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SOIL AMENDMENTS ROLE IN ENHANCING RICE FE AND ZN CONTENT: A PATHWAY TO SUSTAINABLE SOIL HEALTH AND BIOFORTIFICATION

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SUMMARY

Reports of the application of soil improvers, such as zeolite, microbes, and organic fertilizers, stated to provide better nutrition availability and improve the yield, as well as the nutritional content of crop plants' edible parts. This study aimed to investigate the effect of different soil improvers on rice (*Oryza sativa* L.) yield and the iron (Fe) and zinc (Zn) content in the grain. The experiment proceeded according to a split-plot design with three replications under irrigated conditions. The experiment's main factor was soil improvers comprising six types, i.e., inorganic fertilizer as baseline control, while the other treatments had additional microbes, organic fertilizers, zeolites, microbes + organic fertilizers, and microbes + zeolites. The sub-factor comprised five rice cultivars, i.e., Sembada Hitam, Sembada Merah, Inpari 47 WBC, Inpari Arumba, and Ciherang. The soil improvers and rice cultivars significantly affected grain yield. Additional organic fertilizer and zeolite maintained the rice yield; however, the combination of soil improvers did not necessarily increase the yield. Furthermore, the soil improver, especially zeolite, and the rice cultivars have a significant effect on increasing Fe and Zn content in rice grains. Sembada Merah tended to have higher grain Fe and Zn content across the soil improver treatments.

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Keywords: Rice (*O. sativa* L.), iron, microbes, organic fertilizers, pigmented rice, rice, soil improvers, yield, zeolite, Zn

Key findings: Results showed the soil improver and rice (*O. sativa* L.) cultivars have a significant effect on rice yield and grain Fe and Zn content. Sembada Merah had the highest grain Fe and Zn content across various soil improver treatments. Zeolite may associate with the increase of rice grain Fe and Zn content.

INTRODUCTION

Rice (*Oryza sativa* L.), a cereal crop serving as the primary energy source for more than half of the world's population (Chường *et al.*, 2020), is a major concern due to its naturally low zinc (Zn) and iron (Fe) content in the grain. This issue is particularly acute in developing countries, where rice constitutes a significant portion of daily caloric intake, often with little dietary diversification (Liu *et al.*, 2023). Thus, micronutrient deficiencies became a serious threat. Addressing these challenges, the researchers have explored various strategies, including conventional breeding and biotechnology approaches and biofortification to enhance the iron and zinc content of rice.

Conventional breeding efforts have had moderate success in increasing the iron and zinc concentrations in rice grain. Recent studies have also highlighted the potential of biofortification through genetic engineering, where the overexpression of genes involved in metal uptake, chelation, and storage can significantly boost iron and zinc levels in the rice endosperm (Senguttuvel *et al.*, 2023). Biofortification is a promising strategy to address these micronutrient deficiencies, which is achievable through plant breeding and agronomic practices, such as application of appropriate fertilizers. Pigmented rice inherited genetic traits that influence the accumulation of various health-promoting phytochemicals, viz., anthocyanins, carotenoids, and phenolic compounds (Mbanjo *et al.*, 2020).

Soil amendments are crucial in modulating the bioavailability of zinc and iron in crop plants by altering soil properties and influencing metal speciation (Baruah and Gogoi, 2023). Overall, soil improvers, microbial

activity, and biofortification strategies are integral to addressing Zn and Fe deficiencies, thereby supporting sustainable agriculture and improving human health outcomes (Tripathi *et al.*, 2022; Mishra *et al.*, 2023). Pigmented rice cultivars are common in being more nutritious, containing higher levels of minerals, vitamins, and antioxidants than white rice (Zhao *et al.*, 2020). Microminerals, such as iron and zinc contents, appeared higher in black and red rice cultivars than in white rice (Irakli *et al.*, 2016).

A hypothesis states that using pigmented rice in combination with organic matter and zeolite as soil amendments will enhance the bioavailability and accumulation of Zn and Fe in rice grain. This strategy could offer a sustainable biofortification approach, improving the nutritional quality of rice grain while addressing the growing concern of soil contamination by pollutants. The study also aims to explore a biofortification strategy integrating the use of pigmented rice cultivars (black and red rice) alongside white rice to improve the micronutrient content, particularly Zn and Fe. Additionally, this research investigates the use of alternative soil improvers—organic matter and zeolite—as potential strategies to enhance Fe availability in the soil.

In testing this hypothesis, this research sought to investigate a) the influence of pigmented rice cultivars on grain Zn and Fe content compared with white rice and b) the impact of soil improvers on the soil Fe and Zn content. This study also seeks to evaluate the effectiveness of combining pigmented rice cultivars with soil improvers as a biofortification strategy, with the potential to enhance the grain Fe and Zn content.

MATERIALS AND METHODS

Study site and procedure

The experiment on rice (*O. sativa* L.) commenced from April to November 2023 in Sleman Regency, Yogyakarta Province, Indonesia, under irrigated conditions. Geographically, the jurisdiction of Sleman Regency is between 110°15'13" and 110°33'00" East Longitude and from 7°34'51" to 7°47'03" South Latitude.

The experiment ensued following a split-plot design with the main factor as soil improvers application comprising six types, i.e., inorganic fertilizer as control; inorganic fertilizer + microbes; inorganic fertilizer + organic fertilizer; inorganic fertilizer + zeolite; inorganic fertilizer + microbes + organic fertilizers; and inorganic fertilizer + microbes + zeolite. The sub factor was five rice cultivars, namely, Sembada Hitam (black grain rice), Sembada Merah (red grain rice), Inpari 47 WBC (white grain rice), Inpari Arumba (red grain rice), and Ciherang (white grain rice). The first two are local varieties originating from Yogyakarta Province with registration and formal release. Meanwhile, the last three are improved and released cultivars derived from a hybridization breeding program.

A consortium of heavy metal-resistant bacteria, *Pseudomonas putida* (Ginting *et al.*, 2021), *Bacillus megaterium*, and *Stenotrophomonas maltophilia* (Jiwanti, 2016), reached formulation by developing liquid and solid cultures ready for application at 6 kg/ha. The bacterial consortium was applicable by soaking the seedlings for planting for at least one hour with 30% of the dose, with the rest applied in two split doses at the age of seven and 30 days after planting. The organic fertilizer used had a rate of 2 t/ha, with zeolite administered at a dose of 500 kg/ha. The application of organic fertilizers and zeolite as a treatment transpired before planting.

Sample preparation

The preparation for Fe and Zn contents of the rice grain samples continued by dehulling around 50 g of the grain using the Satake mini

dehuller with a modified roller on polypropylene material. Soil samples' preparation occurred from a composite (collecting the soil samples from the soil's depth of 1–20 cm from five different points).

Observed variables

The recorded data included various agronomic and yield-related traits in different rice cultivars. These are plant height, leaf length, number of productive tillers, leaf width, panicle length, filled grains per panicle, empty grains per panicle, 100-grain weight, grain weight per clump, and grain yield (t/ha at 14% moisture content). In the experimental soil and rice grain, the Fe and Zn contents' quantification used the XRF Epsilon 4 from Malvern PANalytical at the Radiation Laboratory of BRIN, Yogyakarta, Indonesia.

Data analysis

The analysis of data utilized the PROC GLM with SAS (SAS Institute, Inc., Cary, NC, USA), as well as a model statement appropriate for a split-plot design. Variance analysis preceded the Duncan's multiple range test (DMRT) for post-hoc analysis at the significance level of $p < 0.05$.

RESULTS

Agronomic traits

The analysis of variance revealed the soil improver treatments significantly affected panicle length, filled grains/panicle, 100-grain weight, grain weight/clump, and yield (Table 1). However, soil improver treatments had a nonsignificant difference in the panicle length and empty grains per panicle. Meanwhile, the variety treatment exerts a noticeable influence on all the observed variables, except empty grains/panicle. The interactions between the variety and the soil improvers were not evident.

Additional microbes and organic fertilizers gave comparable yield with inorganic fertilizer as a baseline treatment. However, the

Table 1. Performance of rice cultivars over soil improver treatments.

Treatments		Panicle length (cm)	Filled grains/panicle	Empty grains/panicle	100-grain weight (g)	Grain weight/clump (g)	Yield (t/ha)
Soil improver (M)	Inorganic fertilizer	24.16	137.65	31.77	2.59	46.66	7.08
	Inorganic fertilizer + Microbes consortia	24.06	147.49	24.2	2.61	47.22	6.73
	Inorganic + Organic fertilizer	23.91	134.81	27.65	2.55	45.04	6.64
	Inorganic fertilizer + Zeolite	23.58	140.03	29.95	2.48	39.04	4.93
	Inorganic + Organic fertilizer + Microbes consortia	24.04	135.53	31.03	2.59	43.69	5.66
	Microbes + Zeolite	23.51	128.84	24.92	2.52	36.97	3.92
Variety (V)	Sembada Hitam	24.30	132.38	21.81	2.64	47.40	4.84
	Sembada Merah	21.77	122.37	12.30	2.49	38.48	5.68
	Inpari 47 WBC	25.18	166.93	41.29	2.47	45.15	6.93
	Inpari Arumba	24.67	130.12	41.78	2.56	37.31	4.98
	Ciherang	23.46	135.17	24.03	2.61	47.19	6.71
<i>p</i> -value	Microbes + soil improver (M)	0.228	0.04*	0.096	0.037*	0.0013*	0.0001*
	Variety	0.0001*	0.0001*	0.0001*	0.0005*	0.0001*	0.0001*
	M x V	0.2883	0.1056	0.9698	0.1339	0.496	0.9768
CV (%)		3.35	11.25	30.96	4.64	17.28	18.26

Values with the same letters are not significantly different according to DMRT at $\alpha = 0.05$.

*Indicate $P < 0.05$ (significant).

addition of other soil improver treatments gave a lower yield instead (Table 1). This had also happened in almost all other observed variables, such as filled grains/panicle, 100-grain weight, and grain weight/clump. It indicated that microbes and organic fertilizer were able to maintain yield. Comparison among cultivars showed that Inpari 47 WBC (6.93 t/ha) had the highest yield, comparable with Ciherang (6.71 t/ha), followed by Sembada Merah (5.68 t/ha), Inpari Arumba (4.98 t/ha), and Sembada Hitam (4.84 t/ha) (Table 1). This study's results signify pigmented rice cultivars have a lower yield than the white grain rice cultivars (Inpari 47 WBC and Ciherang).

Grain Fe and Zn contents

Results indicated a significant influence of soil improvers, rice cultivars, and their interactions on the grain Fe and Zn contents (Figures 1 and 2). Among the treatment combinations, the

highest Fe content resulted in the Inpari Arumba variety with the addition of zeolite treatment (14.9 ppm), followed by Sembada Merah with the addition of microbes (14.8 ppm) and Sembada Merah with the application of microbes + zeolite (13.9 ppm) treatment. Additional microbial treatment caused the maximum average of varietal grain Fe content. These results indicate that microbes had a potential role in increasing the grain Fe content in rice plants. Conversely, the highest grain Zn content was evident in the control treatment (inorganic fertilizer) in the Sembada Merah variety (19.6 ppm), followed by adding microbial + zeolite, both in Ciherang (18.90 ppm) and Sembada Merah (17.50 ppm) cultivars.

Soil Fe and Zn contents

Comparing soil analysis after harvesting to the base condition before planting showed a decrease in soil Fe and Zn contents in most of

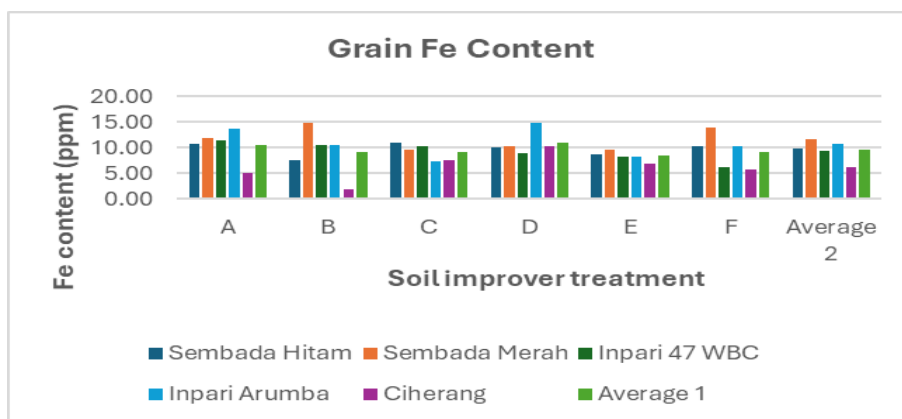


Figure 1. Fe content of five rice cultivars that underwent six different soil improver treatments (ppm).

Remarks: A. Control (inorganic fertilizers); B. Inorganic fertilizers + Microbial treatment; C. Inorganic + Organic fertilizer from cow dung waste (2 t/ha); D. Inorganic fertilizers + Zeolite soil improver (500 kg/ha); E. Inorganic fertilizers + Microbial treatment + Organic fertilizer, and F. Inorganic fertilizers + Microbial treatment + Zeolite. Average 1: average over variety in each soil improver treatment; Average 2: average over soil improver treatment in each variety.

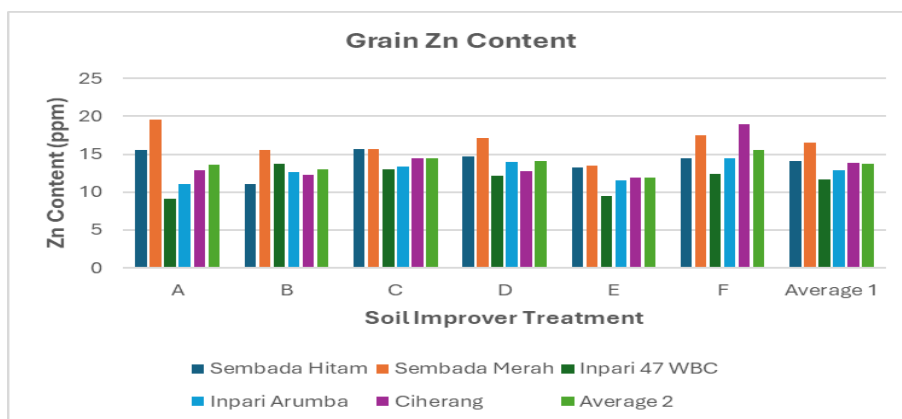


Figure 2. Zn content of five rice cultivars that underwent six different soil improver treatments (ppm).

Remarks: A. Control (inorganic fertilizers); B. Inorganic fertilizers + Microbial treatment; C. Inorganic + Organic fertilizer from cow dung waste (2 t/ha); D. Inorganic fertilizers + Zeolite soil improver (500 kg/ha); E. Inorganic fertilizers + Microbial treatment + Organic fertilizer, and F. Inorganic fertilizers + Microbial treatment + Zeolite. Average 1: average over variety in each soil improver treatment; Average 2: average over soil improver treatment in each variety.

the soil improver treatments (Table 2). Exceptions were notable in the addition of zeolite treatment, which increased soil Fe content, and adding microbial treatment, which raised soil Zn content.

Correlation of grain Fe and Zn contents and rice productivity

Correlation analysis revealed a positive association between grain Fe and Zn contents

Table 2. Fe and Zn content of soil before planting and after harvesting.

Treatments	Fe soil (%)	Zn soil (ppm)
Before planting (Base condition)	12.35	218.20
After Treatment		
Inorganic fertilizer (A)	11.95	226.20
Microbial treatment (B)	12.06	247.80
Organic fertilizer (C)	11.94	202.50
Zeolite (D)	13.67	213.20
Microbial treatment + Organic fertilizer (E)	11.09	190.40
Microbial treatment + Zeolite (F)	10.73	177.8
CV (%)	7.89	11.23

Values with the same letters are not significantly different according to DMRT at $\alpha = 0.05$.

Table 3. Correlation between rice productivity and Fe and Zn contents.

Variables	Productivity	Fe	Zn
Productivity	1		
Fe	-0.0162	1	
Zn	-0.1854	0.4483*	1

* = significant at $\alpha 0.05$.

($r = 0.4483$) (Table 3). This means that higher Fe levels tend to coincide with higher Zn levels, and vice versa. These results align with the positive association observed in rice cultivars under irrigated conditions by Anusha *et al.* (2021). Interestingly, the yield had no correlations with grain Fe ($r = 0.0162$) and Zn ($r = 0.1854$) contents (Table 3).

DISCUSSION

This study did not detect any soil improver treatment giving a higher yield than the baseline inorganic fertilizer treatment. It may be due to soil and environmental complexity affecting yield altogether during the trial. The role of microbial consortia as fertilizers had reports on barley (Al Methyeb *et al.*, 2023), maize (López-Montañez *et al.*, 2024), and rice (Ríos-Ruiz *et al.*, 2020). In contrast, cow dung-originated organic fertilizer also had findings of being able to maintain yield in rice (Atman *et al.*, 2018). Similarly, zeolite has statements of significantly increasing rice yield (Li *et al.*, 2022). Nevertheless, combinations among microbes, organic fertilizers, and zeolite did not automatically increase yield. Synergistic among organic fertilizer and other soil improvers that

further increase in yield resulted from some factors, such as the soil condition, including pH (Al Methyeb *et al.*, 2023), and specific agronomic treatments and environmental conditions (Rahmani *et al.*, 2023). Advanced studies need to unravel the problem supposed to help further improve yield and grain Fe and Zn contents in specific locations and other settings with similar conditions.

The highest yield resulted in Inpari 47 WBC (6.93 t/ha) and Ciherang (6.71 t/ha) varieties. Inpari Arumba (4.98 t/ha), a red aromatic rice variety released from the hybridization breeding program, had a moderate yield. Inversely, Sembada Merah (5.68 t/ha) and Sembada Hitam (4.84 t/ha) had lower yields. Both Sembada Merah and Sembada Hitam are colored grain local rice cultivars from Yogyakarta Province, with formal registration and release. Meanwhile, Inpari 47 WBC and Ciherang are white-grain, high-yielding released rice cultivars obtained through a hybridization breeding program (Sastro *et al.*, 2021). This study revealed white rice cultivars have higher yields. Nevertheless, yield and pigmented grain may not have a natural correlation. Lap *et al.* (2024) reported the negative correlation between pericarp color intensity and yield, but further study is

necessary on selective breeding to transgressive segregants on both traits, indicating the success of obtaining high-yielding, high-pericarp color intensity lines. It showed both traits sustained control from distinct genes that randomly segregate in the progenies. The negative correlation between grain color and yield may be because anthocyanin deposition reduces the chlorophyll content in spikelets, decreasing the photosynthetic and grain-filling rates (Rahman *et al.*, 2015). Historical selection of the cultivars could become an additional factor resulting in the yield performance of white and colored rice used in this study.

This study revealed significant variations in rice grain Fe and Zn contents across different treatments. Sembada Merah and Inpari Arumba are the red-pigmented rice cultivars used in this study. The results indicated that red-pigmented rice genetically provides a higher Fe content than the other cultivars, as influenced by the treatment of soil improver zeolite. Each genotype has a unique potential with effects from the environment for Zn content. Anthocyanin and grain Zn concentrations also vary according to the rice genotype (Fongfon *et al.*, 2021). Higher Fe and Zn in local black rice versus red rice was a previous report (Manwan *et al.*, 2023).

This research disclosed that inorganic fertilizer and zeolite applications may increase grain Fe content. Montoya *et al.* (2020) reported that the urea fertilizer application indicated a link to increased grain Zn and Fe contents in wheat grain. This finding may be similar to this study's result. Zeolite increasing Fe content might be due to its characteristics, such as enhancing cation exchange and Fe availability in the soil (Cataldo *et al.*, 2021).

On the other hand, organic fertilizers and zeolite application tend to increase grain Zn content. Previous research findings, which demonstrated that adding organic matter, such as straw manure and P fertilizer, significantly boosted the levels of micronutrients, i.e., Cu, Zn, and Fe, in the soil (Ben-Yin *et al.*, 2010). Long-term application of manure and fertilizers has proven to develop a positive balance for micronutrients like Fe and Zn by improving soil properties; however, these benefits manifest

gradually, influencing nutrient uptake over time (Shahid *et al.*, 2016). Moreover, long-term bioorganic fertilization has occurred to improve the availability of essential nutrients and raise soil enzyme activities, further enhancing micronutrient uptake in crops (Zheng *et al.*, 2024).

Microbial biofortification is gaining attention as an innovative approach to enhance plant metal uptake by leveraging soil microbes, particularly through the production of siderophores. Siderophores are specialized molecules produced by certain bacteria that bind to metals, facilitating their uptake by crop plants. This mechanism is particularly effective in enhancing the availability of essential trace elements like iron, zinc, and selenium in the soil, which are crucial for plant growth and human nutrition (Majewska *et al.*, 2024). Plant growth-promoting rhizobacteria (PGPR) improve the iron uptake and plant growth by producing siderophores in various crops (Ahmad *et al.*, 2023).

This study revealed a reduction of soil Fe and Zn contents during the cultivation season. It could indicate that continuous cultivation practices without any effort to maintain and even increase the soil mineral content may result in a big negative impact from a long-time agricultural perspective. Chemical fertilizer poorly maintains soil Fe content, but zeolite application performs otherwise. Zeolite increases soil Fe content through various ways, such as enhancing cation exchange, pH stabilization, and improved moisture retention (Cataldo *et al.*, 2021). It may further increase Fe availability for the plant. Thus, zeolite is apparently a valuable soil amendment to improve soil nutrient management and crop productivity. Contrastingly, this study indicated that microbial treatment increases soil Zn content. It may refer to the bacteria consortium, which increases Zn availability in the soil. A report stated the ability of some microbes in solubilizing soil Zn for easier plant uptake (Srithaworn *et al.*, 2023). It is indicative of the importance of searching for other soil improvers and their best combination to maintain mineral contents, especially Fe and Zn, in the soil for their sustainability in the

long-term land utilization for agricultural practices.

It is a suspicion that a correlation exists between the anthocyanin and the mineral content of Fe and Zn in rice grain. In line with this, Jaksomsak *et al.* (2021) observed a significant positive association between the anthocyanin and Zn content in rice grain. The higher the anthocyanin content, the higher the Zn content. However, Fe and Zn contents are irrespective of their aleurone color (Gogoi *et al.*, 2024). Significant differences in Zn content appeared between two black rice cultivars of South Sulawesi, whereas non-waxy black rice was higher in Zn than the waxy black rice type (Manwan *et al.*, 2023). Therefore, the variation in Fe and Zn contents in rice grain must also consider multiple factors, including environmental effects on plant growth and development.

The results received further support from other studies reporting positive correlations between Zn and Fe contents in rice. Susanto *et al.* (2021) stated the significant correlation between Zn and Fe in rice grain (0.725 and 0.276, respectively). Importantly, none of these studies identified a significant correlation between mineral concentrations and the grain yield. Similarly, no significant relationship between Zn levels and grain yield was evident (Rohaeni *et al.*, 2023), reinforcing this pattern. Anusha *et al.* (2021) said there was a potential negative correlation between Fe and Zn concentrations in parental rice lines and yield under varying conditions. These findings highlight the complexity of the relationship between Zn and Fe contents, grain yield, and other factors influencing the rice quality, warranting further research to better understand these interactions.

CONCLUSIONS

The soil improvers and rice cultivars significantly affected the grain yield and its components without any interaction between both factors. Additional organic fertilizer and zeolite maintain the yield, but the combination of soil improvers did not necessarily increase

the yield. Furthermore, the soil improver, especially zeolite, organic fertilizer, and even inorganic fertilizer, notably impacted increasing Fe and Zn contents in the rice grain. Sembada Merah tended to have higher grain Fe and Zn contents across the soil improver treatment. A positive correlation between grain Fe and Zn contents emerged, but no correlation between yield and grain Fe or Zn content occurred. Further research is essential to determine appropriate soil improvers for maintaining soil mineral contents and enhancing grain Fe and Zn contents that are efficacious, economical, and consistent between cultivars and the environment.

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