



JERANGO (*ACORUS CALAMUS* L.) WITH PHYTOREMEDIATION ROLE IN MERCURY-CONTAMINATED RICE FIELDS TO IMPROVE PRODUCTIVITY AND REDUCE MERCURY CONTENT IN RICE PLANTS

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SUMMARY

The novel study aimed to determine the optimal population density of jerango (*Acorus calamus* L.) plants as the phytoremediators and examine the heavy metal absorption by rice plants under mercury-contaminated soil stress conditions. The experiment, carried out in a two-factorial randomized design, had the first factor being the number of Jerango plants per square meter with six phytoremediation treatments, viz., F₀ (control), F₁ (1 plant/m²), F₂ (2 plants/m²), F₃ (3 plants/m²), F₄ (4 plants/m²), and F₅ (5 plants/m²). The second factor was the levels of HgCl₂-treated soil medium with seven treatments, i.e., K₀ (0 ppm), K₁ (3 ppm), K₂ (6 ppm), K₃ (9 ppm), K₄ (12 ppm), K₅ (15 ppm), and K₆ (18 ppm). The experiment had two repetitions to recognize the variations in rice growth at the early vegetative stage under mercury-contaminated soil stress conditions. The results showed phytoremediation treatment with three jerango plants/m² reduced the soil mercury content below the threshold level, improved growth and production, and lessened mercury content in rice roots, stems, leaves, and grains below the quality standard threshold. No studies have yet reported jerango (*A. calamus* L.) as a phytoremediation plant.

Keywords: Jerango (*A. calamus* L.), amalgamation waste, growth traits, heavy metals, mercury-contaminated soil, tolerant rice cultivar

Key findings: Phytoremediation with three plants/m² of jerango (*A. calamus* L.) considerably reduced the soil mercury content below the threshold level, increased rice growth and production, and reduced the mercury content in the rice roots, stems, leaves, and grains.

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: January 08, 2026; Accepted: June 20, 2026.

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Citation: Syahril M, Rosmayati, Rahmawati N, Siregar DS (2026). Jerango (*Acorus calamus* L.) with phytoremediation role in mercury-contaminated rice fields to improve productivity and reduce mercury content in rice plants. *SABRAO J. Breed. Genet.* 58 (5) 2316-2326. <http://doi.org/10.54910/sabrao2026.58.5.31>.

INTRODUCTION

In the North Sumatra Province, Indonesia, the Mandailing Natal (Madina) Regency has a total area of 662,070 ha, wherein the paddy field area is 22,625 ha, observed with the production of 95,562 tons of rice in 2021 (BPS, 2024). The said area has a labor force population of 228,248, in which 50% is a rice farming community, which uses the harvest for personal consumption while selling the rest (BPS, 2024). The arable land in Madina is irrigated using water from the Batang Gadis River.

The Batang Gadis River, flowing along 180 km in the District Madina, plays a remarkable role as the source of life for the existing population, providing drinking and irrigation water and protein-rich food in the form of fish. Unlicensed gold mining, such as PETI in the Madina District, particularly the subdistricts Muara Sipongi, Hutapungkut, Hutabargot, and Nagajuang, generates mercury waste and discharges into this river, posing health risks. The mercury content in ricefields of the sub-district of Hutabargot, with irrigation from amalgamation waste, has reached 14.26 ppm (Syahril *et al.*, 2024). Triyanti *et al.* (2024) reported that the mercury content in ricefields exposed to amalgamation waste in the Mandailing Natal Regency exceeds the threshold level, and these contents were the highest in subdistricts Hutabargot and Nagajuang (14.24 and 13.26 ppm, respectively). These mercury contamination levels in the agroecosystem exceed the recommended threshold of 0.03 mg kg⁻¹ dry matter (Indonesia National Agency of Drug and Food Control, 2018).

This problem needs thorough attention and a solution as it adversely affects human health and agroecosystems. Mercury contamination in human food can occur through daily consumption of several foodstuffs. However, rice grown on mercury-contaminated soils is the major source of mercury poisoning (Du *et al.*, 2021). Of the total mercury consumed by humans, rice contributes the highest (94%–96%), and only an estimated 1%–2% originates from fish

(Giwa *et al.*, 2022). Zhang *et al.* (2019) reported that mercury concentrations in rice grains can reach 569 µg kg⁻¹.

Phytoremediation is one of the most time-efficient solutions for reducing and even eliminating mercury contamination in rice grains. Other proposed solutions are either time-consuming or incurring high costs, with some even adversely affecting soil quality, such as the assembly of low-mercury rice cultivars, which takes at least six years (Tiodar *et al.*, 2021). Bioremediation with biochar enriched with Fe₃O₄ also requires relatively high costs and causes Fe accumulation in rice fields, and the biochar enriched with sulfur eventually increases soil acidity in the long term (Giwa *et al.*, 2022; Triyanti *et al.*, 2025). Therefore, phytoremediation seemed to be the most effective mercury remediation method.

The conduct of intensive research is progressive on phytoremediation to overcome environmental pollution caused by various heavy metals. Past studies have shown parupuk plants (*Phragmites karka*) can absorb 90.74% of mercury from contaminated soils (Ni'mah *et al.*, 2022), and mustard plants (*Brassica juncea*) can survive up to 20 mg of mercury kg⁻¹ soil because of the viable antioxidant defense system (Suganthi and Avudainayagam, 2022). Bortoloti and Baron (2022) also reported the same results with mustard and water hyacinth plants to phytoremediate the wastewater (Nazir *et al.*, 2020). Moreover, guava (*Psidium guajava* L.), lempuyang gajah (*Zingiber zerumbet* L.), and harendong bulu (*Clidemia hirta* L.) plants can accumulate mercury from gold mining waste (Mariwy *et al.*, 2021). Although several phytoremediation studies have reported success in reducing heavy metal contamination in ex-mining lands, phytoremediation application in rice cultivation continues to be rare because phytoremediation plants often engage in nutrient competition with the main crop.

Based on the above circumstances, the pioneering study aimed to determine the optimal population density of jerango (*Acorus calamus* L.) plants for the phytoremediation of mercury-contaminated soil stress conditions.

In addition, it sought to examine the ability of *A. calamus* plants to better absorb heavy metals from mercury-contaminated planting media compared with the water hyacinth (*Eichhornia crassipes*), kiambang (*Pistia stratiotes*), and water jasmine plant (*Echinodorus palaefolius*) (Syahril *et al.*, 2025).

MATERIALS AND METHODS

Experimental site and procedure

This study, conducted between July and October 2024, took place in the greenhouse at the University of North Sumatera, Medan, Indonesia. The research's main objective was to determine the most optimal population density of jerango (*A. calamus* L.) plants as a phytoremediator to reduce the mercury uptake in rice grains while maintaining the highest productivity. The materials used in this study included concentrated HNO_3 and H_2SO_4 solutions, HgCl_2 , aquabidest, dithizone, Whatman 42 filter paper, and seeds of the rice cultivar IR64 being mercury tolerant (Syahril *et al.*, 2025). The tools used in this study comprised an ultraviolet-visible (UV-Vis) spectrophotometer with the addition of dithizone at a wavelength of 495 nm and some other tools that support mercury analysis in the laboratory (Ahmad *et al.*, 2021).

Jerango (*A. calamus* L.) plants used as phytoremediators in this study incurred analysis for mercury content in their rhizomes in the laboratory. Mercury extraction from *A. calamus* rhizomes proceeded following the method of Du *et al.* (2021) as follows: weighing 2 g of mashed plant samples before placing them into a 250 ml round-bottom flask; subsequently, gradually adding 65% concentrated HNO_3 and 95% concentrated H_2SO_4 solutions ensued, followed by heating until the solution became clear. The solution underwent cooling and filtering through a Whatman 42 filter paper. In the extraction solution, measuring the mercury metal content continued after adding dithizone at a wavelength of 495 nm using a UV-Vis spectrophotometer (Ahmad *et al.*, 2021).

This study used a two-factorial randomized design, with the first factor being the number of *A. calamus* phytoremediation plants/ m^2 , with six phytoremediation treatments, i.e., F_0 (control), F_1 (1 plant/ m^2), F_2 (2 plants/ m^2), F_3 (3 plants/ m^2), F_4 (4 plants/ m^2), and F_5 (5 plants/ m^2). The determination of the phytoremediator plant to be tested was according to the results of the first year of study, with the most optimal treatment of the *A. calamus* plants at a mercury contamination level of 14.26 ppm. The second factor was the mercury contamination levels, with seven treatments comprising K_0 (0 ppm), K_1 (3 ppm), K_2 (6 ppm), K_3 (9 ppm), K_4 (12 ppm), K_5 (15 ppm), and K_6 (18 ppm). Treatment determination of concentration levels was reliant on various levels of mercury contamination in the Madina District.

In the planting media, the mercury content determination proceeded after applying HgCl_2 according to different concentrations. The study had two repetitions, with the planting media filled in a circular tub with an 80-cm diameter; the number of plants per research unit was 13, with three plants used as destructive samples. *A. calamus* plants entailed planting four weeks before using with the population according to the treatment, then placing them among the rice plants.

Traits studied and analysis

The data recorded were on various parameters comprising soil mercury content 1–4 weeks after planting the phytoremediator plants, mercury content in phytoremediator plants (*A. calamus* rhizome), plant height, and the number of rice tillers in the early vegetative phase. Data recording also included the number of productive tillers, the number of filled grains, the number of empty grains, production per m^2 , and mercury content in rice roots, stems, leaves, and grains. The data obtained underwent the analysis of variance at a 95% confidence level. If the treatment has a significant effect, the analysis continues with the Duncan's multiple range test (DMRT) at a 5% significance level. The data analysis used SAS 9.4 software.

RESULTS AND DISCUSSION

The phytoremediation results showed a considerable decrease in mercury content in the research media, which emerged in line with the increase in the number of phytoremediator plants and absorption time (weeks after planting or WAP) (Figure 1). The soil mercury content with 6 ppm and the phytoremediation treatment with 3 plants/m² of *A. calamus* could reduce soil mercury content and keep it below the threshold level at 4 WAP. Meanwhile, those with 4 and 5 plants/m² of *A. calamus* reduced the mercury content to below the threshold level at 3 and 2 WAP, respectively. However, the soil mercury content of 9 ppm and the minimum number of *A. calamus* (4 plants/m²) necessitated the mercury content reduction and keeping it below the threshold level in 4 WAP. In contrast, with soil mercury contents of 12, 15, and 18 ppm, none of the phytoremediation treatments could reduce the mercury content below the threshold level at 4 WAP. Ansari *et al.* (2021) also reported that 10 different genotypes of Indian mustard (*Brassica juncea*) could accumulate mercury in their roots and crowns within 20 days, and the plants' mercury content rose with an increased mercury content in the planting medium. Specifically, regarding *Acorus calamus*, no studies have yet reported its use as a remediation plant.

The results further revealed *A. calamus* plants could absorb mercury from the contaminated soil media. Phytoremediation can help reduce mercury content in mercury-contaminated soils. For example, the water hyacinth plants can phytoremediate wastewater (Nazir *et al.*, 2020); guava (*Psidium guajava* L.), lempuyang gajah (*Zingiber zerumbet* L.), and Harendong bulu (*Clidemia hirta* L.) have reports of the ability to accumulate mercury from gold mining waste (Mariwy *et al.*, 2021); and parupuk plants (*Phragmites karka*) can absorb 90.74% of mercury from contaminated soil (Ni'mah *et al.*, 2022). Moreover, mustard plants (*Brassica juncea*) can survive up to 20 mg mercury kg⁻¹ soil due to their antioxidant defense system (Suganthi and Avudainayagam, 2022) and can

accumulate it in their tissues (Shomali *et al.*, 2024).

Treatments could reduce mercury content below the threshold level of 4 WAP. These are phytoremediator treatments of 1 *A. calamus* plant/m² at 6 ppm mercury content, minimum populations of 2 plants/m² at 9 ppm mercury content, and at least 5 *A. calamus* plants/m² at 12 to 15 ppm mercury content. However, with soil mercury content of 18 ppm, phytoremediation treatment with 5 plants/m² could not reduce mercury content at 4 WAP. These results revealed the need for a special treatment to overcome mercury-contaminated land with mercury content of above 18 ppm. However, phytoremediation technology effectively remediates mercury contents of up to 15 ppm.

The *A. calamus* plants were evidently potential phytoremediators because of their ability to accumulate mercury, which increased with higher content of Hg in the soil (Figure 2). Mercury uptake by *A. calamus* plants was higher in lower populations; however, the said ability decreased with an increased population density of *A. calamus*. The ability of *A. calamus* to accumulate mercury also rose with a higher level of heavy metal pollution, recognizing the *A. calamus* plants as potential hyperaccumulators. However, further evaluation is essential as no study exists recognizing *A. calamus* plants as mercury hyperaccumulators (Liu *et al.*, 2021). Few past studies have reported certain plants as potential Hg hyperaccumulators. Some studies have reported that potential hyperaccumulator plants must have a translocation factor (with heavy metal concentration in shoots/roots) above 1.0 (Liu *et al.*, 2021).

Study results showed a considerable decrease in rice growth for the number of tillers at the early vegetative phase (1–3 weeks) (Figure 3). The phytoremediation treatments with 1 and 2 *A. calamus* plants/m² provided a nonsignificant difference from the control treatment (without phytoremediation) because these treatments failed to cause a significant reduction in soil mercury content. The highest increase in the number of tillers per plant in rice resulted from the

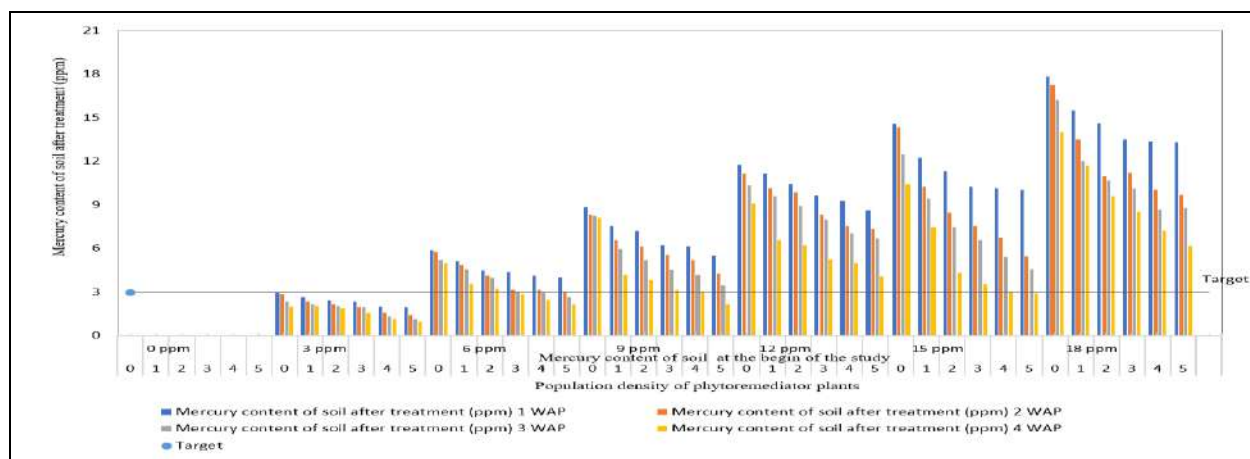


Figure 1. Mercury content in soil media at 1–4 weeks after planting phytoremediator plants.

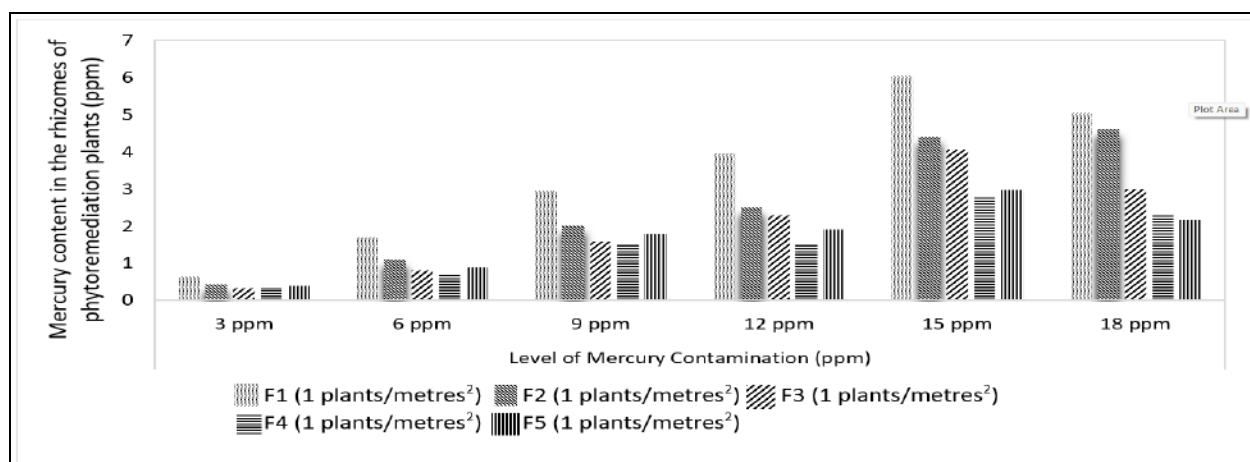


Figure 2. Mercury content in the rhizomes of phytoremediation plants.

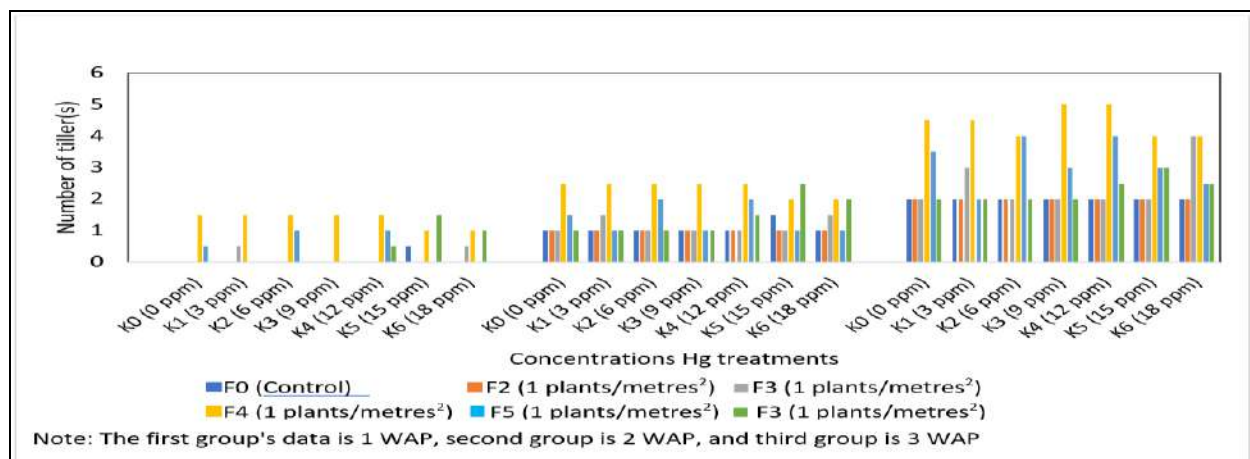


Figure 3. Number of tillers at the early vegetative phase of rice.

phytoremediation treatment with 3 plants/m², as this treatment substantially reduced the soil mercury content. These findings confirmed the soil mercury content analysis, which revealed that soil mercury content significantly decreased in the phytoremediation treatment with 3 *A. calamus* plants/m². Although the phytoremediation treatments with 4 and 5 *A. calamus* plants/m² were also able to reduce the soil mercury content, they induced nutrient competition between the phytoremediator and rice plants, thereby disrupting the growth of rice plants as the main crop.

Reduction in plant growth caused by a disruption in nutrient absorption and photosynthesis was because of the heavy metal contamination. An imbalance in metal content in photosynthetic cells causes inhibition of the photosynthetic process by disrupting the carbon reaction (Shomali *et al.*, 2024). The increased concentration of heavy metals, such as Hg, beyond the threshold level disrupts the process of water absorption and homeostasis (Saini *et al.*, 2021). Moreover, heavy metals disrupt the status of nutrients in the soil, thereby affecting the absorption of other nutrients acquired by the crop plants (Asgher *et al.*, 2023). Particularly potassium is the channel for the entry of Hg into plants, with the similar entry of K⁺ ions into crop plants (Kudryashova *et al.*, 2021; Šestak *et al.*, 2022). Tetraethylammonium (TEA) is a plant compound that inhibits mercury entry into plants. TEA closes the Hg entry channel and simultaneously closes the entry of K⁺ into crop plants (Kudryashova *et al.*, 2021). In crop plants, the potassium deficiency disrupts the balance of anions and cations, as well as the synthesis of proteins and enzymes that act in response to stress conditions, such as altering the formation of the antioxidant ascorbic acid (De-Luca *et al.*, 2021; Kudryashova *et al.*, 2021).

The phytoremediation treatment with 3 *A. calamus* plants/m² increased the number of productive tillers, the number of filled grain, and the production of rice per m². The phytoremediation treatment with 3 plants/m² also showed a decrease in the number of empty grains (Figure 4). The highest yield was evident in the treatment without mercury

stress and without phytoremediation (1431.80 g/m²), which was not significantly different from the treatment with 3 ppm mercury stress combined with phytoremediation using 2 plants/m² (1390.35 g/m²) and 3 plants/m² (1423.59 g/m²). It also did not differ significantly from the 9 ppm stress treatment with phytoremediation at 3 plants/m² (1380.60 g/m²). In addition to interfering with K⁺ nutrient uptake, Hg interferes with Fe uptake by plants. Disruption of Fe metal absorption causes leaf chlorosis, which interferes with photosynthesis and reduces the plant biomass. With the disruption of the photosynthesis process, rice biomass reduction can be notable with the decrease in the number of tillers in the treatment with higher mercury content without phytoremediation. Suganthi and Avudainayagam (2022) reported that Hg inhibits Fe and induces chlorosis in leaves, which has deleterious effects on chlorophyll metabolism. Heavy metal toxicity reduces micronutrients necessary for plant growth and development. Owing to metal stress, the pigment levels' decrease is one of the main causes of impaired photosynthesis. Gomes *et al.* (2016) also reported heavy metal stress reduced physiological functions in pea plants, and Hg, both organic and inorganic, caused the depletion of potassium, magnesium, and manganese, as well as iron accumulation.

Mercury contamination at concentrations above the threshold level can cause disturbances in growth and development and physiological processes of plants. By exposing plants to Hg, the most common symptoms were the root growth inhibition, photosynthesis limitation, and disruption of water transport and uptake systems, with leaf chlorosis and yield reduction (Liu *et al.*, 2021). In this present study, by exposure of rice plants to mercury, the observed negative effects were the decrease in the number of tillers per plant. A reduction in the number of tillers will ultimately result in a lower crop yield because of the significant positive correlation between the number of tillers and rice production (Syahril *et al.*, 2025).

The results detailed the mercury content was below the threshold level in rice plant organs, particularly in rice grains, which

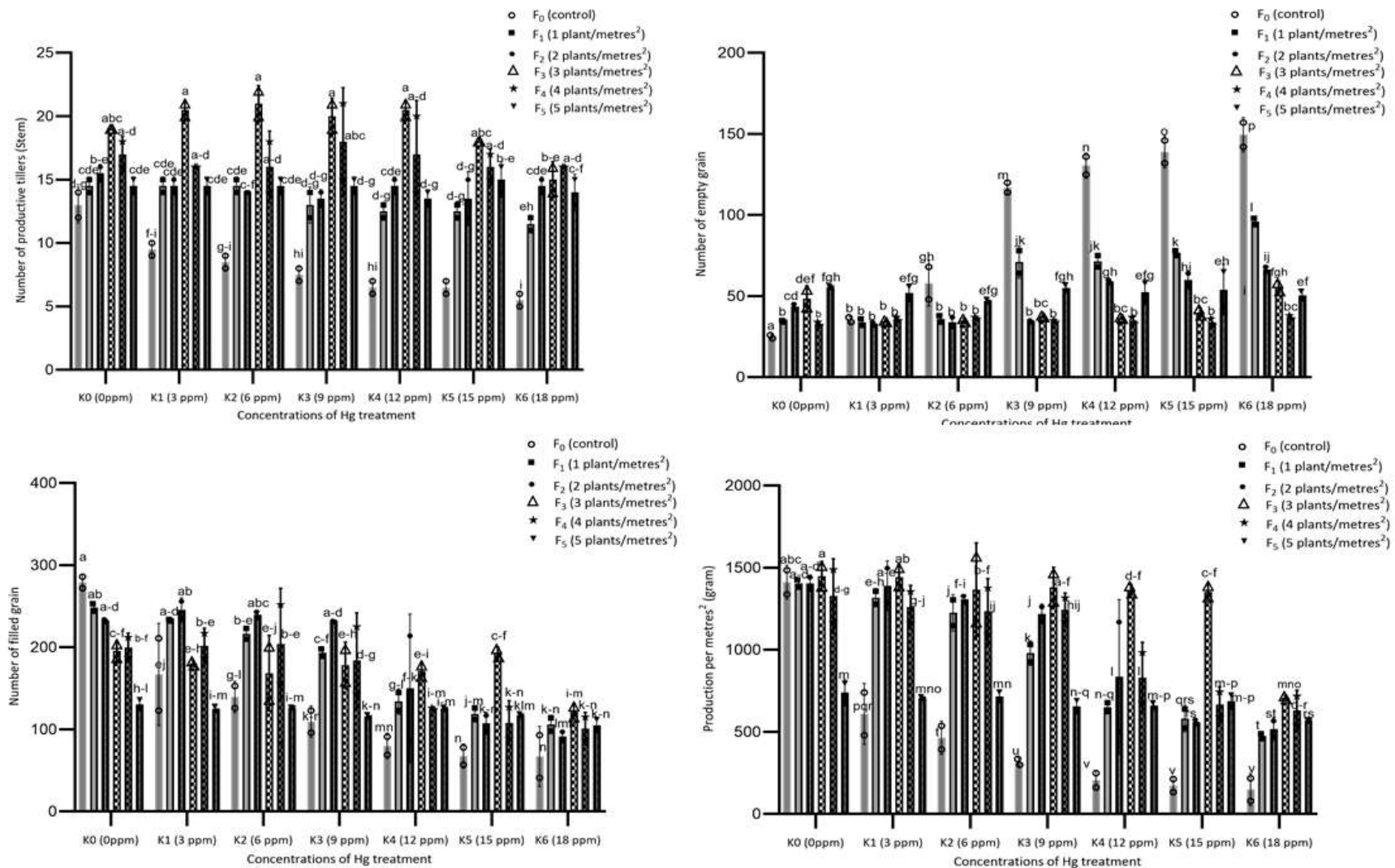


Figure 4. Number of tillers, number of empty and filled grains, and production per m² in rice plants. Note: Histograms with the same letter indicate no significant difference according to the DMRT at the 5% significance level.

recognized that phytoremediation had a significant effect on mercury content in rice plants (Figure 5). Without phytoremediation, the mercury content in rice plant roots was entirely above the threshold level. For Hg concentrations of 3–6 ppm, phytoremediation treatment was able to reduce the mercury content in roots below the threshold level. With an Hg concentration of 9 ppm, only the 5 *A. calamus* plants/m² ably reduced the mercury concentration in roots below the threshold. Meanwhile, at Hg concentrations above 9 ppm, phytoremediation treatment was unable to reduce the mercury content in rice roots below the threshold (Figure 5a). In rice plant stems without phytoremediation, the mercury content was entirely above the threshold level. Concerning Hg concentrations of 3–6 ppm, phytoremediation treatment was able to reduce stem mercury content below the threshold. With an Hg concentration of 9 ppm, phytoremediation treatments of 4 and 5 *A. calamus* plants/m² enable the reduction of the rice stems' mercury content and keep them below the threshold. With Hg concentrations of above 12 ppm, only the 5 plants/m² could reduce the mercury content in rice stems below the threshold. However, at the said concentration (12 ppm), phytoremediation treatment was unable to reduce the root mercury content and keep it below the threshold level (Figure 5b).

In rice plants without phytoremediation, leaf mercury content was entirely above the threshold. With Hg concentrations of 3–6 ppm, phytoremediation treatment was successful in reducing the mercury content in rice leaves and keeping it below the threshold level. At the Hg concentration of 9 ppm, phytoremediation treatments of 4 and 5 *A. calamus* plants/m² were able to reduce the mercury content in rice leaves below the threshold. With Hg concentrations above 12 ppm, only the 5 *A. calamus* plants/m² could reduce the leaf mercury content and keep it below the threshold level. At a mercury concentration of above 12 ppm, phytoremediation treatment was unable to reduce the leaf mercury content below the threshold (Figure 5c).

The mercury content in rice grains of the untreated group was entirely high and above the threshold level. With Hg concentrations of 3–6 ppm, all the phytoremediation treatments were able to reduce leaf mercury content below the threshold. At an Hg concentration of 9 ppm, phytoremediation treatments of 3, 4, and 5 *A. calamus* plants/m² were able to reduce the grain mercury content below the threshold. Meanwhile, at concentrations above 12 and 15 ppm, the phytoremediation treatments of 3, 4, and 5 plants/m² could reduce the mercury content in rice grains below the threshold. With an Hg concentration of above 18 ppm, phytoremediation treatments of 4 and 5 plants/m² can reduce mercury content in rice grains below the threshold. However, the phytoremediation treatments of 1, 2, and 3 plants/m² were unable to reduce mercury content in rice grains below the threshold level (Figure 5d). *A. calamus* plants can grow and adapt to soils contaminated with heavy metals, such as mercury, without damaging growth and development and showing no symptoms of toxicity. Ramadan and Mohamed (2022) also reported the ability of canary grass (*Phalaris minor* Retz.) and rabbit foot grass (*Polypogon monspeliensis* L.) as phytoremediators for managing heavy metals.

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 (Mora *et al.*, 2022). Although in soil, the mercury contamination can have detrimental effects on plant growth and development, and even cause plant death, some plant species show tolerance and can thrive in mercury-contaminated soils (Qu *et al.*, 2019). The rice plant organs with a reduced mercury content revealed the effectiveness of *A. calamus* plants as phytoremediators, with the best treatment being the 3 *A. calamus* plants per square meter. Based on soil contamination in the Mandailing Natal District, Indonesia, with an Hg concentration of 15 ppm, the optimal density of phytoremediation plants was 3

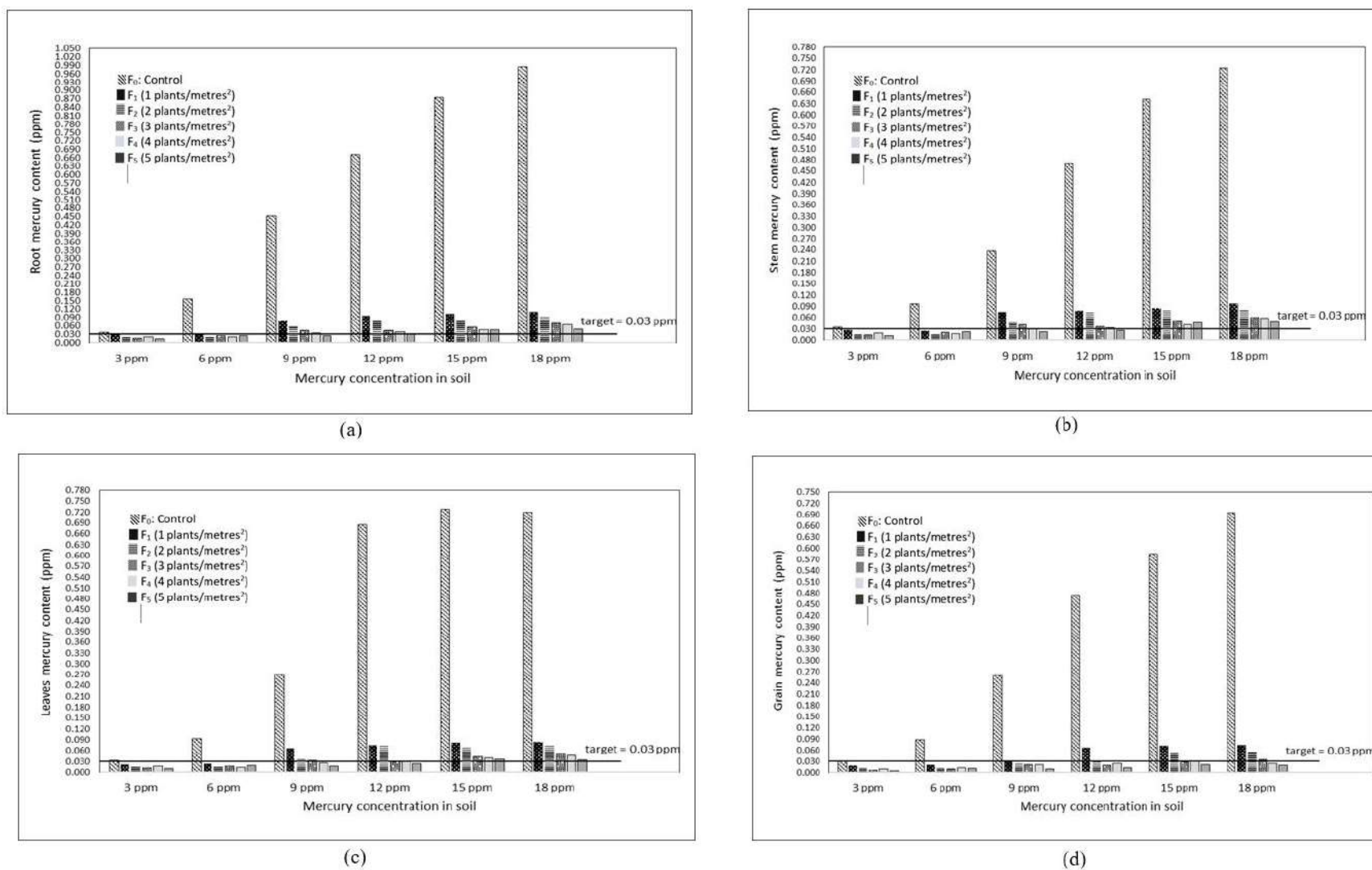


Figure 5. Mercury content in rice plants (ppm), a) roots, b) stems, c) leaves, and d) grains.

plants per square meter. Phytoremediation is an environmentally friendly technology with advantages, such as low cost, simple operation, and in situ remediation.

CONCLUSIONS

Based on the promising study, one can conclude that phytoremediation with a population density of 3 *A. calamus* plants/m² is effective and optimal to reduce the soil mercury content below the threshold level. The said treatment increased growth and development and production, while reducing the mercury content of the rice plant's roots, stems, leaves, and grains below the threshold level. Phytoremediation treatments with *A. calamus* population densities above 3 *A. calamus* plants/m² caused nutrient competition with primary plants. Inversely, the population densities of fewer than 3 *A. calamus* plants/m² proved ineffective in reducing the soil mercury content and keeping it below the threshold level.

ACKNOWLEDGMENTS

The authors thank the Ministry of Education, Culture, Research, and Technology through the DRTPM for funding this research through the Fundamental Research Grant Program under contract number 093/E5/PG.02.00. PL/2024 for the 2024 fiscal year.

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