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Biofilm biofertilizer modulates heavy metals in soil–plant systems to produce high-quality rice

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Abstract

High concentrations of toxic heavy metals (THM) and low micronutrients in rice grains adversely affect human health. In this research, we investigated the potential of using biofilm biofertilizer (BFBF) in managing THM and micronutrients in rice by conducting field experiments that compared BFBF practice with the practice of using chemical fertilizer (CF) alone in Sri Lanka. Bioaccumulation and translocation factors were evaluated to assess THM and micronutrient distribution in soil–plant systems. The human health risk was also estimated. The BFBF practice showed a significant reduction in estimated daily intakes in the range of ca. 0.08–0.99 μg kg^{-1} day^{-1} for THM such as As, Co, Cd, and Cr compared to the range of 0.16–1.40 μg kg^{-1} day^{-1} when using CF alone. Thus, there were significantly low values of hazard quotient (HQ) and hazard index (HI) in the BFBF practice over CF indicating lower health risk. In the CF practice, the translocation of As from panicle to rice seed was significantly increased, and As in rice seeds is reported to exceed the safe level in some cases in Sri Lanka. On the contrary, reduced translocation of As and increased translocation of Cr within the safe level to rice seeds were observed with the BFBF application. Interestingly, the HI had been kept below the threshold value of 1.0 by significantly reducing the HQ values of each THM, only in the BFBF practice. These results highlight the role of increased microbial diversity and abundance induced by the BFBF, in mitigating the health risks and enhancing the sustainability of the soil–plant system.

Keywords: *biofilm biofertilizer, grain quality, rice paddy, toxic heavy metals*

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1. Introduction

Consumption of metal-contaminated foods has been reported as the main route of transmitting toxic heavy metals (THM) from soils to the human body [1]. Once the human body is exposed to THM in the long term, many adverse effects are exerted on human health [2]. For example, exposure to arsenic (As) may induce harmful effects on the cardiovascular and hematopoietic system, and exposure to lead (Pb) can significantly elevate blood Pb level, a causative factor in renal impairment [1, 3]. THM can also lower energy levels and damage the brain, lungs, kidneys, liver, blood vessels, and other vital organs. In addition, long-term exposure to such elements can cause physical, muscular, and neurodegenerative processes that mimic diseases such as chronic sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Prolonged exposure to certain elements and their compounds can even cause cancer [4].

Globally, a considerable extent of paddy lands has been reported to have excessive amounts of THM and low concentrations of non-THM or micronutrients, resulting from the predominant contribution of chemical inputs, such as chemical fertilizer (CF) and agrochemicals, and other anthropogenic activities [5–8]. THM could be absorbed by plants and could accumulate in edible portions, thus increasing the risk of chronic toxicity [6, 9]. Among agricultural produce, rice (*Oryza sativa* L.) is the principal food source for over half of the global population and could be a significant dietary source of THM, especially in Asia [5, 6, 10]. In the Sri Lankan context, annual rough rice production is ca. 2.7 Mt, and the per capita consumption fluctuates around 100 kg $year^{-1}$ [11]. Therefore, reducing the accumulation of THM in rice grains via reduced contamination of agricultural soils and reduced transmission into the plant is crucial to food safety [5, 6, 12, 13].

The micronutrients govern plant growth, life cycle completion, electron transfers, and other important metabolic processes [14–17]. They also play a major role in the synthesis of nucleic acids, proteins, and photosynthetic pigment and contribute to the structural and functional integrity of cell membranes [18]. Nevertheless, at elevated concentrations, micronutrients also produce toxicity symptoms in plants [19–21].

However, the knowledge on heavy metal (HM) distribution and accumulation in rice is still limited. In this context, the involvement of microbes in metal remediation for reducing the bioavailability of

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THM in rice grains [22–24] has been emphasized [1, 17]. That is because microbes can counteract HM stress using diverse defensive systems, such as compartmentalization, exclusion, formation of complexes, and the synthesis of binding proteins [25]. Microbial processes play a significant role in bioremediation, particularly in solubilizing and immobilizing metals. These processes, which occur both *in situ* and *ex situ*, have been recognized for their potential for environmental cleanup. In addition, extracellular polymeric substances (EPS) produced by microbes are important in metal biosorption [23, 26]. The application of such a fungal–bacterial biofilm–based suppliant, i.e., biofilm biofertilizer (BFBF), can facilitate these functions in agricultural systems. The National Institute of Fundamental Studies (NIFS), Sri Lanka, conducted a long-term research [27, 28] in this regard. It is interesting to note that microorganisms in biofilm mode, protected by an extracellular matrix, are proven as an efficient and safer strategy in bioremediation because the results are more significant than the conventional microbial bioremediation [29–32]. For instance, the use of BFBF showed the potential to remediate THM in cropland soils [30], thus showing its feasibility in reducing THM concentrations in edible parts of the crop. The BFBF practice is used by farmers in paddy cultivation [33–35] due to its ability to convert plant-unavailable organic and mineral nutrients into available forms for ensuring plant growth and physiology to increase the crop yield [36, 37]. Therefore, this study was designed to assess the effect of BFBF practice on the translocation of HMs in the paddy soil–plant system and the health risk of their accumulation in rice grains compared to that of the conventional practice of CF-alone application.

2. Materials and methods

2.1. Experimental design

The field experiments were carried out during the wet season 2021–2022 in Kurunegala (7°48′N 80°36′E) district, Sri Lanka. Two consecutive, uniform paddy plots (each ca. 0.4 ha) were taken as a randomized complete block design in each location, and three field locations were used as replicates, viz. *Thalwita*, *Minuwangete*, and *Galgamuwa*, to compare these major two fertilizer practices: (1) BFBF practice [2.5 l of BFBF with 225 kg NPK (Urea 150, TSP 33, and MOP 42 kg ha^{-1})] and (2) CF-alone practice [340 kg NPK (Urea 225, TSP 55, and MOP 60 kg ha^{-1}), recommended by the Department of Agriculture, Sri Lanka (2013)]. These practices are being used by farmers in Sri Lanka. The microorganisms contained in the BFBF are extracted from rice rhizosphere. As this is a patented product [Sri Lanka Patent No. 15958 (2013)], exact composition cannot be disclosed due to intellectual property right reasons. Irrigation water was managed separately for each plot, and pesticides were not needed due to the bio-controlling effect of the BFBF [33–35, 38]. The research sites had a typical subtropical climate with a mean annual rainfall of ca. 2,000 mm and consisted of variable soil types, particularly red-yellow podzolic and low-humic gley [39], which have been categorized as anthroposols under the World Reference Base for Soil Resources (WRB) soil classification [40]. Plots equal in depth were selected at the same ground level, and bunds were built to half a foot in height to prevent fertilizer from mixing with nearby or entering from outside. Paddy was broadcasted, and irrigation water was managed separately in the three fields, without mixing from the surrounding fields. All practices used the same rice variety (BG 360) and were installed on the same day.

2.2. Sample collection and preparation

Six plants (hills) were carefully uprooted at 50% flowering stage from random positions in each plot by excavating around the root zone without causing damage to the root system. The soil was separated from plant samples and allowed to air dry and then crushed and sieved (<0.5 mm). The plant samples were rinsed thoroughly with tap water followed by deionized water to remove dust. Air-dried plant samples were ground to a fine powder, stored in sealed polyethylene bags, and kept in a desiccator before analysis. A ground subsample of 0.25 g was put in a digestion tube and treated with 3 mL of trace metal grade HNO_3. The treated plant samples were digested for 15 min at 180°C on a microwave digester while soil samples were kept at 210°C for 20 min [41]. After the samples reached room temperature, they were added to Milli-Q water to a volume of up to 25 mL. Then, the samples were filtered using cellulose acetate filters (pore size 0.45 μm). A blank was also prepared for every sample in the same way to be free from the interferences of sample processing.

Arsenic (As), cadmium (Cd), lead (Pb), cobalt (Co), chromium (Cr), and nickel (Ni) were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, iCPA 7000, Thermo Scientific, Waltham, MA). The equipment was calibrated by using Standard Reference Materials of the elemental mixture. All samples were analyzed in duplicates. As per the ISO/IEC 17025 Standards, two food samples of known concentrations of HMs were analyzed as Proficiency Testing to detect the accuracy of the analysis.

2.3. Human risk assessment methods

2.3.1. Noncarcinogenic risk assessment

The health risk assessment model generated by the United States Environmental Protection Agency (USEPA) was employed to assess the human health risk of HMs to adults [42–44]. In accordance with this guideline, humans' exposure to HMs via consumption has been considered for the evaluation. The hazard quotient (HQ) was used to evaluate the noncarcinogenic risk assessments by chronic exposure to an individual THM. The factors were defined based on Eqs. (1) and (2) as follows [45, 46]:

$$\mathrm{EDI}_i = \frac{C_i \times \mathrm{IR} \times \mathrm{ED} \times \mathrm{EF}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-3} \tag{1}$$

$$\mathrm{HQ}_i = \frac{\mathrm{EDI}_i}{\mathrm{RfD}_i} \tag{2}$$

Here, EDI_i is the estimated daily intake (mg kg^{-1} day^{-1}) of HM i, C_i (mg kg^{-1}) is the concentration of HM i in the rice grain, IR (g person^{-1} day^{-1}) is the daily average consumption of rice in the region, BW (kg person^{-1}) represents the body weight, EF is the exposure frequency (365 days year^{-1}), ED (year) is the exposure duration, and AT is the average time (**Table 1**). HQ_i is the hazard quotient of HM i. The applied chronic oral reference doses (RfD_i) for the HQ calculation were 0.0003, 0.001, 0.3, 0.003, and 0.02 (mg kg^{-1} day^{-1}) for As, Cd, Co, Cr, and Ni, respectively [47, 48].

Table 1 • The definitions and values of exposure parameters of heavy metals for rice

Parameter	Definition	Value	Reference
C_i	Heavy metal content in grain (mg kg^{-1})	Observed value	
EF	Exposure frequency (day)	365	[10]
ED	Exposure duration (years)	70	[10]
BW	Body weight (kg person^{-1})	60	[49]
AT	Average time (day years)	365 × 70	[10]
IR	Ingestion rate (g person^{-1} day^{-1})	293.15	[50]

The hazard index (HI) was used to evaluate the overall non-carcinogenic risk posed by more than one toxicant [51]. For several hazardous elements, the HI is calculated as the summation of HQ of the individual toxic element. If the value of HQ or HI is less than 1, it is assumed that it does not create harmful health effects for the exposed HMs, and hence believed to be safe. Otherwise, it is detrimental, and higher values increase the likelihood of the occurrence of adverse health effects [49, 48]. The HI through daily average rice consumption for a human being was calculated according to Eq. (3) [46, 52] as follows:

$$\mathrm{HI} = \sum_{n=1}^{i} \mathrm{HQ}_i \tag{3}$$

2.3.2. Carcinogenic risk assessments

The likelihood of developing cancer throughout a lifetime of exposure to a carcinogen was calculated by Eq. (4). The THMs As, Cd, Cr, and Ni were considered potential carcinogenic contaminants, based on the order of classification group defined by the International Agency for Research on Cancer [53]. For chemical carcinogens of As, Cd, Cr, and Ni ingestion, oral carcinogenic slope factors (SFs) were considered as 1.5 [10, 50, 54], 15, 0.5, and 0.91 mg kg–1 day–1, respectively [53, 55].

In the presence of multiple carcinogenic elements, the carcinogenic risks from all carcinogens are summed as explained by Zeng et al. in 2015 [56]. According to the USEPA, the value of cancer risk in the range from 10^{-6} to 10^{-4} is under the acceptable or tolerable risk, and a risk of less than 10^{-6} can be ignored. However, a risk exceeding 10^{-4} was considered to be unacceptable [48]. The factors were defined based on the following equations:

$$\mathrm{CR}_i = \mathrm{EDI}_i \times \mathrm{SF}_i \tag{4}$$

$$\mathrm{CR}_t = \sum_{n=1}^{i} \mathrm{CR}_i \tag{5}$$

Here, CR_i is the carcinogenic risk of metal i, SF_i is the oral carcinogenic slope factor (mg kg^{-1} day^{-1}) of metal i, and CR_t is the total carcinogenic risk.

Bioaccumulation factor and translocation factor

The translocation behaviors of the HMs in the soil–rice system were studied by bioaccumulation factor (BaF) and translocation factor (TF) [57]. They were defined based on the following equations:

$$\mathrm{BaF}_{i,s-r} = C_{i,\mathrm{root}} / C_{i,\mathrm{soil}} \tag{6}$$

$$\mathrm{TF}_{i,r-s} = C_{i,\mathrm{stem}} / C_{i,\mathrm{root}} \tag{7}$$

$$\mathrm{TF}_{i,s-l} = C_{i,\mathrm{leaf}} / C_{i,\mathrm{stem}} \tag{8}$$

$$\mathrm{TF}_{i,l-p} = C_{i,\mathrm{panicle}} / C_{i,\mathrm{leaf}} \tag{9}$$

$$\mathrm{TF}_{i,p-g} = C_{i,\mathrm{grain}} / C_{i,\mathrm{panicle}} \tag{10}$$

Here, C_{root}, C_{soil}, C_{stem}, C_{leaf}, C_{panicle}, and C_{grain} are the concentrations of HM i in the root, paddy soil, stem, leaf, panicle, and grain, respectively. $\mathrm{BaF}_{i,\,s-r}$ is the bioaccumulation factor of HM i from paddy soil to root. $\mathrm{TF}_{i,\,r-s}$, $\mathrm{TF}_{i,\,s-l}$, $\mathrm{TF}_{i,\,l-p}$, and $\mathrm{TF}_{i,\,p-g}$ are the translocation factors of HM i from root to stem, stem to leaf, leaf to panicle, and panicle to seed, respectively [1, 43, 57].

A schematic diagram of the methodology is depicted in **Figure 1**.

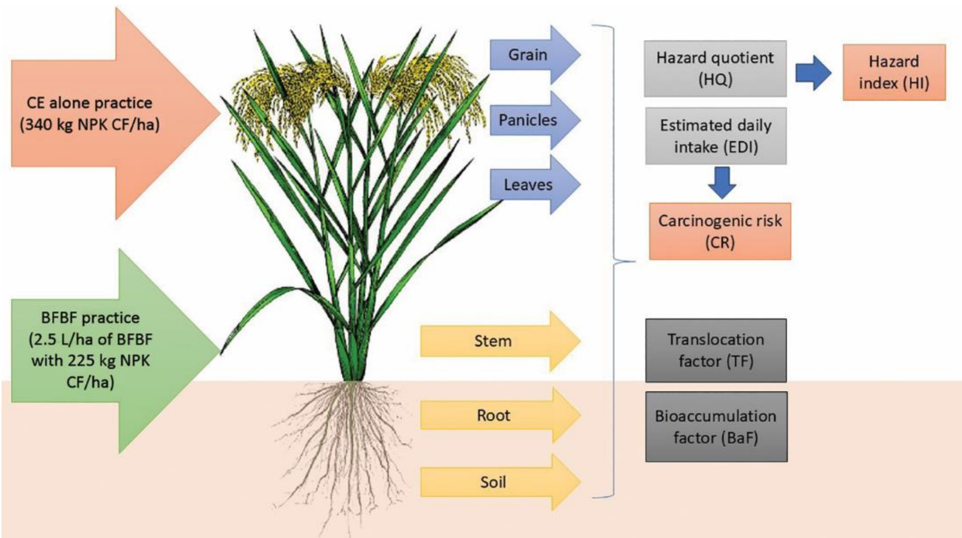

Figure 1 • A schematic diagram of the methodology followed in the study.

2.4. Statistical analysis

Data were analyzed using the statistical package Minitab, version 17. Analysis of variance followed by Tukey's Honestly Significant Difference (HSD) test was performed to compare the means. The probability of <0.05 was used as the threshold for significance.

3. Results and discussion

3.1. Heavy metal concentrations in the root-zone soils of biofilm biofertilizer and chemical fertilizer–alone practices

The concentrations of all HMs analyzed (**Table 2**) were within the previously observed ranges in rice soils of Sri Lanka [58]. The differences in their concentrations were not significant between the BFBF and CF practices ($P > 0.05$). This indicated that the two practices had similar concentrations of HMs in the root-zone soil. The soil's physicochemical properties and other parameters of BFBF and CF varied due to microbial actions [34].

Table 2 • Different heavy metals in the root-zone soils of biofilm biofertilizer and chemical fertilizer–alone practices

Element	Heavy metal concentration (μg kg⁻¹)		
	CF alone	BFBF	Difference
As	1,443.6 ± 49.23	1,607 ± 101.46	163.4 (0.157)
Cd	ND	ND	-
Pb	5,314 ± 129.64	5,323 ± 179.12	9 (0.968)
Co	6,584 ± 1,061.86	5,697 ± 839.95	887 (0.517)
Cr	22,340 ± 2,172.95	21,093 ± 1,663.94	1,247 (0.652)
Ni	7,078 ± 738.85	6,942 ± 606.95	136 (0.887)

Mean ± standard error. Values within parentheses are probability levels at which the differences are significant. CF, chemical fertilizer; BFBF, biofilm biofertilizer; ND, not detected.

3.2. Bioaccumulation factor and translocation factor

Both BaF and TF are important parameters for assessing the potential risks of contaminants in the environment and are used in regulatory frameworks to set limits on the concentration of contaminants in the environment. By understanding the BaF and TF of different THMs in the paddy plant, we would be able to better realize their potential impacts on human and environmental health and take steps to minimize those impacts.

The BaF of As, Pb, and Cr, except Co and Ni, from soil to plant roots were higher in the BFBF practice than in the CF-alone practice (**Table 3**). This could be attributed to the triggering of binding HM by HM-complexation [21, 59], as revealed by the strong correlations among As, Pb, and Cr for their enhanced bioaccumulation [60]. Generally, negatively charged soil EPS synthesized by the applied BFBF induces the chelation/binding of these HMs in the soil [61]. However, the BFBF practice caused increased bioaccumulation of the three HMs in the roots against their soil chelation. This shows that ecosystem intelligence has played a role in selectively removing As and Pb, in particular, from the soil, and to store them in the roots in the BFBF practice, because the two HMs are activated by the microbes, and in turn adversely affect them [62–64]. The selective removal of the two HMs has taken place under similar concentrations in the soil of the two practices (**Table 2**), reiterating the role of intelligence [64].

The application of BFBF enhanced the translocation of Co, whereas the CF increased the translocation of Pb and Cr at significant paces from root to stem ($P < 0.01$). Even in the absence of Cd in soils, its end-up with the rice grain is probably due to long-term accumulation and grain-filling mechanism changes. Once in the soil environment, even at extremely low concentrations, Cd can mobilize and become bioavailable due to soil conditions such as low pH, high salinity, or poor cation exchange capacity [65]. These factors facilitate the uptake of Cd by rice plants through root systems, often because of similarities in ionic charge and radius between Cd and essential nutrients such as Zn or Ca [66, 67]. In the CF-alone practice, Co and Pb showed greater TF from stem to leaf, whereas Pb showed greater TF from leaf to panicle, leaving Co in leaves. This might tend to concentrate Co in the leaves causing chlorosis and/or necrosis [68]. Low chlorophyll content in rice leaves in the CF practice compared to the BFBF practice is a frequent observation reported in the field (Ekanayake et al. in manuscript). In the CF practice, the TF of As from panicle to seed was significantly increased, while that of Cr was significantly decreased ($P < 0.01$) in comparison to the BFBF practice. The increased translocation of As from panicle to seed might have been caused by the increased translocation of Pb from leaf to panicle, because As and Pb are positively correlated in plants [60]. In rice consumed in Sri Lanka, As is frequently reported to exceed the maximum permissible level in some cases, whereas Cr does not show toxicity [69]. Further, As accumulation in rice has been reported to reduce the levels of essential micronutrients manganese (Mn), Ni, and selenium (Se) [70]. At its safety levels, Cr is beneficial for human brain health and insulin regulation [71]. In this manner, reduced translocation of As and increased translocation of Cr to rice grains with the BFBF application imply a sign of intelligence in the soil–plant system [64].

The BFBF significantly enhanced the bioaccumulation of As, Pb, and Cr. This could be attributed to the fact that BFBF triggered the synthesis of phytochelatins (PCs) by itself and the plant, enabling the binding of HM by HM-complexation, compartmentalization, and sequestration into vacuole for enhanced bioaccumulation of toxic metals [72, 73]. It is interesting to note that PCs are also trafficking essential HMs of Co and Ni though not at a significant rate, as demonstrated in this study. Moreover, negatively charged EPS synthesized by BFBF is believed to induce the chelation of these HMs [74]. Consequently, the BFBF's natural chelating abilities regarding toxic and essential HMs emphasize that biofilms can act as biosensors, thus detecting the substrate efficiently. Moreover, the immobilization of HMs in the soil via biosorption, precipitation, and biofilm formation, while chelating agents such as siderophores and organic acids bind metals, limiting their uptake [75–77]. Microbes also alter HM bioavailability through redox transformations, compete with plants for metal absorption, and enhance root defense mechanisms such as efflux pumps and selective nutrient transport [78]. In addition, they sequester metals within root tissues, regulate xylem and phloem transport, and promote plant growth to reduce HM stress [79, 80]. These interactions ensure reduced accumulation of toxic metals in edible plant parts, offering a sustainable approach to minimizing HM contamination in agriculture while producing healthy food.

Table 3 • Bioaccumulation and translocation factors of the different heavy metals in the biofilm biofertilizer and chemical fertilizer–alone practices

Factor	Treatment	As	Cd	Pb	Co	Cr	Ni
BaF_{s-r}	BFBF	1.414 ± 0.10	-	0.344 ± 0.02	2.275 ± 0.26	0.302 ± 0.03	0.147 ± 0.01
	CF alone	1.166 ± 0.05	-	0.225 ± 0.01	2.584 ± 0.40	0.190 ± 0.03	0.130 ± 0.02
	P-value	0.032		0.000	0.519	0.004	0.369
TF_{r-s}	BFBF	-	-	0.720 ± 0.05	0.186 ± 0.04	0.666 ± 0.05	2.257 ± 0.17
	CF alone	-	-	1.350 ± 0.87	0.077 ± 0.00	1.534 ± 0.07	2.531 ± 0.48
	P-value			0.000	0.004	0.000	0.598
TF_{s-l}	BFBF	-	-	2.117 ± 0.15	0.600 ± 0.10	1.752 ± 0.28	1.752 ± 0.28
	CF alone	-	-	2.410 ± 0.21	0.882 ± 0.08	0.981 ± 0.08	1.499 ± 0.13
	P-value			0.026	0.035	0.468	0.412
TF_{l-p}	BFBF	-	-	0.434 ± 0.07	1.009 ± 0.21	0.430 ± 0.06	0.511 ± 0.13
	CF alone	-	-	0.739 ± 0.13	0.664 ± 0.04	0.508 ± 0.02	0.650 ± 0.07
	P-value			0.049	0.188	0.229	0.359
TF_{p-g}	BFBF	0.050 ± 0.01	0.173 ± 0.02	-	0.083 ± 0.02	0.166 ± 0.01	1.540 ± 0.43
	CF alone	0.093 ± 0.01	0.218 ± 0.03	-	0.117 ± 0.02	0.125 ± 0.00	0.734 ± 0.18
	P-value	0.003	0.176		0.165	0.000	0.090

Mean ± standard error. *P*-values indicate the probability levels at which the differences between the biofilm biofertilizer and chemical fertilizer–alone practices are significant. BaF_{s-r} is the bioaccumulation factor of a given heavy metal from paddy soil to root. TF_{r-s}, TF_{s-l}, TF_{l-p}, and TF_{p-g} are the translocation factors of a given heavy metal from root to stem, stem to leaf, leaf to panicle, and panicle to seed, respectively. CF, chemical fertilizer; BFBF, biofilm biofertilizer.

3.3. Human risk assessment

3.3.1. Noncarcinogenic risk assessment

The highest and lowest EDI values were observed in Ni and Cd, respectively, in both practices (**Table 4**). The application of BFBF pointed out that the daily intake of As, Co, Cd, and Cr could be reduced significantly compared to the CF-alone practice ($P < 0.05$). It is reported that reducing the daily intake of THM such as Cd and As can lessen serious diseases such as lung cancer, bone defects, and also bronchitis [81]. The reduced EDI of the THM in the BFBF practice seems to have played a role in ecosystem intelligence [64].

All the HQ values were less than one, except As in CF-alone practice (**Figure 2**), which has reached the noncarcinogenic health risk level. The HQ is a measure of the potential risk associated with exposure to a single THM [82, 83]. In the BFBF practice, As, Cd, Co, and Cr showed significantly lower HQ values, possibly due to the binding of the HM to fungal cell walls [50] with the increased microbial diversity and abundance under the BFBF application [38, 84]. As such, the HQ values were significantly reduced by the BFBF intervention, except for the micronutrient Ni (**Figure 2**). Even if it is nontoxic as a single HM, we consume the HM collectively. Thus, HI value that depicts the potential health risk associated with exposure to multiple THM is a better parameter [85].

Interestingly, the HI of the BFBF practice had been kept below the threshold value (**Figure 3**) by significantly reducing the HQ values of the single HM though the values were well below the threshold of the HQ (**Figure 2**). This is clear evidence for the action of ecosystem intelligence with the BFBF practice compared to that of the CF practice [64].

Table 4 • Estimated daily intake of the different heavy metals in the biofilm biofertilizer and chemical fertilizer–alone practices

Heavy metal	EDI (μg kg^{-1} day^{-1})	
	BFBF	CF alone
As	0.12 ± 0.016	0.32 ± 0.014
	(0.000)	
Cd	0.08 ± 0.0085	0.16 ± 0.023
	(0.004)	
Pb	-	-
	-	
Co	0.12 ± 0.026	0.20 ± 0.024
	(0.027)	
Cr	0.99 ± 0.038	1.40 ± 0.058
	(0.000)	
Ni	3.79 ± 1.0	4.40 ± 1.1
	(0.688)	

Mean ± standard error. Values within parenthesis are probability levels at which the differences between the biofilm biofertilizer and chemical fertilizer–alone practices are significant. EDI, estimated daily intake; CF, chemical fertilizer; BFBF, biofilm biofertilizer.

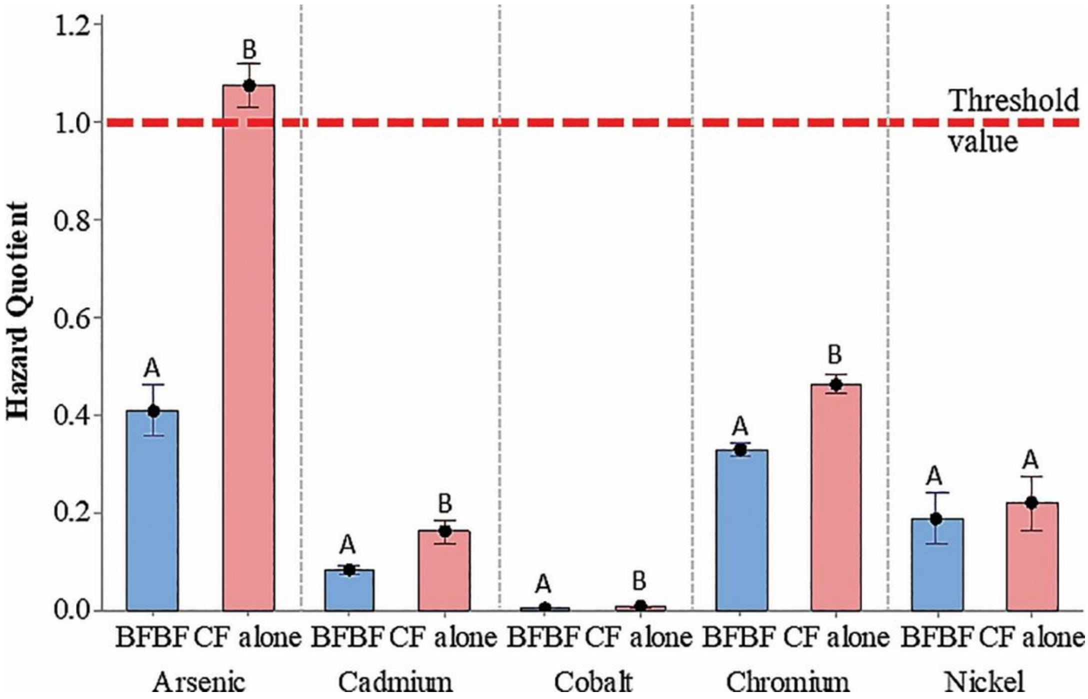

Figure 2 • Hazard quotients of different heavy metals in both biofilm biofertilizer and chemical fertilizer–alone practices and the threshold value of the quotient. CF, chemical fertilizer; BFBF, biofilm biofertilizer.

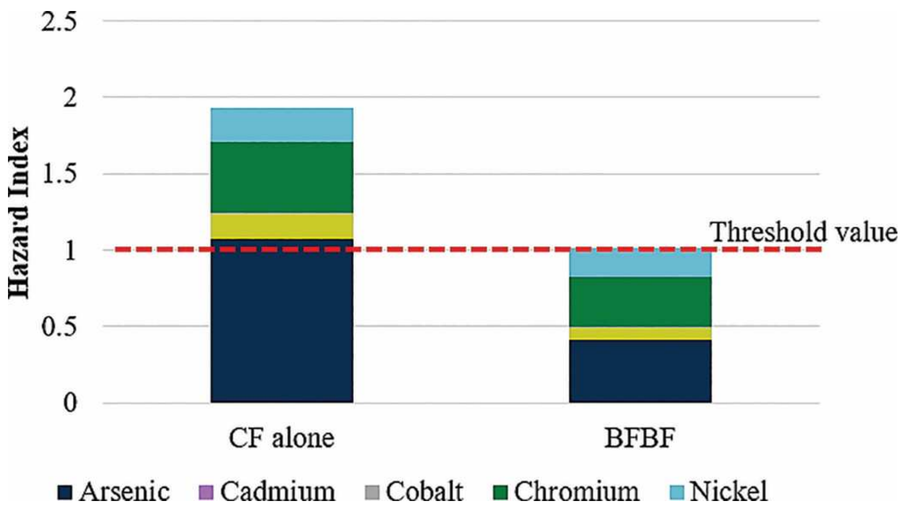

Figure 3 • Hazard index values in the biofilm biofertilizer and chemical fertilizer–alone practices. CF, chemical fertilizer; BFBF, biofilm biofertilizer.

Ecosystem intelligence is an outcome of the complex signaling among microbes, plants, and animals in the system for sustainability [64, 86]. Microbes are the focal point of ecosystem intelligence. When the microbial cells commune in great numbers, their startling collective talents for solving problems and controlling their environment have been observed through awareness, understanding, or other capacities implicit in real intellect [87–89]. As such, increasing microbial diversity and abundance contributes immensely to reinstating the intelligence in degraded ecosystems, in particular, for beneficial outcomes, as was seen with the BFBF application [64].

4. Conclusions

This study demonstrated that the application of BFBF could effectively manipulate THM levels and micronutrients within the soil–plant system, resulting in the production of high-quality rice. This indicates a level of ecosystem intelligence. Furthermore, the use of HQ and HI is expected to provide enhanced health benefits in both short and long run. On the other hand, the CF-alone application blunted this intelligent feature. This clearly shows the importance of introducing microbial interventions like BFBF to paddy cultivation for a healthy plate of rice. It is concluded from this study that the BFBF should be developed and tested globally in paddy cultivations to minimize the health risks of rice consumption for healthy human generations ahead.

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Author contributions

Conceptualization, M.P. and G.S.; methodology, M.P. and G.S.; software, D.W. and S.E.; validation, D.W. and S.E.; formal analysis, D.W., S.E. and M.P.; investigation, D.W., S.E. and M.P.; resources, M.P. and G.S.; data curation, D.W. and S.E.; writing—original draft preparation, D.W., S.E. and M.P.; writing—review and editing, G.S., M.P. and Z.X.; visualization, G.S., M.P. and Z.X.; supervision, M.P. and G.S.; project administration, G.S. and M.P.; funding acquisition, G.S. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Competing financial interests

The authors have no relevant financial or nonfinancial interests to disclose.

Data availability statement

Data supporting these findings are available within the article, at https://doi.org/10.20935/AcadEnvSci7532, or upon request.

Institutional review board statement

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