Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.2.001382

RESEARCH ARTICLE

Heavy Metal Mercury Accumulation Using Taro Plant (Colocasia esculenta)

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ARTICLE INFO

Received: Oct 17, 2024

Accepted: Dec 7, 2024

Keywords

Phytoremediation,

Bioaccumulation

Translocation Factor

Heavy Metals

Epidermis

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ABSTRACT

An easy-to-implement and environmentally friendly technology in accumulating mercury (Hg) from contaminated soil is phytoremediation using plants. Certain plants called hyperaccumulators have been shown to be resistant to heavy metals contamination and can accumulate high concentrations of ions without experiencing significant cell damage due to poisoning. Therefore, this study aims to determine the capacity of mercury accumulation using taro plant (Colocasia esculentava) in a glass reactor. Observations of physical form and measurements of plant height were carried out on days 1, 10. 20 and 30 during the phytoremediation process, while the concentration of mercury in soil, root, and leaves samples was measured using mercury analyzer. Subsequently, analysis of the cell structure of root and leaves samples was performed using a light microscope. The observation results of physical form and measurements of plant height showed that the growth process of taro plant was not disturbed despite being under mercury stress. The concentration of mercury in reactors A1 and A2 showed that soil samples had higher levels when compared to roots and leaves. For reactors B1 and B2, the highest concentration was found in roots and leaves. The analysis results of cell structure using a light microscope revealed that epidermis and endodermis tissues of roots and epidermis and cuticle of leaves in reactors A1, A2, B1, and B2 were thicker when compared to root and leaves samples of taro in control reactor. Meanwhile, the calculation of bioconcentration factor (BCF) and translocation factor (TF) showed that taro plant was categorized as accumulators with the mechanism of mercury translocation to leaves being phytoextraction.

INTRODUCTION

Concerns about environmental pollution and its impacts have driven the development of appropriate technologies to assess the presence and mobility of metals in soil, water, and wastewater (Shtangeeva *et al.*, 2004)This effort is very important as part of the process of saving the environment and the inherent ecosystems (Gratalo *et al.*, 2005). One technology that is easy to apply and environmentally friendly in accumulating heavy metals from contaminated soil is phytoremediation using plants (Tangahu *et al.*, 2011).

According to previous, phytoremediation facilitates the reclamation of land and water by plants. The reclamation is carried out by plants through the accumulation of organic or inorganic pollutants, followed by extraction of the surface (Alberto & Sigua., 2013). In addition, an essential factor in the successful implementation of the technology is the detoxification of pollutants (Thakur *et al.*, 2016).

During the implementation process, phytoremediation is majorly divided into 5 types, including phytoextraction, rhizofiltration, phytostabilization, phytodegradation, and phytovolatilization (Surriya *et al.*, 2015).

Several studies have also reported that phytoremediation is an alternative ecological technology that comprises the use of plants to clean or restore soil contaminated with toxic metals (Gratalo *et al*, 2005). Certain plants called hyperaccumulators have been shown to be resistant to heavy metals contamination and can accumulate high concentrations of ions without experiencing significant cell damage due to poisoning (Berti & Cunningham, 2000, Yan *et al.*, 2020)

A major plant that has been studied for its effectiveness as an accumulator of heavy metals in wastewater and land is taro (*Colocasia esculenta*). The results of a study by Bindu *et al.,* 2008 showed that taro plant was able to reduce nitrate concentrations and had the ability to remove mercury (Hg) from contaminated soil. The results of a study by Asare *et al* 2021 also showed that it was effective at removing mercury from contaminated soil. This indicates that taro plant is suitable for use as phytoremediation agent to reduce heavy metals content in polluted land and water. Therefore, this study aims to 1) analyze the amount of absorption of mercury by taro plant (*Colocasia esculenta*), 2) assess the relationship between the contact time of mercury metal with soil, roots and leaves of genjer plant with cell damage in roots and leaves of taro plant, 3) determine the BCF (*Bioconcentration factor*) and TF (*translocation factor*) values of taro plant

MATERIALS AND METHOD

Sampling and phytoremediation process in a greenhouse

A 2-month-old taro plant samples were obtained from Sanleko Village, Buru Island, ensuring a similar phenotypic appearance (plant height and number of leaves). These samples were acclimatized in a greenhouse for 2 months. For the phytoremediation process, a single taro plant sample was placed in 1 control reactor outside the greenhouse while 4 plant samples were placed in 4 test reactors inside the greenhouse. Furthermore, 2 test reactors were labeled A1 and A2 to observe the difference in the concentration of mercury solution given (A1 = 10 ppm. A2 = 20 ppm) while, another 2 test reactors were labeled B1 and B2 to observe the difference in contact time with mercury solution (B1 = 20 days, B2 = 30 days). The process of watering mercury solution was conducted with the following mechanism, namely reactor A1 with 250 mL mercury solution with a concentration of 10 ppm, reactor A2 mercury 250 mL with a concentration of 20 ppm, while reactors B1 and B2 with 250 mL mercury solution each and a concentration of 10 ppm. Taro plant samples in the glass reactor were watered with distilled water every day while the measurement and observation of the physical form of the plants such as height, color, and number of leaves was conducted on the first day and every 10 days of the phytoremediation period. On the 20th day, soil and plant samples were collected from reactor B1 to be analyzed in the laboratory using a mercury analyzer and light microscope, while on the 30th day, soil and plant samples were obtained for control reactor. All reactors in group A and reactors in group B were to be analyzed in the laboratory using a mercury analyzer and light microscope.

Sample preparation and destruction process

Soil sample was heated in an oven at 40°C and ground until smooth, then 2 grams were obtained and a mixture of concentrated HNO_3: HCl (3:1) of 10 ml was added while stirring. Subsequently, the solution was heated at 100°C for 1 hour, and 5 ml of 30% H_2O_2 was added until the solution was clear. This solution was cooled and filtered using Whatman filter paper. Measurement of mercury metal levels at a wavelength of 253.7 nm was carried out using a Mercury Analyzer. For root and leaves samples, the plants were cleaned with distilled water, then dried in an oven at 40°, cut into small pieces, and ground using a mortar and pestle. The ground sample weighed 2 grams and was placed in a 250 ml round-bottom flask. Furthermore, 30 ml of 65% concentrated HNO_3 solution and 10 ml of 95% concentrated H_2SO_4 solution were added successively gradually. The mixed solution was heated at 100 °C for 1 hour and 5 ml of 30% H_2O_2 was added in bits until the solution was clear, then cooled and filtered with Whatman filter paper. After this procedure, mercury metal content was measured at a wavelength of 253.7 nm using a Mercury Analyzer (Mariwy *et al.*, 2021)

Making mercury standard solution

The 1000 ppm mercury stock solution was made by weighing 1.3539 g of anhydrous $HgCl_2$, dissolving it in 1 M HCl, and diluting it to the limit mark, which was 100 ppm. The standard solution was made from the 100 mg/L mercury stock solution by pipetting 1 mL of the 100 ppm mercury stock solution. This was placed into a 100 mL measuring flask, and distilled water was added to the limit mark, which contained 1000 ppb mercury solution. Subsequently, from this stock solution, 1 mL was pipetted and placed in a 10 mL measuring flask. The solution was adjusted to the limit mark with distilled water, containing 100 ppb mercury solution. Serial dilution into standard mercury solutions with concentrations (ppb): 0.5; 1.00; 2.50; 7.50; 10.00; 15.00 and 20.00 by pipetting each (mL) 0.05; 0.10; 0.25; 0.75; 1.00; 1.50, and 2.00 from a 100 ppb mercury solution. Furthermore, each was placed in a 10 mL measuring flask and adjusted to the limit mark with distilled water. The prepared solution was transferred into a test tube and 0.1 mL of 5% KMnO4 was added and shaken thoroughly. Another 0.1 mL of 10% hydroxylamine hydrochloride was added, followed by shaking, and 0.5 mL of 10% SnCl2 was added. Each of these solutions was measured for its absorbance at a wavelength of 253.7 nm using a Mercury Analyzer (Mariwy *et al.,* 2019)

Analysis of the anatomical structure of taro plant root and leaves cells

Taro root and leaves samples were cleaned, washed, and soaked in distilled water and then semi-permanent preparations were made. Furthermore, the samples were cut as thin as possible using a sliding microtome that had been fitted with a razor blade. The sample was inserted into the cork hole or split and then the sample and cork cylinder were inserted into the sliding microtome holder. This was then sliced using a microtome and transferred to a petri dish filled with distilled water. Subsequently, the slices were transferred to a glass object while being dripped with glycerin solution which functioned to coat the preparation to ensure that the color did not fade. In this study, the preparation was observed using a light microscope. The important parameters observed were the profile of the anatomical structure of roots and leaves with the primary focus being thickness of epidermis and endodermis of roots, as well as cuticle and epidermis of leaves (Tupan *et al.*, 2014)

Data analysis

BCF and TF value calculation

Bioaccumulation Concentration Factor (BCF) analysis. BCF calculation formula by Yoon *et al* 2006., as follows:

$$\text{BCF} = \frac{plant\ parts\ mg/kg}{soil\ mg/kg)} \qquad (1)$$

Plant categories were divided into 3, namely :

1. Accumulator: when BCF value > 1
2. Excluder: when BCF value < 1
3. Indicator: when BCF value approaches 1

The analysis of TF to calculate the process of translocation of heavy metal mercury from roots to leaves used the equation:

$$\text{TF} = \frac{in\ the\ leaf\ (\frac{mg}{kg})}{in\ the\ roots\ (mg/kg)} \qquad (2)$$

TF value according to Baker 1981, has categories, namely:

TF >1: Phytoextraction mechanism

TF <1: Phytostabilization mechanism

RESULTS

Acclimatization and phytoremediation process

Taro plant samples obtained from Sanleko Village, Namlea District, Buru Regency, were acclimatized into the phytoremediation laboratory. Acclimatization referred to a stage to adjust plant samples to the greenhouse environment where the phytoremediation process occurred. This method was to

place taro plant in a plastic tub for 1 month before being transferred to a glass reactor. The goal was for plants to adapt to the conditions in the greenhouse where phytoremediation process occurred. After the acclimatization stage, the next process was to transfer genjer and taro plant samples into a glass reactor consisting of 1 control reactor and 4 test reactors that were filled with soil that was from Sanleko Village. One of the important things in the stage of selecting plant samples to be moved in a glass reactor for the phytoremediation process is to have physical similarities such as plant height, number, and color of leaves. Plant height was measured every 10 days, as well as the observations of leaves color. Furthermore, the number of leaves was counted, and soil, root, as well as leaves samples were collected for analysis in the laboratory. Taro plant samples in phytoremediation process can be seen in Figure 1.

Figure 1: Taro plant samples in the phytoremediation process

Physical changes in plant samples in the phytoremediation process

Data on physical changes in taro plant samples, including plant height, number, and color of leaves in control reactor and 4 test reactors indicated a daily increase in plant height. By the 20th day in reactor B1 and the 30th day in control reactor, A1, A2, and B2 the average plant height in control and test reactors was 53 cm. Data on the height of taro plant samples during phytoremediation process was presented in Table 1.

Table 1: Data on taro plant height during the phytoremediation process

Day	Taro plant height					Unit
	Control reactor	Reactor A1	Reactor A2	Reactor B1	Reactor B2	
1	45	47	46	46	47	cm
10	47	49	48	48	48	cm
20	48	53	51	51	51	cm
30	52	55	53	-	53	cm

These data showed that mercury heavy metal stress did not affect the growth of taro plant during the phytoremediation process. In this study, it was evident from the results of observations of the color of taro plant sample leaves that the color of all taro leaves in both control and test reactors remained green, new shoots were growing on taro plant in all reactors, and on the 20th and 30th days there were wilted and dead leaves in all reactors. Data on taro plant height was seen in Figure 2.

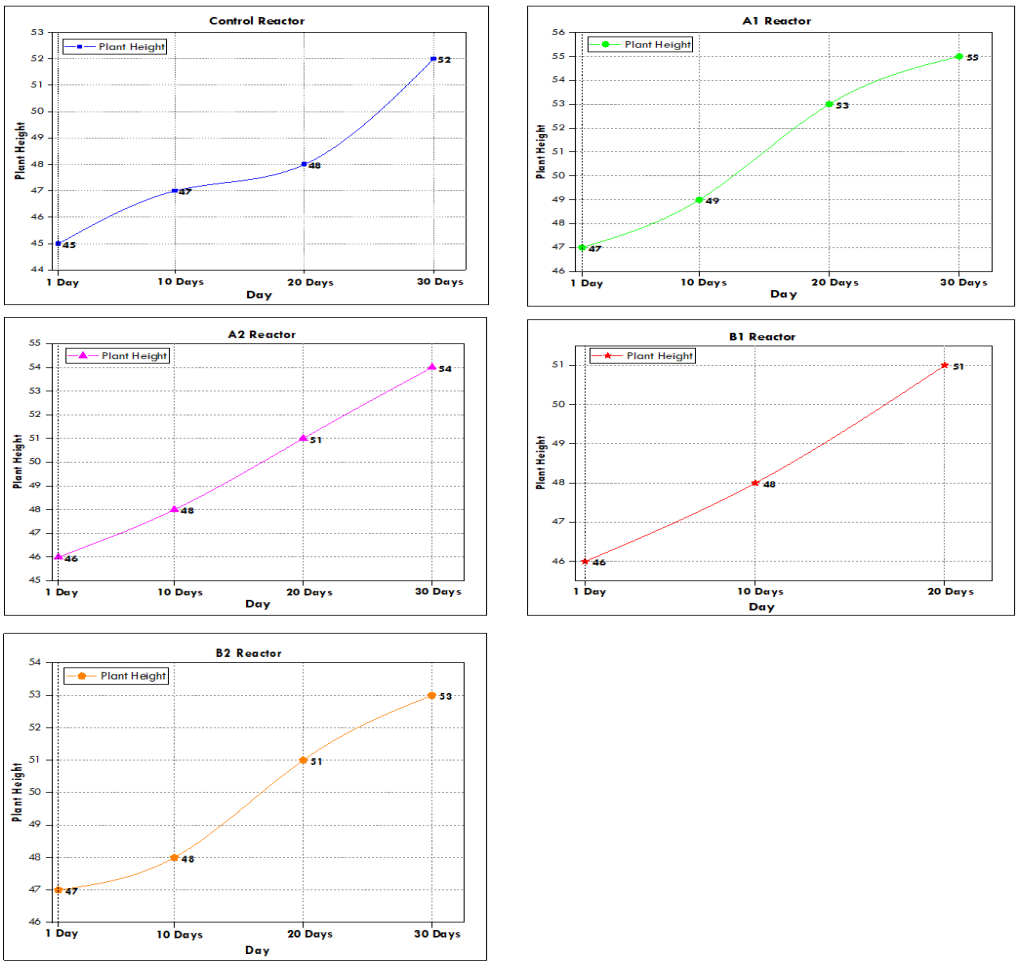

Figure 2: Graph of taro plant height during phytoremediation process

Mercury concentration in soil, root, and leaves samples of taro plant

Measurement of mercury concentration in soil, root, and leaves samples of taro plant in all reactors was conducted using a mercury analyzer with the calibration curve method. Based on the results in control reactor, mercury concentration in soil sample was 0.30 ppm, roots 0.01 ppm, and leaves 0.03 ppm. Mercury concentrations in soil, root, and leaves samples in reactor A1, included 2.96 ppm, 1.48 ppm, and 0.31 ppm, respectively, those in reactor A2 were 2.38 ppm, 0.40 ppm, and 0.131 respectively, and those in B1 were 2.77 ppm, 1.11 ppm, and 6.42 ppm respectively. Furthermore, the concentration of mercury in soil, root, and leaves samples in reactor B2 was 1.46 ppm, 1.45 ppm, and 1.96 ppm respectively. Mercury concentration data in each reactor was depicted in Table 2.

Table 2: Mercury concentration in samples

Sample Type	Mercury concentration for each reactor					Unit
	Control reactor	Reactor A1	Reactor A2	Reactor B1	Reactor B2	
Soil	0.30	2.96	2.38	2.77	1.46	ppm
Roots	0.01	1.48	0.40	1.11	1.45	ppm
Leaves	0.03	0.31	0.11	6.42	1.96	ppm

The data in Table 2 showed that soil samples in control reactor contained mercury at quite high concentrations. Meanwhile, for the test reactor, the largest mercury concentration in reactors A1 and A2 was soil and the samples with the largest mercury concentration in reactors B1 and B2 were leaves. Mercury concentration data in taro plant samples was indicated in Figure 3.

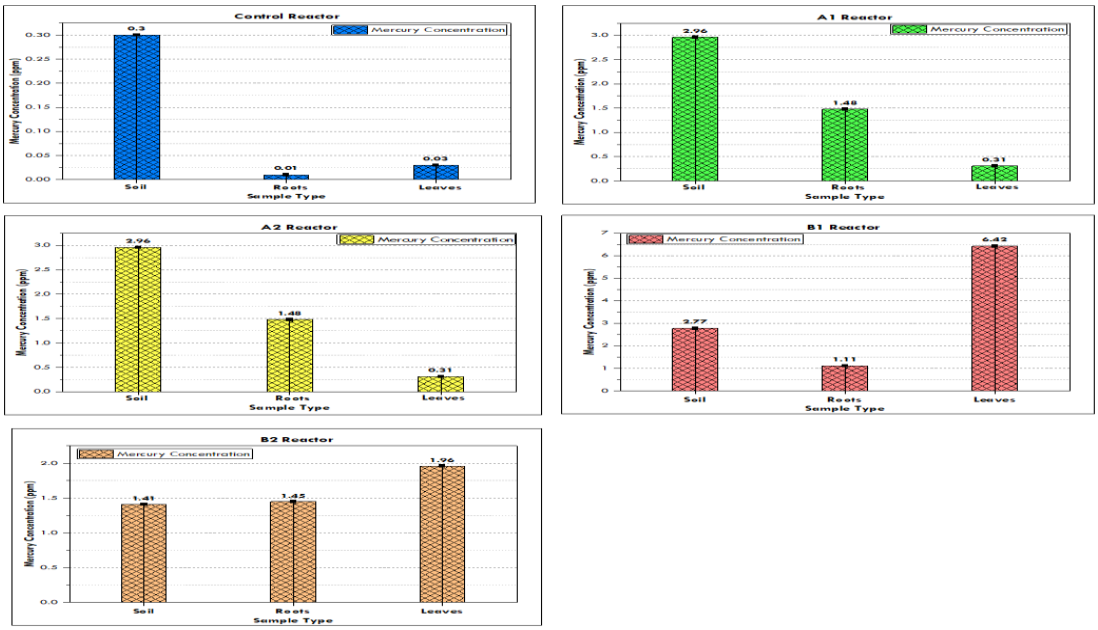

Figure 3: Graph of mercury concentration in taro plant samples

Analysis of cell anatomical structure

Changes in the anatomical structure of taro root and leaves samples in each reactor as a result of mercury stress were observed by comparing the anatomical characteristics of roots and leaves of control with test plants. Analysis of the anatomical structure of cells was conducted using a light microscope while measurements of cell thickness, both epidermis, endodermis, and cuticle, were carried out with the Ruster image program. The morphological analysis of cuticle and epidermis layers of the samples in reactor A1, subjected to a mercury concentration treatment of 10 ppm with a contact time of 30 days revealed a thickening of these layers compared to control reactor. In control group, cuticle thickness was 3.30 µm and epidermis layer was 0.53 µm. Taro leaves samples in reactor A1 were 4.46 µm and epidermis layer was 1.20 µm. Meanwhile, the morphological analysis results of epidermis and endodermis layers of taro root samples in reactor A1 with the same concentration and contact time treatment showed that there was a thickening of both layers compared to those in control reactor. The measurement results showed that thickness of epidermis layer of taro root samples in control reactor was 2.15 µm and endodermis layer was 2.15 µm, while thickness of epidermis layer in reactor A1 sample was 2.37 µm and endodermis layer was 2.25 µm. Furthermore, the anatomy of taro leaves and root samples in control reactor and reactor A1 was observed in Figure 4.

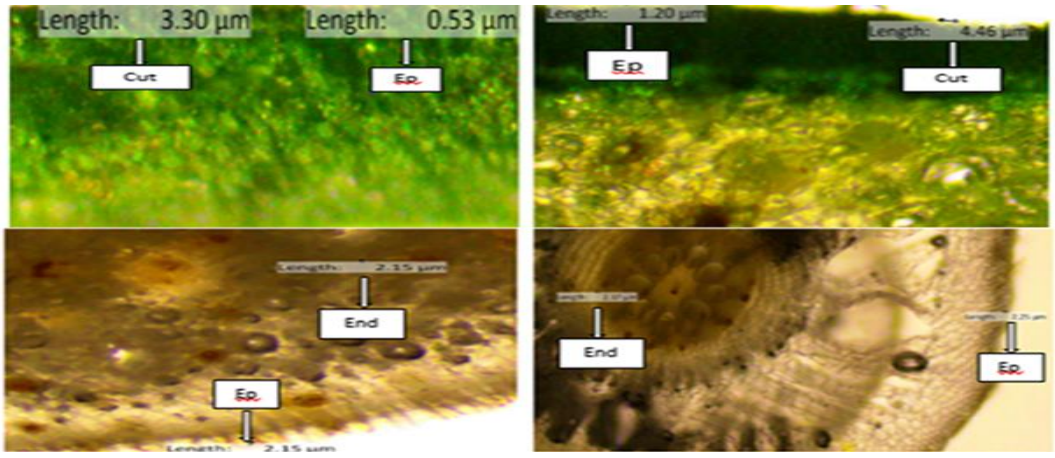

Figure 4: Anatomy of taro leaves samples in control reactor and reactor A1 (top), and taro roots in control reactor and reactor A1 (bottom)

Thickness measurement of cuticle and epidermis layers of taro leaves samples in reactor A2, treated with 20 ppm mercury for a contact time of 30 days, revealed significant thickening compared to control reactor. In control reactor, cuticle layer was 3.30 µm and epidermis layer was 0.53 µm. Meanwhile, cuticle in reactor A2 was 5.34 µm and epidermis layer was 1.27 µm. The analysis of epidermis and endodermis layers of taro root samples in reactor A2 indicated a thickening compared to control reactor. Furthermore, the measurement results showed that epidermis layer in control reactor A was 2.15 µm and endodermis layer was also 2.15 µm, while epidermis layer in reactor A2 sample was 2.23 µm and endodermis layer was 2.26 µm. The anatomy differences in taro leaves sample in control reactor and reactor A2 were demonstrated in Figure 5.

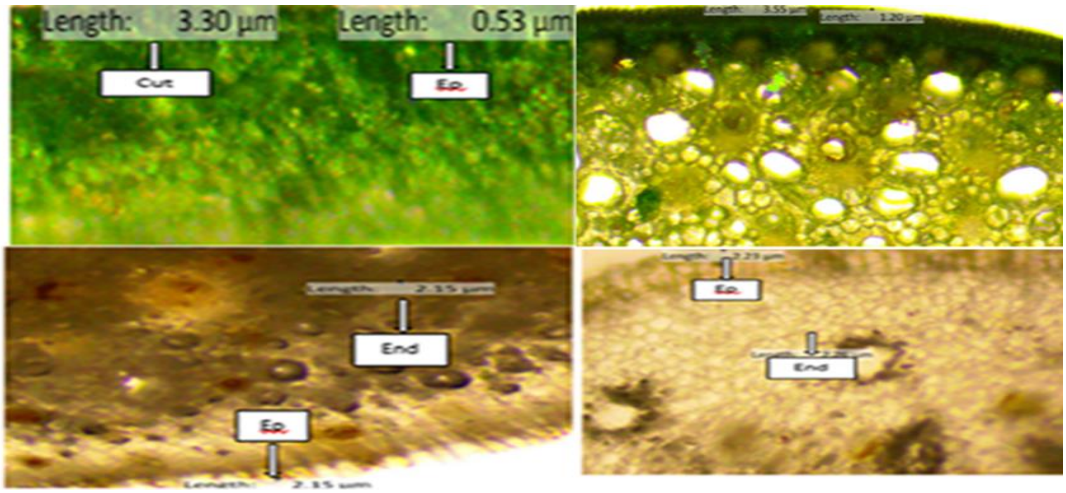

Figure 5: Anatomy of taro leaves samples in control reactor and reactor A2 (top), and taro roots in control reactor and reactor A2 (bottom)

Using the Ruster imaging program, measurements of cuticle and epidermis layers of taro leaves samples were taken in reactor B1, and treated with 10 ppm mercury for 20 days. The results revealed a thickening of these layers compared to control reactor. In control reactor, cuticle layer was 3.30 µm and epidermis layer was 0.53 µm, while cuticle layer in reactor B1 was 7.44 µm and epidermis layer was 1.20 µm. Meanwhile, the results of epidermis and endodermis layers thickness analysis in reactor B1 with a mercury concentration treatment of 10 ppm for 20 days showed that there was a thickening of both layers compared to thickness of epidermis and endodermis layers in control reactor where of epidermis layer in control reactor was 2.15 and endodermis layer was also 2.15 µm, while epidermis layer in reactor B1 sample was 2.22 µm and endodermis layer was 2.25 µm. The anatomy of taro leaves samples in control reactor and reactor B1 were displayed in Figure 6.

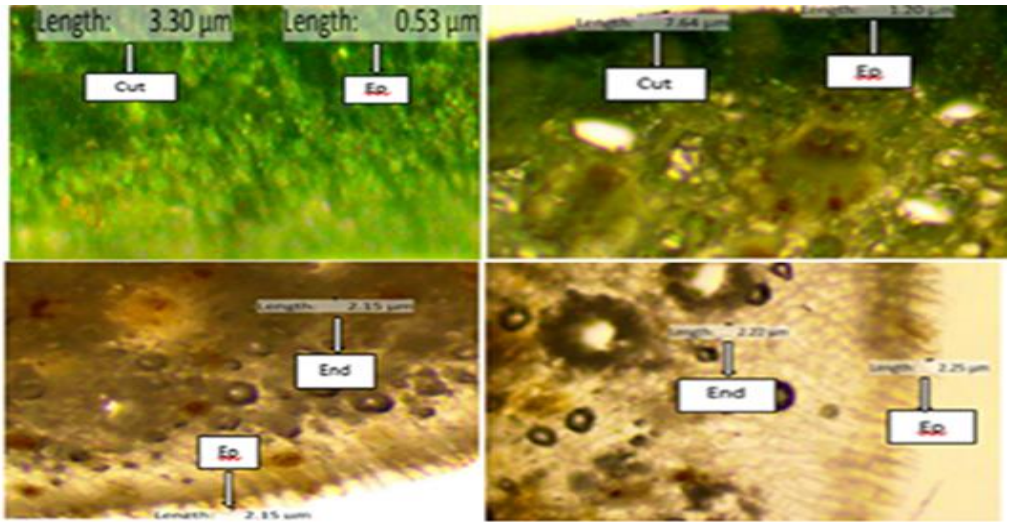

Figure 6: Anatomy of taro leaves samples in control reactor and reactor B1 (top) and taro roots in control reactor and reactor B1 (bottom)

Thickness measurements of cuticle and epidermis layers of taro leaves samples using the Ruster image program in reactor B2 with a mercury concentration treatment of 10 ppm and a contact time of 30 days indicated that there was a thickening of both layers compared to thickness of cuticle and epidermis layers of the samples in control reactor. The measurement results revealed that thickness of cuticle and epidermis layer of taro leaves sample in control reactor was 3.30 µm and 0.53 µm, respectively, while thickness of cuticle and epidermis layer in reactor B2 sample was 5.54 µm and 1.27 µm, respectively. Furthermore, thickness analysis of epidermis and endodermis layers of taro root samples in reactor B2 showed that there was a thickening of both in control reactor where thickness of epidermis layer was 2.15 µm and thickness of endodermis layer was also the same, while thickness of epidermis and endodermis layer in reactor B2 sample was 2.01 µm and 2.33 µm, respectively. The anatomy of taro leaves sample in control reactor and reactor A2 was displayed in Figure 7.

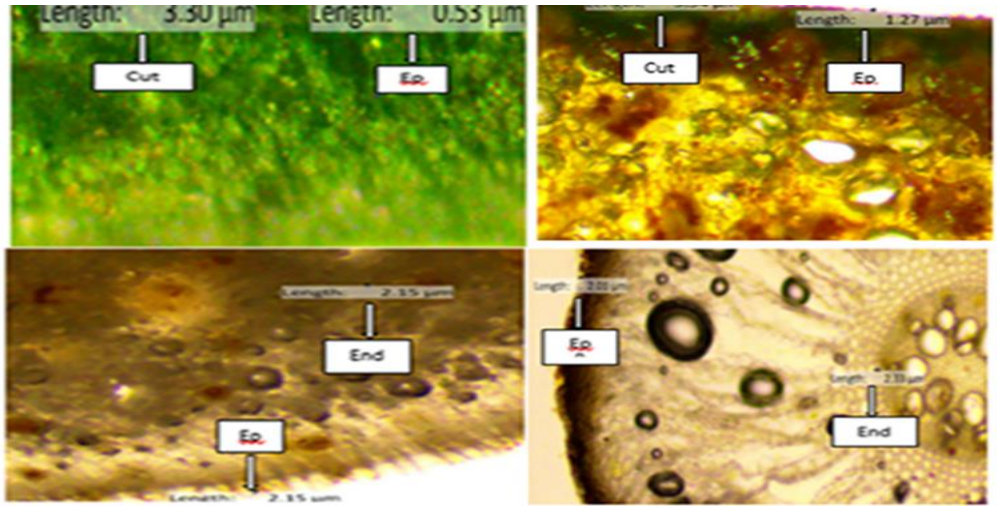

Figure 7: Anatomy of taro leaves samples in control reactor and reactor B2 (top) and taro roots in control reactor and reactor B2 (bottom)

Bioconcentration factor (BCF) and translocation factor (TF) of taro plant samples

The results of calculating BCF and TF values of the samples for each test reactor showed that taro plant samples in reactor A1 were categorized as accumulators because BCF value = 1.60, those in reactor A2 were categorized as excluders because BCF value = 0.21 was exhibited, those in reactor B1 and B2 were categorized as accumulators because BCF value = 2.71 and 2.41, respectively. The results of calculating BCF and TF values in taro plant samples in test reactors A1, A2, B1, and B2 were presented in Table 3.

Table 3: BCF and TF values of taro plant samples

Reactor	BCF Value	TF Value
A1	1.60	0.20
A2	0.21	0.27
B1	2.71	5.70
B2	2.41	1.55

DISCUSSION

Phytoremediation was an effort to decontaminate waste and environmental pollution problems either ex-situ using artificial ponds, reactors or in-situ (directly in the field) on soil or areas contaminated with waste using plants. Plants used as phytoremediation agents must meet 3 main requirements, namely 1. The plant's roots must be able to absorb contaminants from the soil, 2. The plant must be able to physically and chemically bind the contaminants into its root tissue, and 3. The plant must be able to transport the contaminants to leaves (translocation process) and prevent the contaminants from leaving soil (Alberto & Sigua., 2013)

The samples used in this study were taro plant and the results of observations of the physical form of the plant such as height and leaves color indicated that mercury metal stress did not affect their

growth. According to the results of the study by Asare *et al*., 2021, taro plant were very good at removing mercury from contaminated soil. This was because it contained secondary metabolites found in leaves. Rustiani *et al.,* 2021 indicated that the ethanol extract of taro stems and leaves contained flavonoid compounds of 3.18% and 4.33%, respectively, while the terpenoid content was 7.10% and 8.39%. These secondary metabolites could bind to the heavy metal mercury. Furthermore, the reaction between flavonoids (phenolic compounds found in various plants) and mercury metal produced a complex known as flavonoid-mercury complex (Kulshreshta & Saxena., 2016). The reaction between catechin, one of flavonoid compounds, and mercury metal was depicted in Figure 11.

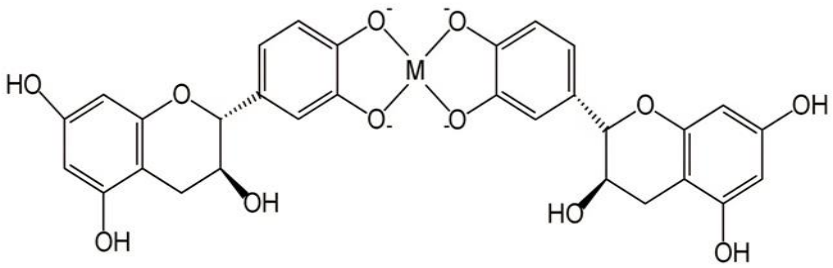

Figure 11: The reaction between catechin, a flavonoid compound, and mercury metal (Yedda *et al*., 2022)

Based on the results of measuring thickness of cuticle and epidermis layers of taro leaves samples as well as epidermis and endodermis of taro root samples using the Ruster image program, there was a thickening of the 4 layers compared to thickness of the same layer in taro leaves and root samples in control reactor. The increase in thickness of cuticle and epidermis layers of taro leaves samples indicated that the plants conducted anatomical adaptations that were very sensitive to environmental changes (Naz *et al*., 2016). An environment with high mercury stress caused changes in leaves anatomy as observed in cuticle which was the first line of defense in dealing with exposure to pollutants (Kulshrestha & Saxena., 2016). Meanwhile, thickening of epidermis layer provided a physical barrier to heavy metal penetration and could slow or inhibit the entry of heavy metals into root tissue (Gomes *et al*., 2011). Endodermis layer was an apoplastic barrier that played an important role for plants as protection against several types of stress (Enstone *et al*., 2003). Metals that entered root tissue through the apoplastic pathway were then blocked in endodermis layer by the Casparian strip and excreted by the plant detoxification system (Pourrut *et al*, 2011). This occurred because most plants gradually form self-defense mechanisms and tolerance to heavy metal stress environments. Furthermore, these self-defense mechanisms affected heavy metal mobility and microbial activity through root exudates, fine isolation, and regionalization of cell walls, cell membranes, and vacuoles (Anjum *et al*. 2014). Plants had developed mechanisms to combat heavy metal stress including immobilization, plasma membrane exclusion, restriction of absorption and transport, synthesis of certain heavy metal carriers, induction of stress proteins, chelation, and sequestration by certain ligands (Kumar *et al*., 2019, Yavaş *et al*., 2022).

Surface micrographs of the sample characterization results using SEM in all test reactors as presented in Figures 7 and 9 revealed the shape of taro leaves surface with heterogeneous characteristics. This was because it consisted of circles and polygons that began to thicken and were irregular when compared to the shape of taro leaves surface in control reactor where thickening of epidermis layer was related to its role as the primary protector of plant leaves against extreme environmental changes such as drought, ultraviolet light, and heavy metal stress (Javelle *et al*., 2011).

The calculation results of BCF value of taro plant samples for each test reactor showed that taro plant samples in reactor A1 were categorized as accumulators because BCF value = 1.60 was exhibited. Taro plant samples in reactor A2 were categorized as excluders because of BCF value = 0.21, those in reactor B1 were categorized as accumulators because BCF value = 2.71, while those in reactor B2 were also categorized as accumulators because BCF value = 2.41. Meanwhile, the calculation results of TF value showed that the metal translocation mechanism in reactors A1 and A2 was phytostabilization and the metal translocation mechanism in reactors A1 and A2 was phytoextraction. The phytostabilization mechanism was carried out by plants by absorbing metals in

the rhizosphere through adsorption and precipitation of metals into more soluble forms such as metal carbonates and sulfides, metal complexes with organic compounds, metal adsorption on root surface and metal accumulation in root tissue (Muhammad *et al*., 2014). Furthermore, the phytoextraction mechanism allowed the plant to consistently absorb heavy metal from the soil and move it into shoot or leaves organs (Raheleh *et al*., 2022). This also ensured detoxification by plants through natural chelation processes such as phytochelatins (PC) during translocation and absorption processes (Ghori, *et al*., 2016).

CONCLUSION

In conclusion, the results of this study indicated that taro plant was used as phytoremediation agents for the rehabilitation process of land that had been contaminated with mercury metal. The growth process of this plant was not disturbed, although it was under mercury stress. Furthermore, the increasing thickness of cuticle and epidermis layers of leaves samples and epidermis and endodermis layers of root samples indicated that taro plant could carry out anatomical adaptations that were very sensitive to environmental changes. The results of BCF and TF calculations revealed that taro plant was categorized as accumulator with the mechanism of mercury metal translocation to leaves being phytoextraction.

Author's contribution

AM: Conceptualization, writing manuscript, DAJS: Research methodology, YTM: Research design, CIT: Data analysis

Acknowledgments

The author was grateful to the Southwest Maluku Regency Government, the Maluku Provincial Government, the National Research and Innovation Agency (BRIN), and the Chancellor of Universitas Pattimura Ambon for the financial assistance provided, hence, this study can be carried out.

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