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Absorption of Heavy Metal Mercury (Hg) in Long Bean (*Vigna unguiculata* L. Walp) with Variation of Planting Period and Planting Medium

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Abstract: The amalgamation technique of Sekotong gold mining produces heavy metal pollution in agricultural areas. The purpose in this study to find the effect of mine soil variations on growing media on growth and absorption of mercury in long bean (*V. unguiculata* L. Walp). This experimental study uses RCBD with a mixture of katel soil and mine soil media A (100% katel soil), B (80%: 20%), C (60%: 40%), D (40%: 60%) and E (20%: 80%) and 5 replications. Data collection on growth of stem height and number of leaves every week while the absorption of mercury every 1, 2, 3 and 4 months of planting. Data analyzed with One Way Anova test, Mann Whitney test and T-test. Results of analysis showed effect of variation in planting media on stems height and number of leaves from highest to lowest is media A, B, C were significantly different, followed D and E not significantly different. Mercury absorption of media A and C roots, stems, leaves, fruits 1 and 4 months of planting significantly different, not significantly different from root 2 months of planting and roots, stems and fruits 3 months of planting. Total absorption of mercury media A and C was significantly different.

Keywords: Amalgamation; mercury; growth of long bean; absorption.

INTRODUCTION

One of the regions in Indonesia that has gold mining is province of West Nusa Tenggara (NTB). Area of gold mining site located in Sekotong District, West Lombok, West Nusa Tenggara. In gold mining, there is an extraction process and in that process there is technique namely amalagamation. The amalgamation technique uses a mercury that being add to the milling process to obtain amalgam where the amalgam that has been obtained will be burned so that the mercury and gold can be separate. The amalgamation process produces residue in the form of mud (Handayanto *et al*., 2015). The sludge which is the residue of this gold mining process contains mercury and directly discharged into the environment.

Mercury is a heavy metal that easily accumulates in the environment, inhabit underwater surfaces which can easily evaporate and pollutes the air. Mercury can reduce soil quality and water which will indirectly impact to humans and other organisms (Suhadi et al., 2019). Long bean plants are also an agricultural commodity in the West Lombok region. Data obtained by BPS for West Lombok Regency in 2011 showed that the commodity of long bean plants had increased from the previous year, namely in 2010, which amounted to 6,252 kw to 7,780 kw. Heavy metals in the growing medium can be absorbed by plants (Hidayah, 2016) and can accumulate in the roots, stems and leaves of these plants (Ling et al., 2010). Mercury can be absorbed throughout the plant and has a negative impact on the growth of long bean leaves. The negative impact is in the form of leaves experiencing chlorosis and necrosis. Mercury can interfere with photosynthesis because mercury can replace the essential ion in the form of Mg2 + in chlorophyl (Gontia-Mishra et al., 2016). Mercury can also cause disturbances in water balance and growth, one of which is in the form of leaves that cause necrosis and abscission (Ordak et al., 2016) and can be a carrier of mercury get into the human body.

MATERIALS AND METHODS

This research was arranged in a randomized block design with 5 variation of planting media and 5 replications. Planting media in this research is a mixture between katel soil and mining soil of Sekotong gold mining. There are 5 variations of planting media namely media A (100% katel soil), media B (20% of mining soil), media C (40% of mining soil), media D (60% of mining soil) and media E (80% of mining soil). Plants in this research are grown in a 4.5liter bucket. This research is divided into several stages including the preparation of planting media, preparation of planting areas, data collection, and data analysis. Data was analyzed using One Way ANOVA, Mann Whinet Test and Independent T-Test. This Research use Mann Whitney Test because data in this research is not homogeneous and to see which variation that has differences.

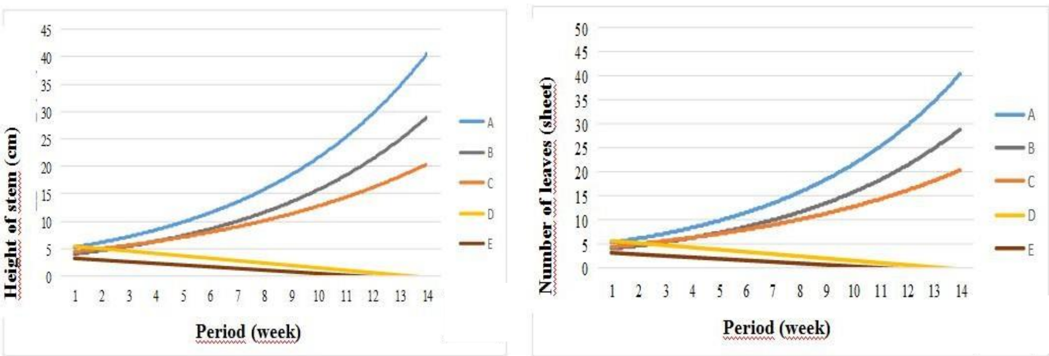
The first stage is the preparation of the planting medium in the form of a mixture of katel soil and sekotong soil with various concentration variations on the two soils where there are five kinds of concentrations indicated by the planting medium A (100% katel soil), B (20% mine soil), C (40 % mining land), D (60% mining land) and E (80% mining land). Katel soil is a mixture of soil Katel and humus brand "Floris" were purchased at the Splendid Market, Malang City with a ratio of 3: 1 (Katel soil: humus). Furthermore, the land was prepared for conducting research. The land or research area is located on the 3rd floor or the rooftop greenhouse building, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang. The container for planting is a bucket with a volume capacity of 4.5 liters.

The second stage is nursery of long bean (*V. unguiculata* L. Walp) varieties of kanton tavi. Seeding is done by soaking long bean seeds for ± 4 hours in warm water. After soaking the seeds, polybags are prepared as seed containers. The seeds that already soaked planted in polybags containing katel soil or control soil (100% katel soil). After that, water with volume ± 100 ml being watered to the plant every day at nine in the morning and at three in the afternoon. The length of time for the seeds to become seedlings with three leaves is ± 7 days. After the seeds grow into three-leaf seedlings, transfer them to a bucket container. Each bucket contains one seed. Then watered with ± 100 ml of water every day at nine in the morning and at three in the afternoon.

Besides watering the long bean plant care, it is also carried out by taking pests. Pest collection is done by taking a tissue. Pest observations were carried out every day at the time of watering. If a pest is found, what is done is to immediately take the pest. Every week of planting, data was collected in the form of soil pH and temperature. Retrieval of data for each planting period in the form of light intensity and wind speed. In addition to the data collection, data collection was carried out every week in the form of stem height and number of leaves. Stem height data were collected using a ruler, while the number of leaves was immediately counted and recorded. Sampling was carried out at each harvest with samples taken in the form of roots, stems, and leaves. Samples that being taken (roots, stems, and leaves) will dried in an oven at 38°C for 2 to 3 days or until dry (not brittle). Furthermore, each part (roots, stems, and leaves) was weighed and recorded and then the data became dry weight data. After that, each part of the long bean plant was tested for the content of heavy metals in the form of mercury (Hg) at the Chemical Laboratory using the Atomic Absorption Spectroscopy (AAS) method.

RESULT

Research that has been done show long bean (*V. unguiculata* L. Walp) that planted at growing medium or planting medium A is the highest in growth of height of stem and number of leaves followed by planting medium B, C, D and the lowest is planting medium E (Figure 1).



(a) (b)

Figure 1. Difference in Growth of Height of Stem (a) Number of Leaves (b) based onVariation of Planting Period and Planting Medium

Growth (height of stem and number of leaves) of long bean (*V. unguiculata* L. Walp) that planted in planting medium A being the highest with average of height of stem is 17,464 cm and average of number of leaves is 24,5 sheets. Followed by long bean (*V. unguiculata* L. Walp) that planted in planting medium B for being the highest with average of height of stem is 12,757 cm and average of number of leaves is 17,9 sheets. Long bean (*V. unguiculata* L. Walp) that being planted in planting medium C has average of height of stem is 10,6 cm and average of number of leaves is 11,1 sheets. Long bean (*V. unguiculata* L. Walp) that being planted in planting medium D has average of height of stem is 2,6 cm and average of number of leaves is 2 sheets. Long bean (*V. unguiculata* L. Walp) that being planted in planting medium E has average of height of stem is 1,2 cmand average of number of leaves is 1 sheet.

Research that has been done also show long bean (*V. unguiculata* L. Walp) that planted at planting medium A is the highest in growth of dry weight of roots, stems, leaves, fruits, and total followed by planting medium B, C, D and the lowest is planting medium E.

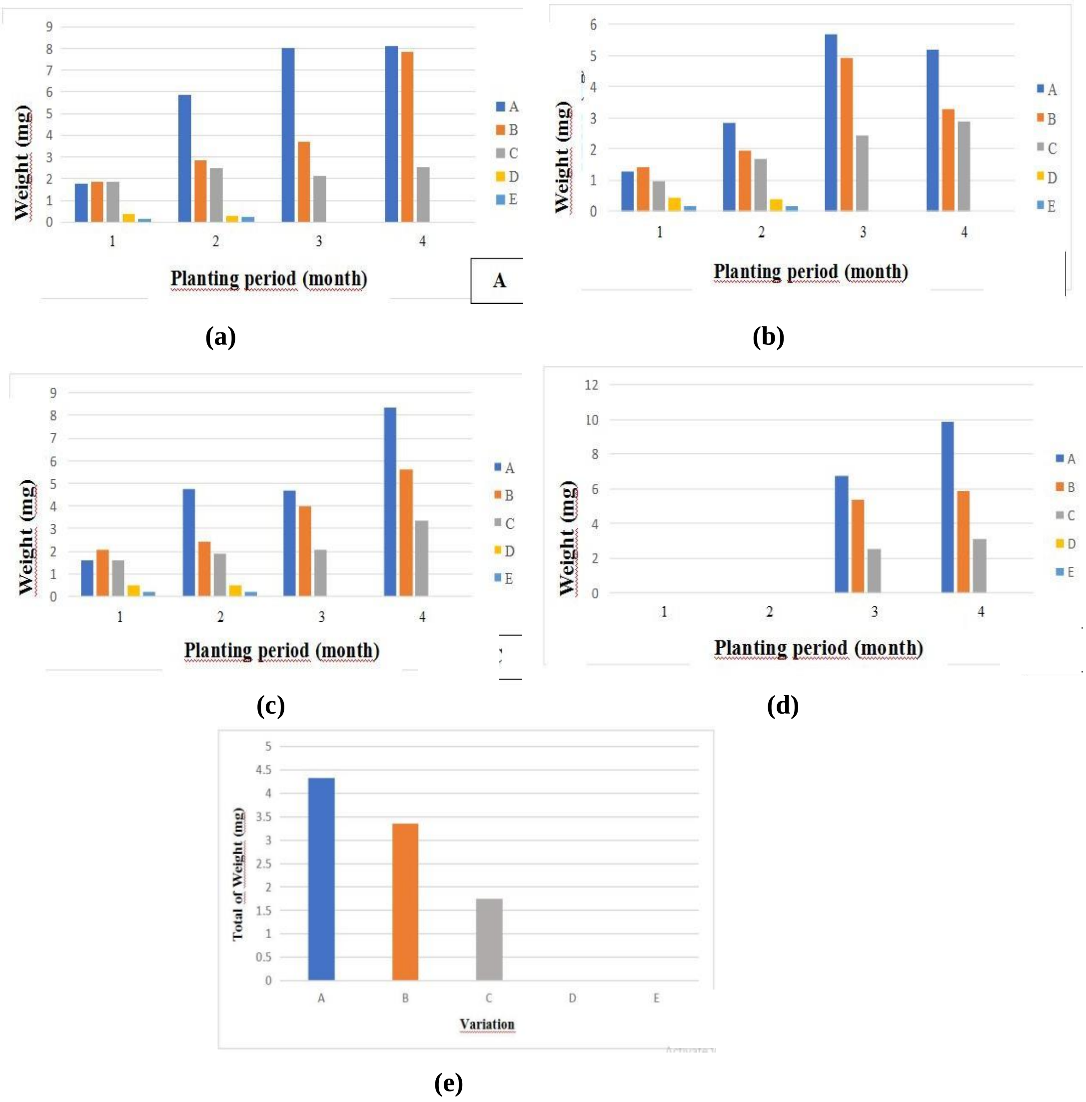


Figure 2. Difference in Growth of Dry Weight of Roots (a), Stems (b), Leaves (c), Fruits and Total Dry Weight (E) of long bean (*V. unguiculata* L. Walp) based on Variation ofPlanting Period and Planting Medium

The highest dry weight is long bean (*V. unguiculata* L. Walp) that planted in planting medium A with average dry weight of roots is 1,8 mg, average dry weight of stem is 1,3 mg, average dry weight of leaves is 1,6 mg, average dry weight of fruits is 1,13 mg and total dry weight is 4,3 mg. Followed by long bean (*V. unguiculata* L. Walp) that planted in planting medium B for being the highest with average dry weight of roots is 0,4 mg, average dry weight of stem is 0,3 mg, average dry weight of leaves is 0,4 mg, average dry weight of fruits is 1,07 mg and total dry weight is 3,3 mg. Long bean (*V. unguiculata* L. Walp) that planted in planting medium C has average dry weight of roots is 0,4 mg, average dry weight of stem is 0,2 mg, average dry weight of leaves is 0,3 mg, average dry weight of fruits is 1,02 mg and total dry weight is 1,7 mg. Long bean (*V. unguiculata* L. Walp) that planted in planting medium D has average dry weight of roots is 0,07 mg, average dry weight of stem is 0,09 mg and average dry weight of leaves is 0,1 mg. Long bean (*V. unguiculata* L. Walp) that planted in planting medium E for being the lowest with average dry weight of roots is 0,03 mg, average dry weight of stem is 0,03 mg and average dry weight of leaves is 0,04 mg.

Research that has been done show there is differences between long bean (*V. unguiculata* L. Walp) that planted at variation of growing medium or planting medium A and planting medium C (Table 1).

Table 1. Result of Mann Whitney Test

Part of plant	Planting period (mounth)			
	1	2	3	4
roots	0,009	0,251	0,251	0,009
stems	0,009	0,009	0,117	0,009
leaves	0,005	0,005	0,005	0.005
fruits	-	-	0,136	0,019

Based on Mann Whitney test that has been done, known that there is a part of the long bean body that not significantly different, that is root in 2 months of planting and root, stem, and fruit in 3 months of planting. Furthermore, this research also shows that there are differences in total absorption of mercury in long bean (*V. unguiculata* L. Walp) that planted on variation of planting medium A and planting medium C (Figure 3).

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TOTAL ABSORPTION	Equal variances assumed	2.215	.187	-2.572	6	.042	-1.04098	.40470	-2.03124	-.05073
	Equal variances not assumed			-2.572	3.568	.069	-1.04098	.40470	-2.22041	.13845

Figure 3. Result of Independent T-test

Total absorption of heavy metal mercury in long bean (*V. unguiculata* L. Walp) was tested by the T-test. The value of 0.042 means that there is a difference between the total absorption of heavy metal mercury on growing media A (100% katel soil) with planting medium C (40% of mine land) during the planting period 1, 2, 3 and 4 months.

DISCUSSION

Planting medium A could be the highest in growth of height of stem and number of leaves because planting medium A only contain katel soil. Katel soil is sediment that can be found at riverbanks and contain sand and high fertilizing soil (Satsiaji, 1991). Roots of plant that grow at planting medium only contain katel soil more developed because katel soil has low level of soil density (Muthahara et al., 2018). Also, roots that grow well will affect upper part of plant will grow well too (Tatik et al., 2014). Planting medium E being the lowest because accumulation of heavy metal mercury can inhibit long bean to grow with prevent plant to absorb nutrition from soil (Putra et al., 2018). Planting medium A could be the highest in growth dry weight of roots, stems, leaves, fruits, and total dry weight followed by planting medium B, C, D and the lowest is planting medium E because roots of plant that grow at planting medium only contain katel soil more developed because katel soil has low level of soil density (Muthahara et al., 2018). Also, roots that grow well will affect upper part of plant will grow well too (Tatik et al., 2014). The higher concentration of mercury can make a damage to the plant.

The dry weight of roots, stems, leaves and fruit on planting media D (60% of mine soil) and E (80% of mine soil) decreased until death occurred in long bean (*V. unguiculata* L. Walp) plants in 3 months of planting and 4-month planting period was caused by the mine soil which had a higher mixed concentration than the ratio of the katel soil, making the soil in the planting medium compact so that it was difficult for water to enter the planting medium. The tailings waste around the planting hole can turn into solid so that water absorption in the soil is bad and has a negative impact on root development. This negative impact is followed by the inability to develop roots as a means of absorbing water and nutrients by the plants so that the plants do not develop normally and the growth of these plants will be stunted (Fauziah, 2009). The reduction in root and stem weight is supported by research which states that mercury also has a negative impact on root and stem growth. *Allium cepa* and *Allium sativum* plants exposed to mercury showed reduced cell mitosis at the root tips and increased frequency of chromosomal aberrations (abnormalities). The increase in chromosome aberration is directly proportional to the concentration present in the soil and the length or duration of exposure to mercury-containing soil roots. So that the higher the level of mercury, the more damaged the roots will be and not as thick as the roots of plants that grow in the planting medium A (Patra & Sharma, 2000).

Table 1 show that absorption of mercury at the roots of plants long bean (*V. unguiculata* L. Walp) were grown on medium A and medium C at the planting period of 1 month and 4 months had a significant difference where the analysis results show that the significance value of the Mann Whitney test during the planting period of 1 month and 4 months, the value was 0.009. This value showed that there was a significant difference in the mercury content of the section root media A and medium C. Heavy metals are absorbed by roots can be caused by plants to produce the enzyme reductase which functions to reduce the heavy metal mercury so that mercury can enter the root (Gosh & Singh, 2005). Furthermore, mercury absorption at 2 and 3 months planting period showed no significant difference between the roots of long bean plants grown on medium A and medium C. This could be due to the possibility of organic matter being added to it the planting medium contains heavy metals in the form of mercury (Erfandi & Juarsah, 2010). Although mercury was in levels low concentrations, if the plant absorbs mercury it will have consequences toxic to the plant (Hamim et al., 2018). Absorption of mercury on the stem of long bean plants (*V. unguiculata* L. Walp) grown on medium A and medium C at planting time 1, 2, and 4 months have a significant difference where the analysis results shows that the significance value of the Mann Whitney test at the planting period is 1, 2, and 4 months shows a value of 0.009. This value indicates a difference the mercury content in the stem section of medium A and medium C. Mercury that is absorbed in the roots can be translocated to the upper part of the organ plant like stems. Addition of organic matter from organic fertilizers can affect absorption mercury in plant roots due to the chelating that is formed. Chelate will affect the mobility of heavy metal ions in the soil. The cation possessed by heavy metals will turn into anions so that heavy metals remain mobile in the ground (Purwadinata & Sutrisno, 2013).

Absorption of mercury in the leaves of long bean plants (*V. unguiculata* L. Walp) grown on medium A and medium C at planting 1, 2, 3, and 4 months have a significant difference in the results of the analysis shows that the significance value of the Mann Whitney test at the planting period 1, 2, 3 and 4 months shows a value of 0.005. This value indicates a difference which is significant in the mercury content in the leaf section of growing media A and media planting C. This can be caused by mercury ions which are mobile can be absorbed easily gets to the plant canopy and can be stored in epidermal cells leaves. The heavy metal mercury is absorbed by the roots will enter the transpiration stream that reaches the leaves so that the leaves can contain mercury (Meagher & Heaton, 2005). Apart from that the heavy metal is capable of mercury evaporates into the atmosphere and can be absorbed by the leaves (Pivetz, 2001).

Absorption of mercury in fruit of long bean plants (*V. unguiculata* L. Walp) grown on medium A and medium C during the planting period 4 months have a significant difference which the analysis results show that the significance value of the Mann Whitney test at the planting period of 4 months shows a value of 0.019. This value indicates that there is a significant difference in mercury content in the fruit section of media A and medium C. Mercury content on the fruit of long bean plants grown on growing media A more lower than the fruit of the long bean plant grown in the planting medium C because the roots can inhibit the absorption of mercury so that mercury does not translocate to other plants such as fruit. Meanwhile, at the 3 month planting period, it shows a significance value of 0.136. The value 0.136 means that there is no significant difference in the fruit of plants grown on growing media A and medium C. The presence of mercury on fruit supported by research conducted by Ross & Stewart (1962) that the mercury in the fruit is the result of translocation of mercury from leaves to fruit.

Figure 3 show that total absorption of heavy metal mercury in long bean (*V. unguiculata* L. Walp) was tested by the T-test. The value of 0.042 means that there is a difference between the total absorption of heavy metal mercury on growing media A (100% katel soil) with planting medium C (40% of mine land) during the planting period 1, 2, 3 and 4 months. Planted long bean (*V. unguiculata* L. Walp) plants on the growing medium C shows that mercury is absorbed throughout plant. This is supported by research conducted by Marrugo-Negrete et al. (2020) that mercury can be absorbed into roots, stems, leaves, and fruits in long bean plants. Also, mercury ions (Hg) are categorized into ion groups that can move (mobile) so that it can be easily translated to plant canopy (Meagher & Heaton, 2005).

CONCLUSION

Stem height, number of leaves, dry weight (roots, stems, leaves and fruit) and total dry weight of cowpea (*V. unguiculata* L. Walp) the highest was in the planting medium A (control) followed by the planting medium B (20% mine land), C (40% of mine land) and has decreased at planting media D (60% mining land) and E (80% mining land) and there is a significant difference in mercury absorption cowpea (*V. unguiculata* L. Walp) grown in planting media A and C the roots, stems, leaves and fruit at the planting period of 1 and 4 months. Not there was a significant difference in the roots at the 2 and 3 month planting period, in the stems during the planting period of 3 months and the fruit at the time planting 3 months. There is a significant difference in total mercury absorption on planted cowpea (*V. unguiculata* L. Walp) media A and planting medium C.

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