triterpenoid	10,0	$C_{30}H_{50}O$	426	-3,52	427, 409, 229, 217	28,41	-24,38	-25,75	-21,76
26	Pomolic acid	Triterpenoid	10.1	$C_{30}H_{48}O_4$	472	-2,54	473, 437, 409, 313	-88,82	13,38	8,40	3,53
27	Hemigossypolone	Triterpenoid	16,2	$C_{15}H_{14}O_5$	274	-2,55	272, 151, 125	-7,14	-90,72	-3,92	86,14
28	Kaempferitrin	Steroid	5.59	$C_{27}H_{30}O_{14}$	578	-2,18	-	70,33	86,97	16,45	-29,34
29	Rhodalin	Steroid	6.19	$C_{20}H_{18}O_{11}$	456	5,06	-	38,33	39,31	-19,52	-19,84
30	Rhoifolin	Steroid	6.30	$C_{27}H_{30}O_{14}$	362	-3,97	-	1,46	18,88	-16,20	17,98
31	3-Dehydroteasterone	Steroid	9.04	$C_{28}H_{46}O_4$	446	1,51	447, 351, 265	13,27	18,44	8,88	21,62
32	4,4-Dimethylzymosterol	Steroid	9.71	$C_{29}H_{48}O$	412	-2,91	413, 395, 241	-86,92	47,01	15,12	14,51
33	4α-Hydroxymethyl-4β-methyl-5αcholesta-8-en-3β-ol	Steroid	9.96	$C_{29}H_{50}O_2$	428	-2,56	429, 219, 191, 165	2,01	5,78	-13,48	-13,46

3.6.6. Steroid groups.

Steroid compounds were identified in *Jatropha* and *Reutealis* extracts of Sunan 3-Dehydroteasterone (31), 4,4Dimethylzymosterol (32), Rhodalin (29), Kaempferitrin (28) 4α-Hydroxymethyl-4β-methyl-5αcholesta-8-en-3β-ol (33) and Rhoifolin (30). At Jatropha, the average increase in band area at all tailings concentrations was 50 and 100%, while in Reutealis, the average experienced a decrease in the band area, but there was also an increase in band area at tailings concentrations of 50 and 100%. The data can be seen in Table 3. This shows that the higher the tailings concentration in the soil, the more it affects the plant's growth.

The steroid identified in the leaves is 4αhydroxymethyl-4β-methyl-5αcholesta-8-en-3β-ol (33), with a negative ion mode, fragmented at m/z 429[M-H], 219$[M\text{-}H\text{-}C14H_{25}\text{-}OH]^-$, 191$[M\text{-}H\text{-}C14H25\text{-}OH\text{-}C2H4]^-$ and 165$[M\text{-}H\text{-}C14H25\text{-}OH\text{-}C2H4\text{-}C2H2]^-$. 3-Dehydrotasterone (31) identified in the positive ion mode, fragmented at m/z $[M+H]^+$ 447$[M+H\text{-}C_7H_{12}]^+$ 351$[M+H\text{-}C_7H_{12}\text{-}C_5H_{10}O]^+$ 265. 4,4- Dimethylzymosterol (32) was identified in the positive ion mode, fragmented at m/z 413$[M+H]^+$, 395$[M+H\text{-}H_2O]^+$, and 241$[M+H\text{-}H_2O\text{-}C_9H_{18}\text{-}C_2H_4]^+$.

4. Conclusions

Tailings treatment of *J. curcas* and *R. trisperma* at concentrations of 50% and 100% resulted in a decrease in morphology and plant growth. The 100% *tailings* treatment had a greater negative impact on both plant species when compared to the 50% *tailings* treatment. Overall, the negative effect of *tailings* treatment on *R. trisperma* was lower when compared to *J. curcas*; this indicated that *R. trisperma* had higher adaptability than *J. curcas*. The content of metal elements contained in the leaves of both plants, namely Ca, K, Fe, Ti, Si, P, Mn, and Mg, in the leaves of *R. trisperma*, while in *J. curcas* namely, Ca, K, Fe, Ti, Mn, P, Si, Mg, and S There is a difference between these 2 plants in that *R. trisperma* does not contain metal sulfur, but *Jatropha* contains metal sulfur because the XRF detection limit is 50 ppm, sulfur in *R. trisperma* may be below 50 ppm or very small, identification of compounds in different treatments on gold-mine tailings from *J. curcas* and *R. trisperma* using UHPLC-MS/MS resulted in 33 Chemometric results revealed significant differences in the geographical location metabolites profiles, which *J. curcas* and *R. trisperma*.

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Conflicts of Interest

The authors declare no conflict of interest.

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