AFSSAAE

Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering 2022, 5(1), 68-76
ISSN 2622-5921

ORIGINAL RESEARCH Open Access

Combination of FOBIO biopesticide and *Brassica rapa* L. as remediator of heavy metal Pb in soil

Surya Ari Widya[1*],Moch. Arifin[2], Sri Wiyatiningsih[3]

Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Jatim, Surabya, Indonesia

KEYWORDS

Brassica rapa L.
FOBIO biopesticide
Heavy metal Pb
Phosphate-solubilizing bacteria
Polluted soil

ABSTRACT

Marginal soil is infertile soil caused by various factors, one of which is the excessive accumulation of heavy metals. FOBIO microorganism-based biopesticide formula containing phosphate solubilizing bacteria is expected to be used to reduce excess heavy metals in the soil. *Brasica rapa* L. is a variety resistant to Pb heavy metal stress and absorbs more heavy metals into root tissue than leaf tissue with the help of microorganisms. This study aimed to determine the combination of *Brassica rapa* L. and FOBIO biopesticide in absorbing Pb in the soil, determine the growth of *Brassica rapa* L. at each Pb concentration, and determine the population of phosphate-solubilizing bacteria in the soil at each level of Pb stress after remediation. This study used variations in the concentration of heavy metals, particularly Pb. Parameters observed included plant growth, Pb concentration in the soil before and after remediation, and the population of phosphate-solubilizing bacteria. The results showed that combining FOBIO biopesticides and *Brassica rapa* L. could reduce Pb concentration in the soil. The growth of *Brassica rapa* L. plants was significantly different after treatment. The findings confirmed that increasing, the concentration of Pb led to a decrease in the population of phosphate-solubilizing bacteria.

Introduction

As the demand for agricultural products in the world increases but the productive land decreases, it is necessary to use marginal lands as a solution to meet agricultural needs. Marginal soil is soil that has low quality due to several limiting factors such as sloping topography, the dominance of parent material, low content of nutrients and organic matter, low moisture content, pH that is too low or too high, and even accumulation of metallic elements that are toxic to plants. If efforts are made to cultivate plants in the soil, the results will be less profitable because only certain types of plants can adapt to the soil (Gerwin et al., 2018). Pb is a heavy metal that is often a problem in the environment because it is colorless and odorless, making it difficult to identify. Indirectly, these heavy metals damage the environment in a short time If they exceed the threshold, heavy metals in the soil will be active and cause ecological damage (Artiningsih et al., 2019). As a result, higher costs are required to manage marginal land to be profitable.

Remediation techniques that are low cost, environmentally friendly, and can maintain soil structure, namely using the addition of suitable soil bacteria on polluted soil, have been proven to increase the population of microorganisms to break up pollution in the soil (Kure et al., 2018). According to Lebrazi and Benbrahim (2018), *Rhizobium sp*. is able to absorb excess heavy metals in the soil. Ameen et al. (2020) suggested that Lactobacillus sp. is very prospect to be developed because it can absorb excess heavy metals in water and soil. Alternatively, the role of phosphate-solubilizing bacteria can convert heavy metals into ions and mobile; thus plants can absorb heavy metals through roots (Aheemad, 2014). The FOBIO biopesticide formula contains microorganisms that have a role as biological agents, decomposers, and *Plant Grow Promotion Rhizobacteria* (PGPR). The

*Corresponding author
E-mail address: suryaaw27@gmail.com

Received on 23 November 2021, revised on 16 January 2022, accepted on 22 June 2022

nutrient content in the formula can increase production/harvest yields while still paying attention to ecology, environment, populist economy, and human health (Hasyidan et al., 2021). *Brasica rapa*. L is used as an indicator plant because, apart from being economically profitable, this plant is a variety that is resistant to heavy metal stress and can translocate heavy metals into root tissue more than the crown tissue with the help of microorganisms (putra, 2020). Therefore, it is necessary to conduct research using a microorganism-based biopesticide formula (FOBIO) on soils that are stressed by heavy metals, especially Pb. Based on the above, the researchers took the title of research on combination of FOBIO biopesticide and *Brassica rapa* L. as remediator of heavy metal Pb in soil. The objectives of this research are:

1. To determine the combination of *Brassica rapa* L. and microorganism-based biopesticides (FOBIO) in absorbing Pb in soil.
2. To determine the growth of *Brassica rapa* L. at each Pb concentration
3. To determine the population of phosphate-solubilizing bacteria before and after remediation.

Research Methods

The research was carried out from July 2021 to September 2021 in Gresik Regency, East Java Province. Geographically, it is located at a position of 7.1 South latitude and 12.1 East longitude. The topography of this village is in the form of medium land, which is about 3m above sea level.

Materials

The materials used in this study include: pakcoy seeds obtained from agricultural shops and, heavy metal Pb sulfate ($PbSO_4.7H_2O$) obtained from chemical stores. Formulations of microorganism-based biopesticides (FOBIO) was made from the rhizosphere of roots of coconut, sugarcane, siwalan, tunjang, and mangroves with the carrier medium for the biopesticide formulation in the form of potato extract, sugar, black sticky rice, and meat.

Experimental design

This research was conducted using a completely randomized design, with three replications, and the treatment plot plan is shown in Figure 1. The treatments were as follows: T0 = Without heavy metal Pb; T1 = 25ppm; T2 = 50ppm; T3 = 75ppm; T4 = 100ppm; T5 = 125ppm; T6 = 150ppm.

Explanation: T = Heavy Metal Pb
U = Repetition

Research Implementation

1. Soil Analysis before remediation

The soil analysis procedure was based on Waoo, et al., (2014). First, the soil sample taken from the location was air-dried. The dried soil was then ground and sieved (2 mm mesh). About 100 g soil powder was placed in a plastic bag and added with Pb solution, according to the treatment. Pb solution was prepared using lead sulfate compounds ($PbSO_4.7H_2O$). The mixture samples were left for 7 days, then analyzed for heavy metal content using Atomic Absorption Spectroscopy (AAS) to determine the interaction of each treatment.

2. Seedling Media Preparation

The soil used for nursery media was taken from the local area of Menganti Gresik, East Java. Before planting the seedlings, the nursery media was composted with a ratio of 1: 1to maintain the soil's moisture and to facilitate the seed's. imbibition process.

3. Nursery

The seeding process was carried out according to the Dexin et al. (2012). After the soil was mixed with compost, the media was transferred to a tray. *Brassica rapa* L. seeds were ready for sowing in trays where, each hole contained 1 seed. Treatment of plant seeds continues to be carried out until they become seedlings, and ready to be transferred to polybags for testing. Plant seeds can be transferred to polybags if they have 3 leaves (10 days). The nursery media was placed in a shady place and watered once a day, in the afternoon.

T2U3	T3U2	T0U3	T3U1	T4U1	T2U1	T6U1
T4U3	T1U1	T3U3	T0U1	T5U2	T6U2	T2U2
T5U1	T1U2	T0U2	T4U2	T6U3	T1U3	T5U3

Figure 1. Treatment plot plan

4. Addition of Heavy Metals to Soil

The addition of heavy metal Pb into the growing media with concentrations of 25ppm (5.165 g/kg), 50ppm (10.331 g/kg), 75ppm (15,495 g/kg), 100ppm (20.661 g/kg), 125ppm (25.826 g/kg) and 150ppm (30,991 g/kg) from each treatment required 15 kg of soil per polybag and each treatment was repeated 3 times. The calculation method for addition is to take into account the material's purity (Borchert et al., 2021).

5. Biopesticide Formula Application

Micro-organism-based biopesticide formulas (FOBIO) were used according to operational standards based on Sukaryorini and Wiyatiningsih et al. (2021). The first step began with spraying biopesticides on the soil. This is intended to sterilize the environmental conditions of the soil against soil-borne pathogens (Soilborne Disease). The implementation of soil sterilization with biopesticide formulations was carried out by turning the soil over. ,for all treatment or all treatment plots, a concentration of 10 mL/L of biopesticide was sprayed over 3 days and repeated up to 3 times. After the plant's transplanting process, the biopesticide formulation was applied weekly with a concentration of 3 mL/L. According to Nakkeeran et al. (2005), for microorganism concentration of 50 mL/5 L requires a land area of 45.6 m^2 11.40 m x 4 m, L x W). Thus, in this study, with a polybag size (30 x 30) and weight of 15 kg required 2 mL/0.2 L of biopesticide formula per polybag for soil sterilization. As for the application of the biopesticide formula every week after planting, it required 0.59 mL/0.05 L per polybag.

6. Transplant

In the previous transplanting process, 21 polybags with a size of 30 x 30 and a soil weight of 15 kg/polybag were labeled according to thetreatments. Each polybag contained 5 plants. Each plot of *Brassica rapa* L. was placed at a distance of ~ 12 cm (Shahin and Valiollah, 2009).

7. Maintenance

a. Watering

Watering was aimed to meet the water availability for *Brassica rapa* L. plants. Wateringwas done twice a day (in the morning and evening), using a sprayer 2. The amount of water sprayed was based on the condition of the plant and the planting medium of the *Brassica rapa* L.

b. Weeding

Weeding was carried out if there are weeds growing around the *Brassica rapa* L. plant. Weeding was done manually by pulling weeds by hand.

c. Control of Plant Pest Organisms (OPT)

Plant-disturbing organisms were controlled by manually and directly taking pests on plants.

d. Fertilization

Fertilization was done twice: at the age of 2 weeks and 6 weeks after planting (Mallick et al., 2021).

8. Harvest

Harvesting was carried out when some plants entered the generative phase (11 weeks). *Brassica rapa* L. was harvested by slowly hand-pulling the plant. The hervested plant was then measured foor moisture content, wet weight, dry weight, and the concentration of Pb.

9. Soil Sample Analysis After Remediation

The Soil samples after the remediation process were taken from the post-harvest *Brassica rapa* L. plant, for all treatment, the soil was taken from the polybags and placed in plastic, then further analyzed the concentration of Pb (Hassan and Ahmad, 2015).

Observation Parameter

Heavy Metal Levels in Plants

Analysis of heavy metal content in *Brassica rapa* L. was taken when the whole plant entered the generative phase (about 10%). According to Faridah et al. (2020). the sample preparation process is carried out by wet digestion. The wet digestion process was performed by drying each sample using an oven at 80^{o}C for approximately 10 hours. It aimed to change the sample into powder to facilitate the destruction process. Then, a 2 g sample was dissolved in 65% HNO_3 and heated until the color of the solution turned brighter. The addition of hot 65% HNO_3 facilitates the oxidation of organic substances contained in the sample. About 70-72% $HClO_4$ was added as a potential oxidizing agent to form perchlorate salts (highly soluble salts) and effiently oxidize the organic material. The mixture solution was filtered using Whatman filter paper (No. 41) to separate solid and liquid fractions. The liquid fraction (or filtrate) was diluted with distilled water in a 100 mL volumetric flask and analyzed for Pb concentration at a wavelength of 283.2 nm. The measurement of (Pb) in *Brassica rapa* L. plant samples starts with measuring the absorbance of a standard solution of Pb using (AAS). The measurements were made based on standard parameters for Pb, as shown in Table 1.

Table 1. Heavy Metal Atomic Absorption Spectrophotometry Parameters for Pb

Parameters	Description
Wavelength (nm)	283.2
On Type	Air-Acytylene
Gap Width (nm)	0.7
Cathode Lamp (mA)	10
Carrier Gas	Acetylene

Heavy Metal Content in Soil

Soil analysis was carried out before and after remediation to determine the combination of the biopesticide formula FOBIO and *Brassica rapa* L. as a Pb bioremediation agent. Furthermore, the soil analysis was also done on the sample with addition of Pb at different concentration, before and after treatment.

Determination of Pb Concentration in Soil

The method used to determine Pb in soil samples followed the procedure carried out by SNI 13-6974-2003 regarding the determination of Pb, Cu, Zn, Fe, Mn, and Cd levels with (AAS). Sample destruction A total of 0.1 g of the prepared soil sample was dissolved with 5 mL of aquaregia (7.5 mL of 10 M HCl and 2.5 mL of 15.6 M HNO_3 or the ratio of HCl and HNO_3 was 3:1) (Karamina et al., 2019).

Phosphate solubilizing Bacteria Population

Soil samples were taken from each treatment polybag around the rhizosphere area of the plant. Soil samples were put in black plastic, placed into an ice flask, and taken to the soil microbiology laboratory. The soil was dried and the pH was measured. The bacteria were isolated and the population was calculated using the petri dish calculation technique (Suliasih and Widawati, 2005). According to Ekamaida (2017), the method of calculating the petri dish is the soil solution and 0.85% NaCl was prepared in duplicate in a dilution series of 10^{-6} – 10^{-8} . Then, about 0.5 mL of the solution was dripped on solid media Nutrient Agar (NA) in a petri dish and flattened using a stick spreader. The petri dishes were incubated in an inverted position for 3-4 days at 37°C. The method used to count the number of colonies is the counting cup method. The petri dishes containing colonies between 30-300 were selected and counted. If no colonies were observed, a colony number close to 300 is chosen. The principle of this method is that if living microbial cells are grown in the media, the microbes will multiply and form colonies, which are visually seen and counted without a microscope. The formula for calculating the number of colonies was based on Ekamaida (2017) as follows:

CFU / :

$$= \frac{\text{Average number of colonies each petri dish jelly x df}}{\text{Volume of dispersed culture suspension}}$$

Where df is dilution factor and CFU is Colony Forming Unit (CFU/mL)

Plant Growth *Brassica rapa L.*

The *Brassica rapa* L. plant growth was measured 10 days after the transfer until several plants in each treatment entered the generative phase. The retrieval of data taken includes:

1. Plant length

The length of the plant was measured using a ruler measuring 30 cm, from the soil surface to the growing point of the *Brassica rapa* L. plant. Measurements were carried out every seven days until some plants entered the generative phase (11 weeks). The measurement results is shown in Table 2.

2. Plant biomass

a. Gross weight

Gross weighing was carried out using an analytical balance after harvesting the plants. For all treatment, The gross weight of the plant was weighed by weighing whole *Brassica rapa* L. plants (with roots) in each polybag.

b. Dry weight

Brassica rapa L. plants (with roots) was cleaned and placed in a perforated brown paper bag, then dried using an oven at 85 ^{o}C for 24 hours. Then, weighed the total dry weight of the sample using. an analytical balance.

Data Analysis

The data were statistically analyzed by analysis of variance (ANOVA) using Ms. Excel. If the F test shows a significant effect, then it is continued with the Least Significant Difference (LSD) test at a 5% significance level.

Table 2. Average Height of *Brassica rapa* L. Plant

Treatment	Weeks after planting (cm)										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
T0	2.2	5.7^{c}	9.1^{a}	16.3^{a}	16.8^{a}	17.2^{a}	17.7^{a}	17.8^{a}	17.9^{a}	18.0^{a}	18.1^{a}
T1	2.4	6.0^{a}	8.7^{b}	16.0^{b}	16.2^{b}	16.4^{b}	16.6^{b}	16.6^{b}	16.7^{b}	16.8^{b}	16.8^{b}
T2	2.7	5.8^{b}	8.7^{b}	15.2^{c}	16.1^{b}	16.3^{c}	16.4^{c}	16.5^{b}	16.6^{b}	16.6^{b}	16.6^{b}
T3	2.5	5.7^{c}	8.2^{c}	15.3^{c}	15.8^{c}	16.0^{d}	16.0^{d}	16.1^{b}	16.2^{b}	16.2^{b}	16.2^{c}
T4	2.3	5.4^{d}	7.9^{d}	14.1^{d}	14.3^{d}	14.4^{e}	14.6^{e}	14.6^{c}	14.7^{c}	14.7^{c}	14.8^{c}
T5	2.0	5.1^{e}	6.9^{d}	13.7^{e}	13.9^{e}	14.0^{f}	14.0^{f}	14.1^{c}	14.2^{c}	14.3^{c}	14.4^{c}
T6	2.2	5.3^{d}	6.4^{e}	12.4^{f}	12.7^{f}	13.0^{g}	13.2^{g}	13.2^{d}	13.3^{d}	13.3^{d}	13.4^{d}
LSD 5 %	**NR**	**0.06**	**0.06**	**0.17**	**0.08**	**0.06**	**0.10**	**0.51**	**0.51**	**0.51**	**0.51**

Note: LSD : Least Significance Different; NR : Not Real. Values with different letter in the same row are significantly different

Results and Discussion

Plant Height

From observations, plant height is one of the parameters used to determine the plant's growth. As the plants continue to grow, it shows that plants are experiencing cell division and enlargement. The observations of plant height were carried out once a week, and The results are shown in Table 2:

Table 2 shows a significant difference in the plant's height from week 2 to week 11, was parallel to the Pb concentrations in each treatment. At week 11, the average *Brassica rapa* L. plant height from T0 treatment (without Pb addition) had the highest plant value of 26.6 cm (or 13.2 cm higher than other treatments). From week 2, the plant's height from treatment T0 was the same as T3, but higher than T4, T5, anf T6. At the same time, T1 and T2 treatment were higher than T0. Then, from week 3 to week 11, the average plant's height from T0 was higher than other treatments. This shows that the addition of Pb significantly influenced the plant's growth. This study confirmed that increasing Pb concentration inhibits, he plant's growth rate. This is in accordance with Giannakoula et al. (2021) that high concentrations of Pb can disrupt membrane integrity, decrease growth and photosynthesis, and inhibit mineral nutrition. Nas and Ali (2018) argue that Pb accumulated in various parts of the plant may negatively impact the plant physiological processes (such as, photosynthesis, respiration, mineral nutrition, membrane structure seed germination, seedling growth, germination percentage, germination index, and root/shoot length). Tolerant plants such as the brassica group can grow well even with a small amount of heavy metal accumulation. Howerver, brassica plants are, more suitable for phytostabilization than hyperaccumulator plants (Naaz and Chauhan, 2019).

Number of leaves

Leaves are organs of the plant to synthesize food for plant needs and as food reserves. Leaves have chlorophyll which functions to carry out photosynthesis. The more leaves there are the more places for photosynthesis. The number of leaves was observed once a week and the results and the results are shown in the Table 3.

Table 3 shows a significant difference in the number of leaves from week 4 to week 11, which was correlated to the concentration of Pb in each treatment.. At week 11, the average number of leaves of *Brassica rapa* L. plants from T0 treatment (without Pb addition) had the highest yield of 13.0. According to

Wiyatiningsih et al. (2021), the application of FOBIO biopesticides can increase the number of leaves. While Bhalerao (2015) stated that an increase in Pb concentration in the soil might reduce the chlorophyll content, leaf surface area, and the number of stomata, thus stunted growth.

Brassica rapa L. Plant Biomass Production

The average production of plant biomass in the form of gross weight and dry weight of *Brassica rapa* L. plants can be seen in Table 4.

Table 4 shows the highest gross and dry weight of plants was from the control *Brassica rapa* L. plant treatment (T0, without Pb Addition), with the value of 63.98 g and 19.99 g, respectively. The lowest gross and dry weight was 26.18 g and 15.51 g, resulting from T6 treatment with a concentration of 150ppm Pb. According to Nas and Ali (2018), Pbabsorbed by the roots into the plant may inhibit the enzyme formation needed for the plant's metabolic process.

Table 3. The Average Number of Leaves of *Brassica rapa* L. Plants

Treatment	Weeks after planting (sheet)										
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**
T0	2.9	4.1	5.9	7.1^{a}	8.5^{a}	9.9^{a}	10.8^{a}	11.4^{a}	12.1^{a}	12.5^{a}	13.0^{a}
T1	2.8	4.0	5.4	6.7^{b}	8.1^{b}	9.3^{b}	10.1^{b}	10.3^{b}	10.5^{b}	10.7^{b}	10.7^{b}
T2	2.9	4.1	5.2	6.6^{bc}	7.9^{bc}	8.9^{bc}	9.8^{bc}	9.9^{b}	9.9^{bc}	9.9^{b}	10.0^{b}
T3	2.8	4.0	5.1	6.4^{bc}	7.3^{cd}	8.5^{cd}	9.2^{cd}	9.3^{b}	9.3^{c}	9.6^{b}	9.6^{b}
T4	2.7	3.7	5.1	6.5^{c}	7.6^{d}	8.7^{d}	9.4^{d}	9.5^{b}	9.7^{c}	9.9^{b}	9.9^{b}
T5	2.8	3.6	4.7	5.7^{d}	6.5^{e}	7.0^{e}	7.7^{e}	8.1^{c}	8.1^{d}	8.3^{c}	8.3^{c}
T6	2.9	4.0	4.8	5.6^{d}	6.5^{e}	6.9^{e}	7.7^{e}	7.9^{c}	8.0^{d}	8.0^{c}	8.0^{c}
LSD 5 %	**NR**	**NR**	**NR**	**0.21**	**0.32**	**0.38**	**0.38**	**1.02**	**0.96**	**1.15**	**1.09**

Note: LSD: Least Significance Dfferent); NR: Nor Real. Values with different letter in the same row are significantly different.

Table 4. Average yield of *Brassica rapa* L. Plant Biomass

Treatment	Gross weight (Gram)	Dry weight (Gram)
T0	63.98^{a}	19.99^{a}
T1	50.02^{b}	18.06^{b}
T2	47.01^{bc}	18.05^{b}
T3	44.98^{c}	17.09^{b}
T4	39.59^{d}	14.57^{c}
T5	38.80^{d}	13.96^{c}
T6	26.18^{e}	10.51^{d}
LSD 5 %	**4.07**	**1.19**

Note: LSD (Least Significance Different). Values with different letter in the same row are signifivantly different.

The metabolic process in the plant includes the respiration process to produce ATP to be further used for photosynthesis. The results from photosynthesis are used for cell division (i.e. height, the number of leaves, and biomass yield). Thus, if inhibition of the metabolic process occurs, this may disrupt the plant's reproduction. The activities of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX) are usually increased to reduce oxidative stress caused by heavy metals. However, a high concentration of heavy metals is present, this may decrease enzymatic activity (Rabelo and Borgo, 2016).

Heavy Metal Content in Plants

Table 5 shows the concentration of Pb before and after the treatment. The results show that the highest Pb concentration was found at concentrations of 150 ppm from T6 treatment. A higher heavy metal absorbed by plants, is parallel to a higher metal accumulation in plant tissues. This condition may cause saturation, thus limiting the absorption and decreasing the ability of plants to absorb the metals (Unadkat and Parikh, 2017).

The Brassica group is a plant that is tolerant of heavy metals because it can reduce the level of damage caused by the disruption of the redox system of cells. This is achieved by reducing metal uptake in cell vacuoles, which can form metal-binding chelators (phytochelatins), and through induction of enzymatic and nonenzymatic detoxification systems on exposure to metal stress (Qodir et al., 2014).

Number of Phosphate-Solubilizing Bacteria

Table 6 shows the number of phosphate-solubilizing bacteria before and after treatment. The results indicate there are bacterial colonies that are still alive. This proves that the role of phosphate-solubilizing bacteria makes Pb easy to transport, thus easily absorbed by *Brassica rapa* L. plants.

Table 5. Pb Concentration in *Brassica rapa* L. Plants and Phosphate-Solubilizing Bacteria

Treatment	Ppm	CFU
T0	0.20^{e}	4.03^{a}
T1	2.11^{de}	3.10^{b}
T2	3.44^{cd}	2.03^{c}
T3	3.97^{bc}	0.60^{d}
T4	4.81^{abc}	0.54^{d}
T5	5.36^{ab}	0.42^{d}
T6	5.95^{a}	0.34^{d}
LSD 5 %	1.82	0.79

Note: LSD (Least Significance Diierent). Values with different letter in the same row are significantly different

Table 6. The Number of Phosphate-Solubilizing Bacteria

Treatment	Repetition		
	U1	U2	U3
T0	3.8×10^4	3.4×10^4	4.9×10^4
T1	2.9×10^4	4.1×10^4	2.3×10^4
T2	1.9×10^4	2.1×10^4	2.1×10^4
T3	6.5×10^3	5.5×10^3	6.1×10^3
T4	5.2×10^3	5.6×10^3	5.3×10^3
T5	4.1×10^3	4.8×10^3	3.8×10^3
T6	3.9×10^3	3.1×10^3	3.2×10^3

According to Hasyidan et al. (2021), adding microorganisms is critical in the remediation process of soil contaminated with heavy metals. Bacteria can strengthen the effect of plant remediation on soil contaminated with heavy metals in two ways. First, bacteria have an adsorption effect on heavy metals and reduce the toxicity of heavy metals to plants in the soil. Second, releasing organic acids and nutrients needed for plant growth increases the absorption of heavy metals by hyperaccumulator plants. Soil inoculation with phosphate-solubilizing microorganisms produces gibberellins compounds that can stimulate the plant's growth. These bacteria also affect the development of root hairs, secretion of sap, lateral development of the plant roots and can correct phosphorus deficiency in plants (Wuryandari et al., 2017). However, if the soil contains a higher concentration of Pb, it will cause a decrease in soil microorganisms, resulting in a lack of soil nutrients further and leading to disruption of the plant's metabolism (Baikhamurova et al., 2020).

Conclusion

Brassica rapa L. and FOBIO microorganism-based biopesticide formula can reduce Pb concentration in the soil. The treatment were significantly different starting from week 2 for the plant's height and week 4 for the number of leaves.all treatments show a significant difference in the plant biomass an increase in the concentration of Pb leads to more inhibition of the plant growth. In each treatment, there was a population of phosphate-solubilizing bacteria. However in treatment T3 (75 ppm), T4 (100ppm), T5 (125ppm). and T6 (150ppm). the number of phosphate-solubilizing bacteria decreased drastically compared to treatment T0 (Control), T1 (25ppm), and T2 (50ppm).

Declarations

Conflict of interests The authors declare no competing interests.

References

Ahemad, M. (2014) 'Phosphate solubilising bacteria assisted phytoremediation of metalliferous soil : A review Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils : A review', *3 Biotech*, 5, pp. 111-121

Ameen, F. A., Hamdan, A. M., and El-Naggar, M. Y. (2020) 'Assessment of the heavy metal bioremediation efficiency of the novel marine lactic acid bacterium, *Lactobacillus plantarum* MF042018', *Scientific Reports*, 10(1), pp. 1–11

Artiningsih, A., Zubair, H., Imran, A. M., and Widodo, S. (2019) 'The potential and contamination of metals Pb and Zn on the soil around Tamangapa Antang landfill', *IOP Conference Series: Earth and Environmental Science*, 314, pp. 1-8

Baikhamurova, M. O., Sainova. G. A., Abseyit. A., Tashmetova, G., and Kalesbayev, K. (2020) 'The influence of lead on the growth and development of various mustard types', *Eurasian Journal of Bioscience*, 14, pp. 57-64

Bhalerao. S. A., Sharma, A. S., and Poojari, A. C. (2015) Toxicity of nickel in plants', *Internasional Journal Pure And Applied Bioscience*, 3(2), pp. 345-355

Borchert, K. B. L., Boughanmi, R., Reis, B., Zimmermann, P., Steinbach, C., Graichen, P., Svirepa, A., Schwarz, J., Boldt, R., Schwarz, S., Mertig, M., and Schwarz, D. (2021) 'Removal of lead, cadmium, and aluminum sulfate from simulated and real water with native and oxidized starches', *Polysaccharides*, 2(2), pp. 429–453

Dexin, S., Evanylo, G., and Goatley, J. M. (2012) 'Effects of compost sources and seeding treatments on germination and emergence of four turfgrass species', *Compost Science & Utilization*, 20(3), pp. 165-170

Ekamaida, E. (2017) 'Counting total bacteria in land organic waste household and land inorganic with total plate count method (TPC). *Jurnal Penelitian Agrisamudra*', 4(2), pp. 87-91

Faridah, D. N., Solihat, I ., and Yuliana, N. D. (2020) 'Validation of mineral (Fe, Zn, and Cu) analysis methods in carbohydrate, protein and fat-rich samples using microwave digestion method' *Indonesia Journal of Chemistry*, 20 (2), pp. 348 - 359

Gerwin, W., Repmann, F., Galatsidas, S., Vlachaki, D., Gounaris, N., Baumgarten, W., and Freese, D. (2018) 'Assessment and quantification of marginal lands for biomass production in Europe using soil-quality indicators', *SOIL*, 4(4), PP. 267–290

Giannakoula, A., Therios, L., and Chatzissavvidis, C. (2021) 'Effect of lead and copper on photosynthetic apparatus in Citrus (*Citrus aurantium L.*) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress', *Plants*, 10(1), pp. 1-14

Hasan, S., and Ahmad. Z. (2015) 'Quantitative analysis of lead (Pb) in soil and grass grown along road side with low, medium and high traffic intensity', *International Research Journal of Biological Sciences*, 4(4), pp. 12-21

Hasyidan, G., Wiyatiningsih, S., and Suryaminarsih, P. (2021) 'Aplikasi biopestisida fobio dan *Streptomyces sp.* untuk mengendalikan penyakit moler pada tanaman bawang merah (Application of biopesticide fobio and *Streptomyces sp.* to control moler disease in onion plants)', *Jurna*l *AGROHITA*, 6(2), pp. 168-173 [In Indonesian]

Karamina, H., Widowati., and Mudjoko, T. (2019) 'The analysis of the heavy metal iron (Fe), cadmium (Cd), copper (Cu) content in crystal guava (*Psidium Guajava L.*) and soil at Batu', *Jurnal Pembangunan dan Alam Lestari*, 10(2), pp. 141-145

Kure, J., Gana, M., Emmanuel, A., Isah, R., and Ukubuiwe, C. (2018) 'Bacteria associated with heavy metal remediation: A review', *International Journal of Applied Biological Research*, 9(1), pp. 134–148

Lebrazi, S., and Benbrahim, K. F. (2018) 'Rhizobium-legume symbioses: Heavy metal effects and principal approaches for bioremediation of contaminated soil', in Meena, R. S., Das, A., Yadav, G. S., and Lal, R. (eds.) *Legumes for Soil Health and Sustainable Management*. Singapore: Springer, pp. 205-233

Mallick, S. K., Jyotirmayee, B and Mahalik, G. (2021) 'Use of organic fertilizer and the potential influence on *Brassica rapa* L. and soil nutrient composition', *Bulletin of Environment, Pharmacology and Life Sciences*, 10(9), pp. 29-35

Naaz, G. and Chauhan, K. L. (2019) 'Lead tolerance and accumulation potential of *Brassica juncea* L. Varieties in imitatively contaminated soil', *Research Journal of Life Science, Bionformatics, Pharmaceutical, adn Chemical Sciences*, 5(2), pp. 436–447

Nakkeeran, S., Fernando, W, G, D., and Siddiqui, Z, A. (2005) Plant growth promoting rhizobacteria formulations and its scope in commercialization for the mangement of pests and diseases in Siddiqui, Z, A. (eds.) *PGPR: Biocontrol and Biofertilization*. Dordrecht: Springer, pp. 257-296

Nas, F. S., and Ali, M. (2018) 'The effect of lead on plants in terms of growing and biochemical parameters: A review', *Ecology and Environmental Sciences*, 3(4), pp. 265-268

Putra, R, M., Sarkawi, M., Ramadhan, A, D., and Shopiar, S, P. (2020) 'Double aeration system on the enhancement of EAPR for removal of lead (Pb) and uptake by pakcoy (*Brassica rapa* subsp. *Chinensis*): An evaluation of using phytomorphology changed', *AIP Conference Proceedings*, 2242(1), pp. 1-5

Qodir, S., Hameed, A.,Nisa, N., Azooz, M. M., Wani, M. R., Hasannuzaman, M., Kazi, A. K., and Ahmad, P. (2014) Brassicas : Responses and

tolerance to heavy metal stress' in Ahmad, P., Wani, M. R., Azooz, M. M., and Tran, L. S. P. (eds.) *Improvement of Crops in the Era of Climatic Changes Vol 2*. New York: Springer, pp 1-35

Rabêlo, F. H. S., and Borgo, L. (2016) 'Changes caused by heavy metals in micronutrient content and antioxidant system of forage grasses used for phytoremediation: An overview', *Ciência Rural*, *46*(8), pp. 1368–1375

Shahin, A., and Valiollah, R. (2009) 'Effects of row spacing and seeding rates on some agronomical traits of spring canola (*Brassica napus* L.) cultivars' *Journal of Central European Agriculture*, 10(1), pp. 115-122

Sukaryorini, P., and Wiyatiningsih, S. (2009) Peningkatan ketahanan kultivar bawang merah terhadap *Fusarium oxysporum* F.sp. cepae penyebab penyakit moler menggunakan formula suspensi mikroorganisme (Increased resistance of shallot cultivars to *Fusarium oxysporum* F.sp. cepae that causes moles using a suspension formula of microorganisms). In: *Prosiding Seminar Nasional Kebijakan Agribisnis Dalam Pemberdayaan Masyakat Tani*, UPN Veteran Jawa Timur, Surabaya, 19 November 2009, pp 78-85 [In Indonesia]

Suliasih and Widawati, S. (2005) 'Isolation and identification of phosphate solubilizing and nitrogen fixing bacteria from soil in Wamena Biological Garden, Jayawijaya, Papua', *Journal of Biological Diversity*, 6(3), pp. 175-177

Unadkat, K., and Parikh, P. (2017) 'A review on heavy metal absorption capacity of aquatic plants: Sources, impact and remediation technique', *International Journal of Allied Practice, Research and Review*, 4(12), pp. 23-30

Waoo, A. A., Khare, S., and Ganguli, S. (2014) 'Extraction and analysis of heavy metals from soil and plants in the Industrial Area Govindpura, Bhopal', *Journal of Environment and Human*, 1(2), pp. 2373-8332

Wiyatiningsih, S., Suryaminarsih, P., and Hasyidan, G. (2021) 'Pemanfaatan fobio dan *Streptomyces sp*. dalam meningkatkan pertumbuhan daun bawang merah (Utilization of phobio and Streptomyces sp. in increasing the growth of shallots)', *Jurnal Sains dan Teknologi Pertanian Modern*, 2021, pp. 39–45 [In Indonesian]

Wuryandari, Y., Wiyatiningsih, S., and Maroeto. (2017) 'Introduksi formula pupuk hayati berbahan aktif pseudomonad flourescent isolat PF-122 untuk meningkatkan pertumbuhan dan produksi tanaman cabai dilapang (The introduction of a biological fertilizer formula with the active ingredient pseudomonad flourescent isolate PF-122 to increase the growth and production of chili plants in the field)', *Jurnal Hama Dan Penyakit Tumbuhan Tropika*, 17(1), pp. 156 - 161 [In Indonesian]