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Soil Heavy Metal Absorption Potential of *Azolla pinnata* and *Lemna gibba* with Arbuscular Mycorrhizal Fungi in Rice (*Oryza sativa* L.) Farming

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Citation: Herath, B.M.; Bamunuarachchige, C.; Stephenson, S.L.; Elgorban, A.M.; Asad, S.; Kumla, J.; Suwannarach, N.; Karunarathna, S.C.; Yapa, P.N. Soil Heavy Metal Absorption Potential of *Azolla pinnata* and *Lemna gibba* with Arbuscular Mycorrhizal Fungi in Rice (*Oryza sativa* L.) Farming. *Sustainability* **2023**, *15*, 4320. https://doi.org/10.3390/su15054320

Academic Editor: Prashant Kaushik

Received: 24 January 2023
Revised: 17 February 2023
Accepted: 18 February 2023
Published: 28 February 2023

Abstract: This study assessed the potential uptake of soil-contaminated heavy metals by *Azolla pinnata* and *Lemna gibba* in combination with and without arbuscular mycorrhizal fungi (AMF) in traditional and improved rice varieties. Total levels of cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) in soil, rice roots, shoots, grains, *A. pinnata*, and *L. gibba* were estimated using ICP-MS. The percentage colonization in AMF-inoculated and AMF-uninoculated rice varied from 1.13–30.67% and 1.33–5.42%, respectively. These findings suggested that AMF provide protection to rice plants against the combined toxicity of Cd, As, Pb, and Hg in rice field soil. The combined interaction of AMF, organic fertilizer, and *A. pinnata* decreased heavy metal accumulation in rice roots, shoots, and grains in both tested varieties. The intake and subsequent accumulation of Cd, As, Pb, and Hg in the rice grains differed significantly ($p < 0.05$) between the two rice varieties. Furthermore, it was revealed that the AMF-inoculated rice plants reduced the translocation of heavy metals from root to shoot. Therefore, it can be concluded that heavy metal absorption and accumulation in rice can be reduced by the application of AMF, organic fertilizer, and *A. pinnata* together in rice farming.

Keywords: AMF; *Azolla pinnata*; heavy metal; *Lemna gibba*; organic fertilizer; rice

1. Introduction

For centuries, rice (*Oryza sativa* L.) has been Sri Lanka's primary food source, and the country's economy, customs, and culture have been deeply influenced by it. In other Asian nations, this is also the case. Rice is grown across the entire island, which has a variety of climates and terrain. There are an estimated 0.77 million hectares (34%) of Sri Lankan farmland allocated to rice farming [1].

However, the use of agricultural pesticides and the irrigation of paddy fields with polluted water can considerably elevate trace element levels in rice, which are generally present in extremely minute amounts [2]. Cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) levels in rice grains from polluted regions were found to be much higher compared to un-polluted areas [3].

It is likely that human health might be negatively impacted by exposure to these trace elements in food chains, which could eventually result in the emergence of certain chronic disorders [4]. As a result, major issues with heavy metal deposition in rice soil have been resolved recently. Cd, As, Pb, and Hg are potentially dangerous trace elements that have been identified in disturbingly high concentrations in rice from various Asian countries [5].

Arbuscular mycorrhizal fungi (AMF) play a vital role in mineral cycling in the soil as well as the breakdown of organic substances [6]. They also have an impact on the health of plants. Mycorrhizal plants are better able to survive toxic metals, root infections, dehydration, salt, high soil temperatures, adverse pH, and transplant shock [7].

AMF have the ability to prevent the transport of non-essential elements such as heavy metals into plants and store them inside the plant roots [8]. To lessen heavy metal hazards, AMF may secrete various compounds that precipitate heavy metals in polyphosphate granules, adsorb the metals to fungal cell walls, and chelate the Cd inside fungal cells [9]. To prevent the transmission of heavy metals to shoots, mycorrhizal colonization of the roots might bind them to the cell walls of fungal hyphae. Plant shoots can be protected against heavy metals by using them as a filtering barrier [10].

Rice plants form a mycorrhizal relationship in upland soil but rarely in submerged ones due to the anaerobic conditions [11]. Vallino et al. [12] and Ruiz-Sánchez et al. [13] found that the anaerobic state of the soil decreased the AMF colonization of host roots for rice growing in a non-flooded environment.

Traditional rice varieties have naturally evolved to tolerate drought, submergence, salinity, and iron toxicity better than newly improved varieties. Traditional rice has wider variation in grain nutrition, texture, appearance, and cooked rice aroma than improved cultivars due to its 10 times larger population size and extensive exposure to natural selection [14]. Improved varieties are cultivated for large grain yields but the yield of the traditional varieties is substantially less. Though traditional rice varieties have health benefits such as low glycemic index, vigorous antioxidant activity, and high fiber content, few studies have shown their nutritional superiority over modified rice [15–17].

Genetically enhanced rice varieties aim to increase grain output, pest and disease resistance, and grain quality. As a result of their modest plant height and upright leaves, they are also resistant to lodging. Furthermore, enhanced rice varieties are highly responsive to fertilizer addition [18,19], and their milled rice output is higher than traditional rice. The BG 300 rice variety was parameterized and assessed for short-duration cultivation under submerged conditions in Sri Lanka. The validated model accurately predicted grain production in diverse agro-climatic zones in Sri Lanka under water-limited farmer-field settings [20]. Amarasingha et al. [21] assumed that BG 300 rice is robust enough to test rice performance under hypothetical climatic scenarios. At the time of the survey, roughly 49% of farmers were growing Suwandel out of nearly 1400 cultural paddy types. Additionally, the majority of farmers in the sample from Colombo (55%) and Anuradhapura (72%) had grown Suwandel [22].

Lemna spp. (duckweeds) occur in temperate and tropical locations and do not require a period of vegetative rest, making them ideal for wastewater treatment throughout the year. *Lemna* spp. are known for their rapid development and colonization of large areas, which leads to the production of thick free-floating mats [23]. With their high and rapid nutrient absorption, duckweeds are well-suited to the phytoremediation of nutrient-rich waterways [24].

The fern *Azolla* is a widely used biofertilizer and source of green manure. The *Azolla–Anabaena* system can be used for tropical rice production due to its mutualistic symbiosis with *Anabaena azollae*, which fixes atmospheric nitrogen more efficiently than other systems. The use of *Azolla* with artificial nitrogen fertilizers has also been successful. Compared to other biofertilizers, the *Azolla* treatment increased rice grain yield. *Azolla's* thick mat reduces weeds and ammonia volatilization in rice fields [25].

Azolla can be used to eliminate phenol from industrial effluents and manage weeds and has been used as a biosorbent for metal-bearing effluents. *Azolla* is abundant in a variety of nutrients, including proteins, amino acids, vitamins (including A, B12, and beta-carotene), growth-promoting intermediates, and minerals [26].

Excess usage of fertilizer and pesticides may cause significant issues regarding the buildup of heavy metals in the soil in which rice plants grow that have been addressed in recent years [27]. In this research, *Lemna gibba* and *Azolla pinnata*, which were inoculated with AMF, were incorporated into the soil as a top dressing during rice cultivation with the aim of the removal of heavy metals from the rice field soil.

2. Materials and Methods

2.1. Experimental Location

A field experiment was conducted in Mihintale (Figure 1b), Anuradhapura, North Central Province, Sri Lanka (8° 23′ 30.12″ N, 80° 39′ 04.24″ E) during the Yala season in 2021. The average annual temperature was between 30 to 35 °C, and the average annual precipitation was 1750 mm. Reddish-brown earth (Alfisols) was found to be the predominant soil type in the experimental field [28].

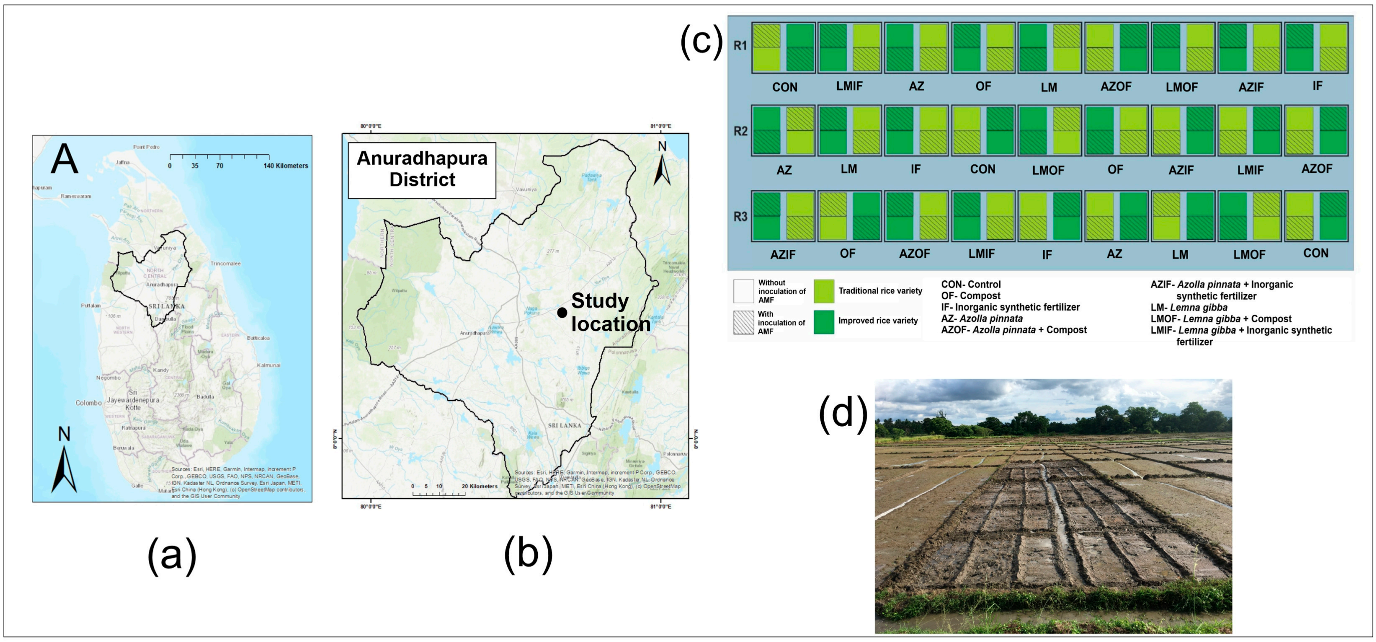


Figure 1. (**a**) Map of Sri Lanka; (**b**) The study location (Mihintale) within the map of Sri Lanka; (**c**) Split-plot experimental design; (**d**) Paddy field prepared according to the split-plot design.

2.2. Preparation of the Native AMF Inoculum

The AMF inoculum was prepared using the trap culture method. The bait plant was maize (*Zea mays* L.). Soil samples with tiny pieces of plant roots were taken from the top 0–15 cm of the soil near the boundary of a natural forest in Mihintale. These root pieces served as an inoculum for the indigenous AMF. After two days of soaking, maize seeds were sown in the pots containing the soil that had been collected from the natural forest boundary.

The roots and shoots of maize plants were separated after one month. The roots were then divided into pieces approximately 5 cm long and added to the rice field plots according to the treatment plan. The mixture of spores, mycelium, soil, and fragments of roots gathered were considered as the AMF inoculum. The AMF colonization percentage was estimated for randomly chosen maize roots using the McGonigle technique before applying root pieces into the field [29]. AMF spores were isolated from the maize trap-cultured soil, adapting the method described by Brundrett et al. [30]. The rhizosphere soil, which typically contained 100 AMF spores per 100 g, and AMF colonized root fragments (about 60–75% potential colonization), was used as the source of the inoculum [31].

2.3. Azolla pinnata and Lemna gibba Collection

Azolla pinnata and *Lemna gibba* were collected from tanks in the North Central Province, Sri Lanka. To eliminate any epiphytes and insect larvae that had formed on the collected *Azolla pinnata* and *Lemna gibba*, they were first washed with tap water, then with 1% (v/v) sodium hypochlorite solution, and finally with deionized water. To help the plants adjust to their new surroundings, they were placed in cement tanks with tank water and exposed to natural sunlight for a week.

2.4. Crop Establishment and Management

2.4.1. Establishment of Treatment

The treatments were laid out in a split-plot design, with a subplot as a full factorial with three replicates. The main plots consisted of nine treatments, and the presence and absence of AMF (1 Mg ha^{-1}) and one improved rice variety, BG 300 (BGSP), and one traditional rice variety, Rath suwandal (TRSP), were used as subplots.

The treatments were CON: control (no application of *Azolla pinnata*, *Lemna gibba*, and soil amendments,); OF: compost (6 Mg ha^{-1}); IF: inorganic synthetic fertilizer (225 N kg ha^{-1}/55, P_2O_5 kg ha^{-1}/60, K_2O kg ha^{-1}/5, $ZnSO_4$ kg ha^{-1}); AZ: *Azolla pinnata* (0.5 Mg ha^{-1}); AZOF: *Azolla pinnata* (0.5 Mg ha^{-1}) + compost (6 Mg ha^{-1}); AZIF: *Azolla pinnata* (0.5 Mg ha^{-1}) + inorganic synthetic fertilizer (225 N kg ha^{-1}/55, P_2O_5 kg ha^{-1}/60, K_2O kg ha^{-1}/5, $ZnSO_4$ kg ha^{-1}); LM: *Lemna gibba* (0.5 Mg ha^{-1}); LMOF: *Lemna gibba* (0.5 Mg ha^{-1}) + compost (6 Mg ha^{-1}); LMIF: *Lemna gibba* (0.5 Mg ha^{-1}) + inorganic synthetic fertilizer (225 N kg ha^{-1}/55, P_2O_5 kg ha^{-1}/60, K_2O kg ha^{-1}/5, $ZnSO_4$ kg ha^{-1}).

The experimental field contained 27 plots. The main plots were divided into bunds that were 45 cm wide and 30 cm high, and there was a drainage canal running in between the two bunds to prevent any cross-contamination of the different treatments. Each plot was divided equally into four subplots: with and without inoculation of AMF, one improved rice variety, and one traditional rice variety. The area of a subplot was 9 m^2, and each was separated by a bund (45 cm in width and 45 cm in height).

2.4.2. Seedling Transplanting

Recently harvested rice seedlings of one improved (BG 300) and one traditional variety (Rath Suwandal), raised in a dapog nursery, were transplanted with two plants per hill with 30 $\times$ 30 cm spacing on the puddled and leveled field.

One day prior to transplanting, the AMF inoculum was sprayed on the top of the soil in the respective subplots and repeatedly applied every 1.5 months. After transplanting rice plants in the field, the collected *Azolla pinnata* and *Lemna gibba* were spread in the respective treatments separately (AZ, LM, AZOF, AZIF, LMOF, and LMIF).

Commercially available compost (1% nitrogen), an organic fertilizer, was applied to the soil as a base dressing one week prior to transplanting. Two split doses, each containing compost, were then applied to the soil in accordance with the treatment (OF, AZOF, and LMOF).

The IF treatment plots (IF, AZIF, and LMIF) were fertilized with inorganic chemical fertilizers one hour before rice transplantation, and the entire amount of phosphorus and zinc was sprayed as the base fertilizer. The application of nitrogen fertilizer and potassium fertilizer was repeated four times after the first application according to the fertilizer recommendation for rice in Sri Lanka. Weed management was performed mainly through water management and regular manual weeding, and pest management was conducted by applying neem *(Azadirachta indica)* extract.

2.5. Soil and Plant Sampling

Before harvesting, 250 g soil samples from each treatment were taken from the different experimental rice field plots, which ranged in depth from 0 to 15 cm. Since the plot's edges were not included in the sampling grid, the composite soil sample comprised sub-samples taken from the center of the 4 m^2 grid. The soil samples were thoroughly homogenized, air-dried, lightly crushed, and then passed through a 2 mm sieve before being packed into sealed polythene bags.

Before harvesting, *Azolla pinnata*, *Lemna gibba*, and the entire rice plant, including the shoots, roots, and panicles containing grains, were all randomly sampled. Within the 4 m^2 experimental plot, plants were randomly chosen from each subplot. To remove soil particles, deionized water was washed over the roots. The plant samples were washed one more time in deionized water and then dried for roughly two days at 65 °C to achieve a constant weight. To ascertain the degree of arbuscular mycorrhizal colonization for each treatment, *Azolla pinnata*, *Lemna gibba*, and rice roots were maintained in a formaldehyde–acetic acid solution containing 7% formaldehyde. Samples of the dried plants roots, shoots, and grains were separated and weighed.

2.6. Analysis of Available Heavy Metal Concentrations in Soil and Plant Samples

Microwave digestion was utilized to digest soil and plant samples. A total of 0.5 g of a dried soil sample was mixed with 12 mL of HNO_3 and HCl in a proportion of 3:1 (v/v). The sample was heated to 180 °C for 30 min using a microwave digestion system (Model: ETHOS EASY-49030, Milestone, Italy) [32]. A further 0.25 g of the ground *Azolla pinnata*, *Lemna gibba*, root, shoot, and rice grain samples were placed in a digestion tube and then we added 6 ml of HNO_3 and H_2O_2 in a proportion of 5:1 (v/v). The microwave digestion system at 200 °C was used to continue acid digestion for 30 min [32]. The contents of Cd, Pb, As, and Hg in the digested samples were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Model: NexION 2000B, PerkinElmer®, Waltham, MA, USA).

2.7. ICP -MS (Inductively Coupled Plasma Mass Spectrophotometer) Analysis

The ICP-MS measurements were performed using a PerkinElmer NexION 2000B device. The calibration standards and blank used 2% traceable HNO_3. When generating standard curves, expected metal concentrations in each test group were considered, and each curve had a correlation coefficient over 0.999. The measured heavy metal isotopes were 111Cd, 91AsO, 208Pb, and 202Hg. Limits of detection (LOD) and limits of quantification (LOQ) were determined according to Şengül [33]. LOD and LOQ values were determined for 111Cd (0.0009, 0.009 mg L^{-1}), 91AsO (0.003, 0.033 mg L^{-1}), 208Pb (0.0007, 0.007 mg L^{-1}), and 202Hg (0.002, 0.022 mg L^{-1}). The operation conditions for ICP-MS in this study are summarized in Table 1.

Table 1. ICP-MS operating conditions.

Instrument Parameter	Standard	Helium KED	Oxygen DRC
Torch		Quartz single pieces touch	
Nebulizer gas flow (L min^{-1})	0.98	0.98	0.98
Nebulizer		Meinhard Concentric	
ICP RF power (W)	1600	1600	1600
Gas flow (L min^{-1})	0	3.8	0.6
Plasma gas flow (L min^{-1})	15	15	15
Auxiliary gas flow (L min^{-1})	1.2	1.2	1.2
Outer gas flow	0.2	0.2	0.2
Sample uptake rate (rpm)	35	35	35
Number of replicates	3	3	3

2.8. Quality Control

HNO_3 (TraceMetal™, Fisher Chemical, USA), H_2O_2 (Suprapur®, E. Merck, Germany), and HCl (Suprapur®, E. Merck, Germany) were used as reagents. Ultrapure water served as the test subject. All vessels used in the experiment were immersed in 20% nitric acid for 48 h and washed three times with ultrapure water. For each run of studies, three sets of blanks and 10% parallel samples were placed aside.

Multi-element standard solution (PerkinElmer Pure Plus Instrument Calibration Standard 2) was used to validate the method parameters [34]. Two dried, homogenized soil and plant samples of the same weight (0. 25 g) were measured. Only one soil and plant sample were spiked with multi-element standard solution concentrations close to the middle of the calibration curve (10 ppb) before adding reagents for digestion, while the other soil and plant samples were not spiked (Normal sample). The above process was repeated 25 times (25 replicates). Table 2 indicates the normal and spiked concentrations of the samples, as well as the analytical quality control parameters. The recoveries of the elements Cd, As, Pb, and Hg in soil and plant samples were within the permitted limit, according to Table 2. The difference between the 25 parallel determinations was less than 10%.

Table 2. Metal concentrations measured in normal and spiked samples (mean SD; mg kg^{-1}) and recovery (%).

Element	Normal Sample		Spike Sample		Recovery (%)	
	Plant (mg kg^{-1})	Soil (mg kg^{-1})	Plant (mg kg^{-1})	Soil (mg kg^{-1})	Plant	Soil
Cd	0.21 ± 0.02	0.23 ± 0.04	5.07 ± 0.51	5.12 ± 0.45	95.86	95.51
As	0.13 ± 0.04	0.15 ± 0.02	2.59 ± 0.39	2.72 ± 0.64	94.98	94.49
Pb	0.15 ± 0.01	0.18 ± 0.03	2.68 ± 0.29	2.75 ± 0.18	94.40	93.45
Hg	0.04 ± 0.01	0.05 ± 0.02	1.35 ± 0.15	1.39 ± 0.1	97.01	96.40

2.9. Percentage Arbuscular Mycorrhizal Colonization

The AMF colonization percentages of rice roots were determined by following the procedures described by Phillips and Hayman [35] and McGonigle et al. [29].

2.10. Soil-to-Plant Transfer Factors

2.10.1. Bioaccumulation Factor (BAF)

The bioaccumulation factor (*BAF*) is a measure of a plant's capability to accumulate a certain metal in relation to the concentration of that metal in the soil [36]. The bioaccumulation factor (*BAF*) was estimated by using the following equation:

$$BAF = \frac{Element\ concentration\ \left(\mu g\ kg^{-1}\right)\ at\ the\ edible\ part\ of\ rice}{Element\ concentration\ \left(\mu g\ kg^{-1}\right)\ in\ soil} \tag{1}$$

2.10.2. Translocation Factor (TF)

The translocation factor (*TF*) was used to determine how much metal was transferred from the soil to the plant's root, from root to the shoot, and from shoot to the grain [37].

$$TF = \frac{Element\ concentration\ \left(\mu g\ kg^{-1}\right)\ in\ root\ or\ shoot\ or\ grain}{Element\ concentration\ \left(\mu g\ kg^{-1} g\right)\ in\ correosponding\ soil\ or\ root\ or\ shoot} \tag{2}$$

2.11. Statistical Data Analysis

The effects of the treatments on the availability of heavy metals in soils and on the amounts of heavy metals in rice tissue were investigated using three-way analysis of variance (ANOVA). Levene's test was used to make sure that the variances were all the same before performing an analysis of variance. Duncan's test revealed a significant difference in mean values between the various treatments ($p < 0.05$). The data that were studied represented an average of the results of three separate replications. The statistical package SPSS version 26.0 was used for all of the statistical analyses, and utilized in the production of all the figures.

3. Results and Discussion

3.1. Percentage AMF Colonization of Rice Roots

Most rice cultivation worldwide occurs in wetland habitats, which have anaerobic environments; it is difficult for AMF to survive in such environments [38]. While research has shown the occurrence of AMF colonization inside rice roots in paddy fields [39], other studies have claimed that AMF are rare or absent in rice plant roots of flooded paddy fields [40,41]. Different rice plant responses to mycorrhizal growth exist, ranging from positive to negative [39,42].

When AMF inoculum is added to rice soil, it can potentially increase the root colonization of AMF in rice plants. Because of this bioaugmentation, there may be an increase in the immobilization of heavy metals by AMF in the rice soil and water. However, the rice was grown in submerged soil conditions, making it difficult for AMF to live in the roots. This difficulty was avoided by incorporating AMF inoculum into the soil at a frequency of one and a half months [43].

Our data indicated that the AMF colonization percentages of rice roots were significantly different ($p < 0.05$) in the AMF, rice variety, and treatment interaction (Figure 2). The treatment that had the highest percentage AMF colonization was the combined interaction of AMF, AZOF, and TRSP (30.67 ± 0.12%), while the combined interaction of IF and BGSP (1.33 ± 0.88%) was the lowest. Adding AMF inoculum has been reported to yield a higher percentage of root colonization in previous studies [44,45]. Furthermore, Purakayastha and Chhonkar [46], Wangiyana et al. [47], and Chareesri et al. [44] reported 2.6%, 3–5%, and 7% of indigenous AMF colonization in non-inoculated rice plants, respectively, in different soil and climatic conditions. Moreover, the literature has demonstrated that environmental factors and agricultural practices, such as fertilizer application and water management, affect the symbiosis and diversity of AMF populations in rice field soil [48].

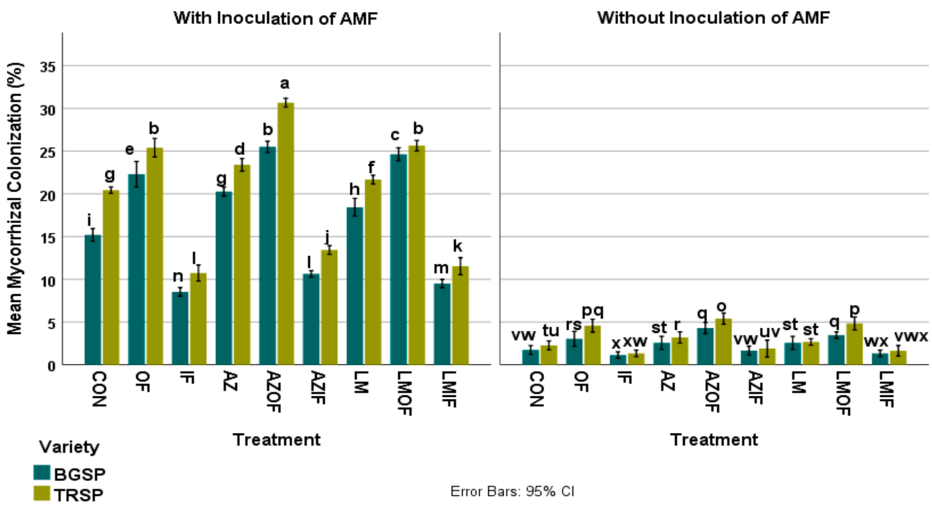


Figure 2. Mean mycorrhizal colonization percentages with and without inoculation of AMF in different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

The functioning of AMF mutualism in paddy fields is influenced by several factors, including AMF species, microbe strains, soil tillage, chemical fertilization, biocides, and climatic characteristics [49]. The harmful elements affecting microbial consortia operation are soil alterations by soil tillage, chemical fertilization, and biocides. The detrimental impact of the factors mentioned above on the field's AMF symbiosis may be mitigated by organic farming. In order to increase the effectiveness of microbial consortia in the field, it is crucial to use the proper agricultural methods [50,51]. When there is a greater amount of plant-available phosphorus (P) in the soil, the host plant provides less carbon to the AMF, which might cause the AMF to become carbon-limited. As a direct consequence, one may anticipate low colonization rates at high P concentrations. Increased levels of P in the soil brought about by the application of phosphorus fertilizer may be the cause of lower root colonization rates in some of the rice fields [52].

Bio-cementation with $CaCO_3$ is one of the sustainable approaches to soil remediation [53], with applications ranging from enhancing soil's geotechnical qualities to removing contaminants. This approach, called bioremediation, involves mechanisms such as microbial-induced calcite precipitation (MICP) to encapsulate heavy metals within precipitated calcium carbonate. The MICP approach stimulates metabolic activity in specific soil bacteria (*Sporosarcina pasteurii*). Another kind of bioremediation is enzyme-induced calcite precipitation (EICP), which employs urease enzymes from plants to precipitate calcium carbonate. Enzymes are helpful since they are non-toxic and environmentally benign [54].

Additionally, HMs become immobilized in AMF fungal hyphae that live with plants in a symbiotic relationship, reducing their availability to plants by keeping the heavy metals in the cell wall, vacuole, or cytoplasm by chelation, hence reducing metal toxicity in the plants [55,56]. AMF have been shown to ingest, translocate, and accumulate heavy metals and increase plant tolerance to heavy metal stress in several ways. These strategies include retaining heavy metals in mycorrhizal roots and external hyphae, stimulating nutrient absorption, sequestering heavy metals in vacuoles, binding them to the fungal cell wall, protecting the reaction center and rectifying gas exchange capacity, increasing plant antioxidant response, chelating heavy metals in the cytosol of fungi, inducing glomalin by AMF, and AMF-mediated phytoremediation [57].

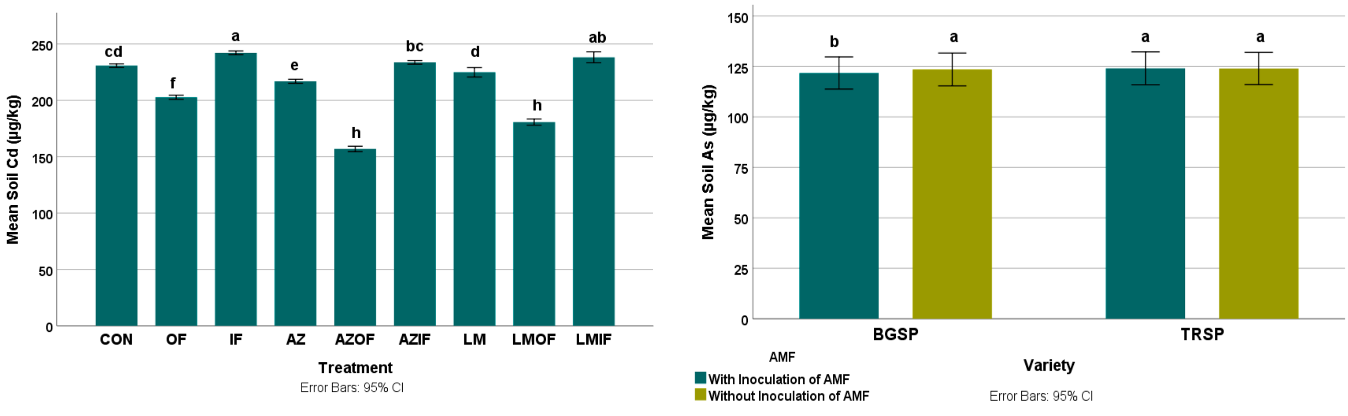
3.2. Heavy Metals in Rice Field Soil

3.2.1. Cadmium in Rice Field Soil

The results obtained showed that soil Cd levels were significantly different ($p < 0.05$) in treatments and depending on the rice variety. However, AMF and interactions among factors (AMF, rice variety, and treatments) were not significantly different ($p > 0.05$) for soil Cd. The highest soil available Cd was observed in the IF treatment (242.13 ± 0.75 μg kg^{-1}). The minimum soil available Cd was observed in AZOF (151.97 ± 1.86 μg kg^{-1}) (Figure 3a). Furthermore, the BGSP rice variety (shoot or root) (212.54 ± 4.06 μg kg^{-1}) showed lower plant-available Cd than the TRSP rice variety (214.59 ± 3.83 μg kg^{-1}). Organic matter possessed several functional groups, including COOH and OH. Heavy metals bind to these functional groups, resulting in the limitation of Cd^{+2} [58].

There is no natural mineral that contains only cadmium. It occurs as CdCOS or CdS in low concentrations in zinc minerals. Less than 1 mg kg^{-1} of Cd has been detected in the Earth's crust [59]. Of all the heavy metals, Cd dissolves the fastest in water. As such, it exhibits high rates of dispersion in the natural world and is not a prerequisite for human survival. Because Cd is soluble in water, plants take it up and it can accumulate in their systems. With cadmium fertilizers and insecticides, it combines well with soils [59].

According to studies by Bandara et al. [60] and Premarathna et al. [61], the triple super phosphate (TSP) utilized by Sri Lankan farmers had P_2O_5 in concentrations ranging from 23.50 to 71.4 mg kg^{-1} of Cd. He noted that bispyribac sodium, a routinely used weedicide in Sri Lankan rice farming, contains 0.5 mg L^{-1} of Cd. Therefore, crops may become unsafe when Cd levels in agricultural soil are high. Many plant species rapidly absorb Cd from their roots and translocate it to their leaves when growing in a Cd-polluted area.



(**a**) (**b**)

Figure 3. (**a**) Mean soil Cd for different treatments; (**b**) Mean soil As with and without inoculation of AMF in the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

3.2.2. Arsenic in Rice Field Soil

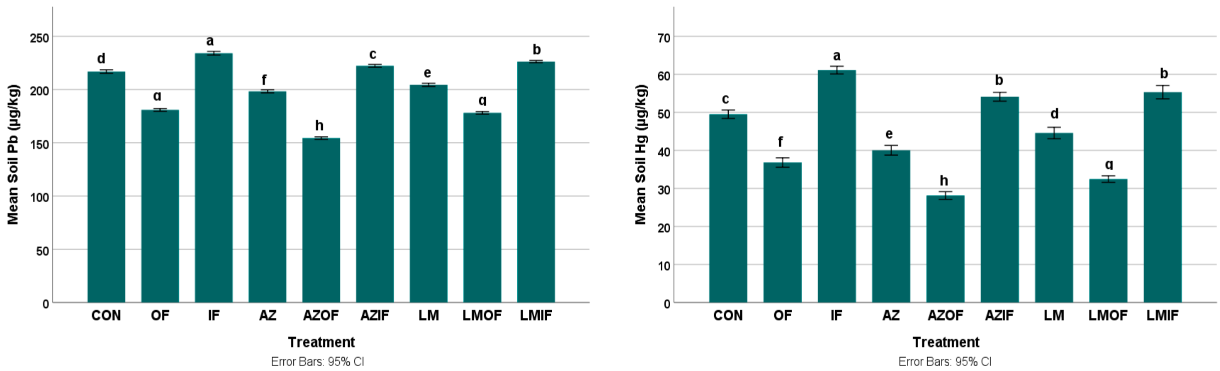
The data from the present study revealed that AMF and rice variety interaction had significantly different soil As levels ($p < 0.05$). The interaction of components in AMF, rice variety, and treatments had no statistically significant ($p > 0.05$) impact on soil As. The combined interaction of AMF and TRSP was found to have the highest plant-available soil As (124.06 $\pm$ 3.97 µg kg^{-1}). The lowest amount of soil As accessible to plants was found in the combined interaction of AMF and BGSP (121.75 $\pm$ 3.89 µg kg^{-1}) (Figure 3b).

Arsenic can be found as a pure elemental crystal or a component of many other minerals. Unlike other metals, arsenic is a metalloid. Despite its many allotropes, only the gray metallic-looking form is commercially relevant. It has been found in soil at concentrations between 0.1 and 40 ppm. When the organic compounds in the soil are oxidized, arsenic is released into the water and eventually absorbed by the plants. As water temperature rises, the concentration of arsenic increases [62]. As is naturally found in paddy soils at levels between 4 and 8 mg kg^{-1}. This level can go up to 83 mg kg^{-1}, as has been reported in many places around the world where As-contaminated groundwater is used to water paddy soils [63]. According to their research data, Singh et al. [64] recorded 7 mg kg^{-1} of As concentration in rice soil in India and Choi et al. [65] found 0.54 mg kg^{-1} of As was accumulated in rice soil in South Korea.

3.2.3. Lead in Rice Field Soil

Our results demonstrated that soil available Pb levels were significantly different ($p < 0.05$) for AMF and treatment interactions. Interactions between AMF, rice variety, and treatments did not significantly affect soil Pb ($p > 0.05$). The IF treatment had the highest plant-available soil Pb (234.05 $\pm$ 0.80 µg kg^{-1}), while AZOF resulted in the lowest plant-available soil Pb concentration (154.39 $\pm$ 0.53 µg kg^{-1}) (Figure 4a). In addition, some rice soil researchers have reported that Pb concentrations had maximum average values exceeding 100 mg kg^{-1} [66]. For example, Payus et al. [67] recorded 8.03 mg kg^{-1} of Pb in rice soil in Malaysia.

Particulate lead compounds are obtained from various sources, including from burning solid and liquid fuels, alkali lead synthesis facilities, lead extraction furnaces, brass mills, lead oxide mills [59]. Soil and environmental factors affect the Pb absorption by plants. Most of a plant's absorbed Pb ends up in its roots. Plants can only take in and use lead at concentrations of 0.05 to 5 ppm in the soil's soluble Pb rather than the total lead. In soil, Pb compounds that are highly soluble change into the Pb compounds that are insoluble [59].



(**a**) (**b**)

Figure 4. Mean soil (**a**) Pb and (**b**) Hg for different treatments. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

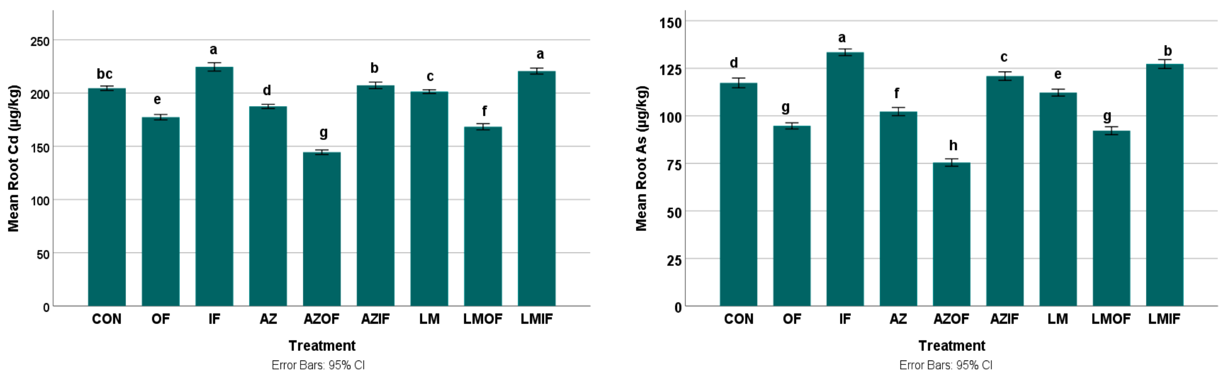
3.2.4. Mercury in Rice Field Soil

Our results showed that soil total Hg levels differed significantly ($p < 0.05$) considering the treatments and the rice variety. The interactions between AMF, rice variety, and treatments had no significant difference for soil Hg ($p > 0.05$). The IF treatment showed the highest plant-available soil Hg (61.11 ± 0.50 µg kg^{-1}) (Figure 4b), whereas the lowest plant-available soil Hg content was recorded in the AZOF treatment (28.13 ± 0.46 µg kg^{-1}). Mercury may become immobilized in the soil, forming insoluble compounds, including phosphate, carbonate, and sulfide [59].

3.3. Heavy Metals in Rice Roots

3.3.1. Cadmium in Rice Roots

There was a statistically significant difference ($p < 0.05$) in the amount of Cd found in the rice roots across AMF, rice varieties, and treatment. However, there was no statistically significant difference ($p > 0.05$) for the interaction of AMF, rice varieties, and treatments on the overall amount of Cd found in rice roots. The highest mean root Cd concentration was observed for the IF treatment (224.52 ± 1.79 µg kg^{-1}). It was also observed that the lowest root Cd concentration was for the AZOF treatment (144.42 ± 0.98 µg kg^{-1}) (Figure 5a). Furthermore, considering the roots of the TRSP rice variety, the Cd accumulation (194.45 ± 3.41 µg kg^{-1}) was higher than that of the BGSP variety (191.22 ± 3.40 µg kg^{-1}). The inoculation of AMF also decreased the root Cd concentrations of the rice (191.59 ± 3.42 µg kg^{-1}) more than the non-inoculation of AMF (194.08 ± 3.40 µg kg^{-1}).



(**a**) (**b**)

Figure 5. Mean root (**a**) Cd and (**b**) As for the different treatments. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

Ahmad et al. [68] recorded the highest Cd (0.05 ± 0.002 mg kg^{-1} dry weight) content in the roots of *Oryza sativa* grown in contaminated soil. Chen et al. [69] reported that AMF could reduce the amount of Cd in the roots by 38% due to the Nramp5 and HMA3 genes being less active in the roots. The application of compost stimulates plant growth, prevents Cd from entering the roots, and boosts plant development, lowering the amounts of Cd in the rice roots. Juang et al. [70] reported that 5% compost (cattle manure and tea waste) could stabilize Cd, and then the phyto-availability of Cd can effectively be reduced.

According to Herath et al. [71], roots in both old and new enhanced rice varieties accumulate the most Cd compared to shoots and grains, and Cd is dispersed in roots, shoots, and grains. BG 300 was the most resilient of the examined kinds of rice, whereas traditional rice cultivars were the least resilient. Generally, there is a positive correlation between soil Cd content and rice Cd uptake [72]. Some metallic elements, especially Na, have been said to enhance plants' ability to absorb Cd [73,74]. In contrast, the absorption of Cd is inhibited by other metallic elements such as silicon (Si), calcium (Ca), magnesium (Mg), manganese (Mn), and potassium (K) [75–77]. When Mn oxides are released in excess in soil solutions, this prevents rice roots from absorbing Cd [75].

The concentration of accessible Cd in the soil is significantly influenced by soil characteristics, including pH [78]. When the soil is quite acidic, more Cd is readily available, which encourages rice roots to absorb more Cd. Rice does not absorb as much Cd when the soil is excessively alkaline because conjugated Cd changes the free Cd in the soil into a less bioavailable form [78]. According to reports, using too much nitrogen fertilizer might cause soil to become more acidic and encourage the uptake of Cd [79].

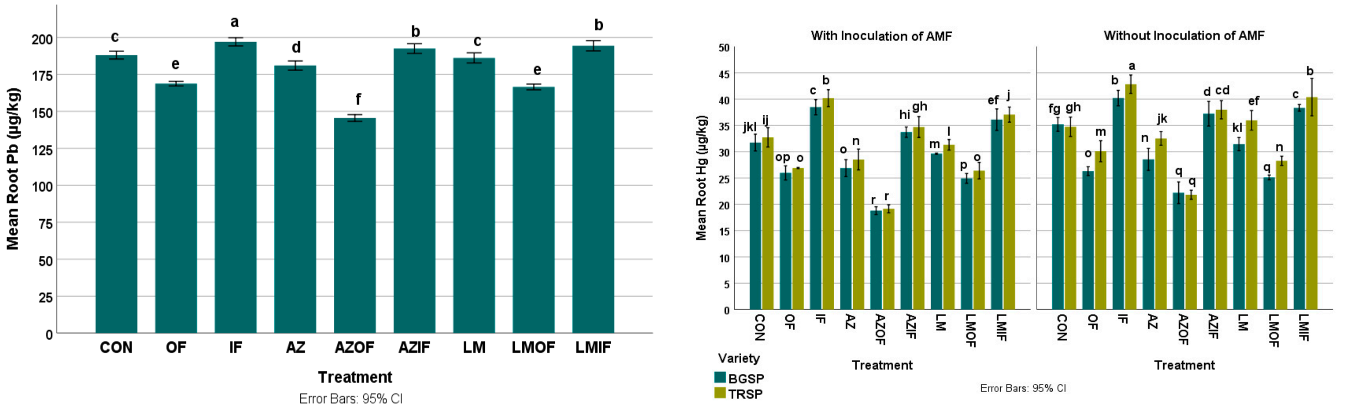
3.3.2. Arsenic in Rice Roots

Although the means for As in the rice roots were significantly different ($p < 0.05$), when considering each factor separately, there was no significant difference ($p > 0.05$) for the interaction of AMF, rice variety, and treatment. The IF treatment had the highest root As level (133.42 ± 0.80 µg kg^{-1}), and the AZOF treatment had the lowest root As level (75.45 ± 0.87 µg kg^{-1}) compared to other treatments and the control (Figure 5b). It was also found that in the non-AMF-inoculated treatment plots, higher root As levels were observed ((109.68 ± 2.47 µg kg^{-1}) compared to the AMF-inoculated plots (107.14 ± 2.44 µg kg^{-1}). Looi et al. [80] found that the highest amount of As (4.62 mg kg^{-1}) was present in the roots when the soil was contaminated with As. This can be explained, as the roots had an iron plague on the surface, strongly linked to As accumulation.

The concentrations and speciation of the pollutants in the soil, soil characteristics, paddy water management, and climatic conditions are some environmental factors affecting rice's ability to absorb As. The biogeochemical cycle of As and Cd is strongly influenced by paddy water management, which also affects the bioavailability of these elements in rice plants [81].

3.3.3. Lead in Rice Roots

There was no significant difference ($p > 0.05$) in the interaction of AMF, rice variety, and treatment for mean Pb concentration in the rice roots. The highest root Pb level was observed in the IF (197.04 ± 1.27 µg kg^{-1}) treatment. Furthermore, the lowest root Pb was recorded in the AZOF treatment (145.52 ± 1.04 µg kg^{-1}) (Figure 6a). In general, the BGSP rice variety exhibited lower levels of root-available Pb (177.81 ± 2.14 µg kg^{-1}) than TRSP (182.18 ± 2.32 µg kg^{-1}). Pb absorption is primarily the responsibility of the young cells located at the root apices. This is because the adsorption of Pb is more significant here than it is over the entire root surface [82]. According to research by Ahmad et al. [83], roots of *Oryza sativa* cultivated in polluted soil had 0.224 ± 0.006 mg kg^{-1} of Pb.



(**a**) (**b**)

Figure 6. (**a**) Mean root Pb for different treatments; (**b**) Mean root Hg with and without inoculation of AMF in different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

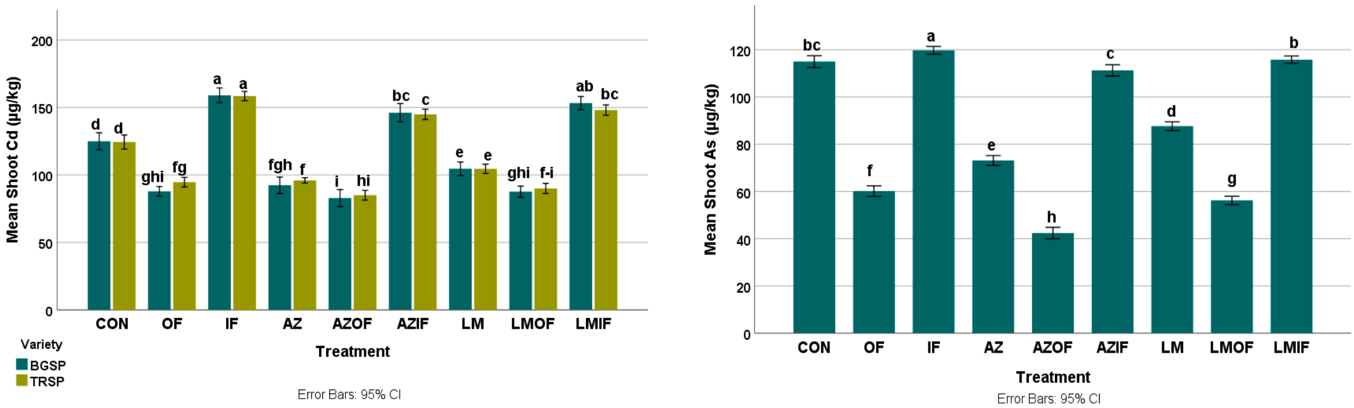
3.3.4. Mercury in Rice Roots

Our results indicated that the interaction of AMF, rice variety, and treatment was significantly different ($p < 0.05$) for means of total Hg in rice roots (Figure 6b). The combined interaction of IF and TRSP was observed to the highest root Hg content (227.15 ± 2.19 µg kg^{-1}). The lowest amount of Hg found in rice root was found in the combined interaction of AMF, AZOF, and BGSP (140.88 ± 0.87 µg kg^{-1}).

3.4. Heavy Metals in Rice Shoots

3.4.1. Cadmium in Rice Shoots

The results showed that the combined interaction of rice variety and treatment had significant differences ($p < 0.05$) in terms of the Cd content in the rice shoot. The highest level of Cd in the shoot was found in the combined interaction of IF and BGSP (159.01 ± 2.13 µg kg^{-1}), whereas the lowest level was found in the combined interaction of AZOF and BGSP (82.86 ± 2.45 µg kg^{-1}) (Figure 7a).



(**a**) (**b**)

Figure 7. (**a**) Mean shoot Cd for different treatments of the two tested rice varieties; (**b**) Mean shoot As in different treatments. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

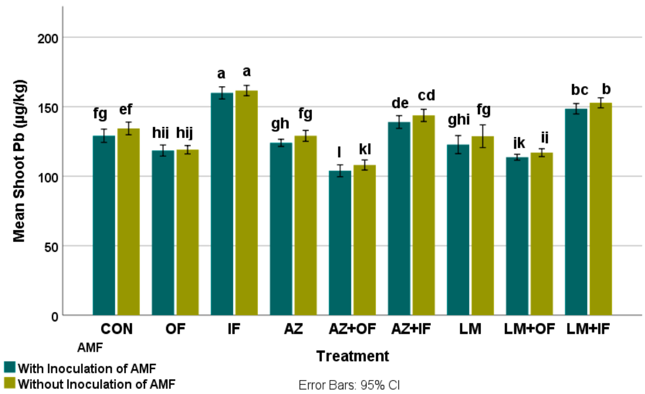
Our data indicate that Cd was quickly accumulated by plants and distributed to other parts in the plants [84]. According to the findings of several studies, the migration of cadmium from the rice root to the tissues above ground is regulated mainly by the transport of the xylem and, phloem [85]. According to Ahmad et al. [83], *Oryza sativa* cultivated in polluted soil had the highest shoot Cd concentration than the other parts of the rice plant (0.035 ± 0.001 mg kg^{-1} dry weight).

3.4.2. Arsenic in Rice Shoots

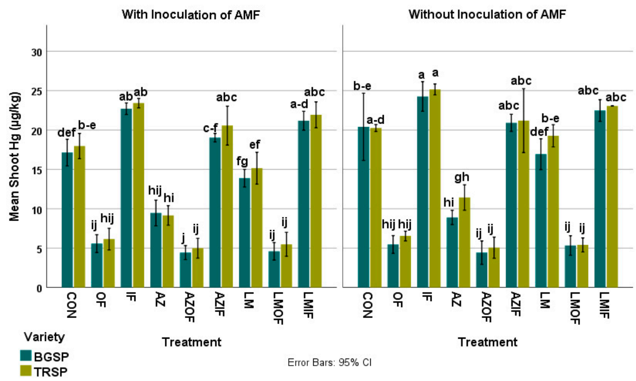
Although the means of As in the rice root were significantly different ($p < 0.05$), when considering each factor separately, there was no significant difference ($p > 0.05$) in the interaction of AMF, rice variety, and treatment. The results also indicated that the IF treatment (119.72 ± 0.75 μg kg^{-1}) had the highest As levels in the rice shoot. The shoot As level was lower for the AZOF (42.41 ± 1.09 μg kg^{-1}) (Figure 7b).

3.4.3. Lead in Rice Shoots

The Pb concentration in rice shoots showed a significant difference ($p < 0.05$) for the combined interaction of AMF and treatment. Higher levels of shoot Pb were recorded in the combined interaction of IF and non- inoculation of AMF (163.71 ± 2.38 μg kg^{-1}), and the lowest was in the combined interaction of AMF and AZOF (102.46 ± 1.52 μg kg^{-1}) (Figure 8a). According to the literature, Pb binding peptides in shoots have been hypothesized to contribute to their Pb concentration [86]. According to Ahmad et al. [83], 0.033 ± 0.004 mg kg^{-1} of Pb concentrations were detected in the shoot of *Oryza sativa* cultivated in polluted soil.



(**a**)



(**b**)

Figure 8. (**a**) Mean shoot Pb of different treatments with and without inoculation of AMF; (**b**) Mean shoot Hg of with and without inoculation of AMF in different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

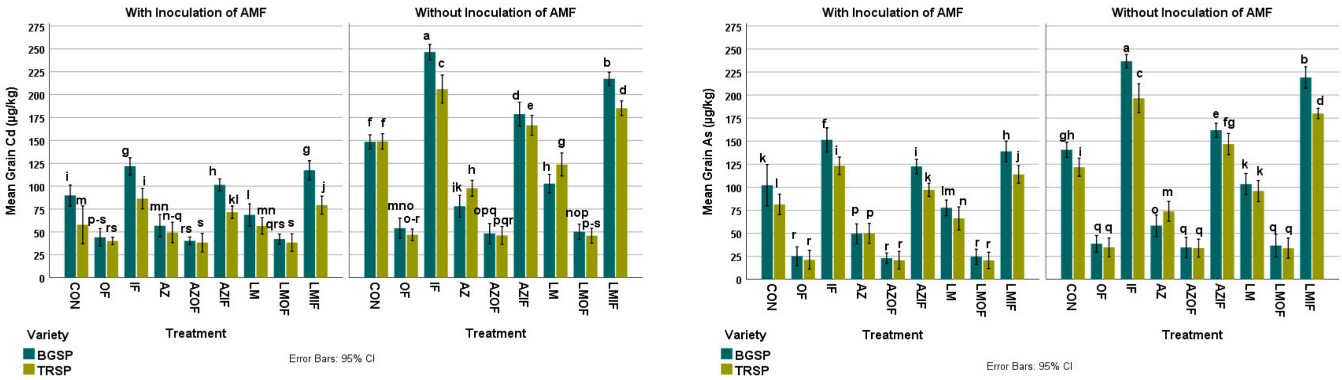
3.4.4. Mercury in Rice Shoots

The interaction of AMF, rice variety, and treatment had a significant difference ($p < 0.05$) effect on the total Hg levels in rice shoots. The highest Hg levels in the shoot were found in the combined interaction of IF and TRSP (24.43 ± 1.29 μg kg^{-1}) and the lowest was in the combined interaction of AMF, AZOF and BGSP (4.43 ± 0.52 μg kg^{-1}) (Figure 8b). Laacouri et al. [87] found a link between the amount of Hg in the leaf and the number of stomatal pores. They found that stomatal absorption of HgO from the atmosphere is the primary mechanism responsible for the buildup of Hg inside leaves.

3.5. Heavy Metals in Rice Grain

3.5.1. Cadmium in Rice Grain

The results indicate that rice grain Cd levels were significantly different ($p < 0.05$) in the combined interaction of AMF, variety, and treatment (Figure 9a). The highest grain Cd was observed in the combined interaction of IF and BGSP (246.53 ± 0.1.92 μg kg^{-1}), and the lowest grain Cd was observed in the combined interaction of AMF, AZOF, and TRSP (38.37 ± 2.38 µg kg^{-1}). However, the maximum concentration of grain Cd did not exceed the standard level of Cd determined by the Codex Alimentarius Commission [88], 400 µg kg^{-1}.



(**a**) (**b**)

Figure 9. Mean grain (**a**) Cd and (**b**) As with and without inoculation of AMF in different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

Soil metal concentrations typically correlate with metal absorption [89]. In general, increased soil Cd concentration led to increased Cd absorption by plants. For example, Liu et al. [90] found Cd contents in rice ranging from 0.22 to 2.86 mg kg^{-1} when cultivated in 100 mg kg^{-1} of Cd-contaminated soil. Murakami et al. [91] found 0.30 mg kg^{-1} of Cd in the grains of rice grown in contaminated soil that had 0.8 mg kg^{-1} of Cd in total. Luo et al. [92] reported that AMF might reduce Cd content in grain, and the mechanism of action of AMF significantly elevated the Cd. Chen et al. [69] recorded that using biological organic fertilizers can decrease the Cd level in rice grains by 52.6% in 0.196 mg kg^{-1} of contaminated paddy soil.

According to Dai et al. [93], Cd stress tolerance varies widely among plant species, and even within genotypes. The mean Cd concentration of rice in Sri Lanka is 81 µg kg^{-1}, behind only Bangladesh's value of 99 µg kg^{-1} Cd [94,95]. Meharg et al. [94] reported Sri Lankan rice contains 81 µg kg^{-1} of Cd (n = 75) which was the highest value, with a median of 24 µg kg^{-1}. The quantity of Cd that rice plants can store in their grains depends on the Cd concentration of the growth media. According to several works within the literature, various Cd treatments resulted in varying levels of Cd in rice grains. For instance, Jinet al. [96] found that for paddy soils containing 0.15 mg kg^{-1} of soil Cd, the mean Cd in unpolished rice grains of 110 cultivars was 0.022 mg kg^{-1}.

According to Areo and Ae [97], in 31 distinct rice cultivars, the mean grain Cd concentration ranged from 2.14 mg kg^{-1} to 7.4 mg kg^{-1} soil Cd. Furthermore, in the same study, they noted that the traditional Sri Lankan cultivar Rathal accumulates between 2.12 and 3.34 mg kg^{-1} Cd in its grains. Additionally, the study by Shi et al. [98] discovered 4.9–367.1 µg kg^{-1} Cd in polished rice grain (n 137). According to Navarathna et al. [99], BG 300 has high amounts of Cd (101.2 ± 4.0 µg kg^{-1}). Additionally, Kuruluthuda, a traditional Sri Lankan cultivar, had the highest Cd level (158.9 ± 90.0 and 126.8 ± 11.7 µg kg^{-1}), both when cultivated organically and with fertilizer. Furthermore Herath et al. [100] found that BG 300 grains only acquired a small quantity of Cd at 100 mg kg^{-1} soil Cd. Therefore, of the evaluated kinds of rice, this one is the best for growing in areas with high Cd pollution.

3.5.2. Arsenic in Rice Grain

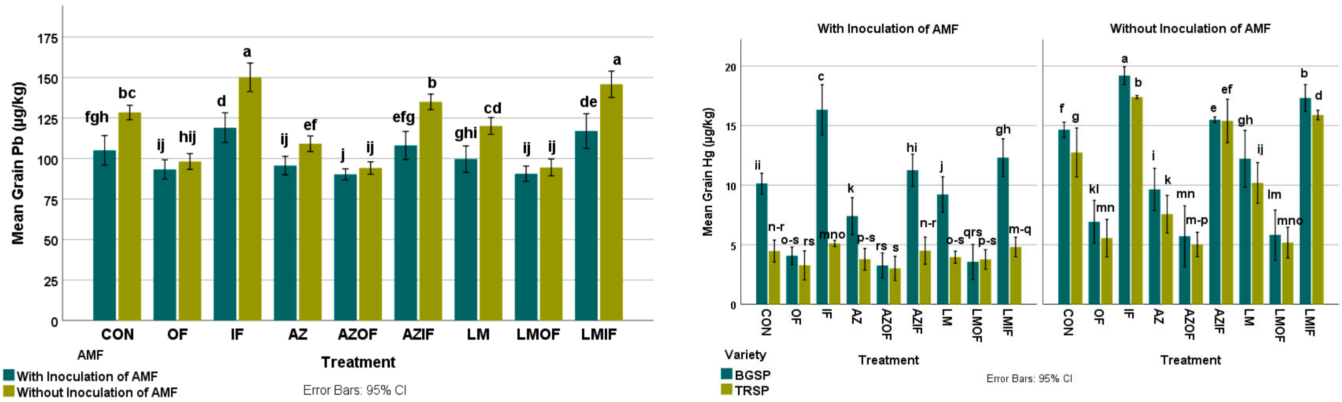
The results showed that the As levels of rice grains were significantly different ($p < 0.05$) in the combined interaction of AMF, rice variety, and treatment (Figure 9b).

The maximum As was found in grains in the combined interaction of IF and BGSP (236.77 ± 1.68 μg kg^{-1}), and the minimum was found in rice grains in the combined interaction of AMF, AZOF, and TRSP (20.59 μg kg^{-1}). Furthermore, the standard level of Cd in rice determined by the Codex Alimentarius Commission [88] is 350 μg kg^{-1} and As content in tested grain samples did not exceed the standard level.

Regarding climatic conditions, recent research has shown a positive correlation between grain As concentration and average air temperature during the middle period of grain filling and temperature increase, and CO_2 concentration in a future climate scenario could increase As concentration in rice grain [101,102]. Rice can absorb As more effectively than other cereals [103]. According to Zeng et al. [104], the physical–chemical characteristics of the soil, especially the equilibrium pH, can also affect As concentration in rice grains. According to past research, the As concentrations of rice grains in Gangneung, South Korea, and Zhejiang Province, China, were recorded as 0.13 mg kg^{-1} and 0.08 mg kg^{-1}, respectively [105,106]. The use of fertilizer is vital in reducing the toxicity of arsenic. In addition, nitrogen-based fertilizers contribute to reducing arsenic intake [107].

3.5.3. Lead in Rice Grain

The results showed that there is a statistically significant difference ($p < 0.05$) between AMF and treatment (Figure 10a). The maximum Pb in grain was found in the IF treatment (156.69 ± 1.85 μg kg^{-1}), and the minimum Pb in grain was found in the combined interaction of AMF and AZOF (90.70 ± 1.99 μg kg^{-1}). Furthermore, the Codex Alimentarius Commission [88] has set a threshold for Pb in rice grain at 200 μg kg^{-1}, and the levels of Pb in the grain of the examined samples did not exceed the standard. Zhou et al. [108] found that 0.21–0.93 mg kg^{-1} of Pb can accumulate in rice grain grown in polluted soil. According to Ahmad et al. [83], 12.03 ± 0.3367 mg kg^{-1} of Pb was recorded in *Oryza sativa* grains which were cultivated in polluted soil.



(**a**) (**b**)

Figure 10. (**a**) Mean grain Pb of different treatments with and without inoculation of AMF; (**b**) Mean grain Hg with and without inoculation of AMF in different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

3.5.4. Mercury in Rice Grain

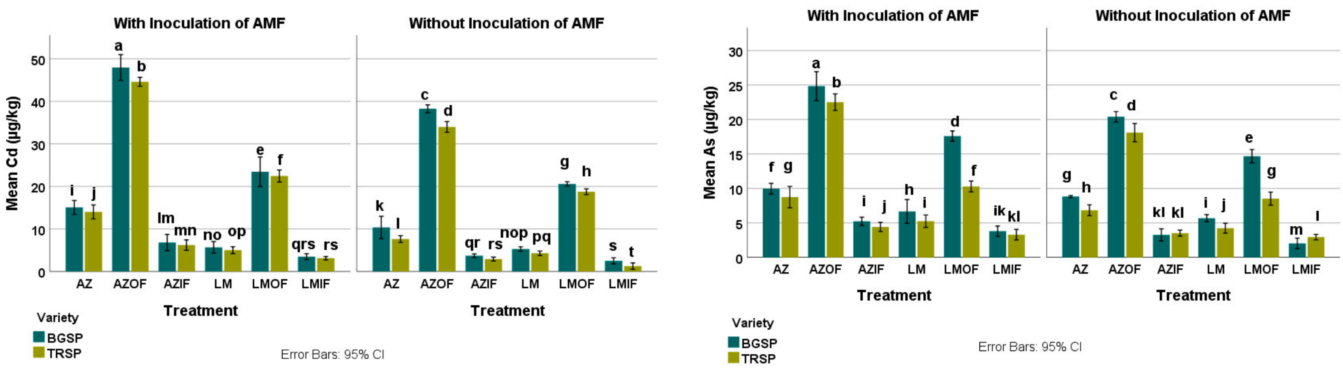
The results showed that the combined interaction of AMF, variety, and treatment was significantly different ($p < 0.05$) for the total Hg level of rice grain (Figure 10b). The total grain Hg concentration was highest in the combined interaction of IF and BGSP (19.19 ± 0.17 μg kg^{-1}) and lowest in the combined interaction of AMF, AZOF, and TRSP (3.02 ± 0.24 μg kg^{-1}). In addition, the Chinese maximum allowable total Hg concentrations of contaminants in foods [109] set a threshold of 20 μg kg^{-1}, and the levels of total Hg in the grain of the examined samples did not exceed the standard. Methyl mercury is more toxic than inorganic mercury, and in the present study, only total Hg was measured. According to Wang et al. [110], the accumulation of methyl Hg in rice grains is more than 800 times greater than inorganic mercury. Nevertheless, Rothenberg et al. [111] gathered rice from the market in 2010 rather than taking samples directly from the fields, and they discovered that the total Hg content was 9.5 ± 8.7 μg kg^{-1}.

3.6. Heavy Metals in Azolla pinnata and Lemna gibba

3.6.1. Cadmium in *Azolla pinnata* and *Lemna gibba*

It was reported that the Cd levels in *Lemna gibba* and *Azolla pinnata* were significantly different ($p < 0.05$) in the interaction of AMF, treatment, and rice variety (Figure 11a). The combined interaction of AMF, LMOF, and BGSP (20.59 ± 0.12 μg kg^{-1}) and LMIF and TRSP (1.25 ± 0.18 μg kg^{-1}) had the highest and lowest observed Cd concentrations in *Lemna gibba*, respectively. The combined interaction of AMF, AZOF, and BGSP (47.96 ± 0.70 μg kg^{-1}) and AZIF and TRSP (2.92 ± 0.11 μg kg^{-1}) had the highest and lowest observed Cd concentrations in *Azolla pinnata*, respectively.

According to the findings of Chaudhuri et al. [112], the Cd content in *Lemna minor* increased intensely from 1647.83 mg kg^{-1} to 4734.56 mg kg^{-1} with an increase in Cd concentration from 0.5 to 2 mg L^{-1} correspondingly. Amare et al. [113] found that Cd could be accumulated in *Lemna* spp. to a maximum of 2.17 mg kg^{-1} dry weight at an initial concentration of 11.33 mg L^{-1} of Cd. Bennicelli et al. [114] found that 310 to 740 mg kg^{-1} of Cd was found in *Azolla*, and according to research by Rai [115], *A. pinnata* was able to remove 70–94% of Cd from alkaline wastewater effluent in India.



(**a**) (**b**)

Figure 11. Mean *Azolla pinnata* and *Lemna gibba* (**a**) Cd and (**b**) As with and without inoculation of AMF in different treatments of the two different tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

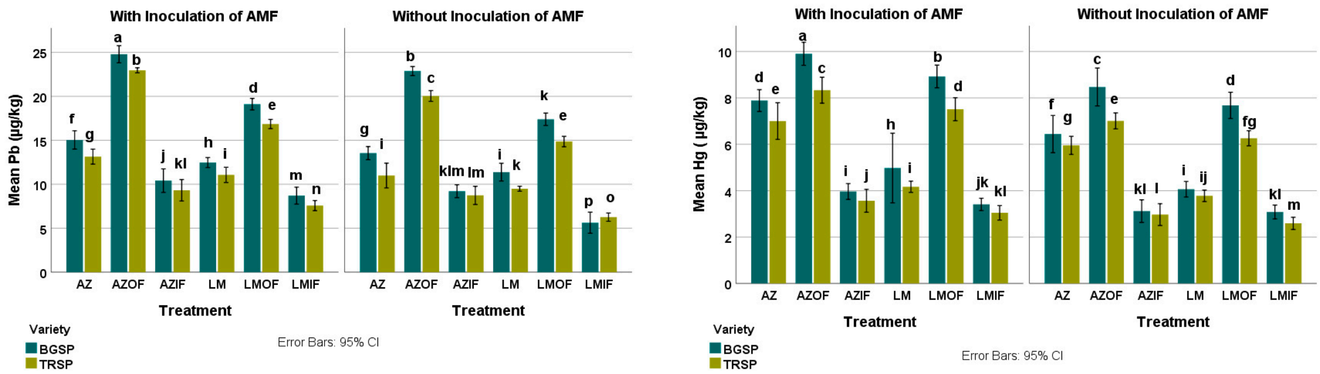
3.6.2. Arsenic in *Azolla pinnata* and *Lemna gibba*

As concentration in *Lemna gibba* and *Azolla pinnata* was significantly different ($p < 0.05$) in the combined interaction of AMF, variety, and treatment (Figure 11b). The highest As concentration in *Lemna gibba* was found in the combined interaction of AMF, LMOF, and BGSP (17.59 ± 0.22 μg kg^{-1}), while the lowest value was found in the combined interaction of LMIF and BGSP (1.89 ± 0.07 μg kg^{-1}). Furthermore, the maximum and minimum As concentrations in *Azolla pinnata* were found in the combined interaction of AMF, AZOF, and BGSP (24.83 ± 0.49 μg kg^{-1}) and the combined interaction of AZIF and TRSP (8.74 ± 0.24 μg kg^{-1}), respectively.

Goswami et al. [116] found that more than 70% of arsenic could be removed by *Lemna* sp. at an initial concentration of 0.5 mg L^{-1} of arsenic solution. According to Zhang et al. [117], the As accumulation in cultivated *Azolla* ranged from 29 to 397 mg kg^{-1} dry mass. Furthermore, they found that *A. caroliniana* had the highest accumulation of arsenic at 284 mg kg^{-1}, while *A. filiculoide* had the lowest accumulation of 0.54 mg kg^{-1}.

3.6.3. Lead in *Azolla pinnata* and *Lemna gibba*

Our data indicate that Pb levels in *Lemna gibba* and *Azolla pinnata* were significantly different ($p < 0.05$) for the combined interaction of AMF, variety, and treatment (Figure 12a). In the combined interaction of AMF, LMOF, and BGSP (19.11 ± 0.15 μg kg^{-1}) and the combined interaction of LMIF and BGSP (5.62 ± 0.28 μg kg^{-1}) the highest and lowest Pb concentrations in *Lemna gibba* were observed, respectively. Furthermore, the maximum and lowest Pb contents in *Azolla pinnata* were observed in the combined interaction of AMF, AZOF, and BGSP (24.79 ± 0.23 μg kg^{-1}) and the combined interaction of AZIF and TRSP (8.74 ± 0.24 μg kg^{-1}), respectively. Removal rates of Pb by *L. minor* have been reported to be 76% [118], 94.19% [119], and 98.55% [120].



(**a**) (**b**)

Figure 12. Mean *Azolla pinnata* and *Lemna gibba* (**a**) Pb and (**b**) Hg with and without inoculation of AMF for different treatments of the two tested rice varieties. Error bars indicate 95% confidence intervals. Means denoted as the same letters do not differ at $p < 0.05$.

3.6.4. Mercury in *Azolla pinnata* and *Lemna gibba*

The results indicated that the interactions between AMF, rice variety, and treatment were significantly different ($p < 0.05$) for total Hg levels in both *Lemna gibba* and *Azolla pinnata* (Figure 12b). The highest and lowest Hg concentrations in *Lemna gibba* were found in the combined interaction of AMF, LMOF, and BGSP (8.92 ± 0.11 μg kg^{-1}) and the combined interaction of LMIF and TRSP (2.59 ± 0.06 μg kg^{-1}), respectively. In addition, *Azolla pinnata* had the highest and lowest Hg concentrations in the combined interaction of AMF, AZOF, and BGSP (9.90 ± 0.12 μg kg^{-1}) and the combined interaction of AZIF and TRSP (2.97 ± 0.11 μg kg^{-1}), respectively. Through the process of rhizofiltration, the *Azolla pinnata* and *Lemna gibba* could accumulate lower mercury levels. The experimental examination of *Lemna* sp. by Cvjetko et al. [121] found that a maximum removal of more than 490 μg g^{-1} of Hg could be achieved at an initial concentration of 0.1–2 mg L^{-1} of Hg. Additionally, Rai and Tripathi [122] found that *Azolla* sp. had a better percentage clearance (80–90% of removal) of Hg, and 940 mg kg^{-1} could accumulate in the fern.

3.7. Bioaccumulation Factor

The bioaccumulation factor (BAF) for rice is calculated by comparing grain heavy metal concentration and soil heavy metal absorption. If the BAF value is greater than 1, crops contain elevated levels of toxic metals [123].

According to these data (Table 3), the combined interaction of AMF, rice variety, and treatment was statistically significant for Cd, As, Pb, and Hg BAF in rice grain ($p < 0.05$). Relatively low BAF values were found for Cd (0.199 ± 0.005) and As (0.191 ± 0.024) in the TRSP rice varieties with OF treatment and AMF inoculation. Furthermore, higher BAF values were found for Cd (1.013 ± 0.01) and As (1.600 ± 0.013) in BGSP rice varieties with IF treatment. Among the treatments, the BAF of both Pb (0.675 ± 0.008) and Hg (0.421 ± 0.013) for the grain in the BGSP rice variety was higher than those of the other treatments. Furthermore, As BAF values for CON (1.052 ± 0.018), IF (1.600 ± 0.013), AZIF (1.119 ± 0.01), and LMIF (1.506 ± 0.014) treatments of the BGSP rice variety and IF (1.316 ± 0.037), AZIF (1.026 ± 0.011), and LMIF (1.239 ± 0.015) treatments of the TRSP rice variety were greater than 1. Additionally, the BAF value of the Cd for the IF treatment (1.012 ± 0.01) in the BGSP rice variety was greater than 1.

Table 3. Bioaccumulation factor for heavy metals in rice grain.

AMF	Rice Variety	Treatment	Cd	As	Pb	Hg
With inoculation of AMF	BGSP	CON	0.392 ± 0.014^{k}	0.782 ± 0.045^{k}	0.519 ± 0.006$^{h–k}$	0.210 ± 0.007$^{g–j}$
		OF	0.218 ± 0.012$^{s–u}$	0.226 ± 0.022st	0.538 ± 0.015$^{g–i}$	0.116 ± 0.006^{no}
		IF	0.506 ± 0.007^{h}	**1.024 ± 0.017^{fg}**	0.542 ± 0.011$^{f–j}$	0.273 ± 0.009^{de}
		AZ	0.265 ± 0.012$^{o–s}$	0.431 ± 0.018^{h}	0.502 ± 0.017$^{i–m}$	0.189 ± 0.01$^{i–k}$
		AZOF	0.260 ± 0.009$^{o–s}$	0.268 ± 0.016^{rs}	0.593 ± 0.014$^{b–d}$	0.123 ± 0.011^{mn}
		AZIF	0.439 ± 0.008^{j}	0.885 ± 0.014^{ij}	0.523 ± 0.01$^{h–k}$	0.216 ± 0.009$^{g–i}$
		LM	0.306 ± 0.012$^{m–o}$	0.625 ± 0.015^{l}	0.518 ± 0.012$^{h–k}$	0.208 ± 0.01$^{g–j}$
		LMOF	0.235 ± 0.006$^{q–u}$	0.244 ± 0.02st	0.523 ± 0.013$^{h–k}$	0.110 ± 0.009^{no}
		LMIF	0.495 ± 0.010^{hi}	0.962 ± 0.015^{g}	0.557 ± 0.011$^{d–h}$	0.225 ± 0.015^{gh}
	TRSP	CON	0.248 ± 0.020$^{p–t}$	0.603 ± 0.027^{l}	0.450 ± 0.006^{o}	0.087 ± 0.002^{o}
		OF	0.199 ± 0.005^{u}	0.191 ± 0.024^{t}	0.491 ± 0.01$^{k–h}$	0.086 ± 0.01^{o}
		IF	0.357 ± 0.01^{kl}	0.824 ± 0.021^{jk}	0.475 ± 0.007$^{m–o}$	0.082 ± 0.002^{o}
		AZ	0.226 ± 0.011$^{r–u}$	0.435 ± 0.027^{n}	0.469 ± 0.007$^{m–o}$	0.095 ± 0.011^{no}
		AZOF	0.249 ± 0.012$^{p–t}$	0.235 ± 0.027st	0.580 ± 0.018$^{b–f}$	0.105 ± 0.006^{no}
		AZIF	0.305 ± 0.008$^{m–o}$	0.667 ± 0.003^{l}	0.454 ± 0.014^{no}	0.082 ± 0.006^{o}
		LM	0.250 ± 0.01$^{p–t}$	0.534 ± 0.019^{m}	0.464 ± 0.009$^{m–o}$	0.090 ± 0.003^{no}
		LMOF	0.212 ± 0.017$^{t–u}$	0.196 ± 0.018^{t}	0.500 ± 0.016$^{j–m}$	0.115 ± 0.005^{no}
		LMIF	0.332 ± 0.01$^{l–n}$	0.784 ± 0.022^{k}	0.477 ± 0.01$^{l–o}$	0.089 ± 0.007^{no}
Without inoculation of AMF	BGSP	CON	0.645 ± 0.011^{f}	**1.052 ± 0.018^{f}**	0.599 ± 0.014^{bc}	0.301 ± 0.008^{cd}
		OF	0.268 ± 0.011$^{o–r}$	0.350 ± 0.013$^{o–q}$	0.556 ± 0.007$^{d–h}$	0.190 ± 0.016$^{i–k}$
		IF	**1.013 ± 0.01^{a}**	**1.600 ± 0.013^{a}**	0.669 ± 0.007^{a}	0.417 ± 0.011^{a}
		AZ	0.359 ± 0.01^{kl}	0.502 ± 0.018^{m}	0.546 ± 0.014$^{e–h}$	0.240 ± 0.009^{fg}
		AZOF	0.341 ± 0.017^{lm}	0.400 ± 0.035^{no}	0.612 ± 0.013^{b}	0.200 ± 0.018$^{h–j}$
		AZIF	0.762 ± 0.017^{d}	**1.119 ± 0.010^{e}**	0.612 ± 0.014^{b}	0.374 ± 0.012^{b}
		LM	0.458 ± 0.014^{ij}	0.832 ± 0.023^{jk}	0.571 ± 0.014$^{c–g}$	0.276 ± 0.016^{de}
		LMOF	0.278 ± 0.007$^{o–q}$	0.353 ± 0.030$^{o–q}$	0.540 ± 0.015$^{f–j}$	0.183 ± 0.018$^{i–k}$
		LMIF	0.913 ± 0.042^{b}	**1.506 ± 0.014^{b}**	0.675 ± 0.008^{a}	0.421 ± 0.013^{a}
	TRSP	CON	0.645 ± 0.014^{f}	0.912 ± 0.011^{hi}	0.586 ± 0.009$^{b–e}$	0.261 ± 0.004^{ef}
		OF	0.229 ± 0.009$^{r–u}$	0.310 ± 0.025^{qr}	0.532 ± 0.018$^{g–k}$	0.150 ± 0.012^{lm}
		IF	0.849 ± 0.014^{c}	**1.316 ± 0.037^{c}**	0.616 ± 0.023^{b}	0.369 ± 0.01^{b}
		AZ	0.450 ± 0.008^{j}	0.638 ± 0.017^{l}	0.547 ± 0.01$^{e–h}$	0.186 ± 0.006$^{i–k}$
		AZOF	0.294 ± 0.013$^{n–p}$	0.386 ± 0.028$^{n–p}$	0.603 ± 0.009^{bc}	0.177 ± 0.017$^{j–l}$
		AZIF	0.709 ± 0.011^{e}	**1.026 ± 0.011$^{f–g}$**	0.598 ± 0.01^{bc}	0.283 ± 0.015^{de}
		LM	0.553 ± 0.034^{g}	0.763 ± 0.031^{k}	0.596 ± 0.006$^{b–d}$	0.226 ± 0.003^{gh}
		LMOF	0.254 ± 0.012$^{p–t}$	0.324 ± 0.022$^{p–r}$	0.516 ± 0.021$^{h–l}$	0.157 ± 0.003^{kl}
		LMIF	0.777 ± 0.004^{d}	**1.239 ± 0.015^{d}**	0.615 ± 0.007^{b}	0.328 ± 0.005^{c}

The values represent the mean ± standard deviation. At $p < 0.05$, different letters in the same row indicate a significant difference.

Crop type, soil features, selectivity of the crops, and permissibility of the metals are just a few variables that affect the accumulation of heavy metals in rice [124,125]. Additionally, Fe, Mn, P, and other crucial components are frequently used by rice to transport and absorb heavy metals, which are then progressively absorbed into the grain [126]. Mineral components may also impact rice's ability to absorb and accumulate heavy metals [127]. The ion concentration in the soil affects the enrichment of heavy metals in the crop, even though the soil medium impacts the heavy metal BAF and capacity to move into the crop under different pH levels [128].

Payus et al. [67] found that the Cd and Pb concentrations were greater than 1, indicated that *Oryza sativa* is a hyperaccumulator plant that can take up many metals from the soil. The cultivars used may also change the amount of accumulation, absorption, and phytotoxicity. According to the findings of Hang et al. [129], the greatest BAF in paddy plants was associated with Cd (0.178), followed by As (0.025), Pb (0.005), and Hg (0.047).

3.8. Translocation Factor (TF)

3.8.1. Cadmium Translocation Factor

The results showed that the inoculation of AMF was significantly different ($p < 0.05$) for soil-to-root Cd translocation (Tables 4–6). The highest soil-to-root Cd translocation was observed for non-inoculated (0.91 ± 0.01) block, and the lowest was in the AMF-inoculated (0.82 ± 0.02) block. Moreover, the combined interaction of AMF and treatment was significantly different ($p < 0.05$) for the root-to-shoot Cd translocation. According to these results, the maximum and minimum root-to-shoot Cd translocation was found in IF treatment (0.82 ± 0.02) and the combined interaction of AMF and AZOF (0.30 ± 0.01), respectively. Additionally, the results showed that the combined interaction of AMF, variety, and treatment was significantly different ($p < 0.05$) for shoot-to-grain Cd translocation. The shoot-to-grain Cd translocation was the highest in the combined interaction of IF and BGSP (0.89 ± 0.05) and the lowest was in the combined interaction of AMF, AZOF, and TRSP (0.60 ± 0.03).

Table 4. Mean Cd, As, Pb, and Hg translocation factor for soil-to-root.

AMF	Cd	As	Pb	Hg
With inoculation of AMF	0.79 ± 0.02^{b}	0.82 ± 0.02^{b}	0.78 ± 0.01^{b}	0.63 ± 0.01^{b}
Without inoculation of AMF	0.89 ± 0.01^{a}	0.91 ± 0.01^{a}	0.90 ± 0.01^{a}	0.79 ± 0.01^{a}

The values represent the mean ± standard deviation. At $p < 0.05$, different letters in the same row indicate a significant difference.

Table 5. Mean Cd, As, Pb, and Hg translocation factor for root-to-shoot.

AMF	Treatment	Cd	As	Pb	Hg
With inoculation of AMF	CON	0.50 ± 0.02^{g}	0.74 ± 0.02^{f}	0.67 ± 0.01^{i}	0.33 ± 0.01^{j}
	OF	0.38 ± 0.03^{hi}	0.51 ± 0.01^{k}	0.51 ± 0.04^{k}	0.31 ± 0.06^{l}
	IF	0.71 ± 0.04^{b}	0.83 ± 0.05^{d}	0.71 ± 0.02^{d}	0.49 ± 0.09^{d}
	AZ	0.35 ± 0.05^{j}	0.63 ± 0.06^{i}	0.50 ± 0.06^{l}	0.30 ± 0.04^{m}
	AZOF	0.30 ± 0.01^{k}	0.45 ± 0.01^{m}	0.43 ± 0.02^{m}	0.24 ± 0.01^{n}
	AZIF	0.62 ± 0.02^{de}	0.82 ± 0.04^{e}	0.62 ± 0.07^{g}	0.47 ± 0.02^{e}
	LM	0.39 ± 0.03^{i}	0.69 ± 0.06^{g}	0.52 ± 0.02^{j}	0.39 ± 0.06^{i}
	LMOF	0.39 ± 0.07^{h}	0.50 ± 0.01^{i}	0.50 ± 0.06^{l}	0.30 ± 0.08^{kl}
	LMIF	0.59 ± 0.06^{e}	0.82 ± 0.05^{e}	0.68 ± 0.08^{f}	0.48 ± 0.04^{de}
Without inoculation of AMF	CON	0.63 ± 0.05^{d}	0.88 ± 0.03^{c}	0.65 ± 0.09^{e}	0.43 ± 0.09^{f}
	OF	0.51 ± 0.03^{fg}	0.63 ± 0.07^{g}	0.61 ± 0.02^{gh}	0.41 ± 0.04^{g}
	IF	0.82 ± 0.02^{a}	0.98 ± 0.02^{a}	0.81 ± 0.01^{a}	0.60 ± 0.01^{a}
	AZ	0.51 ± 0.05^{f}	0.82 ± 0.09^{d}	0.57 ± 0.06^{i}	0.42 ± 0.06^{h}
	AZOF	0.39 ± 0.06^{i}	0.82 ± 0.06^{d}	0.51 ± 0.08^{k}	0.31 ± 0.02^{jk}
	AZIF	0.73 ± 0.09^{bc}	0.62 ± 0.06^{j}	0.74 ± 0.06^{c}	0.55 ± 0.09^{b}
	LM	0.52 ± 0.04^{f}	0.85 ± 0.01^{cd}	0.61 ± 0.02^{gh}	0.52 ± 0.06^{c}
	LMOF	0.52 ± 0.02^{f}	0.68 ± 0.05^{gh}	0.51 ± 0.06^{k}	0.42 ± 0.03^{fg}
	LMIF	0.71 ± 0.04^{c}	0.95 ± 0.04^{b}	0.77 ± 0.03^{b}	0.54 ± 0.04^{b}

The values represent the mean ± standard deviation. At $p < 0.05$, different letters in the same row indicate a significant difference.

Table 6. Mean Cd, As, Pb, and Hg translocation factor for shoot-to-grain.

AMF	Rice Variety	Treatment	Cd	As	Pb	Hg
With inoculation of AMF	BGSP	CON	0.76 ± 0.02^ef	0.62 ± 0.02^g	0.76 ± 0.01^f	0.77 ± 0.01^de
		OF	0.69 ± 0.03^j	0.59 ± 0.05^h	0.67 ± 0.02^h	0.74 ± 0.06^fg
		IF	0.83 ± 0.02^b	0.80 ± 0.03^b	0.85 ± 0.06^b	0.82 ± 0.06^b
		AZ	0.64 ± 0.03^kl	0.57 ± 0.04^i	0.64 ± 0.01^ij	0.68 ± 0.04^h
		AZOF	0.61 ± 0.05^m	0.52 ± 0.02^k	0.62 ± 0.05^l	0.63 ± 0.01^j
		AZIF	0.80 ± 0.03^cd	0.64 ± 0.06^f	0.80 ± 0.06^de	0.79 ± 0.02^cd
		LM	0.72 ± 0.01^g	0.62 ± 0.05^gh	0.75 ± 0.04^g	0.75 ± 0.06^f
		LMOF	0.62 ± 0.05^l	0.56 ± 0.06^j	0.62 ± 0.02^k	0.66 ± 0.09^i
		LMIF	0.82 ± 0.06^bc	0.72 ± 0.07^d	0.83 ± 0.06^c	0.82 ± 0.07^b
	TRSP	CON	0.74 ± 0.08^fg	0.62 ± 0.02^gh	0.75 ± 0.06^g	0.76 ± 0.04^ef
		OF	0.67 ± 0.04^jk	0.58 ± 0.06^hi	0.65 ± 0.04^i	0.72 ± 0.06^gh
		IF	0.82 ± 0.02^bc	0.72 ± 0.04^d	0.80 ± 0.02^de	0.82 ± 0.02^b
		AZ	0.62 ± 0.03^lm	0.56 ± 0.08^ij	0.62 ± 0.03^k	0.67 ± 0.04^hi
		AZOF	0.60 ± 0.03^n	0.50 ± 0.02^l	0.60 ± 0.02^n	0.62 ± 0.01^l
		AZIF	0.78 ± 0.08^de	0.62 ± 0.01^g	0.76 ± 0.08^f	0.77 ± 0.01^de
		LM	0.70 ± 0.02^hi	0.59 ± 0.06^h	0.66 ± 0.01^hi	0.73 ± 0.02^g
		LMOF	0.62 ± 0.06^lm	0.52 ± 0.09^k	0.62 ± 0.06^m	0.63 ± 0.03^j
		LMIF	0.81 ± 0.02^c	0.68 ± 0.04^de	0.79 ± 0.01^e	0.79 ± 0.08^cd
Without inoculation of AMF	BGSP	CON	0.77 ± 0.05^e	0.64 ± 0.05^f	0.78 ± 0.05^ef	0.79 ± 0.06^cd
		OF	0.71 ± 0.03^h	0.62 ± 0.02^g	0.75 ± 0.07^g	0.75 ± 0.04^f
		IF	0.89 ± 0.05^a	0.85 ± 0.10^a	0.88 ± 0.03^a	0.85 ± 0.04^a
		AZ	0.67 ± 0.01^jk	0.59 ± 0.04^h	0.67 ± 0.06^h	0.72 ± 0.06^gh
		AZOF	0.63 ± 0.09^l	0.54 ± 0.03^jk	0.63 ± 0.09^k	0.66 ± 0.04^i
		AZIF	0.82 ± 0.02^bc	0.67 ± 0.05^e	0.84 ± 0.04^bc	0.80 ± 0.06^c
		LM	0.76 ± 0.02^ef	0.63 ± 0.03^fg	0.76 ± 0.01^f	0.76 ± 0.08^e
		LMOF	0.64 ± 0.05^kl	0.57 ± 0.07^i	0.68 ± 0.03^ij	0.68 ± 0.06^h
		LMIF	0.83 ± 0.06^b	0.75 ± 0.03^c	0.84 ± 0.06^bc	0.82 ± 0.09^b
	TRSP	CON	0.75 ± 0.06^f	0.63 ± 0.06^fg	0.75 ± 0.06^g	0.76 ± 0.05^e
		OF	0.70 ± 0.04^hi	0.59 ± 0.04^h	0.67 ± 0.08^h	0.73 ± 0.01^g
		IF	0.86 ± 0.06^ab	0.83 ± 0.06^ab	0.85 ± 0.04^b	0.82 ± 0.02^b
		AZ	0.65 ± 0.06^k	0.58 ± 0.03^hi	0.65 ± 0.05^i	0.68 ± 0.06^h
		AZOF	0.62 ± 0.05^lm	0.52 ± 0.09^k	0.63 ± 0.06^j	0.63 ± 0.03^i
		AZIF	0.79 ± 0.02^d	0.64 ± 0.07^f	0.81 ± 0.07^d	0.78 ± 0.05^d
		LM	0.75 ± 0.06^f	0.62 ± 0.06^gh	0.75 ± 0.02^g	0.75 ± 0.04^f
		LMOF	0.62 ± 0.09^lm	0.56 ± 0.01^j	0.61 ± 0.01^kl	0.63 ± 0.07^k
		LMIF	0.83 ± 0.02^b	0.75 ± 0.03^c	0.82 ± 0.06^cd	0.81 ± 0.05^bc

The values represent the mean ± standard deviation. At $p < 0.05$, different letters in the same row indicate a significant difference.

Only a small amount of Cd is carried to shoots by the phloem. More and more data point to the importance of OsZIP6, OsZIP7, OsLCD, OsHMA2, CAL1, and OsMTP1 channels in mediating Cd transport in rice [130]. Intervascular and xylem-to-phloem transfer are also involved in translocating As and Cd to rice grain. In rice, Cd is transferred from roots to shoots through several transporters [131]. OsHMA2 [132–134] and OsZIP7 [135] are plasma membrane transporters for Zn and Cd that are found in the pericycle of rice roots and parenchyma cells in rice vascular bundles.

Cadmium is a nutrient that is not needed for plant development. Consequently, no particular Cd transporters are anticipated to exist in plants. Singh et al. [64] and Rahimi et al. [136] investigated the soil-to-root translocation in rice plants and discovered that the Cd value for TF was more than 1. This shows that rice roots grown in contaminated environments collected significant amounts of Cd^{2+} [137]. Furthermore, according to the findings of Satpathy et al. [5], the soil-to-root TF values for Cd range from 0.30–0.60. In addition, the TF values for Cd in the root-to-shoot and shoot-to-grain ranges were between 1.30 and 0.09, respectively.

3.8.2. Arsenic Translocation Factor

The findings indicated that soil-to-root As translocation significantly differed with AMF inoculation ($p < 0.05$) (Tables 4–6). The block without AMF inoculation had the highest soil-to-root As transfer (0.89 $\pm$ 0.01) and the block with AMF inoculation had the lowest (0.79 $\pm$ 0.02). For the root-to-shoot As translocation, the combined interaction of AMF and treatment was also significantly different ($p < 0.05$). According to our data, IF treatment (0.98 $\pm$ 0.02) and the combined interaction of AMF and AZOF (0.45 $\pm$ 0.01) had the highest and lowest root-to-shoot As translocation, respectively. Additionally, for shoot-to-grain As translocation, the results showed that the combined interaction of AMF, variety, and treatment was significantly different ($p < 0.05$). The combined interaction of AMF, AZOF, and TRSP had the lowest shoot-to-grain As translocation (0.5 $\pm$ 0.02) and the combined interaction of IF and BGSP had the highest (0.85 $\pm$ 0.1).

The chemical speciation of As in soil and the rhizosphere is essential because roots may take up distinct chemical species via different pathways. Rice roots mainly absorb As (III) through the silicon uptake pathway [138]. Furthermore, it is widely known that phosphate transporters are involved in the uptake of As(V). It has been demonstrated that OsPT1, OsPT4, and OsPT8 are involved in the As(V) uptake by roots in rice [139–142]. Numerous investigations have shown that As is originally absorbed by roots and then transported to other organs by active transport, aiding in the spread of the organelles in the root [143]. According to the findings published by Singh et al. [64], the soil-to-root TF values for arsenic in rice plants were at 11.34. Additionally, the ratio of root to shoot for As was 0.08, and the ratio of shoot to grain for As was 0.01.

3.8.3. Lead Translocation Factor

The results revealed that AMF inoculation was significantly different ($p > 0.05$) for soil-to-root Pb transfer (Tables 4–6). The highest soil-to-root Pb transfer was reported in the block without AMF inoculation (0.90 $\pm$ 0.01), and the lowest in the block with AMF inoculation (0.78 $\pm$ 0.01). Furthermore, for the root-to-shoot Pb translocation, the combined interaction of AMF and treatment was significant ($p > 0.05$). According to the data, IF treatment (0.81 $\pm$ 0.01) and the combined interaction of AMF and AZIF (0.43 $\pm$ 0.02) had the highest and lowest root-to-shoot Pb translocation, respectively. Furthermore, for shoot-to-grain Pb translocation, the combined interaction of AMF, variety, and treatment was significantly different ($p > 0.05$). Pb translocation from shoot to grain was greatest in the combined interaction of IF and BGSP (0.88 $\pm$ 0.03) and lowest in the combined interaction of AMF, AZOF, and TRSP (0.60 $\pm$ 0.02). Singh et al. [64] reported that the soil-to-root TF value for As in rice plants was found to be 0.39, and the shoot-to-grain ratio for As was 0.22, while the root-to-shoot ratio was 0.33.

3.8.4. Mercury Translocation Factor

The inoculation of AMF showed a significant difference ($p < 0.05$) for soil-to-root Hg translocation, as indicated by the findings (Tables 4–6). Hg transfer from soil to roots was highest in the non-AMF-inoculated block (0.79 $\pm$ 0.01) and lowest in the AMF-inoculated block (0.63 $\pm$ 0.01). Moreover, the combined interaction of AMF and treatment was significantly different ($p < 0.05$) for Hg translocation from root to shoot. According to the results, IF treatment (0.60 $\pm$ 0.01) and the combined interaction of AMF and AZIF (0.24 $\pm$ 0.01) exhibited the highest and lowest root-to-shoot Hg translocation, respectively. In addition, the results demonstrated that the combined interaction of AMF, rice variety, and treatment was substantially different ($p < 0.05$) for Hg translocation from shoot to grain. Hg translocation from shoot to grain was greatest in the combined interaction of IF and BGSP (0.85 $\pm$ 0.04) and lowest in the combined interaction of AMF, AZOF, and TRSP (0.62 $\pm$ 0.01). The soil-to-root TF value for As in paddy plants was recorded at 0.96, according to Singh et al. [64]. Additionally, the ratios of As in roots and shoots to grains were 0.71 and 0.95, respectively.

4. Conclusions

This study found that the application of indigenous AMF to *Oryza sativa* grown in rice soil resulted in a considerable decrease in the accumulation of heavy metals (Cd, As, Pb, and Hg) in the roots, shoots, and grains. of rice plants. The root was discovered to have a greater concentration of heavy metals than the rest of the plant. In addition, the data from the study demonstrated that the combination of AMF, organic fertilizer, and *Azolla pinnata* achieved better remediation effects than the application of inorganic fertilizer. The fact that the inorganic fertilizer treatments had a larger BAF than the other treatments further demonstrate this point.

The implementation of AMF in rice production systems may be significantly impacted as a direct result of these findings. It is, therefore, recommended to rice farmers that they adopt and implement the combination of AMF, natural soil amendments such as compost, and *Azolla pinnata* in their farming regimes in order to decrease the bioavailability of heavy metals in the soil. Other natural soil amendments may also be utilized. Further in-depth studies are required to determine the processes involved in the mycorrhizal influence on heavy metals, as well as in the modification of the mycorrhizal association in rice plants.

Author Contributions: Conceptualization, B.M.H. and P.N.Y.; methodology, B.M.H.; software, B.M.H.; validation, N.S., J.K. and P.N.Y.; formal analysis, B.M.H.; investigation, P.N.Y. and C.B.; resources, P.N.Y.; data curation, B.M.H.; writing—original draft preparation, B.M.H.; writing—review and editing, B.M.H., C.B., S.L.S., A.M.E., S.A., J.K., N.S., S.C.K. and P.N.Y.; visualization, C.B.; supervision, P.N.Y. and C.B.; project administration, P.N.Y.; funding acquisition, P.N.Y. and A.M.E. All authors have read and agreed to the published version of the manuscript.

Funding: Financial assistance given by the World Bank Group through the project Accelerating Higher Education Expansion and Development Operation (AHEAD), DOR, Grant No. 79, Rajarata University of Sri Lanka, is highly appreciated. This research was partially supported by Chiang Mai University and the National Natural Science Foundation of China (NSFC 32260004). The authors extend their appreciation to the Researchers Supporting Project number RSP2023R56, King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the Faculty of Applied Sciences, Rajarata University of Sri Lanka, Qujing Normal University, China, and Chiang Mai University, Thailand.

Conflicts of Interest: The authors declare no conflict of interest.

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