Exploration and Potential Test of Fungi Remediator of Lead and Cadmium in Shallot Cultivation in Brebes Regency

Okti Herliana[1*], *Wilis* Cahyani[1], *Ahmad* Fauzi[1], *Rostaman*[2], *Hardi Rahmat Raharjo*[3] *and, Mutiara Annisa*[3]

[1]Jenderal Soedirman University, Faculty of Agriculture, Laboratory of Agroecology, 53122 Purwokerto, Indonesia
[2]Jenderal Soedirman University, Faculty of Agriculture, Laboratory of Plant Protection, 53122 Purwokerto, Indonesia
[3]Jenderal Soedirman University, Master of Agricultural Biotechnology Program, 53122 Purwokerto, Indonesia

Abstract. Fungi have the ability to convert toxic substances into simple substances that are environmentally friendly. This study aims to explore and identify fungi in shallots rhizosphere in Brebes Regency, to test the ability of fungi from exploration in remediation of lead and cadmium contamination, and to test the effect of fungi applications on the growth of rice seeds. The collected isolates were macroscopically and microscopically identified. The fungal resistance was tested in the PDA media with concentrations of Pb and Cd in 5, 10, 25, and 50 ppm. The remediation capability was assessed using the Randomize Complete Design in 100 to 200 ppm of Pb and 25 to 50 ppm of Cd. The effect of fungi applications on rice seeds was tested in vitro. The study has found seven fungi isolates from four different genera, namely, *Aspergillus*, *Trichoderma*, *Penicillium*, & *Curvularia*. The seven fungi are known to be resistant to all concentrations of Pb and Cd. The results of the heavy metal remediation ability test by selected fungi obtained the highest weight E value of 30%, 24%, & 38% on Pb metals and 71%, 73%, & 75% on Cd metals and potentially as bioremediation agents of Pb and Cd.

1 Introduction

Shallots are horticultural commodities which is high demand, due to many consumptions or used as a spice in complementary daily cuisine by the people of Indonesia. Shallots in the production process are inseparable from the use of chemical fertilizers and pesticides. Intensive shallots cultivation models make them tend to apply large amounts of fertilizer and pesticides. Excessive use of chemical fertilizers and pesticides can leave chemical residues such as heavy metals in the soil. The main causes of environmental pollution besides erosion

* Corresponding author: okti.herliana@unsoed.ac.id

and sedimentation are also influenced by the intensity of the use of agro-surrounding materials in the form of chemical fertilizers and pesticides that exceed the limits [1].

Pollutants such as heavy metals have toxic properties and polluted in all environmental media (land, water and soil). Some heavy metals detected toxic in nature, for example lead (Pb) and cadmium (Cd). Heavy metal is a non-essential metal that is not needed by living things. Pb and Cd are metal types whose levels of toxicity are quite high and non-biodegradable [2].

Efforts to control heavy metal contamination must be held. One of the actions that can be taken is bioremediation. Bioremediation is a biological process by utilizing microorganisms that are environmentally friendly to eliminate dangerous pollutants. Microorganism that are often used in the bioremediation process are fungi [2]. An example of fungi that have been used as bioremediation agents is Aspergillus niger, they able to reduce heavy metal Fe with an average decrease of 26.65% [3]. In addition, it is known that Rhizoctonia, Penicillium, Papulaspora, and Sclerotium resistan to Pb to a concentration of 5000 ppm [4].

Based on the description above as an initial effort, it is necessary to conduct a research exploration of fungi on the soil to obtain biological agencies or fungi that have the potential to redeem heavy metals. The purpose of this study was (1) to explore and identify fungi in rhizosfer of shallots in Brebes Regency, the ability test of fungi to be exploratory in the remediation of lead and cadmium [5], then the effect test of fungi applications on the media polluted heavy metal lead and cadmium to the growth of rice seeds [2].

2 Material and methods

Soil sampling was carried out in Songgom Lor Village, Songgom District, and Pandansari Village, Paguyangan District, Brebes Regency, Central Java Province. Soil isolation, fungal identification, mushroom resistance test on lead metals lead and cadmium, the ability to the capability of fungi in remediation of lead metals and cadmium, and the effect of fungal applications on leads to lead metal lead to lead and cadmium on the growth of rice seeds are carried out in the plant protection laboratory, Faculty of Agriculture, Jenderal Soedirman University in January 2022 to October 2022.

The tools used in this study are a 250 ml Erlenmeyer flask (pyrex), test tubes (pyrex), test tubes, a Petri dish (anumbra), bunsen, micropipette, microscope, glass object, glass cover, spatula, cork drill, beker glass, pumpkin, plastic, autoclave, rotary shaker, atomic absorption spectrometry (SSA), laminar air flow (LAF), digital scales, and analytical scales. the materials used in this study are soil samples in onion plantations, rice seed varieties, potato dextrose media (PDA), potato dextrose broth (PDB), lead solids (Pb), cadmium solids (Cd), lactophenol blue, alcohol 70%, aquades, spiritus, aluminum foil, label paper, plastic wrap, sterile cotton, tissue, gloves, opf markers and ordinary markers.

The experimental design used in this study was carried out in 2 stages, namely: soil sampling, analysis in the laboratory for exploration and fungal identification activities, followed by testing the capability of remediation using mushroom isolates obtained with a complete random design (RAL) consisting of 8 treatments for each treatment for each Testing of heavy metals, in this test used 2 types of heavy metals: lead and cadmium.

Fisrt stap are laboratory analysis to soil isolation from exploration area and grown on the PDA media then the fungus obtained on the PDA media is identified. Three isolates were chosen for heavy metal remediation testing on PDB media using a Complete Randomized Design Method (RAL) consisting of the following treatment:

Fungi testing on lead metal
1 = Pb 100 ppm without fungi.
2 = Pb 200 ppm without fungi.
3 = Pb 100 ppm with fungi 1.

4 = Pb 100 ppm with fungi 2.
5 = Pb 100 ppm with fungi 3.
6 = Pb 200 ppm with fungi 1.
7 = Pb 200 ppm with fungi 2.
8 = Pb 200 ppm with fungi 3.
Fungi testing of cadmium heavy metals
1 = Cd 25 ppm without fungi.
2 = Cd 50 ppm without fungi.
3 = Cd 25 ppm with fungi 1.
4 = Cd 25 ppm with fungi 2.
5 = Cd 25 ppm with fungi 3.
6 = Cd 50 ppm with fungi 1.
7 = Cd 50 ppm with fungi 2.
8 = Cd 50 ppm with fungi 3.

The remediation ability test is made with 2 experimental units each, namely the experimental unit with lead metal and the experimental unit with cadmium heavy metals. Experiments in the fungus test on lead metal lead (Pb) and cadmium (Cd) each totaled 8 treatments and were repeated 3 times, so that experiments were obtained on each heavy metal, as many as 24 experimental units. The total number of experimental units is 48 experimental units.

Variables observed and measured include the following:

2.1 Fungi identification

Macroscopically the variables were observed by looking at the character of the colony such as Color and Colonial surface, radial lines from the center of the colony towards the edge of the colony, as well as concentric circles [6].

Microscopic features observed include the presence or absence of conidia, hyphae & visible conidia colors (dark or transparent hyaline), forms of conidia, hyphae growth (branched or branched), whether or not there is a septum in hyphae (insulated or not insulated) [7]. Microscopic observations using the slide culture method.

2.2 Fungal resistance test on lead and cadmium metals

Heavy metal fungi screening is done by growing fungi isolates on PDA media using the concentration of Pb and Cd contamination used which are 5, 10, 25, and 50 ppm. Indications of fungi that are resistant are indicated by the growth of fungal colonies in the PDA media given contamination.

2.3 Test the ability of fungi in remediation of lead and cadmium metals

The test was carried out by inoculating the fungi inoculum into the Erlenmeyer containing 50 ml of PDB media that was given heavy metal contamination with a concentration of heavy metal contamination Pb 100 ppm and 200 ppm, and the concentration of heavy metals Cd were 25 ppm and 50 ppm. Incubation is carried out for 7 days at room temperature (26-28ºC) using a rotary shaker with an agitation speed of 120 rpm.

2.3.1 Dry mycelium weight (biomass)

The biomass of the fungus that grows is then separated by medium using Whatman filter paper. The formula for the calculation of mycelium biomass [8]:

$$\text{The weight of the end of the mycelium biomass} = A - B \tag{1}$$

Description:
A= weight of Whatman paper (g)
B= weight of Whatman paper with fungus mycelium (g)

2.3.2 The number of absorbed metal ions and biosorption efficiency

A heavy metal analysis is done using SSA. Determination of the number of metal ions absorbed by biomass and the efficiency of metal absorption is calculated using the following equation [9]:

$$q = \frac{(Ci - Cf)}{m} x\, V \tag{1}$$
$$E = \frac{Ci - Cf}{Ci} x100 \tag{2}$$

Description:
q= number of metal ions absorbed per gram of biomass (mg/g)
Ci= initial concentration of metal ions (mg/L)
Cf= final concentration of metal ions (mg/L)
M= dry biomass weight (g)
V= media volume (mL)
E= biosorption efficiency (%)

2.4 Test the effect of fungi applications on the media contaminated with lead metal and cadmium on the growth of rice seeds

Rice seeds are soaked with a sample from the remediation test results for 24 hours. The rice seeds are then grown on the ranging paper for 14 days at room temperature. The humidity of the straw paper media is controlled by looking at the state of the paper if the paper media is dry, then it is given distilled water until the media is moist, whereas if the media is moist, it does not need to be given aqua dest. The observed variables are the growth of sprouts, namely sprouts, the length of the root of the sprouts, the weight of the fresh sprouts, and the sprout power. Calculation of the percentage of germination is done on normal germination seeds using the formula:

$$Germination = \frac{number\ of\ seeds\ that\ germinate\ normally}{number\ of\ seeds\ that\ germinate} x100\% \tag{1}$$

Data on the results of exploration and identification of fungi research results are prepared descriptively. The quantitative data obtained were analyzed using SPSS 25 software with the ANOVA method to test significant differences when P <0.05 and further Duncan Multiple Range Test (DMRT).

3 Result and Discussion

3.1 General conditions sampling location

Brebes Regency is located between 6º44'-7º21 'South Latitude and between 108º41'-109º11 'East Longitude. One of the highest rainfalls occurred in December 2020, with an average rainfall of as much as 1,322 mm with an average rainy day of 23 days. The highest amount of rainfall based on the sub-district that occurred in Paguyangan District was 10,761 mm. The highest temperature is in October with an average temperature of 29ºC. Most types of land in Brebes Regency are gray alluvial. Brebes Regency soil texture consists of clay, dust, and sand fractions to form land [10].

Chemical pesticides and chemical fertilizers are an inseparable part of the conventional system of onion cultivation in Brebes Regency, the maintenance is carried out intensively and hereditary for years. Based on the results of Bahar's research [11], the use of chemicals by onion farmers in Brebes Regency is still very intensive. The impact of excessive use of agrochemicals is the presence of dangerous residues left in the soil. The left behind can be in the form of heavy metals. Heavy metal is a dangerous compound when in a high amount an environment. A sampling of soil samples is carried out in the cultivation area of onion with massive application of agrochemicals marked by the pungent chemicals and land that is located next to the highway so that there is a possibility of heavy metal residues on the onion agricultural land in the Songgom and Circle of Friends. Sampling in the land aims to explore microorganisms that adapt or be able to live in the condition of the polluted land. Microorganisms contained in polluted land have the potential to be biological agents in the heavy metal remediation process. The intended microorganism comes from the type of fungus.

3.2 Fungal resistance test on lead and cadmium metals

Soil samples taken from the root area of shallots in Songgom Lor, Songgom, and Pandansari Village, Paguyangan, Brebes, were successfully isolated. Obtained as many as 7 fungi isolated with 4 different genera. The results of the identification of fungi are presented in Table 1.

Table 1. Result of fungi identification.

Place of Origin	Isolate code	Type of fungi
Pandansari Village, Paguyangan, Brebes	PDJ1	*Trichoderma* sp.
	PDJ2	*Penicillium* sp.
	PDJ3	*Penicillium* sp.
Songgom Lor Village, Songgom, Brebes	SLJ1	*Curvularia* sp.
	SLJ2	*Aspergillus niger*
	SLJ3	*Penicillium* sp.
	SLJ4	*Aspergillus* sp.

Note: PD code: comes from Pandansari Village; SL code: Derived from Songgom Lor Village, Code J1: fungi 1, J2: fungi 2, J3: fungi 3

Based on observations from seven fungal isolates characteristics of each fungus that were successfully identified, among others:

3.2.1 *Trichoderma* sp.

PDJ1 fungi isolates based on macroscopic observations found characteristics, green colonies with rings in the middle and along the edge of the cup, the bottom of the pale green colony; The shape of the colony grows evenly to fill the surface of the media; transparent fine cotton colony texture; The spread of hyphae is fast and evenly distributed a slightly thick surface. Based on microscopic observations, the characteristics, of round conidia are obtained; Branching Konidiophores and Fialid forms like tree branches; & hyphae insulated. The results of this observation are following the morphology of *Trichoderma* sp.

Trichoderma sp. has the characteristics of a lot of branching conidiophores not perpendicular; single or group fialid; round conidia; fast growth and green colony [12]. Trichoderma sp. has the characteristics of colonies to form a circle, dark green in each circle, grows evenly on the surface of the media, and conidium is formed on the edge of the petri dish, round conidium, green, hyphae, and hyaline [13]. Morphological characteristics of *Trichoderma sp*. presented in Figure 1.

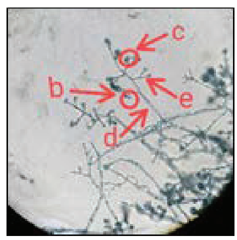

Fig. 1. Morphological characteristics of *Trichoderma* sp. (a) colonies in PDA media, (b) conidia, (c) conidium, (d) conidiophores, (e) fialid (40x magnification) (source: personal documentation).

3.2.2 *Penicillium* sp.

Fungi isolates PDJ2, PDJ3, & SLJ3 based on macroscopic observations have the characteristics of a yellow-green colony at the beginning of growth and then become dark green, the opposite part is slightly reddish; The shape of the round colony and spreads, the surface texture of the velvet. Microscopic observations obtained characteristics, chain rounded conidia; conidium has 2-3 metulas and 3-4 filaid; branching and insulated conidiophores; & hyphae insulated. Isolates PDJ2, PDJ3, & SLJ3 based on these characteristics are thought to be *Penicillium* sp. The results of this observation are following the morphology of *Penicillium* sp.

Penicillium sp. has the characteristics of conidiophores from a single or more mycelium, branching near the peak, ending in the fialid group; One -celled conidia, the form of conidia is mostly round or ovoid in a dry basic chain [12]. The morphological Characteristics of *Penicillium* sp. are presented in Figure 2.

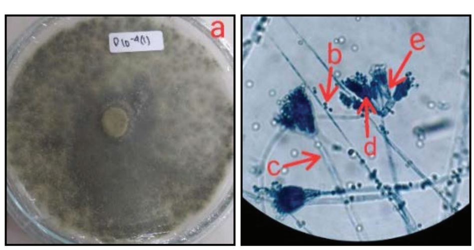

Fig. 2. Morphological characteristics of *Penicillium* sp. (a) colonies in PDA media, (b) conidia, (c) conidiophores, (d) fialid, (e) metula (40x magnification) (source: personal documentation).

3.2.3 Curvularia sp.

SLJ1 fungi isolates based on macroscopic observations have the characteristics of blackish-brown colonies, colonies that grow evenly on the surface of the media, and have a cotton texture. Microscopic observations found characteristics, the form of cylindrical conidia is slightly curved, with 3-4, with a protruding middle cell, brown conidia, single or group conidium, and hyphae. Based on these characteristics, SLJ1 fungi isolates are suspected to be *Curvularia* sp.

Curvularia sp. has the characteristics of a grayish-brown colony, and has a texture like cotton to velvet. A single or branched brown conidiophore; Hifa insulated. Conidia is cylindrical to an ellipse with the hilus that stands out at the bottom, and the form of conidia is straight or slightly curved [14]. Morphological characteristics *Curvularia* sp. presented in Figure 3.

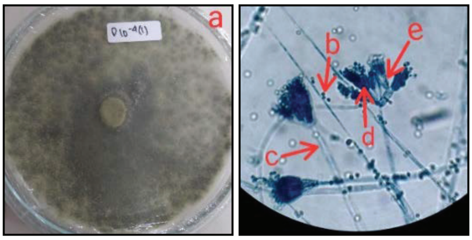

Fig. 3. Morphological characteristics *Curvularia* sp. (a) colonies in PDA media, (b) conidia, (c) conidiophores (40x magnification) (source: personal documentation).

3.2.4 Aspergillus sp.

Found two fungal isolates originating from the genus *Aspergillus* namely, isolates SLJ2 & SLJ4. Based on the observations of macroscopic isolates SLJ2 has the characteristics of the color of the white colony and is packed with a black conidia head, mycelium has a texture like fine cotton. Microscopic observations found characteristics, forms of round black-colored conidia with coarse textured; the long form of conidiophores and hyaline, fine-walled; The peak of the conidiophore is enlarged or called vesicles, large vesicles and filled with fialid and metulas; Hifa insulated. SLJ2 isolates are suspected of *Aspergillus niger*. The *Aspergillus niger* has the characteristics of a slow-growing colony, the white mycelium grows upright and cramped, the structure of the black conidia covers the entire colony, the head of large and black conidia, fine conidiophores and hyaline. The morphological characteristics of *Aspergillus niger* are presented in Figure 4 [15].

Based on macroscopic observations, SLJ4 isolate has a characteristic bluish-green colony color and granular and spreading colony shape. Microscopic observation showed that the conidiophores were erect, insulated, and unbranched, the ends of the conidiophores swelled to form vesicles, the conidia were green, the heads of the conidiophores were semi-spherical and cylindrical, the walls of the conidiophores were smooth, and the hyphae were insulated. SLJ4 isolate was identified as *Aspergillus* sp. Identification results are colored conidiophores erect, simple or rarely branched, smooth on the surface; bearing dark green cylindrical or clavate conidial heads consisting of dense catenulate (chain) conidia borne on uniseriate filaid developed on subglobose or clavate vesicles [15]. Phialosporous conidia, pale green, globose, rough on the surface. The morphological characteristics of *Aspergillus* sp. are presented in Figure 5.

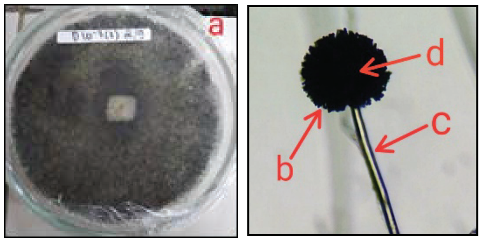

Fig. 4. Morphological characteristics of *Aspergillus niger* (a) colonies on PDA media, (b) conidia, (c) conidiophores, (d) conidia (40x magnification) (source: personal documentation).

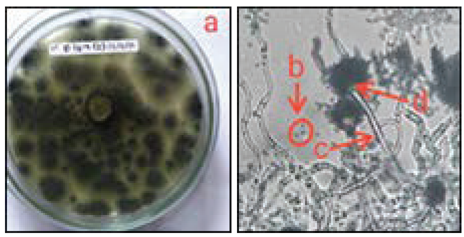

Fig. 5. Morphological characteristics of *Aspergillus* sp. (a) colonies on PDA media, (b) conidia, (c) conidiophores, (d) vesicles (40x magnification) (source: personal documentation).

3.3 Test of fungal resistance to Pb and Cd heavy metals

Seven isolates from the genera *Trichoderma*, *Penicillium*, *Curvularia*, and *Aspergillus* were tested for resistance using PDA media which were given concentrations of the heavy metals lead and cadmium, namely 5, 10, 25, and 50 ppm, then incubated for seven days and observed for growth at seventh day. Based on the research results, it was found that the seven types of Fungi tested were able to survive or be resistant to the heavy metals lead and cadmium up to a concentration of 50 ppm.

The following is a table of the results of tests of resistance of isolates to heavy metals Pb and Cd with various concentrations of 5, 10, 25, and 50 ppm.

Table 2. Isolate resistance test on heavy metals Pb and Cd with various concentrations.

Isolate code	Heavy Metal							
	Pb (ppm)				Cd (ppm)			
	5	10	25	50	5	10	25	50
PDJ1	√	√	√	√	√	√	√	√
PDJ2	√	√	√	√	√	√	√	√
PDJ3	√	√	√	√	√	√	√	√
SLJ1	√	√	√	√	√	√	√	√
SLJ2	√	√	√	√	√	√	√	√
SLJ3	√	√	√	√	√	√	√	√
SLJ4	√	√	√	√	√	√	√	√

Note: √: capable of growing, x: not growing.

Observation of cadmium and lead-resistant fungi in each isolate. Colonies of *Trichoderma* sp. were capable of filling the entire surface of the petri dish with heavy metal Pb contamination up to a concentration of 50 ppm and colonies were able to fill the surface of the dish with a Cd concentration of 10 ppm, after that concentration cadmium became toxic and slowed the growth of the fungal mycelium. Colonies of *Trichoderma* sp. turn white on Cd exposure. Ecotoxicity to fungi when exposed to heavy metals can cause changes in hyphae morphology [16].

Isolate *Penicillium* sp., was able to grow to fill half of the petri dish on Pb contamination up to a concentration of 50 ppm and the isolate was able to fill the surface of the petri dish on Cd contamination up to a concentration of 10 ppm, but at higher concentrations, it showed stunted growth as indicated by a larger colony diameter. decreased at higher concentrations. Cadmium has an inhibitory effect on the diameter of fungal coloniesc [17].

Isolate *Curvularia* sp. on Pb contamination it was able to grow to fill half of the petri dish up to a concentration of 25 ppm, the same thing happened to Cd exposure the isolate was able to fill the surface of the dish up to a concentration of 25 ppm, but at higher concentrations, it showed stunted growth which was characterized by a smaller colony diameter at higher concentration. *Curvularia* and *Aspergillus* show great tolerance to metals (Cd, Cu, and Ni) [15].

Aspergillus niger isolate was able to fill half of the petri dish when exposed to Pb up to a concentration of 50 ppm, CD contamination caused growth inhibition in the isolate, and the isolate diameter decreased at concentrations of 25 ppm and 50 ppm while *Aspergillus* sp. filled half of the petri dish on Pb contamination up to a concentration of 50 ppm, on CD contamination the isolates were able to grow up to a concentration of 25 ppm but growth inhibition occurred, the diameter of the colonies decreased at concentrations of 25 ppm and 50 ppm.

Several isolates from different genera and having higher adaptability were then tested for their ability to remediate heavy metals. SLJ2 (*Aspergillus niger*), PDJ1 (*Trichoderma* sp.), & SLJ1 (*Curvularia* sp.) isolates had higher adaptability and survival ability in heavy metal resistance testing on PDA media compared to other isolates. was chosen to know more about its ability to remediate lead and cadmium heavy metals.

3.4 Fungi remediation ability test

The selected fungi were then tested on PDB media treated with lead and cadmium heavy metal stress. The following table shows the results of the remediation of lead and cadmium heavy metals by fungi.

Table 3. Test results for remediation of heavy metal lead by fungi.

No.	Treatment	Biosorption (mg/L)	Biosorption Efficiency (%)	q (mg/g)	Biomass (g)
1	Pb 100 ppm without fungi	0,20 a	0 a	0 a	0 a
2	Pb 200 ppm without fungi	2,72 b	1 a	0 a	0 a
3	Pb 100 ppm with *Aspergillus niger*	23,85 c	24 e	3,74 b	0,32 cd
4	Pb 100 ppm with *Trichoderma* sp.	30,50 d	30 f	5,81 c	0,26 bc
5	Pb 100 ppm with *Curvularia* sp.	37,65 e	38 g	4,73 b	0,41 de
6	Pb 200 ppm with *Aspergillus niger*	29,83 d	15 b	4,22 b	0,36 cd
7	Pb 200 ppm with *Trichoderma* sp.	36,22 e	18 c	10,34 d	0,18 b
8	Pb 200 ppm with *Curvularia* sp.	39,82 f	20 d	4,45 b	0,48 e
Coefficient of Diversity (*CV*)		3%			19%

Note: q: ion absorption per gram of biomass, the numbers in the column followed by the same letter are not significantly different according to the DMRT test.

Based on the data analysis of the final heavy metal content of lead in the samples added with fungi, it is known that there has been a decrease in lead heavy metal from initial levels of 100 ppm and 200 ppm. The decrease in average initial lead level of 100 ppm by fungi decreased by more than 20%. The lowest reduction was found in the addition of *Aspergillus niger* with a biosorption efficiency of 24%, ion absorption per gram of biomass was 3,74 mg/g, fungi biomass was 0,32 g and the highest was found in the addition of *Curvularia* sp with a biosorption efficiency of 38%, ion absorption per gram biomass 4,73 mg/g, biomass 0,41 g, while the decrease in the average initial lead content of 200 ppm by fungi decreased by more than 10%. The lowest decrease was found in the addition of the *Aspergillus niger* fungus with a biosorption efficiency of 15%, ion absorption per gram of biomass was 10,34 mg/g, biomass was 0,36 g and the highest was found in the addition of the fungus *Curvularia* sp. with a biosorption efficiency of 20%, ion absorption per gram of biomass is 4,45 mg/g, and biomass is 0,48 g.

Table 4. Test results for remediation of heavy metal cadmium by fungi.

No.	Treatment	Biosorption (mg/L)	Biosorption Efficiency (%)	q (mg/g)	Biomas s (g)
1	Cd 25 ppm without fungi	1,11 a	4 a	0 a	0 a
2	Cd 50 ppm without fungi	1,01 a	2 a	0 a	0 a
3	Cd 25 ppm with *Aspergillus niger*	9,45 b	38 b	1,31 a	0,36 d
4	Cd 25 ppm with *Trichoderma* sp.	11,98 d	48 d	5,26 b	0,11 b
5	Cd 25 ppm with *Curvularia* sp.	10,71 c	43 c	1,16 a	0,46 e
6	Cd 50 ppm with *Aspergillus niger*	36,68 ef	73 ef	7,95 c	0,23 c
7	Cd 50 ppm with *Trichoderma* sp.	35,45 e	71 e	14,12 d	0,13 b
8	Cd 50 ppm with *Curvularia* sp.	37,63 f	75 f	5,00 b	0,38 d
(CV)		4%			11%

Note: q: ion absorption per gram of biomass, the numbers in the column followed by the same letter are not significantly different according to the DMRT test.

Based on the data analysis of heavy metal content of cadmium in the samples added with fungi, there was a decrease on cadmium from the initial levels of 25 ppm and 50 ppm. The decrease in average initial cadmium content of 25 ppm by fungi decreased by more than 35%. The lowest decrease was found in the addition of *Aspergillus niger* with a biosorption efficiency of 38%, ion absorption per gram of biomass was 1.31 mg/g, fungi biomass was 0.36 g and the highest was found in the addition of *Trichoderma* sp. with a biosorption efficiency of 48%, absorption of ions per gram of biomass was 5,26 mg/g, biomass was 0,11 g, while the decrease in the average initial cadmium content of 50 ppm by fungi decreased by more than 70%. The lowest decrease was found in the addition of *Trichoderma* sp. with a biosorption efficiency of 71%, absorption of ions per gram of biomass of 14,12 mg/g, the biomass of 0,13 g, and the highest found in the addition of *Curvularia* sp. with a biosorption efficiency of 75%, ion uptake per gram of biomass is 5,00 mg/g, and biomass is 0,38 g.

An increase in the amount of biomass causes a decrease in the balance of heavy metal absorption which is known directly from the results of the calculation of the biosorption capacity. This happens because biomass has a certain maximum absorption capacity, when the absorption has reached the maximum point there will be saturation in the biomass so that the absorption of metal ions will stop . This is because the surface of the cell wall has been covered by a layer of adsorbed metal ions resulting in saturation or cessation of absorption of metal ions.

Based on the data in Table 3 and 4, it is known that the higher the value of the biomass produced, the lower the biosorption efficiency of heavy metals. This is occurs due to a decrease in the contact surface area of the biosorbent to bind metals The greater biomass value causes the concentration of the cell suspension to be large and makes the cells bind to each other, thereby reducing the contact surface area and cell contact with heavy metals contained in the solution.

Based on the data in table 4, it is known that treatment with a lower initial concentration of heavy metal Pb has a greater absorption percentage efficiency compared to treatment with a high initial concentration of heavy metal Pb. A high initial concentration results in a low percentage of metal absorption efficiency, while at a low initial concentration the metal can improve the performance between the binding sites and metal ions so that the biosorption efficiency becomes optimal.

3.5 Germination test of rice seeds on lead and cadmium contaminated media remediated by fungi

Rice seeds grown on remediated media showed growth trends that were not significantly different (Table 5). Rice seeds show good stem growth and do not experience necrosis, this indicates that the fungus works to remediate or reduce levels of contamination so that the media used to grow rice seeds provides the same good carrying capacity as media that is not polluted by heavy metals. The results of bioremediation are carbon dioxide, water, and cell biomass . These compounds can be used by rice seeds for the growth of rice seeds.

Table 5. Germination of rice seeds on lead and cadmium heavy metals

No.	Treatment	HS (cm)	RLS (cm)	WoS (g)	PG (%)
1	without heavy metals and fungi	3,68	3,03	0,17	96,67
2	without heavy metals and with *Aspergillus niger*	2,79	0,79	0,10	96,67
3	without heavy metals and with *Trichoderma* sp.	2,71	2,04	0,11	66,67
4	without heavy metals and with *Curvularia* sp.	1,93	1,36	0,06	90,00
5	Pb 100 ppm with *Aspergillus niger*	2,80	1,35	0,10	66,67
6	Pb 100 ppm with *Trichoderma* sp.	2,43	1,13	0,06	73,33
7	Pb 100 ppm with *Curvularia* sp.	1,99	1,38	0,05	76,67
8	Pb 200 ppm with *Aspergillus niger*	2,56	0,82	0,10	93,33
9	Pb 200 ppm with *Trichoderma* sp.	3,04	2,45	0,05	76,67
10	Pb 200 ppm with *Curvularia* sp.	2,64	1,15	0,06	90,00
(CV)		36%	56%	56%	26%
No.	Treatment	HS (cm)	RLS (cm)	WoS (g)	PG (%)
1	without heavy metals and fungi	3,68	3,03	0,17	96,67
2	without heavy metals and with *Aspergillus niger*	2,79	0,79	0,10	96,67
3	without heavy metals and with *Trichoderma* sp.	2,71	2,04	0,11	66,67
4	without heavy metals and with *Curvularia* sp.	1,93	1,36	0,06	90,00
5	Cd 25 ppm with *Aspergillus niger*	1,84	0,97	0,07	93,33
6	Cd 25 ppm with r *Trichoderma* sp.	3,27	1,49	0,12	90,00
7	Cd 25 ppm with *Curvularia* sp.	2,17	1,36	0,09	96,67
8	Cd 50 ppm with *Aspergillus niger*	2,75	1,27	0,05	66,67
9	Cd 50 ppm with *Trichoderma* sp.	3,84	2,29	0,14	100,00
10	Cd 50 ppm with *Curvularia* sp.	2,73	1,55	0,08	83,33
(CV)		38%	57%	57%	29%

Note: HS: sprout height, RLS: sprout root length, WoS: fresh sprout weight, PG: germination percentage.

Fungi are known to be able to accumulate heavy metals in external hyphae and can also be through the mechanism of complexing these metals by secretion of external hyphae, this indicates a filtration mechanism so that toxic materials are not absorbed by plants [18]. The non-pathogenic properties of *Aspergillus niger*, *Trichoderma* sp., and *Curvularia* sp. on rice seed germination indicate that the three fungi have potential as bioremediation agents.

4 Conculsion

The results of the study found seven fungal isolates from four different genera, namely, *Aspergillus*, *Trichoderma*, *Penicillium* & *Curvularia*. Those all fungi were known to be resistant to all test concentrations of the heavy metals Pb and Cd up to a concentration of 50 ppm. test results for heavy metal remediation by selected fungi, namely *Trichoderma* sp., *Aspergillus niger*, & *Curvularia* sp. the highest heavy metal E values were obtained respectively 30%, 24%, & 38% for Pb metal and 71%, 73%, & 75% for metals and have potential as bioremediation agents for heavy metals Pb and Cd.

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