

# Abundance of fungi on rice straw compost and husk biochars by in-vitro heavy metals mycosorbent

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Manuscript received: 6 July 2024. Revision accepted: 23 April 2025.

**Abstract.** Palupi NP, Kesumaningwati R, Sripati AB, Hardi EH, Fahrunsyah, Rahayu DE, Nugroho RA, Darma S, Idris SD. 2025. Abundance of fungi on rice straw compost and husk biochars by in-vitro heavy metals mycosorbent. *Biodiversitas* 26: 1983-1990. In Samarinda, all paddy fields are contaminated by heavy metals with concentrations exceeding the critical threshold and has disrupted human health and worsened the quality of the agroecosystem of rice fields. This study aims to evaluate the effectiveness of compost and biochar application in absorbing heavy metals in paddy field soil in Samarinda. The research method involves the application of compost, biochar, and their combination on paddy field soil contaminated with heavy metals using incubation experiment with 5 treatments and 4 replications, completely randomized design, and measured parameters (pH, Fe, Zn Cd, and *Aspergillus niger*). The results indicate that the use of compost significantly increases the population of *A. niger* and reduces the concentration of heavy metals such as Fe, Zn, and Cd in the soil. On the other hand, biochar has also proven effective in reducing the content of heavy metals, supported by its high adsorption capacity. The combination of compost and biochar shows better results compared to individual applications, indicating synergy between organic nutrients and adsorption capabilities. The correlation between the population of *A. niger* and heavy metals demonstrates a significant relationship, confirming the role of microbes in soil bioremediation. The practical implication of this research underscores the importance of organic fertilizer use in reducing heavy metal contamination, supporting sustainable agriculture, and reducing dependence on synthetic chemicals. Future research is recommended to further understand the mechanisms of bioremediation and soil microbe interactions in this context. In conclusion, the application of compost and biochar offers a holistic approach to effectively address heavy metal contamination issues, enhance soil quality, and support environmentally friendly agriculture.

**Keywords:** *Aspergillus niger*, biosorbent, heavy metals, husk biochar, paddy field soil, rice straw compost

## INTRODUCTION

Heavy metals are elements with high density and are toxic at more than critical limits. In the soil, heavy metals can come from various sources, including industrial activities, the use of pesticides, synthetic fertilizers, domestic waste, and parent material of soil (Alengebawey et al. 2021). Rice plants can absorb heavy metals that accumulate in rice fields, enter the food chain, and ultimately endanger human health (Uddin et al. 2021). Therefore, effective and sustainable efforts are needed to reduce heavy metal content in agricultural soil.

One promising approach to overcome this problem is using organic materials such as compost and biochar. Compost, product of fermentation process in certain conditions, is the result of the decomposition of organic material by microorganisms, which not only increases soil fertility but can also help in the bioremediation process of heavy metals (Lin et al. 2022). Compost contains organic

material that is rich in nutrients and supports the growth of beneficial soil microorganisms, such as *Aspergillus niger*, which is known to have the ability to bind heavy metals through adsorption and precipitation processes (Ayilara et al. 2020), can form complexes with heavy metals, reducing their mobility and bioavailability in the soil (Gao et al. 2023). This is very important in mitigating heavy metal contamination in agricultural soil.

Biochar is charcoal produced from the pyrolysis of biomass material under conditions of little or no oxygen. Biochar has a high pore structure and large surface area, which provides significant adsorption capacity (Medyńska-Jurasze et al. 2020) with some agronomic and environmental benefits. First, biochar can increase soil fertility by increasing Cation Exchange Capacity (CEC), water retention, and nutrient availability (Sakhiya et al. 2020). Biochar can also increase soil pH, especially in acidic soils, which in turn can increase nutrient availability for plants, and improve soil structure by increasing soil

aggregation and reducing soil density (Haider et al. 2020).

A combination of compost and biochar can provide a synergistic effect in improving soil quality and reducing heavy metal content. The use of both not only increases soil adsorption capacity but also improves soil structure, water retention, and soil biological activity (Nugraha et al. 2020). Compost and biochar are two soil amendments that have great potential to improve soil quality and reduce heavy metal contamination. Compost provides nutrients and improves soil microbiological activity, while biochar increases water retention and heavy metal adsorption capacity. The combination of these two materials can provide a more effective synergistic effect in the remediation of soil contaminated with heavy metals (Wang et al. 2022b).

The remediation process of course also involves microbiological dynamics in soil contaminated with heavy metals. *Aspergillus niger*, plays a key role in the bioremediation process (Singh et al. 2021), the combination of compost and biochar increases the abundance of microbes in rice field soil producing organic acids and enzymes that can convert heavy metals into less dangerous forms (Behera and Samal 2022). This fungus produces various extracellular enzymes, including amylase, cellulase, and pectinase, which break down complex organic compounds into simpler forms that can be absorbed by plants and other microorganisms (Wang et al. 2022a). *Aspergillus niger* can tolerate high concentrations of heavy metals, making it an important subject in bioremediation research. This fungus can accumulate heavy metals in hyphae through various mechanisms, including biosorption, bioaccumulation, and biotransformation (Chaurasia et al. 2023). Biosorption is the process in which fungal cells bind heavy metals to the cell walls through ionic and covalent interactions. Bioaccumulation involves the uptake of heavy metals into cells and binding to proteins or peptides, such as metallothionein or phytochelatin, for detoxification. Biotransformation involves changing the chemical form of heavy metals to forms that are less toxic or more easily precipitated, often through reduction or oxidation (Priyadarshini et al. 2021).

This research was conducted to evaluate the effectiveness of the application of compost and biochar, both separately and in combination, in absorbing heavy metals in rice fields in Samarinda. Specifically, the research will measure changes in heavy metal concentrations (Fe, Zn, and Cd) in soil after the application of various treatments, as well as examine the relationship between *A. niger* populations and reductions in heavy metal content.

## MATERIALS AND METHODS

### Study area

This research was carried out from November 2023-February 2024 and took place at the Soil Science Laboratory, Faculty of Agriculture, Universitas Mulawarman, Samarinda, Indonesia. Rice field soil samples were taken in 2 locations, Loa Janan Ilir Sub-district, Kutai Kartanegara District, East Kalimantan, Indonesia and North Samarinda Sub-district, Samarinda, East Kalimantan, Indonesia, where heavy metals

contaminated the soil. The collection of straw and rice husks was carried out at several random points in the rice fields according to the soil sampling. Incubation was carried out at the Soil Science Laboratory of the Faculty of Agriculture with soil analysis. Chemical analysis of rice straw compost and rice husk biochar was carried out at the Soil Science Laboratory, Faculty of Agriculture, Water Resources Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Mulawarman. Microbial analysis was carried out at the Plant Pest and Disease Laboratory, Faculty of Agriculture, Universitas Mulawarman.

The materials used in this research were rice straw and rice husks obtained from rice fields in Samarinda, then their chemical and physical composition were characterized. The rice straw was processed into compost and the rice husks were processed into biochar. Another ingredient is rice field soil as an incubation medium obtained from rice fields that are included in the actual rice field map in Samarinda. Chemicals for rice straw compost analysis, rice husk biochar analysis, soil chemical analysis, microbial analysis, and heavy metal uptake analysis were obtained from the Soil Science Laboratory and Plant Pest and Disease Laboratory, Faculty of Agriculture, Universitas Mulawarman and from other laboratories in the Faculty at the University.

The results of the phase I survey became the basis for determining the phase II experimental plot by entering each data into 3 (three) category classes, namely category 1-2 highest heavy metals, and category 3-4 highest heavy metals. The highest heavy metal categorization of 5-6 heavy metals present in the soil was also done. After categorizing, 10% of each category was selected to be used as a sample. Each sample was given treatment. Experiments on incubation pot media were carried out outdoors with minimum light, namely in the Faculty of Agriculture, Universitas Mulawarman. Rice straw compost and rice husk biochar were mixed homogeneously at a rate of 2 and 4% (w/w) with dry air soil. Plastic pots with a soil capacity of 7 kg were filled with prepared soil, in addition to the control (soil without biochar and compost). Rice straw compost and rice husk biochar are applied by mixing them with soil in the following ratio: (i) K0 = control without rice straw compost and rice husk biochar; (ii) K1 = soil with rice straw compost (100%); (iii) K2 = soil with biochar rice husks (100%); (iv) K3 = soil with rice straw compost (25%): rice husk biochar (75%); (v) K4 = soil with rice straw compost: 50% rice husk biochar each; (vi) K5 = soil with rice straw compost (75%): rice husk biochar (25%). The pots were arranged in a completely randomized design with five replications. After knowing the best dose, the research continued with rice planting following the application of the best dose above on intensive rice fields.

### Processing, analysis, and layout creation

Soil samples were analyzed pH to determine the soil acidity level using a pH meter, content of Fe (SNI 6989.4:2009, Zn (SNI 6989.7:2009), and Cd (SNI 6989.16:2009 by Indonesian National Standart using the AAS instrument. Soil media preparation is the incubation container in the form of a plastic pot and provides 10

samples per sample with 5 treatments, labeling each container according to the paddy field soil sample and treatment, each container is filled with 500 g of soil. Each container filled with paddy field soil samples according to the treatment and stir the ingredients until evenly mixed, then add 20 to 30 mL of water to keep the incubated soil from drying out. Covering the incubation media tightly aims to prevent soil samples from being contaminated. Store the incubated media indoors and away from direct and indirect sunlight. After a month of incubation, rice field soil samples were taken, 100 g for 50 samples and 30 g for 50 samples.

#### Heavy metals and microbial analysis

The soil samples were analyzed for the content of heavy metals (Fe, Zn and Cd<sup>2+</sup>) at the Agricultural Faculty of Agriculture and Water Resources Laboratory of the Faculty of Fisheries and Marine Sciences of Universitas Mulawarman. The content of heavy metals was determined by measuring the description of soil samples destroyed by wet ashing method with a mixture of strong acids HNO<sub>3</sub> (65% pa) and HClO<sub>4</sub> (60% pa) using Atomic Absorption Spectroscopy (SSA) at wavelengths of 248.3 for Fe, 228.8 nm for Pb, 283.3 nm for Cd, 357.9 nm for Cr, 279.5 nm for Mn, 213.9 nm for Zn, and 324.7 nm for Cu, regarding SNI 8910:2021 (BSN 2021). *Aspergillus flavus* was analyzed in the Water Resources Laboratory of the Faculty of Fisheries and Marine Sciences of Universitas Mulawarman by TPC Method.

#### Data analysis

The collected data is analyzed statistically with analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) to determine the impact of each application treatment and correlation tests to determine the level of relationship between the presence of *A. niger* and the heavy metal content in paddy soil. The results were categorized based on evaluation criteria established by Balai Penelitian Tanah (2005).

## RESULTS AND DISCUSSIONS

#### Rice straw and husk biochar

Initial characteristics of paddy fields in Samarinda show variations in soil pH and heavy metal content (Table 1). Soil pH values ranged from 3.69 to 6.68, with most samples showing acidic soil conditions. The content of heavy metals analyzed including iron (Fe), zinc (Zn) and cadmium (Cd) also showed variations. Fe concentrations in soil samples ranged from 10910.10 to 16317.11 ppm. The sample with code 74 has the lowest Fe content, while the sample with code 53 has the highest Fe content. The Zn content varies from 59.91 to 129.77 ppm. The sample with code 21 shows the lowest Zn content, while the sample with code 64 shows the highest Zn content. Cd concentrations in soil ranged from 0.04 to 0.74 ppm. The sample with code 74 has the lowest Cd content, and the sample with code 57 has the highest Cd content.

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The application of compost showed varying effects on the population of the fungus *A. niger* as well as the absorption of the heavy metals Fe, Zn, and Cd in paddy soil in Samarinda. *Aspergillus niger* fungus populations and heavy metal concentrations were measured in various soil samples with different codes. The population of fungi and the concentration of heavy metals after the application of compost fertilizer can be seen in Tables 2 and 3.

The rice field soil sample with code 6 showed an *A. niger* population of 3.10<sup>5</sup> CFU and a Fe content of 2735.6 ppm, Zn of 19.02 ppm, and Cd of 0.89 ppm. The sample with code 13 had a higher fungal population, namely 13.10<sup>5</sup> CFU, with a Fe content of 3052.3 ppm, Zn of 13.94 ppm, and Cd of 0.90 ppm. In sample code 53, there was the highest fungal population, namely 16.10<sup>5</sup> CFU, with Fe content reaching 3591.1 ppm, Zn of 9.86 ppm, and Cd of 1.35 ppm, which is the highest Cd value among all samples.

**Table 1.** Soil acidity and heavy metals content of rice fields

| Sample code | Soil acidity | Heavy Metals Content (ppm) |        |      |
|-------------|--------------|----------------------------|--------|------|
|             |              | Fe                         | Zn     | Cd   |
| 6           | 6.68         | 13779.12                   | 122.58 | 0.60 |
| 13          | 5.40         | 14459.20                   | 75.05  | 0.58 |
| 14          | 5.20         | 12401.00                   | 107.74 | 0.64 |
| 21          | 5.28         | 13397.44                   | 59.91  | 0.52 |
| 24          | 4.66         | 16149.59                   | 76.12  | 0.54 |
| 53          | 4.32         | 16317.11                   | 91.54  | 0.74 |
| 57          | 4.58         | 15648.36                   | 72.23  | 0.77 |
| 63          | 4.76         | 13983.37                   | 97.08  | 0.62 |
| 64          | 4.10         | 13509.86                   | 129.77 | 0.65 |
| 74          | 3.69         | 10910.10                   | 121.78 | 0.04 |

**Table 2.** Condition of rice fields after compost application

| Sample code | Fungi population         | Heavy metals (ppm) |       |      |
|-------------|--------------------------|--------------------|-------|------|
|             | (×10 <sup>5</sup> CFU)   | Fe                 | Zn    | Cd   |
|             | <i>Aspergillus niger</i> |                    |       |      |
| 6           | 3                        | 2735.6             | 19.02 | 0.89 |
| 13          | 13                       | 3052.3             | 13.94 | 0.90 |
| 14          | 4                        | 3055.5             | 18.80 | 0.88 |
| 21          | 7                        | 3121.5             | 19.44 | 0.71 |
| 24          | 9                        | 2907.9             | 15.62 | 0.74 |
| 53          | 16                       | 3591.1             | 9.86  | 1.35 |
| 57          | 0                        | 3124.0             | 13.11 | 0.79 |
| 63          | 0                        | 3028.2             | 13.76 | 0.84 |
| 64          | 29                       | 293.0              | 18.11 | 0.73 |
| 74          | 15                       | 2818.3             | 15.42 | 0.64 |

**Table 3.** Condition of rice fields after biochar application

| Sample code | Fungi population<br>( $\times 10^5$ CFU) | Heavy metals (ppm) |       |      |
|-------------|--|--------------------|-------|------|
|             | <i>Aspergillus niger</i>                 | Fe                 | Zn    | Cd   |
| 6           | 9  | 2455.00            | 13.85 | 0.53 |
| 13          | 3  | 3177.00            | 13.98 | 0.79 |
| 14          | 5  | 3195.80            | 17.65 | 0.70 |
| 21          | 4  | 3101.90            | 17.69 | 0.85 |
| 24          | 3  | 3132.20            | 17.53 | 0.90 |
| 53          | 4  | 3612.20            | 10.20 | 1.60 |
| 57          | 3  | 3291.20            | 13.45 | 0.83 |
| 63          | 1  | 3126.90            | 14.73 | 0.65 |
| 64          | 6  | 3062.40            | 19.81 | 0.85 |
| 74          | 7  | 2823.70            | 14.74 | 0.58 |

In addition, samples with codes 57 and 63 showed no *A. niger* population (0 CFU), with Fe contents of 3124.0 and 3028.2 ppm respectively, Zn of 13.11 and 13.76 ppm, and Cd of 0.79 and 0.84 ppm. The existence of this variation shows that the application of compost fertilizer can significantly influence the population of soil microorganisms and the absorption of heavy metals. This condition shows that rice field soil in Samarinda has variations in acidity levels and heavy metal content. The high heavy metal content indicates a potential risk of heavy metal contamination which can affect plant health and the quality of agricultural products in rice fields. This analysis is an important basis for evaluating the effectiveness of compost and biochar applications in reducing heavy metal concentrations in paddy soil.

The application of biochar showed a significant effect on the population of the fungus *A. niger* as well as the absorption of the heavy metals Fe, Zn, and Cd in paddy soil in Samarinda. *Aspergillus niger* fungus populations and heavy metal concentrations were measured in various soil samples with different codes. The rice field soil sample with code 6 had an *A. niger* population of  $9.10^5$  CFU, with a Fe content of 2.46 ppm, Zn of 13.85 ppm, and Cd of 0.53 ppm. In the sample with code 13, the fungal population was recorded at 3 CFU, with a Fe content of 3.18 ppm, Zn of 13.98 ppm, and Cd of 0.79 ppm. In addition, the sample with code 53 showed the highest Fe content, namely 3612.2 ppm, with a fungal population of  $4.10^5$  CFU, Zn of 10,20 ppm, and Cd of 160 ppm, which is the highest Cd value among all samples.

Interestingly, the sample with code 63, which has the lowest population of *A. niger* fungus ( $1.10^5$  CFU), shows a Fe content of 3126.9 ppm, Zn of 14,732 ppm, and Cd of 0.65 ppm. The sample with code 57 which had a fungal population of  $3.10^5$  CFU showed a Fe content of 3291.2 ppm, Zn of 13.45 ppm, and Cd of 0.83 ppm. The application of a combination of compost and biochar in various proportions was also tested on *A. niger* fungus population and the absorption of heavy metals in paddy soil in Samarinda. The three proportions tested were the application of 50% compost and 50% biochar, 25% compost and 75% biochar, and 75% compost and 25%

biochar. The results obtained from each combination can be seen in Tables 4, 5, and 6.

The combination of 50% compost and 50% biochar showed *A. niger* populations varying from 0 to  $35.10^5$  CFU. For example, a sample with code 63 shows the highest population of  $35.10^5$  CFU with a Fe content of 3116.06 ppm, Zn of 12.03 ppm, and Cd of 0.48 ppm. In contrast, the sample with code 57 did not show any fungal population (0 CFU) with a Fe content of 3227.33 ppm, Zn of 8.82 ppm, and Cd of 0.53 ppm. These results suggest that this combination can significantly increase fungal populations, potentially improving heavy metal uptake.

The combination of 25% compost and 75% biochar showed *A. niger* populations varying from 1 to 40 CFU. The sample with code 74 has the highest population of  $40.10^5$  CFU with a Fe content of 2936.27 ppm, Zn of 17.36 ppm, and Cd of 0.26 ppm. On the other hand, the sample with code 57 has the lowest population of  $1.10^5$  CFU with a Fe content of 3371.89 ppm, Zn of 14.24 ppm, and Cd of 0.71 ppm. This combination showed an increase in fungal populations in some samples, which could influence the absorption of heavy metals in paddy soil.

**Table 4.** Condition of rice fields after application of 50% compost fertilizer and 50% biochar

| Sample code | Fungi population<br>( $\times 10^5$ CFU) | Heavy metals (ppm) |       |      |
|-------------|--|--------------------|-------|------|
|             | <i>Aspergillus niger</i>                 | Fe                 | Zn    | Cd   |
| 6           | 4  | 2610.80            | 17.42 | 0.33 |
| 13          | 24                                       | 3211.57            | 12.95 | 0.74 |
| 14          | 6  | 3209.56            | 17.24 | 0.85 |
| 21          | 16                                       | 3154.80            | 16.67 | 0.56 |
| 24          | 5  | 3147.67            | 14.44 | 0.66 |
| 53          | 1  | 3622.88            | 8.66  | 0.98 |
| 57          | 0  | 3227.33            | 8.82  | 0.54 |
| 63          | 35                                       | 3116.06            | 12.03 | 0.48 |
| 64          | 0  | 3066.42            | 12.14 | 0.71 |
| 74          | 7  | 2958.73            | 15.55 | 0.35 |

**Table 5.** Condition of rice fields after application of 25% compost fertilizer and 75% biochar

| Sample code | Fungi population<br>( $\times 10^5$ CFU) | Heavy metals (ppm) |       |      |
|-------------|--|--------------------|-------|------|
|             | <i>Aspergillus niger</i>                 | Fe                 | Zn    | Cd   |
| 6           | 8  | 2057.75            | 18.05 | 0.36 |
| 13          | 34                                       | 3230.74            | 13.92 | 0.34 |
| 14          | 14                                       | 3232.79            | 18.56 | 0.48 |
| 21          | 36                                       | 3135.53            | 15.48 | 0.45 |
| 24          | 6  | 3196.89            | 16.65 | 0.68 |
| 53          | 11                                       | 3653.58            | 9.57  | 1.28 |
| 57          | 1  | 3371.89            | 14.24 | 0.71 |
| 63          | 3  | 3190.71            | 17.90 | 0.63 |
| 64          | 7  | 3050.46            | 19.36 | 0.43 |
| 74          | 40                                       | 2936.27            | 17.36 | 0.26 |

**Table 6.** Condition of rice fields after application of 75% compost fertilizer and 25% biochar

| Sample code | Fungi population<br>( $\times 10^5$ CFU) | Heavy metals (ppm) |       |      |
|-------------|--|--------------------|-------|------|
|             | <i>Aspergillus niger</i>                 | Fe                 | Zn    | Cd   |
| 6           | 7  | 2752.53            | 19.25 | 0.25 |
| 13          | 1  | 3265.17            | 13.19 | 0.33 |
| 14          | 8  | 3201.75            | 17.26 | 0.03 |
| 21          | 4  | 3101.37            | 18.92 | 0.46 |
| 24          | 5  | 3251.78            | 16.38 | 0.51 |
| 53          | 22                                       | 3706.62            | 10.02 | 1.00 |
| 57          | 0  | 3349.65            | 13.54 | 0.45 |
| 63          | 1  | 3197.37            | 16.88 | 0.33 |
| 64          | 1  | 2939.67            | 16.26 | 0.21 |
| 74          | 24                                       | 2811.16            | 15.42 | 0.13 |

The combination of 75% compost and 25% biochar showed *A. niger* populations varying from 0 to  $24.10^5$  CFU. The sample with code 74 showed the highest population of  $24.10^5$  CFU with a Fe content of 2811.16 ppm, Zn of 15.42 ppm, and Cd of 0.13 ppm. Meanwhile, the sample with code 57 did not show any fungal population (0 CFU) with a Fe content of 3349.65 ppm, Zn of 13.54 ppm, and Cd of 0.45 ppm. These results indicate that this proportion is also effective in increasing fungal populations and reducing heavy metal concentrations in some samples. This research also evaluates changes that occur in the condition of paddy fields before and after the application of compost, biochar, and a combination of both. The results showed a significant increase in *A. niger* fungus population and a decrease in heavy metal content (Fe, Zn, Cd) after the application of various treatments based on the average. This comparison can be seen in Table 7.

In the initial soil conditions, there was no population of *A. niger* fungus with Fe metal content of 14055.51 ppm, Zn of 95.38 ppm, and Cd of 0.57 ppm. After the application of compost fertilizer, the fungal population increased to  $96.10^5$  CFU, while the Fe metal content decreased to 3032.75 ppm (78.43% decrease), Zn decreased to 15.71 ppm (83.54% decrease), but Cd increased to 0.85 ppm (an increase of 48.29%). Biochar application increased the fungal population to  $4.5.10^5$  CFU. The Fe metal content after biochar application was 3097.82 ppm (77.95% decrease), Zn was 15.36 ppm (83.89% decrease), and Cd increased to 0.83 ppm (445% increase). The combination of 50% compost and 50% biochar increased the fungal

population to  $9.8.10^5$  CFU, with Fe metal content of 3132.58 ppm (77.70% decrease), Zn of 13.59 ppm (85.74% decrease), and Cd of 0.620 ppm (8.64% increase). The combination of 25% compost and 75% biochar produced the highest fungal population, namely  $16.10^5$  CFU. The Fe metal, content after application of this combination was 3175.66 ppm (77.40% decrease), Zn was 16.11 ppm (83.11% decrease), and Cd decreased to 0.56 ppm (1.69% decrease). The combination of 75% compost and 25% biochar increased the fungal population to  $73.10^5$  CFU, with Fe metal content of 3157.71 ppm (77.54% decrease), Zn of 15.71 ppm (83.54% decrease), and Cd decreased to 0.37 ppm (35.7% decrease).

This research also tested the correlation between the presence of the fungus *A. niger* and the content of heavy metals (Fe, Zn, Cd) in contaminated rice field soil. Based on the results of the correlation analysis presented in Table 8, it was found that the presence of *A. niger* had a negative correlation with the heavy metal content Fe and Zn, with correlation coefficients of -0.71 and -0.71 respectively. This shows that the increase in *A. niger* population is correlated with a decrease in the Fe and Zn metal content in the soil.

The negative correlation between *A. niger* and Fe and Zn metals indicates that this fungus has the potential to play a role in the bioremediation process, helping to reduce heavy metal concentrations in the soil. Based on the classification of correlation values in Table 8, the correlation coefficients of -0.71 and -0.71 are included in the strong correlation category, which means there is a significant relationship between the increase in *A. niger* population and the decrease in Fe and Zn concentrations. In contrast, the correlation between *A. niger* and Cd content was not significant, with a correlation coefficient of -0.05. This value is included in the very weak correlation category, which indicates that the presence of *A. niger* does not directly affect Cd levels in paddy soil.

**Table 8.** Correlation value of the presence of *Aspergillus niger* on the heavy metal content of paddy soil

| Heavy metals | Fungi population ( $\times 10^5$ CFU) |
|--------------|---------------------------------------|
|              | <i>Aspergillus niger</i>              |
| Fe           | -0.71                                 |
| Zn           | -0.71                                 |
| Cd           | -0.05                                 |

**Table 7.** Comparison of treatments based on means

| Treatments               | Fungi population ( $\times 10^5$ CFU) | Heavy metals (ppm) |       |      |
|--------------------------|---------------------------------------|--------------------|-------|------|
|                          | <i>Aspergillus niger</i>              | Fe                 | Zn    | Cd   |
| Initial soil conditions  | 0.00                                  | 14055.51           | 95.38 | 0.57 |
| Compost                  | 9.60                                  | 3032.75            | 15.71 | 0.85 |
| Biochar                  | 4.50                                  | 3097.82            | 15.36 | 0.83 |
| Compost 50%, Biochar 50% | 9.80                                  | 3132.58            | 13.59 | 0.62 |
| Compost 25%, Biochar 75% | 16.00                                 | 3175.66            | 16.11 | 0.56 |
| Compost 75%, Biochar 25% | 7.30                                  | 3157.71            | 15.71 | 0.37 |

Application of compost significantly increased the population of *A. niger*, which is known to have bioremediation capabilities against heavy metals. The research results showed that the use of compost fertilizer was able to increase the fungal population by up to  $96.10^5$  CFU and reduce the Fe, Zn, and Cd contents by 78.43, 83.54, and 48.29% respectively. Compost increases the availability of organic nutrients and soil microbes which help decompose and stabilize of heavy metals. Organic acids produced from compost decomposition can chelate heavy metals, thereby increasing their mobility and facilitating removal through leaching or absorption by plants (Barker and Bryson 2020). Apart from that, compost can also improve soil physical properties such as structure, porosity, and water-holding capacity, all of which contribute to reducing heavy metals (Wang et al. 2019).

The combination of compost and biochar proved to be more effective than a single application of each treatment. The combination of 50% compost and 50% biochar increased the fungal population up to  $9.8.10^5$  CFU and showed a significant reduction in Fe, Zn, and Cd content. This combination takes advantage of the advantages of each material, compost provides organic nutrients that increase microbial activity (Ayilara et al. 2020), while biochar provides a large adsorption surface for binding heavy metals (Li et al. 2021). These results show the synergy between compost and biochar in improving the bioremediation process in rice fields contaminated with heavy metals.

Other combination applications such as 25% compost and 75% biochar also show effective results. This combination increased the fungal population to  $16.10^5$  CFU, with a significant decrease in Fe, Zn, and Cd content. The higher increase in fungal populations compared to other combinations may be due to the increased availability of adsorption surfaces and optimal microbial nutrition from this combination (Batool et al. 2022). On the other hand, the combination of 75% compost and 25% biochar also showed good results with an increase in fungal populations of up to  $7.3.10^5$  CFU and a significant reduction in heavy metal content. Even though the resulting mushroom population is lower than the combination of 25% compost and 75% biochar, this result still shows high effectiveness in reducing heavy metal content.

On the other hand, biochar application also shows significant results in reducing heavy metal concentrations in the soil. Biochar, which is a product of biomass pyrolysis under low oxygen conditions, has a complex pore structure and large surface area, making it an effective adsorption agent for heavy metals (Qiu et al. 2022). The research results showed that biochar was able to increase *A. niger* population by up to  $4.5.10^5$  CFU, with a decrease in Fe, Zn and Cd contents of 77.95, 83.89, and 44.95%, respectively. The pore structure of biochar allows the adsorption of heavy metals through physical mechanisms such as adsorption and ion exchange. In addition, the negatively charged surface of biochar can bind heavy metal cations, thereby reducing its biological availability and reducing its toxicity for plants (Song et al. 2022)

This combination treatment shows that using the right proportion of compost and biochar is very important to achieve optimal bioremediation results. A higher proportion of biochar tends to increase the adsorption and exchange capabilities of heavy metal ions, while a higher proportion of compost increases microbial nutrient availability and biological activity. Therefore, determining appropriate proportions based on specific soil conditions and the level of heavy metal contamination is essential to optimize the effectiveness of bioremediation (Sharma et al. 2020).

### **Relationship between *Aspergillus niger* and heavy metals in paddy soil**

This study revealed a significant relationship between the presence of *A. niger* and heavy metal content in contaminated rice field soil. *Aspergillus niger* is a type of fungus that is known to have bioremediation capabilities, namely the ability to reduce or remove pollutants from the environment, including heavy metals (Talukdar et al. 2020). Heavy metals such as Fe, Zn, and Cd are pollutants commonly found in rice fields due to agricultural, and industrial activities and weathering of the parent rock (Zakaria et al. 2021). The presence of these heavy metals can endanger the health of plants, animals, and humans, so understanding the mechanism for reducing heavy metals by *A. niger* is very important (Sharma and Kumar 2021).

The results showed that *A. niger* had a significant negative correlation with Fe and Zn metal content, with correlation coefficients of -0.71 and -0.71, respectively. This negative correlation indicates that an increase in *A. niger* populations in soil is associated with a decrease in Fe and Zn metal content. Based on the classification of correlation values, the correlation coefficients of -0.71 and -0.71 are included in the strong correlation category, which means there is a significant relationship between the increase in *A. niger* population and the decrease in Fe and Zn concentrations (El-Mahdy and Mohammed 2022).

The decrease in heavy metal concentrations in soil by *A. niger* can be explained by several mechanisms. First, *A. niger* can emit organic acids such as citric acid and oxalic acid, which can chelate heavy metals, making them easier for plants to take up or convert into less dangerous forms. This organic acid can dissolve heavy metals from soil particles, thereby reducing the concentration of heavy metals that are strongly bound to the soil (Pathak et al. 2021). Second, *A. niger* is also able to absorb heavy metals directly into its fungal biomass through a bioaccumulation process. This process involves the binding of heavy metals to fungal cell walls or the entry of heavy metals into cells through active transport mechanisms (Priyadarshini et al. 2021).

Another possible mechanism is biosorption, in which *A. niger* uses its cell wall components such as glucans, chitin, and proteins to bind heavy metals. This process does not require metabolic energy and can occur in dead and living cells (Goutam et al. 2021). Thus, *A. niger* can play an important role in reducing heavy metal concentrations in the soil through various physicochemical and biochemical mechanisms (Kumar dan Dwivedi 2020).

Meanwhile, the correlation between *A. niger* and Cd content showed different results. The correlation coefficient between *A. niger* and Cd is -0.05, which is included in the very weak correlation category. This shows that increasing *A. niger* population does not significantly affect Cd levels in paddy soil (Qiu et al. 2021). This difference may be due to the different chemical properties of Cd from Fe and Zn, or perhaps because *A. niger* has a lower capacity to chelate or adsorb Cd (Cabrera-Barjas et al. 2020).

Cd is known as a very toxic heavy metal with chemical properties that are different from Fe and Zn (Pan et al. 2020). Although, *A. niger* can excrete organic acids and carry out biosorption, the ability of this fungus to bind or chelate Cd may be limited compared to Fe and Zn (Dusengemungu et al. 2021). Additionally, other fungal species or soil microorganisms may be more effective in reducing Cd concentrations. Therefore, to address Cd contamination, a different approach or the use of additional bioremediation agents may be required.

This research also shows that the combined application of compost and biochar can increase *A. niger* populations in paddy soil, which in turn contributes to reducing heavy metal content. The combination of 25% compost and 75% biochar showed the highest effectiveness in increasing fungal populations up to  $16.10^5$  CFU, as well as reducing Fe, Zn, and Cd contents. Although there was a slight increase in some heavy metals after application, the overall reduction remained significant compared to initial soil conditions (Wu et al. 2022).

The use of compost and biochar individually also provides positive results in reducing heavy metal content in paddy soil. Compost increased the fungal population by up to  $9.6.10^5$  CFU and reduced the Fe, Zn, and Cd contents by 78.43, 83.54, and 48.29%, respectively. Meanwhile, biochar application increased the fungal population by up to  $4.5.10^5$  CFU, with a decrease in Fe, Zn, and Cd contents of 77.95, 83.89, and 44.95%, respectively. However, the combination of these two ingredients has proven to be more effective than their use alone.

The research concluded that *A. niger* can be an effective bioremediation agent from straw compost and rice husk biochar that a lot of availability in Samarinda for certain heavy metals absorption such as Fe and Zn but does not show the same effectiveness against Cd. Therefore, the use of *A. niger* in a remediation strategy for soil contaminated with heavy metals needs to consider the type of heavy metal that is the target of remediation, as well as the potential effectiveness of the bioremediation agent used.

In conclusion, this research shows that the application of compost and biochar fertilizer separately or in combination is effective in reducing heavy metal content in rice field soil in Samarinda. Compost increases *A. niger* population and reduces the concentration of Fe, Zn, and Cd, while biochar reduces the heavy metal content with a large adsorption capacity. The best combination is straw compost and husk biochar (1:1) indicating synergy between organic nutrients and adsorption capabilities. The correlation between *A. niger* populations and heavy metals showed a significant relationship, indicating the role of

microbes in the bioremediation process in reducing heavy metals by more than 50% compared with control. The practical implication of this research is the importance of using organic fertilizer to reduce heavy metal contamination, support sustainable agriculture, and reduce dependence on synthetic chemicals.

## ACKNOWLEDGEMENTS

The authors are very grateful to the Ministry of Higher Education, Science and Technology, Indonesia for the financial support for doctoral research in 2024.

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