

# Phytoremediation of Mercury and Cyanide Contaminated Soils by Physic Nut (*Jatropha curcas* L.) and Citronella Grass (*Cymbopogon nardus*)

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## ABSTRAK

Merkuri dan sianida merupakan senyawa yang berpotensi mencemari lingkungan, keduanya terdapat dalam limbah tailing pertambangan emas rakyat. Tujuan penelitian ini untuk mengetahui kemampuan jarak pagar (*Jatropha curcas* L.) dan serai wangi (*Cymbopogon nardus*) dalam menyerap merkuri dan sianida di tanah tercemar limbah tailing berdasarkan nilai faktor transfer. Penelitian ini melakukan remediasi tanah tercemar tailing amalgamasi dan sianidasi selama 28 hari. Pengambilan sampel tanah dilakukan setiap 7 hari selama 28 hari, sampel akar dan daun dilakukan pada hari ke 28, analisis kandungan merkuri dan sianida pada tanah dan tanaman menggunakan spektrofotometer serapan atom (AAS) dan spektrofotometer UV-Visible. Setelah 28 hari remediasi, kadar merkuri dan sianida dapat menurun dalam tanah hingga 93,7% untuk merkuri dan 81,8% untuk sianida. Penurunan tersebut dapat disebabkan oleh penyerapan dan akumulasi pada tanaman, dimana akumulasi merkuri maupun sianida lebih banyak pada tanaman jarak pagar daripada serai wangi. Tanaman jarak pagar dan serai wangi memiliki nilai faktor transfer <1 terhadap merkuri dan sianida sehingga merupakan tanaman excluder, kecuali akumulasi merkuri pada tanaman jarak pagar dari tanah tailing sianidasi yang memiliki nilai faktor transfer >1 sehingga merupakan tanaman akumulator.

**Kata kunci:** jarak pagar, limbah tailing, merkuri, serai wangi, sianida

## ABSTRACT

Mercury and cyanide are compounds that have the potential to pollute the environment, both of which are found in the tailing waste of artisanal and small-scale gold mining (ASGM). The purpose of this study was to determine the ability of physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*) to absorb mercury and cyanide in soils polluted with tailings waste based on the value of transfer factors. During this research stage, the remediation of soil polluted with amalgamation tailings and cyanide tailings was carried out for 28 days. Soil sampling was carried out every seven days for 28 days, while root and leaf sampling was carried out on day 28, analysis of mercury and cyanide content in soil and plants using atomic absorption spectroscopy (AAS) and UV-visible spectrophotometer. After 28 days of remediation, mercury and cyanide levels may decrease in soil by 93.7% for mercury and 81.8% for cyanide. The decrease can be caused by absorption and accumulation in plants, where mercury and cyanide accumulate more in physic nut than citronella grass. Physic nut and citronella grass have a transfer factor value of <1 for mercury and cyanide, so they are an excluder plant, except for the accumulation of mercury in physic nut from cyanide tailings soil, which has a transfer factor value of >1, which is an accumulator plant.

**Keywords:** physic nut, tailing waste, mercury, citronella grass, cyanid

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## 1. INTRODUCTION

Gold is one of the most abundant natural resources in Indonesia. Gold mining activities can be carried out by communities traditionally. This traditional gold

mining activity uses available natural resources, such as processed gold, and is a communal mine with simple equipment. The process of gold ore extraction traditionally carried out by these societies uses

amalgamation and cyanidation. The method of amalgamating involves using mercury as a binder for the gold component during the extraction of the gold ore. Both methods produce gold and waste, commonly called tailing, that is left to mount in unmanaged environments (Adinata et al., 2015; Sumarjono et al., 2020).

Artisanal and small-scale gold mining (ASGM) tailings contain mercury and cyanide, which are dangerous substances. Mercury (Hg) is a toxic element that can damage ecosystems and even living creatures, including humans, animals, and plants (Mirdat et al., 2013). Mercury can evaporate and even be washed away by rainwater. It will enter and accumulate in the soil. Cyanide produced by gold mining activities is a hazardous substance, in addition to mercury, that can damage the environment. The cyanide ion (CN<sup>-</sup>) released by any substance is highly toxic. Without proper management, mercury and cyanide can accumulate mercury and cyanide in soil, which can hurt the soil around communal mines.

Mercury and cyanide can pollute water and soil, damaging and poisoning plants and animals nearby. If plants and animals that accumulate contaminants are consumed, and their water is used for community activities, these contaminants can hurt health. The harmful effects of mercury and cyanide on health include death, genetic abnormalities, damage to body tissue, and miscarriages (Puspita et al., 2019).

Hyperaccumulator plants can tolerate and accumulate contaminants in the soil so that they can be used in phytoremediation to improve the quality of soil that has been contaminated by tailing gold mining (Siahaan et al., 2014; Van Oosten & Maggio, 2014). Hyperaccumulator plants are plants that can tolerate and accumulate contaminants in the soil. The quality of water and soil can be affected by environmental degradation caused by mercury and cyanide in gold mining.

Plants that can be used for phytoremediation are physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*). These plants have hyperaccumulative properties and effectively absorb heavy metals in contaminated soil tailing waste. Furthermore, these two plants are widely distributed in Indonesia and require little maintenance.

The research conducted by Pranoto & Budianta (2020) revealed an intriguing fact: physic nut plants can accumulate a higher concentration of Pb than spinach plants. The Pb metal content in root physic nut plants was 81 ppm at first harvest and grew to 92 ppm at harvest time, demonstrating their potential for heavy metal absorption. Similarly, the study by Marrugo-Negrete et al. (2015) found that despite high amounts of Hg in the soil, physic nut crops showed growth and development, with a maximum accumulation value of 7.25 µg Hg/g in plants. Another interesting finding was from the research by Sulastri et al., (2019), which showed that citronella grass

absorbed a total of 20.29 ppm of Cd heavy metals, further highlighting its phytoremediation potential.

The accumulation of mercury and cyanide in plants can decrease the levels of mercury and cyanide in the soil. A study conducted by Juhriah and Alam (2016) found that soil remediation using *Celosia plumose* (Voss) Burv reduced the soil's mercury level by 81.25% to 98.68%. The ability of plants to accumulate contaminants is known from the value of the pollutant transfer factor from soil to plant. If the transfer factor value is more than 1, then the plant is an accumulator plant, whereas if the value is less than 1, then it is an excluder plant (Mariwy et al., 2020; Tang et al., 2019; Yulianti, 2021). Chang et al. (2014) showed that physic nut plants have transfer factor values >1 for Cd, Ni, and Cu metals, whereas for Cr, Zn, and Pb metals, the transfer factor value is <1. Israila et al. (2015) showed citronella grass had a transfer factor value >1 against Cd metals.

Left unmanaged, the accumulation of Artisanal and small-scale gold mining (ASGM) waste in the province of Banten could have a negative impact because of the accumulations of mercury and cyanide in the soil. Soil phytoremediation efforts should be carried out using a physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*). Then, the concentrations of mercury and cyanide in the soil and the plants were analyzed so that the ability of physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*) plants in the phytoremediation of contaminated soil tailing waste was known based on the value of the transfer factor.

## 2. METHODS

### 2.1. Plant Acclimatization

In this study, the first phase was the acclimatization of plants for ±14 days. At this stage, the physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*) adapted to the new growing medium, which is the uncontaminated soil in the polybag, with the addition of 1 gram of NPK fertilizer (16-16-16) to each polybag (Ratnawati & Fatmasari, 2018).

### 2.2. Soil Remediation

Four kilograms of plant media made from contaminated soil are a mixture of 70% of the soil from the Green House Integrated Laboratory Center UIN Syarif Hidayatullah Jakarta and 30% of tailing waste from the artisanal and small-scale gold mining (ASGM). The physic nut (*Jatropha curcas* L.) and citronella grass (*Cymbopogon nardus*) that have acclimatized are transferred to the polybag according to the combination of treatments contained in Table 1. An ammonium thiosulfate solution is added two weeks before harvest to stimulate the absorption of mercury in plants (Wang et al., 2013). Ligan is added in the form of a solution and waters the plant media without direct contact with the plant. Plant maintenance includes daily watering and checking the

pH and soil temperature. Remediation lasted 28 days. Soil samples were collected on days 7, 14, 21, and 28 to measure the amounts of cyanide and mercury. On day 28, roots and leaves were collected (Pratiwi et al., 2016).

### 2.3. Analysis of Mercury Levels in Soils and Plants

Mercury measurements in soil and plant using atomic absorption spectroscopy (AAS), which is calibrated by measuring mercury standard solutions with concentrations of 0, 2, 4, 6, 8, and 10 µg/L. The results of measurements using a standard solution were then obtained, and a standard curve was plotted, showing the correlation coefficient value of 0.9997. Mercury level analysis begins with the destruction of soil and plant samples to be analyzed. The sample weighing 1 gram is put into an Erlenmeyer of 250 mL, and then 10–15 mL of HNO<sub>3</sub> is added until the sample is soaked and stained for a night. Then, it was heated until the brown-colored nitrate vapor disappeared, and then it was cooled to room temperature. Then, we added 5 mL of HCl and 3 mL of H<sub>2</sub>O<sub>2</sub> to help with the process of sample destruction. Then, it was filtered and diluted using aquades for up to 50 mL. Prepare a solution of SnCl<sub>2</sub> as a reducer and aquades as an acid. Then, the capillary pipe for the sample is submerged into the test solution, and its absorption is immediately measured using CV-AAS at a wavelength of 253.7 nm. Then, the mercury level is calculated using the following formula (IK.LP-04.12-LT-1.0):

$$\text{Hg (mg/kg)} = \frac{V \times b}{W} \quad (1)$$

V is the sample volume, b is the mercury concentration read by the instrument, and W is the sample weight.

### 2.4. Analysis of Cyanide Levels in Soils and Plants

Cyanide measurements in soil and plant using UV-Vis spectrophotometer. Which is calibrated by measuring cyanide standard solutions with concentrations of 0,00, 0,005, 0,010, 0,020, 0,040, 0,080, 0,120, 0,160, and 0,200 ppm. The results of measurements using a standard solution were then obtained, and a standard curve was plotted, showing the correlation coefficient value of 0.9996. The analysis of the levels of cyanide in soil and plants began by dissolving the sample in NaOH at 0.1 N. The sample of 2 grams was dissolved in 100 mL of NaOH and then homogenized using a water bath shaker for 1 hour. After dissolving with NaOH, a sample solution

of 20 mL is piped into a 50 mL volumetric flask, and then 0.5 mL of acetate buffer, 1 mL of T chloramine, and 2.5 mL of barbiturate-pyridine acid are homogenized. The aquades are added to the volumetric flask to the threshold, and then the solution is inhaled for 8 minutes. After that, the absorption values of the sample and blank are read using a UV-Vis spectrophotometer at a wavelength of 578 nm. Once the concentration is obtained, the cyanide level is calculated using the following formula (IK.LP-03.25-LT.1.0):

$$\text{CN (mg/kg)} = \frac{A \times B}{C} \quad (2)$$

A is the cyanide concentration read by the instrument, B is the sample volume, and C is the sample weight.

### 2.5. The Transfer Factor Value

The ability of plants to accumulate pollutants is known from the value of the transfer factor, where a value greater than 1 is an accumulator plant, while a value smaller than one is an excluder plant (Mariwy et al., 2020; Yulianti, 2021). The transfer factor value (TF) is calculated using the following formula:

$$\text{TF} = \frac{\text{Hg or CN in plants (mg/kg)}}{\text{Hg or CN in soil (mg/kg)}} \quad (3)$$

## 3. RESULTS AND DISCUSSION

### 3.1. Soil and Plant Conditions during Remediation

The pH and temperature observations for 28 days of phytoremediation on contaminated soil are shown in Table 2.

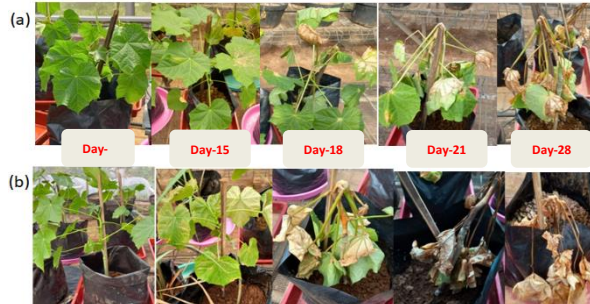
The optimum temperature range for the growth of physic nut plants is 20–26 °C. Too high (>35°C) or too low (<15°C) of temperature will inhibit the growth of jatropha plants. At the same time, citronella plants have an optimal temperature range of 20–30 °C and can tolerate temperatures ranging from 16–36 °C (Abobatta, 2019; Kaur et al., 2021). The average temperature of the plant medium for 28 days of remediation ranges between 29 and 33°C. The temperature of the plant medium during the study is still in the range that allows the physic nut or citronella plants to grow and survive so that the temperature does not inhibit the plant's growth or cause disruption. According to Priherdityo et al. (2016), soil temperatures that are too high can affect plant growth and development as they can rapidly produce root dryness due to water loss from plant tissue.

**Table 1.** Combination of Soil Remediation Treatment

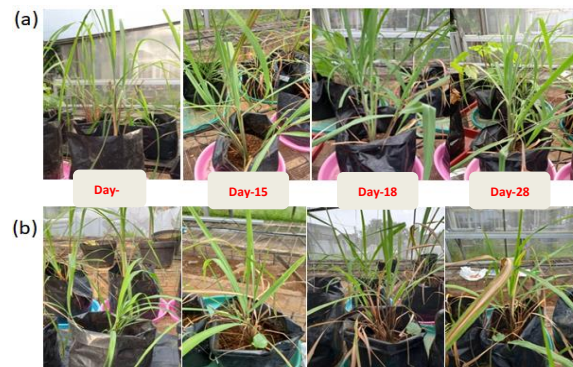
Code	Description
T0L1	Amalgamation tailing
T0L2	Cyanide tailing
T1L1	Physic nut + amalgamation tailing + 4 g ammonium thiosulfate kg <sup>-1</sup> medium
T1L2	Physic nut + cyanide tailing + 4 g ammonium thiosulfate kg <sup>-1</sup> medium
T2L1	Citronella grass + amalgamation tailing + 4 g ammonium thiosulfate kg <sup>-1</sup> medium
T2L2	Citronella grass + cyanide tailing + 4 g ammonium thiosulfate kg <sup>-1</sup> medium

**Table 1.** Soil pH and Temperature

No	Treatment	pH				Temperature(°C)			
		Day				Day			
		7	14	21	28	7	14	21	28
1.	T1L1	5,6	5,6	5,4	5,0	32,14	32,57	32,29	33,14
2.	T2L1	5,6	5,6	5,5	5,0	32,86	32,71	33,43	32,14
3.	T1L2	5,5	5,6	5,4	5,0	31,86	32,29	29,57	32,86
4.	T2L2	5,5	5,4	5,6	5,3	32,00	32,86	33,43	32,14



**Figure 1.** Growth of Jatropha Plants during 28 Days of Phytoremediation (a) T1L1 (b) T1L2



**Figure 2.** Growth of Citronella Grass during 28 Days of Phytoremediation (a) T2L1 (b) T2L2

The condition of the physic nut (*Jatropha curcas*) on the amalgamation tailing soils (T1L1) and the cyanide tailing soils (T1L2) during the 28 days of remediation can be seen in Figure 1. The condition of the citronella grass (*Cymbopogon nardus* L.) on the amalgamation tailing soils (T2L1) and the cyanide tailing soils (T2L2) during the 28 days of remediation can be seen in Figure 2.

The physic nut will grow well if the acidity (pH) of the soil is about 5.5–6.5 (Kaur et al., 2021). An optimal pH range for plants helps prevent bacterial infection of the roots in addition to supplying nitrogen in the growing medium, which can ultimately result in increased harvest yields (Abobatta, 2019; Priherdityo et al., 2016). The medium planted on all sample treatments after observation for 28 days had soil pH values that belonged to the soil's optimal pH range for the growth of physic nut and citronella grass until the 14th day of remediation and decreased its pH after the addition of ammonium thiosulfate, which became smaller than the optimal pH range. This can be one of the factors inhibiting the growth of plants characterized by physic nut plants, citronella grass, and leaves undergoing chlorosis and yellowing, as shown in Figures 1 and 2.

When the metal is transferred to other parts of the plant, the concentration on the roots decreases and

accumulates on the leaf parts (Mariwy et al., 2020; Zulfikah et al., 2014). Metals can enter cells and stick to the catalytic enzymes, causing chemical reactions to be disrupted in cells as a result of mercury's interaction with the sulphhydryl (-SH) group, which is the active side of the enzyme. As a result, plants may experience conditions that cause necrosis and chlorosis of the spongy epidermal tissue, as experienced by plants in this study (Arisusanti & Purwani, 2013).

The Mg element, the macro element of the chlorophyll molecule, has been replaced by mercury (Hg) so that when the concentration of mercury increases, the level of chlorophyll decreases. Figure 3 shows the interaction of chlorophyll with mercury (Hg), which can affect nutrients such as magnesium (Mg), resulting in a change in the color of the leaf (Borolla et al. 2019; Kilikily et al., 2020).

These Mg elements are easily replaced by other cations, so a small amount of heavy metal absorbed can easily replace Mg in chlorophyll. The structure of chloroplasts is highly dependent on nutrients such as magnesium, so as the concentration of mercury (Hg) increases, the intake of nutrients will decrease, resulting in damage to the structure of the chloroplast by causing green matter in the stems and leaves to

undergo chlorosis, yellowing, and losing its green color (Borolla et al. 2019; Kilikily et al.i, 2020).

### 3.2. Mercury Concentration in Soil and Plants

The material is first destroyed using a wet destruction method before being tested for mercury levels using AAS (Anggraini et al., 2018). Destruction is used to break the bond between the organic chemical and the metal being analyzed into its elements so that it can be analyzed. Soil and plants are destroyed using a mixture of nitric acid and chloric acid to produce compounds of nitrosyl chloride and chlore, which are powerful oxidators. In addition to the acid, the high temperature will increase the average kinetic energy (EK), thus increasing the impact between the acid and the sample. Increased oxidation can be achieved with the addition of powerful oxidants, such as peroxide solutions, to obtain perfect destruction results (Amalia et al., 2020). Here's the reaction that happened during the destruction (Suci et al., 2020):

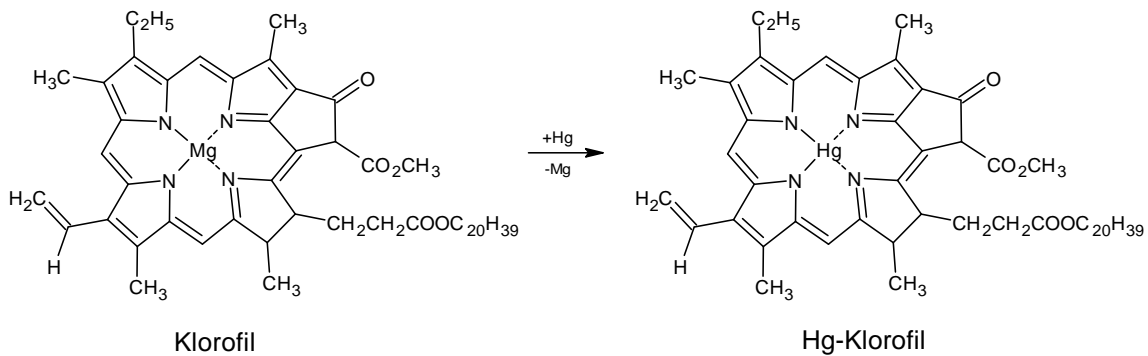
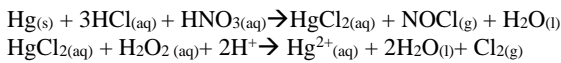
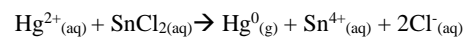


Figure 3. The Interaction of Chlorophyll with Mercury (Hg)

The reduction is required as part of the preparation process when measuring metal Hg with an atomic absorption spectrophotometer. The reducer converts the positive Hg element into an uncharged or neutral Hg in the form of Hg vapor gas, which is then propelled by the N<sub>2</sub> gas towards the absorptive atomic photometry spectroscopic cell, where it interacts with the light from the Hg cathode lamp. The interaction manifests as light absorption, which appears on the screen as absorbance (Prasetiawati et al., 2022; Rohaya et al., 2017). SnCl<sub>2</sub> solutions act as reducers and aquadest as acids. The sample measured its absorption using CV-AAS at a wavelength of 253.7 nm. The reaction between Hg and SnCl<sub>2</sub> solution is



#### 3.2.1. Mercury Concentration in Amalgamation Tailings Soil

Mercury levels in the amalgamation tailing soil per 7 days for 28 days of phytoremediation with physic nut (T1L1) and citronella grass (T2L1) are presented in Figure 4.

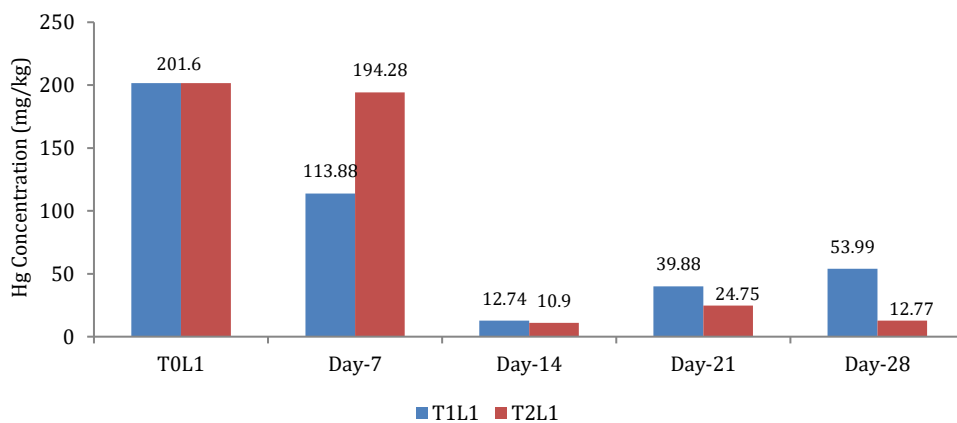


Figure 4. Mercury Concentration in Amalgamation Tailing Soil

The decreasing concentration of heavy metals like mercury in the soil can be caused by the transfer of metals through diffusion and osmosis, where there is a transfer of mass of matter from high-concentration media (ground) to low-concentration media (plants) (Ratnawati & Fatmasari, 2018). On the 14th day, the concentration of mercury decreased considerably, which meant that the plant's quantity increased, thereby increasing the toxicity of the plant. As a result, plants experience symptoms of chlorosis and necrosis that can affect physiological processes so that there is exudation of the roots, which is the exudation of the cell content carried out by plants to draw soil organisms around the burning that can help the process of phytoremediation. This root exudation process causes the mercury to be re-released by plants and sink into the soil so that the concentration of mercury in the soil on the 21st day increases (Kilikily et al., 2020).

Ammonium thiosulfate serves to initiate the absorption of mercury by plants (Wang et al., 2012). Pratiwi et al. (2016) reported that in comparison to remediation without the addition of ligands, there was an increase in mercury accumulation in *Lindernia crustacea* roots by 15% and 11% and in shoots by 61% and 27% with the addition of 4 g and 8 g of ammonium thiosulfate. The increase in such accumulation is because mercury (II) is a soft Lewis acid that easily forms a complex with soft Lewis bases like S-reduced ligands (Wang et al., 2013). Increased accumulation of Hg, especially in the treatment of the addition of ammonium thiosulfate, occurs because mercury has a strong affinity with the thiol groups, especially sulfide and bisulfide complexes (Pratiwi et al., 2016; Sugiono et al., 2014).

Decreases in soil concentration can occur again, related to the plant's ability to reduce toxicity or tolerance to heavy metals (Patandungan et al., 2016). The physic nut and citronella plants develop tolerance to heavy metals by increasing phytochelatin synthesis. If the plant does not produce phytochelatin, then it will

result in death. Phytochelatin works to form a complex compound with heavy metals in the plant's body and then transport it to the cells by active transport, which serves as plant detoxification from the heavy metal. Phytochelatin binds to Hg and forms sulfide bonds at the sulfur ends of the Sistine so that plants tolerate heavy metals and can survive and reabsorb the mercury metal in the soil (Mariwy et al., 2020; Patandungan et al., 2016; Raya & Rahmah, 2012).

### 3.2.2. Mercury Concentration in Cyanidated Tailings Soil

Mercury levels in the cyanide tailing soil per 7 days for 28 days of phytoremediation with physic nut (T1L1) and citronella grass (T2L1) are presented in Figure 5. It shows that the mercury concentration in the soil decreases after phytoremediation using physic nut and citronella plants, where the mercury concentration before remediation was 40.48 mg/kg. After 28 days of remediation, the amount decreased to 7.85 mg/kg in T2L2.

In the remediation treatment using a physic nut plant, the mercury level in the soil increased until the 14th day. This can be caused by the increased toxicity of plants, so that root exudate occurs, which is the excision of the cell contents carried out by plants to attract soil organisms around the field, which can help the phytoremediation process. This root exudation process causes the mercury to be re-released by plants and sink into the soil, increasing the mercury concentration in the soil (Kilikily et al., 2020). The accumulation of Hg in soil is associated with the deposit of anthropogenic activity through the biosphere or atmosphere. Mercury is a volatile metal that can easily evaporate into the air or atmosphere and then be reabsorbed back into the soil, thereby increasing the mercury concentration in the soil (Hindersah et al., 2018).

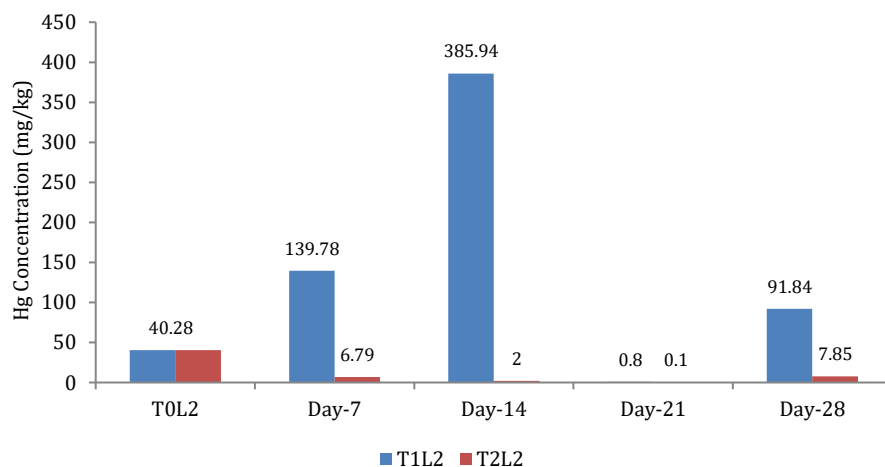


Figure 5. Mercury Concentration in Cyanide Tailings Soil

Ammonium thiosulfate functions to initiate mercury absorption by plants (Wang et al., 2012). The effect of adding ammonium thiosulfate is visible on the 21st day when mercury levels in the soil decrease significantly. This is also demonstrated by the fairly significant change in leaf color in the fence and scented plants that became pale, yellow, and brown after adding ammonium thiosulfate. The increase in the accumulation of mercury in plants increases the toxicity of plants, which is characterized by the condition of the plants becoming more pale, and brown leaves can cause the occurrence of exudate roots and the return of mercury into the soil as a plant defense reaction against the toxicity of mercury so that the mercury levels in the soil can rise again.

On the 28th day, the amalgamation tailings soil's mercury levels dropped by 73,2% and 93,7%, respectively, in both treatments utilizing citronella and jatropha plants. Only in the treatment with citronella plants did mercury levels in the cyanide tailings soil drop from the control on day 28, specifically by 80,6%. This indicates that mercury levels disappear more in the amalgamation tailings than in the cyanide tailings soil. Decreased levels of mercury in the soil can be caused by plant absorption, inland decomposition, evaporation of mercury from the soil, and mechanisms of plant decommissioning such as phytodegradation, phytovolatilation, and phytoextraction (Cristaldi et al., 2017; Kilikily et al., 2020; Zulfikah et al., 2014).

### 3.2.3. Mercury Concentration in Plants

Leaves can also absorb metals from plants through the stomata through adsorption processes where there is a cohesive (attractive) between external leaf tissue and the metals present in the environment (Mariwy et al., 2020). According to Puspita et al. (2014), the roots, leaves, and stomata are the organs in which the absorptive process occurs. When Hg is taken by the plant from the soil, about 80% is bound to the cell walls of the root to avoid toxic effects on the top of the plant. In addition, the plant leaves can absorb Hg from the atmosphere and indirectly from the ground through Hg vapor, which is gradually released from the earth. Through cell membranes, plants can take ions from their surroundings. Because of these characteristics, plants can accumulate heavy metals to a certain concentration or even increase the amount of metal ions in the medium (Chen & Yang, 2012; Marrugo-Negrete et al., 2016).

The mercury levels obtained in plants (roots and leaves) on the 28th day of phytoremediation

with physic nut (T1L1 and T1L2) and citronella grass (T2L1 and T2L2) are presented in Table 3.

The total concentration of mercury in plants mostly occurred in physic nut plants with an amalgamation tailing soil medium (T1L1) of 80,31 mg/kg, followed by treatment with a cyanide tailing medium (T1L2) of 40,7 mg/kg, then in citronella with an amalgamation tailing medium of 17,33 mg/kg (T2L1), and the least in citronella with a cyanide tailing medium of 16,19 mg/km (T2L2). Cyanide tailing contains two contaminants, mercury and cyanide, and concentrations tend to be lower than amalgamation tailing, which contains only mercury contaminants. This suggests that mercury absorption by plants is more effective if the contamination in the growing medium is not more than one contaminant.

From Table 3, it is also known that the most accumulated mercury is in the root; since the roots of the plant are the parts of it that are in the soil, the mercury that contaminates the soil is immediately attached to them by previous research that resulted in the highest concentration of mercury in the root part (Dulanlebit et al., 2021; Marrugo-Negrete et al., 2015; Marrugo-Negrete et al., 2016). In addition, mercury (Hg) is included in the metals that are slowly translocated to other parts of the plant. When Hg is taken by the plant from the soil, about 80% is bound to the cell wall of the root to avoid toxic effects on the top of the plant, especially the development of necrosis and chlorosis on the leaves (Anugroho et al., 2020; Marrugo-Negrete et al., 2016). The largest mercury concentration in these roots indicates that the physic nut and citronella plants have phytoremediation techniques that use phytostabilization mechanisms in the absorption and buildup of mercury. At phytostabilization, pollutants are mobilized and accumulated in the root system through root absorption (Ali et al., 2013).

Physic nut plants accumulate the most mercury compared to citronella plants, which can also be seen in the plant conditions during phytoremediation, where the physic nut undergoes chlorosis on its entire leaves and is also more flattened. However, the least mercury remaining in the growing medium is found in citronella plants. It is related to the mechanisms of mercury decomposition plants carry, such as phytodegradation, phytovolatilation, and phytoextraction. In addition, plant irrigation can also remove mercury from plants because the residue of water that plants do not house also carries mercury, affecting the mercury concentration in the soil (Kilikily et al., 2020; Zulfikah et al., 2014).

**Table 3.** Mercury Concentration in Plants on the 28<sup>th</sup> Day

Treatment	Hg Concentration (mg/kg)		
	Root	Leaf	Total
T1L1	80,21	0,1	80,31
T2L1	7,47	9,86	17,33
T1L2	37,18	3,52	40,7
T2L2	16,16	0,03	16,19

Ammonium thiosulfate improves soil quality by increasing microbial activity, cation exchange capacity, moisture retention, aeration, and nutrient capacity for plant growth (Dadashi et al., 2019). The analysis characterizes these results on treatments T2L1 and T2L2, where plants only absorb mercury slightly and have small mercury residues in the soil. In addition to the mercury evaporative properties or the mechanism of the decomposition of mercury by plants that can be a factor in the loss of mercury levels, soil can also perform such decommissioning. According to Cristaldi et al. (2017), the natural presence of microorganisms in the soil is capable of degrading and reducing organic and inorganic contaminants. The degradation by microorganisms in the soil is commonly called photodegradation. Rhizodegradation is the degradation of organic contaminants in soil areas called the rhizosphere.

Phytochelatin is essential to cope with higher levels of heavy metals in plants by binding them in complexes and absorbing complexes inside their cells. When the plant absorbs the metal, the roots produce phytochelatin, a regulating protein that acts as a binding agent. According to Arisusanti and Purwani (2013), phytochelatin is a peptide with 2–8 amino acids: cysteine as its central component and glutamate acid and glycine.

If the plant does not produce phytochelatin, then it will result in death. Phytochelatin works to form complex compounds with heavy metals in the plant's body and then transport them to the cells by active transport, serving as plant detoxification of heavy metals. In order to reach the cell surface, phytochelatin first develops inside the nucleus and then moves through the endoplasmic reticulum, the Golgi tract, and the secretory vesicles. Hg is transported or translocated into plant tissues through transportation tissues, mainly xylem and phloem; by the way, phytochelatin binds to Hg and forms sulfide bonds at the sulfur ends of cysteine so that complex compounds are formed, as shown in Figure 6 (Kilikily et al., 2020; Mariwy et al., 2020).

In plants, mercury causes damage to enzymes, polynucleotides, and the nutrient transport system and disrupts the integrity of cell membranes (Moldovan et al., 2013). Symptoms of mercury toxicity, in general, are the inhibition of seed and root

growth and the suppression of photosynthesis, which in turn reduces plant production. In addition, mercury accumulated in the plant's roots can inhibit K (calium) absorption by the plant. Mercury absorbed by plants can cause some enzymes to become inactive due to mercury fusion into sulfhydryl peroxide through the formation of reactive oxygen genes compounds, such as superoxide, hydroxyl radicals, and hydrogen peroxides (Chen & Yang, 2012; Ustiatik et al., 2020).

The interaction of mercury with the sulphihydril (-SH) group, which is the active side of the enzyme, can disrupt the chemical reaction in the cell. Mercury can enter cells and stick to enzymes that act as catalysts. (Arisusanti & Purwani, 2013; Kilikily et al., 2020). Mercury interferes with plant metabolism due to the high affinity of Hg to the sulfhydryl group, which is capable of strongly binding the amino acids of several proteins and enzymes. For example, Hg stress can inhibit the activity of NADPH, affecting the biosynthesis of chlorophyll. In addition, Hg produces inhibition of aquaporin, a protein that is involved in water transport within plants and reduces plant growth. Some metabolic processes, namely photosynthesis and respiration, are also affected by the absorption of Hg by plants (Chen & Yang, 2012; Marrugo-Negrete et al., 2016).

### 3.3. Cyanide Concentration in Soil and Plants

Soil and plant samples are dissolved in the NaOH solution. NaOH absorbs the cyanides in the sample. The acetate buffer functions to regulate the pH stable at pH 7. It is believed that chloramine-T reacts at a pH  $\leq 8$ , forming cyanogen chloride (CNCl) when reacting with a cyanide ion. Cyanogen-chloride added with barbiturate-pyridine acid produces a glossy compound, which is then measured for absorption using UV-Vis spectroscopes at 578 nm. The intensity of color is relative to the quantity of cyanide in the solution (Arisanti et al., 2018; Salimon et al., 2012).

#### 3.3.1. Cyanide Concentration in Cyanidated Tailings Soil

Cyanide levels in the cyanide tailing soil per 7 days for 28 days of phytoremediation with physic nut (T1L1) and citronella grass (T2L1) are presented in Figure 7.

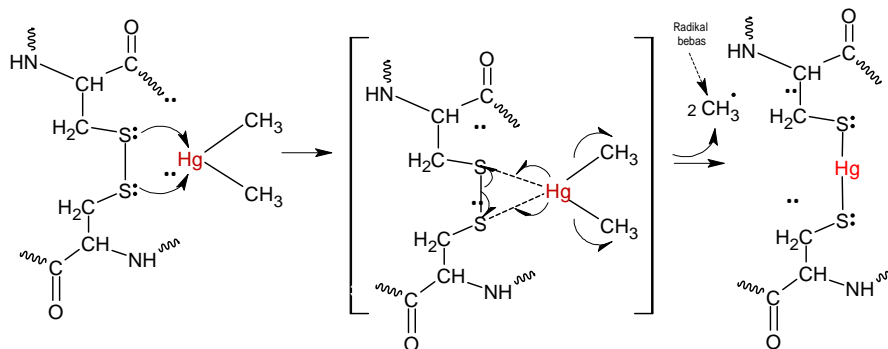


Figure 6. Reaction of Cysteine with the Compound Dimethyl Mercury



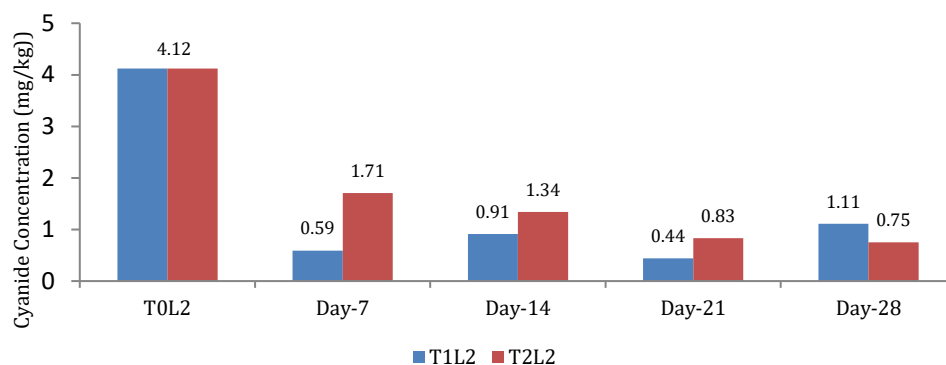


Figure 7. Cyanide Concentration in Cyanidation Tailings Soil

Based on Figure 7, it is known that the concentration of cyanide in the soil decreased after phytoremediation using physic nut and citronella plants, where the cyanide concentration before remediation was 4.12 mg/kg. After 28 days of remediation, the amount decreased to 1.11 mg/kg in T1L2 and 0.75 mg/kg in T2L2. The results of the analysis of the concentrated cyanides in the medium cyanide tailing soil with physic nut plants per 7 days for 28 days of observation fluctuated.

The decreased concentration of cyanide in the soil can be caused by diffusion and osmosis, where there is a transfer of mass of material in the medium from a high concentration (soil) to a low concentration (plants). On the seventh day, there is a decrease in the concentration of cyanides in the soil, with the greatest quantity compared to other days. This is due to the process of transferring the cyanide and the ability of plants to absorb it. There is a fairly large difference between the two media because the cyanide in the soil has high values before remediation while having low values in plants. As a result, plants can absorb the maximum amount of cyanide from the soil in the first week. Absorption is lower from the 14th to the fourth day due to the presence of more cyanides in plants, or it can be said that the toxicity of plants has increased (Ratnawati & Fatmasari, 2018). The increased levels of cyanide in the soil can be caused by the occurrence of root exudate, which is the release of cell content by plants to attract soil organisms around the field, which can help the phytoremediation process. The root exudation process causes the cyanide to be re-released by the plant and sink into the soil, increasing the concentration of cyanides in the soil (Kilikily et al., 2020).

Cyanide levels in cyanide tailing soil decreased from control on the 28th day, both in treatment using physic nut and citronella plants, decreasing by 73% and 81.8%, respectively. Decreased cyanide levels in soil can be caused by plant absorption, inland decomposition, and the evaporation of cyanide from the soil (Cristaldi et al., 2017; Kilikily et al., 2020; Zulfikah et al., 2014). Soil bacteria and fungi have several biochemical pathways for cyanide degradation and/or assimilation, and these organisms

may opportunistically use cyanides as a source of nitrogen (Yu, 2015).

Soil particles do not bind cyanide well; it has great potential to harm plants and other soil inhabitants. Soil microorganisms convert cyanides into hydrogen cyanides and other chemicals at low concentrations, evaporating from the soil. However, at high concentrations, the cyanide is toxic to microbes that usually turn it into a form of evaporation. As a result, cyanide not only stays in the soil, which can harm plants, but can also easily enter groundwater (Kumar et al., 2017).

### 3.3.2. Cyanide Concentration in Plants

The total accumulation of cyanide in plants is highest in physic nut (T1L2), 2.38 mg/kg, while citronella plants (T2L2) accumulated cyanide of  $\pm 2.21$  mg/kg. Table 4 shows that physic nut accumulates slightly more cyanide than citronella plants. In physic nuts, cyanide accumulates more in the leaves (1.62 mg/kg) than in the roots (0.76 mg/kg). In the citronella plant, cyanide is more accumulated in the roots (2.21 mg/kg) than in the leaves (<0.1 mg/kg). This can also be seen in the conditions of the physic nut, whose leaves are all yellowed and splintered, while the citronella plant still has green and fresh leaves.

Plants have different abilities for tolerating and accumulating pollutants like cyanide. In this study, the physic nut absorbs the cyanide through the roots, distributes it, and accumulates it in the leaves without holding the contaminants in the root to prevent the toxicity from spreading to other parts of the plant's body. The largest accumulation of cyanide on these leaves suggests that the physic nut has phytoremediation techniques that use phytoextraction mechanisms in the absorption and buildup of the cyanides. While the citronella absorbs and tries to contain pollutants so as not to be channeled into the bodies of other plants, such as leaves, to accumulate at the roots, The largest accumulation of cyanide in the root suggests that the plant has a phytoremediation technique that uses a mechanism of phytostabilization in the absorption

and accumulation of the cyanides (Ali et al., 2013; Cristaldi et al., 2017).

Once the root absorbs, the cyanide can remain unchanged in its original form, bind to other compounds, or be modified through degradation, detoxification, or assimilation. Phytotoxic effects on plants will only appear when the cyanide species' assimilation rate is much slower than the accumulation rate. The transformation and assimilation of cyanide in plants are most likely through specified pathways, such as the  $\beta$ -cyanoalanine pathway in vascular plants. It is known that CN can move through the root membrane with simple diffusion and eventually transform into asparagine through  $\beta$ -cyanoalanine pathways in plants (Au et al., 2018; Yu et al., 2011).

The transformation and assimilation of cyanide in plants is generally done through the following three pathways:  $\beta$ -cyanoalanine, sulfur transferase, and formamide hydrolase. All vascular plants have the enzyme  $\beta$ -cyanoalanine synthase, which is the primary enzyme for catalyzing the detoxification of cyanides in their metabolism. At the first step of metabolism (Figure 8), hydrogen sulfide and  $\beta$ -cyanoalanine are produced from HCN and cysteine precursors by the enzyme  $\beta$ -cyanoalanine synthase. Enzymes with nitrilase and nitrile hydratase activity mediate the next step. The activity of nitrilase results in the formation of asparagine, while nitrile hydratase forms aspartate and ammonia (Au et al., 2018; Flematti et al., 2013; Kumar et al., 2017; X. Z. Yu, 2015).

The second pathway in plants for cyanide assimilation is through the sulfur transferase pathway, also called the rhodanese pathway, converting cyanides (CN<sup>-</sup>) into the less toxic form of the thiocyanate (SCN<sup>-</sup>) under the catalysis of the enzyme rhodanese (Au et al., 2018; Yu, 2015).

Cyanide metabolism through the formamide hydrolase pathway is shown in Figure 9. The cyanide in the path was first converted to formamide under the enzyme formamide hydrolase catalysis and then produced the formation of formamide acid and ammonia with formaldoxime as a compound between them (Yu, 2015). Formamide can also be converted to formamide and ammonia through direct hydrolysis under the enzyme formamide amidohydrolase catalysis (Au et al., 2018; Yu, 2015).

The phytotoxicity of cyanide is seen to have many effects on plants, ranging from decreasing growth rates (growth rates depend on transpiration rates, chlorophyll content, biomass, etc.) to inhibition of plant enzymatic pathways. In the case of cyanide accumulation, although roots are generally the primary site for cyanide accumulations, different types of cyanide in plants tend to be different because different types are likely to have different distribution patterns in plant material. In terms of phytotoxicity of cyanides in plants, cyanide is known to cause disturbances in plant metabolism primarily through binding to sulfur, iron, and copper-containing enzymes and proteins or blocking the mitochondrial electron transport system through the formation of complexes with iron in the cytochrome (Au et al., 2018).

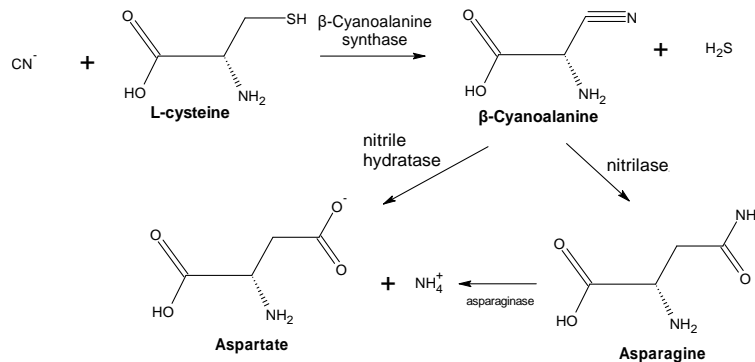


Figure 8.  $\beta$ -cyanoalanine Pathway

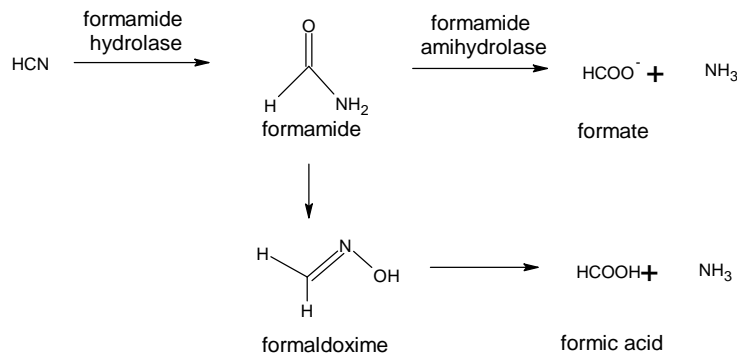


Figure 9. Formamide Hydrolase Pathway

### 3.4. The Transfer Factor Value

The ability of plants to absorb and accumulate mercury and cyanide from contaminated waste soil can be seen from the value of the transfer factor, which is obtained from the ratio of the concentration of mercury or cyanide in plants to the concentration of mercury or cyanide in the soil. The mercury concentration in amalgamation tailings soil was 201.6 mg/kg, with mercury concentrations in jatropha and citronella plants at 80.31 mg/kg and 17.33 mg/kg, respectively. Meanwhile, the mercury concentration in cyanide tailings soil was 40.48 mg/kg, with mercury concentrations in jatropha and citronella plants of 40.7 mg/kg and 16.19 mg/kg, respectively. Therefore, physic nut and citronella plants have a transfer factor value <1 in the accumulation of mercury from the contaminated soil of tailing waste, so they are an excluder plant, i.e., pollution accumulations occur mainly in the roots because they limit the transfer of pollutants from root to leaf as an adaptive mechanism to the pollutant. However, on the mercury in the cyanidation tailing soil, the physic nut plant has a transfer factor value >1, so it is an accumulator plant that accumulates mercury in the plant tissue in large quantities, even exceeding the amount found in the soil, where this physic nut plant accumulates the most mercury at the roots (Mariwy et al., 2020; Sidauruk & Sipayung, 2015; Tang et al., 2019).

Mercury (Hg) belongs to the metals that are slowest to be translocated into other parts of the plant. When Hg is taken by the plant from the soil, about 80% is bound to the cell walls of the root to avoid toxic effects on the top of the plant, especially the development of necrosis and chlorosis on the leaf (Anugroho et al., 2020). In a study, Marrugo-Negrete et al. (2015) stated that the highest concentrations of Hg accumulate in the root, followed by leaves and stems on the physic nut plant. In addition to mercury (Hg), the metals Pb and Cd are also included in metals that are slowly translocated into other parts of the plant. According to a study by Ultra et al. (2022), which stated that citronella accumulates the highest Pb and Cr metals in the roots.

The cyanide concentration in cyanide tailings soil was 4.12 mg/kg, with cyanide concentrations in jatropha and citronella plants at 2.38 mg/kg and 2.31 mg/kg, respectively. Therefore, physic nut and citronella plants have a transfer factor value <1 in the cyanide accumulation from contaminated soil waste tailing, so they are plant excluders. This is by Au et al. (2018), which state that the roots are generally the primary site for cyanide accumulation. Excluder prevents pollutants from entering the top of the plant at a low and constant metal concentration at various metal concentrations in the medium, limiting the pollutant in its roots (Khalid & Ganjo, 2021).

## 4. CONCLUSION

Mercury levels decreased in soil polluted with amalgamation tailings waste in both jatropha and

citronella treatments with a decrease of 73.2% and 93.7% respectively. Meanwhile, mercury levels decreased in soil polluted with cyanidation tailings waste only when treated with citronella plants, with a decrease of 80.6%. In cyanided tailings soils, cyanide levels decreased in both jatropha and citronella treatments, decreasing 73% and 81.8%, respectively. Physic nut (*Jatropha Curcas* L.) and citronella grass (*Cymbopogon Nardus*) have a transfer factor value of <1, so they are excluder plants in accumulating mercury and cyanide, except for jatropha plants with cyanidation tailings soil media, which have a transfer factor value of >1 so that they are accumulator plants in accumulating mercury.

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