PHYTOREMEDIATION ROLE IN INCREASING RICE (*Oryza Sativa* L.) PRODUCTION WITH REDUCED MERCURY CONTENT IN MERCURY CONTAMINATED SOIL

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ABSTRACT

This study aimed to obtain phytoremediator that have better effectiveness to accumulating mercury so as to reduce mercury content in grains, research was conducted on mercury-contaminated rice fields. The study was conducted withim randomized block design with 3 replications. First factors are varieties namely Ciherang, IR-64, Siganteng (local variety), and Inpari-32, the second are the type of phytoremediator, namely: Kiambang, Eceng Gondok and Jerango and third factor are population of phytoremediator, namely: without phytoremediator, 5, 10 and 15 plants per plot. The plot size used was 1 metre x 2 meters with 20 rice plants per plot. The results showed that there was a decrease in mercury content in rice grains of all varieties below the quality standard threshold in eceng gondok and jerango treatments with population 10 and 15 plants per plot. The results obtained to reduce the mercury content below the quality standard threshold with high production can be done by the application of jerango phytoremediators with population 10 plants per plot with the best variety is IR-64.

Keywords: Food, Hg, Heavy Metal, Pollution, food safety

دور المعالجة النباتية في إنتاج أرز (*Oryza sativa* L) منخفض الزئبق في التربة الملوثة بالزئبق

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المستخلص

هدفت هذه الدراسة إلى الحصول على معالج نباتي له فعالية أفضل في تراكم الزئبق لتقليل محتوى الزئبق في الحبوب ، تم إجراء بحث على حقول الأرز الملوثة بالزئبق. أجريت الدراسة بتصميم بلوك معشاة مع 3 تكرارات. العوامل الأولى هي أصناف وهي Ciherang و IR-64 و Siganteng (صنف محلي) و Inpari-32 ، والثاني هو نوع المعالج النباتي ، وهي: Kiambang و Eceng Gondok و Jerango والعامل الثالث هو سكان الوسيط النباتي ، وهي: بدون معالج نباتي ، 5 و 10 و 15 نباتا لكل قطعة أرض. كان حجم قطعة الأرض المستخدمة 1 متر × 2 متر مع 20 نبتة أرز لكل قطعة أرض. أظهرت النتائج أن هناك انخفاضا في محتوى الزئبق في حبوب الأرز من جميع الأصناف دون العتبة القياسية للجودة في علاجات eceng gondok و jerango مع عدد 10 و 15 نبتة لكل قطعة أرض. يمكن تحقيق النتائج التي تم الحصول عليها لتقليل محتوى الزئبق إلى ما دون عتبة الجودة القياسية مع الإنتاج المرتفع عن طريق استخدام المعالجات النباتية jerango التي يبلغ عدد سكانها 10 نباتات لكل قطعة أرض مع أفضل صنف هو IR-64.

الكلمات المفتاحية: الغذاء، الزئبق، المعادن الثقيلة، التلوث، سلامة الغذاء

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INTRODUCTION

Mandailing Natal (Madina) Regency is administratively located in North Sumatra province with an area of 662,070 hectares and a paddy field area of 17,159 hectares with a total production of 72.323 thousand tons and a labor force population of 221,126 people with 43.08% of them are rice farmers with the harvest is for personal consumption and the rest is sold. Agriculture in Madina is mostly irrigated agriculture with water sources coming from the Batang Gadis river. The Batang Gadis River, which flows along 180 km in Madina district, is a source of life for the population as a source of drinking water, irrigation and protein food in the form of fish. Unlicensed gold mining in Madina district, especially in Muara Sipongi, Hutapungkut, Hutabargot, and Nagajuang sub-districts, produces mercury waste through the almalgamation process which is entirely discharged into the Batang Gadis river which can be a source of pollution that is harmful to health (42). Mercury content in rice fields of District Hutabargot with irrigation source from amalgamation waste reached 14.26 ppm (37). Another research also reported that rice fields affected by amalgamation waste in Mandailing Natal Regency have mercury content above the quality standard and the highest contamination was found in the Districts Hutabargot as much as14.24 ppm and Naga Juang as mush as 13.26 ppm (39). These values indicates that the agroecosystem is contaminated with mercury exceeding the quality standard threshold (0.03 mg kg^{-1} dry matter) (3, 4, 14). The presence of mercury over the threshold can trigger various health problems such as damage to the nervous system, lungs, heart, liver, kidneys and skin, and affect fetal health with children and infants being more susceptible to central nervous system damage (42). The problem needs serious handling to avoid worsening health cases. Mercury contamination in humans can occur through several foodstuffs consumed daily. Rice produced from mercury-contaminated land can be a major source of mercury poisoning (8, 29, 34, 45). It is estimated that of the total mercury consumed by humans, rice contributes the highest to 94-96%, and only 1-2% is estimated to come from fish (15, 46) reported that mercury concentration in rice grains can reach 569 μg/kg. (43) also reported that rice produced from Hg mining areas contained levels >100 μg/kg. Research to reduce or even eliminate the effects of mercury can be done with the use of phytoremediation. The use of phytoremediation is estimated to be the fastest and most time-efficient solution when compared to other solutions such as: The assembly of low mercury rice varieties is estimated to take at least 6 years (38), the use of bioremediation with biocar enriched with Fe3O4 (15) which is considered to require high costs and has another side effect of Fe accumulation in rice fields, the use of biocar enriched with sulfur (11) is feared to increase soil acidity in the long run. The use of carbonized gun is effective for absorbing heavy metals, but the pH level varies depending on the type of heavy metal, Therefore, phytoremediation is considered the most effective mercury remediation method. Phytoremediation research is currently being carried out to overcome environmental pollution caused by various heavy metals such as the use of parupuk plants (*Phragmites karka*) which can absorb 90.74% of mercury (27), mustard plants (*Brassica juncea*) which can survive up to 20 mg Hg kg^{-1} of soil because they have antioxidant defense (36), water hyacinth plants (26). Mariwy et al. (2021) (22) also reported the ability of guava plants (*Psidium guajava* L.), lempuyang gajah (*Zingiber zerumbet* L.), and harendong bulu (*Clidemia hirta* L.) in accumulating Hg gold mining waste. Some phytoremediation studies are generally able to reduce the level of Hg pollution on ex-mining land but research on the use of phytoremediation in conjunction with rice paddy planting or the use of phytoremediation to overcome mercury pollution on rice fields is still rarely studied. This is related to the incompatibility of fithoremediator plants planted together with rice plants such as parupuk, guava, lempuyang gajah and harendong bulu plants that will even occur nutrient competition and sunlight competition. For this reason, it is necessary to research suitable phytoremediators to reduce mercury content in paddy fields without disturbing the growth and development of rice plants. This study aims to reduce mercury uptake in rice plants by reducing mercury content in paddy field soil. Specifically, this research aims to: 1. obtain the best type of phytoremediator to overcome mercury pollution in paddy fields in Mandailing Natal district, 2. obtain mercury-tolerant and high-yielding rice varieties.

MATERIALS AND METHODS

The study was conducted on rice fields in Madina district with the location determined based on preliminary analysis of mercury content. The location used as the research site was the rice field with the highest mercury content (14.26 ppm). The research was conducted from July to October 2023. This material used in this study were HNO_3 PA, H_2SO_4 PA, H_2O_2 solution (30%), and four rice varieties Ciherang, IR 64, Siganteng and IR 32. The tools used were UV-Vis spectrophotometer, glass cup, measuring flask, and several other tools that support in the analysis of Hg. The study was conducted withim randomized block design with 3 replications. First factors are varieties namely Ciherang, IR-64, Siganteng (local variety), and Inpari-32, the second are the type of phytoremediator, namely: Kiambang, Eceng Gondok and Jerango and third factor are population of phytoremediation plants, namely: P_0: without phytoremediation, P_1: 5 plants/plot, P_2: 10 plants/plot and P_3: 15 plants per plot. The plot size used was 1 metre x 2 meters with a total of 20 rice plants per plot and 4 plants used as destructive samples. The research also included four control treatments varieties without phytoremediator. The parameters observed were plant height, number of tillers, number of productive tillers, flowering age, harvest age, number of empty grains, number of filled grains, production per plot, and Hg content in rice roots, stems, leaves, and grains. For extraction of Hg from plant tissues, the method of Du *et al.* (2021) was followed (8). Measurement of Hg metal content was made by using UV-Vis spectrophotometer with the addition of Dithizonate at a wavelength of 495 nm (1; 44). The data obtained from the spectrophotometer was compiled and interpreted with simple linear regression equation obtained from the blank sample

RESULTS AND DISCUSSION

The results showed that there was a significant decrease in the parameters of plant height and the number tillers in the treatment without phytoremediation (control) (Table 1). Plant responses to environmental stress, such as toxic metalloids including mercury, can alter growth parameters such as plant height (24, 9). At the molecular level, there are mechanisms such as the production of antioxidant enzymes and signalling compounds that can be produced by exposed parts to protect plants from environmental stress. For example, gibberellins are known to play an important role in determining plant height in various species (19). Genes associated with gibberellin biosynthesis and signalling pathways have been identified as major contributors to plant height variation (13). The parameters of flowering age and harvest age showed that there was a significant decrease in the treatment without phytoremediation (Table 1). The results of this study indicate that plants that are not given the fithoremediation treatment will experience mercury stress as indicated by the adaptation process in the form of decreased plant growth and decreased flowering age and harvest age. Plants that experience stress conditions, including mercury stress, will try to avoid stress conditions including by shortening plant life, accelerated senescence and leaf abscission are the plant 'escape strategies' against stress conditions (20; 10,). Stress conditions in plant can accelerate the senescence process. As a result of the senescence process in plants, it will have an impact on the disruption of the photosynthesis process which has implications for reducing yield (31). The decreases in plant growth occurs because the presence of heavy metals causes disruption of nutrient absorption and photosynthesis processes. The imbalance of metal content in photosynthetic cells causes inhibition of the photosynthetic process by disrupting carbon reactions (35) and Chlorophyl content also decreased significantly linearly with the increasing the concentration of heavy metal Pb and Cr (4). The increase of heavy metals beyond the threshold disrupts the process of water absorption and homeostasis (32), the presence of heavy metals in the soil also disrupts the status of nutrients in the soil (6), disrupting the absorption of other nutrients needed by plants (5) especially the element potassium, this happens because the channel for Hg entry into the plant is the same as the channel for K^+ entry into the plant (33).

Table 1. Plant height, number of tillers, flowering age and harvesting age of 4 varieties under phytoremediation treatments.

Treatments	Varieties			
	V_1 (Ciherang)	V_2 (IR-64)	V_3 (Siganteng)	V_4 (Inpari-32)
		Plant height (cm)		
Control	92.22b	92.60c	91.87b	91.73b
P stratiotes 5 plants/plot	101.93a	96.53b	99.40a	101.47a
P stratiotes 10 plants/plot	101.67a	100.27a	99.93a	103.80a
P stratiotes 15 plants/plot	100.53a	101.73a	99.60a	101.47a
E crassipes 5 plants/plot	103.27a	99.73a	101.27a	101.00a
E crassipes 10 plants/plot	102.27a	102.13a	101.07a	102.67a
E crassipes 15 plants/plot	101.33a	101.53a	102.27a	101.80a
A calamus 5 plants/plot	100.07a	101.20a	100.67a	101.73a
A calamus 10 plants/plot	102.47a	101.33a	100.40a	103.20a
A calamus 15 plants/plot	101.93a	101.73a	102.20a	104.87a
		Number of tillers		
Control	16.62b	16.53b	18.51b	16.27b
P stratiotes 5 plants/plot	24.73a	24.87a	23.40a	24.93a
P stratiotes 10 plants/plot	25.00a	25.33a	25.00a	25.00a
P stratiotes 15 plants/plot	25.67a	24.47a	25.00a	25.07a
E crassipes 5 plants/plot	24.80a	25.00a	24.73a	24.80a
E crassipes 10 plants/plot	24.80a	24.80a	24.73a	25.00a
E crassipes 15 plants/plot	24.60a	25.20a	24.67a	25.07a
A calamus 5 plants/plot	25.20a	24.87a	24.47a	25.40a
A calamus 10 plants/plot	24.40a	24.93a	25.20a	24.80a
A calamus 15 plants/plot	25.07a	24.47a	24.87a	24.87a
		Flowering age (day)		
Control	51.76b	52.27b	50.02b	58.31b
P stratiotes 5 plants/plot	56.20a	56.13a	53.53ab	61.40a
P stratiotes 10 plants/plot	57.93a	57.67a	55.73a	62.07a
P stratiotes 15 plants/plot	57.93a	58.53a	55.73a	62.67a
E crassipes 5 plants/plot	56.67a	55.27a	55.27a	62.00a
E crassipes 10 plants/plot	58.07a	57.60a	55.53a	62.80a
E crassipes 15 plants/plot	58.13a	58.13a	56.20a	62.80a
A calamus 5 plants/plot	55.73a	57.20a	54.67a	61.27a
A calamus 10 plants/plot	58.07a	58.27a	55.60a	62.73a
A calamus 15 plants/plot	58.53a	58.13a	55.73a	62.87a
		Harvesting age (day)		
Control	87.71b	87.27c	85.02c	93.31d
P stratiotes 5 plants/plot	91.20a	91.13b	88.53bc	96.40c
P stratiotes 10 plants/plot	92.93a	94.87ab	96.80a	102.20b
P stratiotes 15 plants/plot	92.93a	95.40a	99.27a	106.33b
E crassipes 5 plants/plot	91.67a	90.27b	90.27b	97.00c
E crassipes 10 plants/plot	93.33a	95.33a	96.40a	105.40b
E crassipes 15 plants/plot	93.13a	94.93a	99.13a	106.67b
A calamus 5 plants/plot	91.67a	92.20b	89.67b	96.27c
A calamus 10 plants/plot	93.20a	95.27a	96.40a	106.67b
A calamus 15 plants/plot	93.53a	97.40a	99.27a	112.27a

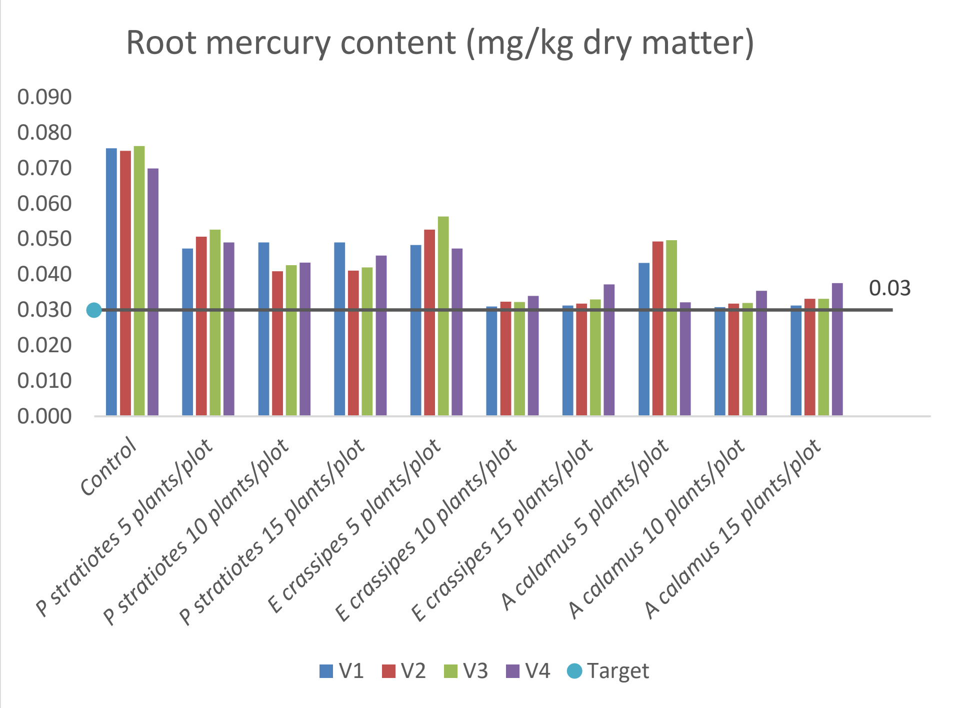
Note: numbers followed by the same letter in the same parameter indicated not significant differences according to duncan's multiple range test (DMRT) at α=0.05.

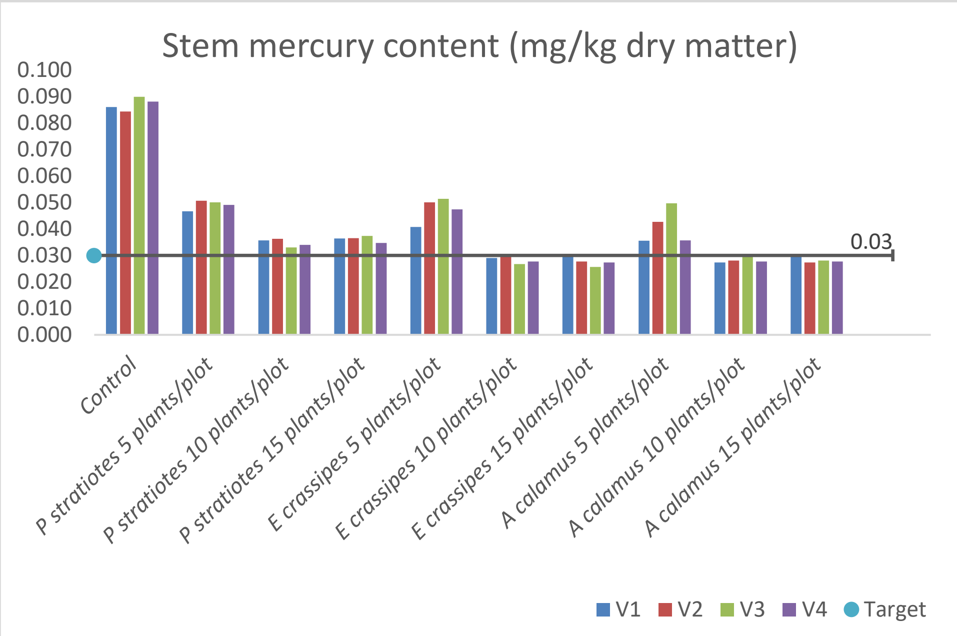
The compound produced by plants to inhibit the entry of Hg into plants is Tetraethilamonium (TEA). The TEA compound will close the channel of Hg entry and at the same time will also close the entry of K^+ in plants (21). K deficiency in plants will cause disruption of the balance of anions and cations, and disruption of protein synthesis and enzymes that act as a response to stress such as the formation of the antioxidant ascorbic acid (7; 18). The results of the analysis of the mercury content of roots, stems, leaves and grain showed a significant effect of the type and population of phytoremediation treatment. Mercury content in the treatment without phytoremediation and phytoremediation with population 5 plants per plot showed a content that was still above the quality standard threshold of above 0.03 mg/kg dry matter (Figure 1). Another research (43) also reported that rice produced from Hg mining areas contained levels >100 µg/kg. While the treatment with a frequency of phytoremediation of 10 plants and 15 plants per plot for all types of phytoremediation showed mercury content that was already below the quality standard threshold. These results indicate that fithoremediation with population above 10 plants per plot can reduce mercury content below the quality standard threshold. Several studies have also reported that the use of phytoremediation can reduce mercury content in mercury-contaminated soil such as the use of parupuk plants (*Phragmites karka*) which can absorb 90.74% of mercury (27), mustard plants (*Brassica juncea*) which can survive up to 20 mg Hg kg^{-1} soil because they have antioxidant defenses and are able to accumulate in their tissues (36), water hyacinth plants, (26). Another research (15) also reported the ability of guava (*Psidium guajava* L.), lempuyang gajah (*Zingiber zerumbet* L.) and harendong bulu (*Clidemia hirta* L.) plants to accumulate gold mining waste Hg.

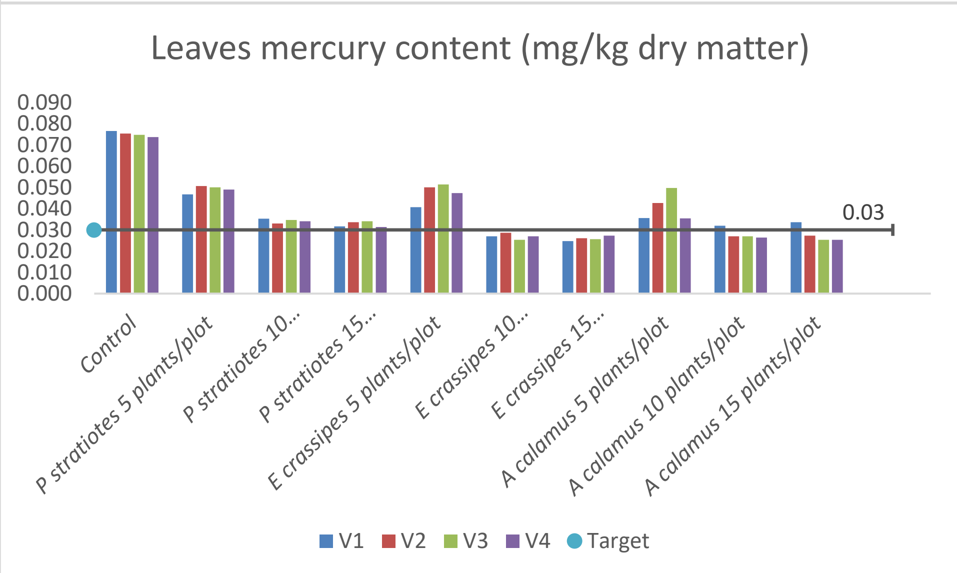
Table 2. Number of filled and empty grains of four rice varieties under phytoremediation treatment.

Treatments	Varieties			
	V_1 (Ciherang)	V_2 (IR-64)	V_3 (Siganteng)	V_4 (Inpari-32)
		Number of filled grain		
Control	87.78m	101.44m	105.00m	99.89m
P stratiotes 5 plants/plot	194.33c-f	176.00fgh	127.67l	192.00def
P stratiotes 10 plants/plot	150.33jk	211.00abc	134.00kl	215.00ab
P stratiotes 15 plants/plot	214.33abc	177.00fgh	192.33def	182.33efg
E crassipes 5 plants/plot	174.00fgh	191.67def	215.33ab	209.00a-d
E crassipes 10 plants/plot	224.67a	215.00ab	228.67a	183.33efg
E crassipes 15 plants/plot	178.33fgh	178.33fgh	169.00g-j	177.00fgh
A calamus 5 plants/plot	209.33a-d	201.67b-e	160.67hij	187.33efg
A calamus 10 plants/plot	126.67l	199.33b-e	202.00b-e	208.00bcd
A calamus 15 plants/plot	183.67efg	214.00abc	171.33ghi	152.67ijk
		Number of empty grain		
Control	131.56g	118.67g	122.89g	124.44g
P stratiotes 5 plants/plot	54.33c-f	57.33def	45.00a-f	53.67b-f
P stratiotes 10 plants/plot	49.67a-f	40.00a-e	65.67ef	21.67a
P stratiotes 15 plants/plot	19.00a	56.33def	25.33abc	33.67a-d
E crassipes 5 plants/plot	72.00f	41.67a-e	25.33abc	26.33abc
E crassipes 10 plants/plot	21.67a	30.67a-d	22.67a	33.67a-d
E crassipes 15 plants/plot	36.00a-e	63.00ef	33.67a-d	27.67a-d
A calamus 5 plants/plot	24.00ab	31.67a-d	53.67b-f	46.00a-f
A calamus 10 plants/plot	49.67a-f	56.00def	30.67a-d	23.00ab
A calamus 15 plants/plot	49.67a-f	30.00a-d	28.67a-d	32.33a-d

Note: numbers followed by the same letter in the same parameter indicate not significant differences according to duncan's multiple range test (DMRT) at α=0.05.


Root mercury content (mg/kg dry matter)
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.03
Control
P stratiotes 5 plants/plot
P stratiotes 10 plants/plot
P stratiotes 15 plants/plot
E crassipes 5 plants/plot
E crassipes 10 plants/plot
E crassipes 15 plants/plot
A calamus 5 plants/plot
A calamus 10 plants/plot
A calamus 15 plants/plot
V1 V2 V3 V4 Target


Stem mercury content (mg/kg dry matter)
0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.03
Control
P stratiotes 5 plants/plot
P stratiotes 10 plants/plot
P stratiotes 15 plants/plot
E crassipes 5 plants/plot
E crassipes 10 plants/plot
E crassipes 15 plants/plot
A calamus 5 plants/plot
A calamus 10 plants/plot
A calamus 15 plants/plot
V1 V2 V3 V4 Target


Leaves mercury content (mg/kg dry matter)
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.03
Control
P stratiotes 5 plants/plot
P stratiotes 10...
P stratiotes 15...
E crassipes 5 plants/plot
E crassipes 10...
E crassipes 15...
A calamus 5 plants/plot
A calamus 10 plants/plot
A calamus 15 plants/plot
V1 V2 V3 V4 Target

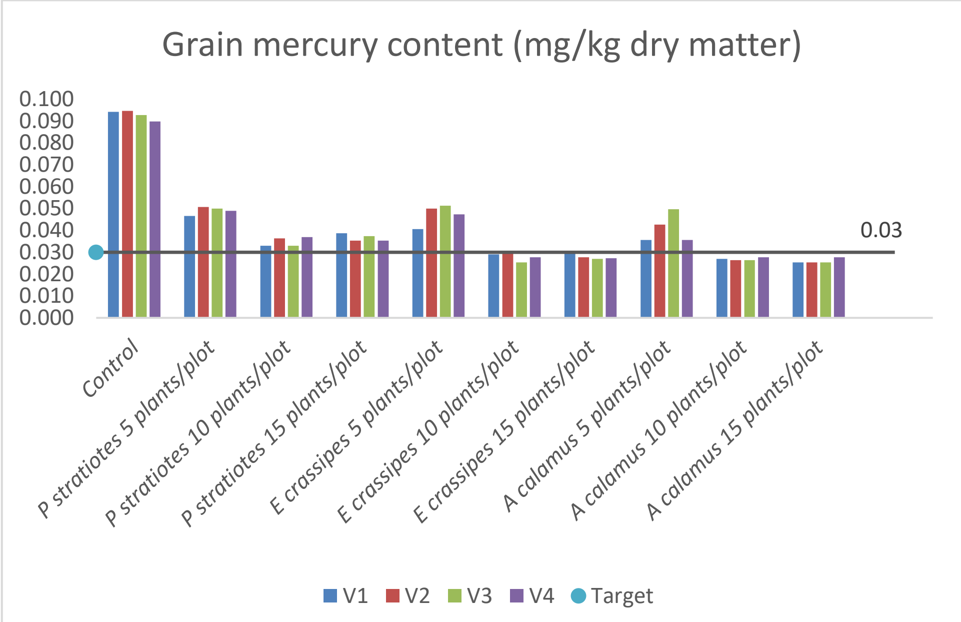


Figure 1. Histogram of mercury content of roots (a), stems (b), leaves (c) and rice grains (d) in phytoremediation treatment.

The results show an increases in production in all types of phytoremediators up to a population of 10 plants per plot while at population 15 plants per plot the results showed a downward trend (Table 3). The increase in production due to phytoremediation treatment is due to the negative effect of mercury metal on rice plants can be minimized. Phytoremediator plants are able to absorb mercury metal so that mercury exposure to rice plants is reduced. The presences of mercury metal in plant roots can reduce the uptake of K metal for plants. For plants, the most common symptoms of mercury exposure are root growth inhibition (40; 41), photosynthesis inhibition and yield reduction (25), disruption of water transport and uptake systems, leaf chlorosis, and inhibition of potassium uptake (33). The negative effects of mercury exposure on rice plants can be seen in the increase in the number of empty grains and the decrease in the number of filled grains (Table 2). The highest number of empty grains was found in the control treatment which was significantly different from the phytoremediation treatment while the lowest number of empty grains was found in Ciherang variety with phytoremediation of *P stratiotes 15* plants/plot. In the phytoremediator treatment with population 15 plants/plot, production started to decline (Table 3). This is related to nutrient competition between the main plant and the phytoremediator plant. If it is associated with mercury content in roots, stems, leaves and grains, the best research result to reduce mercury content below the quality standard threshold with high production is the IR-64 variety planted with *A calamus* phytoremediators with population 10 plants per plot (2 meter^2). *A calamus* has the ability to grow and adapt to soils contaminated with heavy metals, such as mercury, without suffering damage or impaired growth, or showing symptoms of toxicity. Rice et al. (2014) (30) suggested that this adaptive trait is due to the ability of plants to absorb heavy metals from the soil through their roots and then translocate these metals to other parts of the plant, including stems, leaves, and fruits. The process of bioaccumulation and translocation of heavy metals by plants, particularly mercury, is a critical aspect of phytoremediation, where plants aid in the removal or control of pollutants from the environment (23). Although mercury contamination in soil can have detrimental effects on plant growth and even cause plant death, some plant species show tolerance and the ability to thrive in mercury-contaminated environments (28).

Table 3. Production per plot of 4 varieties in phytoremediation treatment (gram)

	Varieties			
	V_1 (Ciherang)	V_2 (IR-64)	V_3 (Siganteng)	V_4 (Inpari-32)
Control	242.44jkl	179.00n	233.67kl	201.56mn
P stratiotes 5 plants/plot	296.67fgh	312.67fg	294.00fgh	301.67fgh
P stratiotes 10 plants/plot	288.00ghi	324.00ef	217.33lm	361.67d
P stratiotes 15 plants/plot	314.67fg	259.00ijk	281.00hi	265.67ij
E crassipes 5 plants/plot	312.67fg	378.00d	324.00ef	352.33de
E crassipes 10 plants/plot	349.33de	350.00de	319.00f	364.00d
E crassipes 15 plants/plot	195.33n	228.33klm	201.67mn	177.33n
A calamus 5 plants/plot	521.00a	429.00bc	448.67b	359.67d
A calamus 10 plants/plot	422.33bc	526.00a	434.67bc	416.00c
A calamus 15 plants/plot	304.33fgh	497.33a	415.67c	404.00c

Note: numbers followed by the same letter indicated not significant differences according to Duncan's multiple range test (DMRT) at α=0.05.

Reduced production due to mercury stress indicates physiological disturbances caused by mercury exposure. Heavy metals at toxic levels can inhibit normal plant functions and act as inhibitors of metabolic processes in a variety of ways, including disruption or displacement of building blocks of protein structures, arising from the formation of bonds between heavy metals and sulfhydryl groups (16), inhibiting functional groups of important cellular molecules (17), replacing or interfering with essential metal functions in biomolecules such as pigments or enzymes (2), adversely affecting the strength of cytoplasmic membranes (12), resulting in the disruption of various important processes in plants such as photosynthesis, respiration, and enzymatic activity (17).

CONCLUSION

1- There was a decrease in mercury content in the roots, stems, leaves and grains of rice plants with phytoremediation treatment. Mercury content in grains that are below the quality standard threshold is found in the phytoremediation treatment of *P stratiotes* or *A calamus* with a population of more than 10 plants/meter2.

2- The best treatment to reduce mercury content below the quality standard threshold with high production was IR-64 variety treatment with *A calamus* phytoremediation with population 10 plants per meter^2.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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