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THE POTENTIAL OF *Zinnia elegans* AND *Bacillus* sp. as Lead (Pb) BIOREMEDIATION AGENTS

Potensi *Zinnia elegans* dan *Bacillus* sp. sebagai Agensia Bioremediasi Timbal (Pb)

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ABSTRACT
Lead (Pb) pollution in soil is a serious problem that reduces crop productivity. One approach to address this issue is through bioremediation and Phytoremediation, using microorganism and plants that can tolerate and bind heavy metals. This study examine the potential of Zinnia elegans (Jacq.) and Bacillus sp., and in combination to reduce Pb in soil. The experiment lasted 60 days using a completely randomized design with six treatments: P0 (control, Pb-contaminated soil without plants or bacteria), P1 (Z. elegans only), P2 (Bacillus sp. only), P3 (combination), P4 (non-contaminated soil with Z. elegans and Bacillus sp.), and P5 (non-contaminated soil with Z. elegans only). The results showed that treatment P3 reduced Pb concentration by 62.31 ppm, while no significant differences were observed among treatments in terms of plant growth parameters. Combining Z. elegans with Bacillus sp. may serve as a practical approach to enhance Pb remediation in contaminated soils.

Keywords: *Bacillus sp., Bioremediation, Lead (Pb), Phytoremediation, Zinnia elegans*

ABSTRAK
Pencemaran timbal (Pb) pada tanah merupakan masalah besar yang dapat menurunkan
Pencemaran timbal (Pb) pada tanah merupakan masalah besar yang dapat menurunkan produktivitas tanaman dan mengancam kualitas lingkungan. Salah satu cara untuk mengatasi masalah ini adalah melalui bioremediasi dan fitoremediasi, yang mengandalkan mikroba dan tanaman yang mampu mentoleransi serta mengikat logam berat. Penelitian ini berfokus pada peran *Zinnia elegans* (Jacq.) dan *Bacillus* sp., yang diaplikasikan secara tunggal maupun kombinasi, dalam menurunkan Pb di tanah. Percobaan dilakukan selama 60 hari dengan rancangan acak lengkap yang terdiri dari enam perlakuan: P0 (kontrol, tanah tercemar Pb tanpa tanaman dan bakteri), P1 (*Zinnia elegans* saja), P2 (*Bacillus* sp. saja), P3 (kombinasi *Z. elegans* dan *Bacillus* sp.), P4 (tanah tidak tercemar dengan *Z. elegans* dan *Bacillus* sp.), dan P5 (tanah tidak tercemar dengan *Z. elegans* saja). Parameter yang diamati meliputi kadar Pb tanah, tinggi tanaman, jumlah daun, dan biomassa tanaman. Hasil penelitian menunjukkan bahwa P3 menurunkan kadar pb sebanyakk 62,31 ppm, namun pada parameter pertumbuhan tanaman tidak berbeda nyata antar perlakuan. Hasil ini menunjukkan bahwa kombinasi tanaman dengan bakteri yang menguntungkan dapat menjadi pendekatan praktis untuk meningkatkan remediasi Pb pada tanah tercemar.

Kata Kunci: *Bacillus sp., Bioremediasi, Timbal (Pb), Fitoremediasi, Zinnia elegans*

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INTRODUCTION

The agricultural industry in Indonesia has grown rapidly in recent decades. This growth has been supported by efforts to optimize land use including the use of synthetic pesticides. Although this measure has been able to boost national food productivity, this practice also has consequences for environmental quality. Synthetic pesticides are used to control plant pests to increase agricultural production, but uncontrolled use of pesticides tends to leave residues in the soil. Heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As) are the dangerous substances found in many insecticides (Silviani et al., 2022).This might happen because some of the chemicals that make insecticides effective also contain the heavy metal lead (Pb). Liquid pesticide formulations generally use active ingredients dissolved in solvents such as xylene, naphthalene, and kerosene. kerosene is a product of crude oil distillation mixed with carrier substances such as kaolin, lime, sand, and clay. The combination of these ingredients has the potential to contribute to the presence of heavy metals, including lead (Pb) in pesticides. Some pesticides containing lead heavy metals are Antracol 70 WP, Dithane M 45 80 WP, Furadan 3G, Goal 240 EC, Buldog 25 EC, Hostathion 200 EC, and Profile 430 EC (Karyadi et al., 2011 in Arlinda et al., 2023).

Lead is a non-essential metal that becomes toxic to plants when accumulated at high concentrations. Excessive lead uptake in plant tissues reduces the absorption of essential elements such as magnesium (Mg), iron (Fe), and nitrogen (N) which are key components in chlorophyll formation due to competition for cation exchange capacity. The deficiency of these elements causes chlorosis symptoms in plant tissues and reduces the volume and number of chloroplasts (Ariyanti et al., 2015) This leads to a reduction in plant quality and quantity, which impacts land productivity. To address this issue, physical and chemical remediation methods such as ion exchange, precipitation, reverse osmosis, evaporation, and chemical reduction have been applied, but they are costly and environmentally harmful (Yulianti, 2021) A more economical, simple, and efficient soil remediation method is bioremediation, which utilizes living microorganisms, and phytoremediation, which utilizes hyperaccumulator plants.

Zinnia elegans is commonly used as a refuge plant (Anita & Haryadi, 2022) *Z. elegans* is a hyperaccumulator plant, meaning it can absorb or tolerate heavy metals such as lead (Pb) and chromium (Cr) through its roots, making it a potential agent for phytoremediation of contaminated soil (Ehsan et al., 2016). According to juhriah et al., (2023), *Z. elegans* can absorb more than 50% of Pb from contaminated soil. *Bacillus* sp. alone can degrade or accumulate heavy metals, making it a potential agent for effective bioremediation. Hasyimuddin et al., (2018) reported that *Bacillus* sp. can reduce heavy metal concentrations such as Pb due to the bacterial cell wall's ability to bind these metals. Rahadi et al., (2020) demonstrated that *Bacillus* sp. could reduce Pb by up to 69.1% in a bacterial reduction test using NB media. The *Bacillus* sp. isolate applied to Pb-contaminated soil in this study was obtained from Purkan et al.,(2017), isolated from Malang agriculture and tested for mercury (Hg) resistance due to its ability to produce mercury reductase enzymes. This isolate has also undergone antagonistic testing against the pathogen Ralstonia solanacearum and has been shown to suppress its growth (Prasetyawati et al., 2023). The interaction between *Bacillus* sp. and *Z. elegans* is expected to reduce heavy metal concentrations in lead-contaminated soil, making it suitable for agricultural use and supporting plant growth. This study aimed to determine the potential of *Bacillus* sp. and *Zinnia elegans* to reduce lead (Pb) content in growing media and to analyze their effect on plant growth.

MATERIALS AND METHODS

Location and Time

The research was conducted from March to June 2025 at Plant Health Laboratory 1 and the greenhouse of the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" East Java, Indonesia.

Materials

The tools used in this research included an autoclave, Erlenmeyer flasks, measuring cups, hot plates with stirrers, shakers, tissues, AAS instruments, sprayers, incubators, colony counters, measuring pipettes, Petri dishes, stoves, laminar air flow cabinets, and shovels.

The materials used in this study included *Bacillus* sp. bacteria from the collection of Dr. Dra. Endang Triwahyu Prasetyawati, M.Si, soil, NA and NB media, *Zinnia elegans* seeds, lead nitrate [$Pb(NO_3)_2$], polybags, seedling trays, and 5% formalin.

Methods

This study used a Completely Randomized Design with a non-factorial arrangement, consisting of six treatments and three replications, resulting in 18 experimental units. The treatments were as follows: P0 (Pb-contaminated soil (control)); P1 (Pb-contaminated soil + *Zinnia elegans*); P2 (Pb-contaminated soil + *Bacillus* sp.*)*; P3 (Pb-contaminated soil + *Bacillus* sp*.* + *Z. Elegans)*; P4 (Non-sterilized, uncontaminated soil + *Bacillus* sp*.* + *Z. Elegans)*; P5 (Non-sterilized, uncontaminated soil + *Z. Elegans)*. Observed parameters included soil Pb content and plant growth (plant height, number of leaves, fresh weight, and dry weight). Data were analyzed statistically using Analysis of Variance (ANOVA), followed by an Honestly Significant Difference (HSD) test at the 5% significance level when significant differences were detected.

Preparation of *Bacillus* sp.

Bacillus sp. is purified by taking the isolate with inoculating needle and streaking it on slanted Nutrient Agar medium in a test tube using the streak plate method. The purification process is carried out aseptically using a Bunsen burner in a Laminar Air Flow. The medium containing the isolate is then incubated at room temperature for 48 hours before use (Wati et al., 2021).

Preparation of heavy metals (Pb) stock solutions

The metal stock solutions used were 40, 60, 80, and 100 ppm. The Pb stock solution was prepared by dissolving 1.598 g of $Pb(NO_3)_2$ in 1000 mL of distilled water. The stock solution was then diluted as required using the following formula (Nisak, et al., 2013)

$$V1 \times N1 = V2 \times N2$$

Note:
V1 = volume of stock solution required
N1 = concentration of stock solution required
V2 = known stock solution volume
N2 = known stock solution concentration

***Bacillus* sp. Range Finding Test**

The resistance of *Bacillus* sp. to Pb was tested to confirm bacterial resistance as a preliminary step before bioremediation application. Pure *Bacillus* sp. cultures were grown in nutrient broth containing Pb at concentrations of 40, 60, 80 and 100 ppm and incubated for 72 hours. The cultures were then diluted to 10^{-8}, plated on nutrient agar, incubated for 24 hours, and colony numbers were determined using the colony count formula with the Total Plate Count (TPC) method. Colony counting was performed using the following formula (Putri and Kurnia, 2018):

Number of colonies (CFU/mL) = Number of colonies per plate x $\frac{1}{Dilution\ factor}$

Plant and Soil preparation

Plant preparation was carried out by sowing *Zinnia elegans* seeds in plastic seedling trays containing a planting medium composed of a 1:1 mixture of soil and compost. After three weeks, seedlings were moved into polybags containing the treated soil. The soil used is a mixture of soil and compost in a 1:1 ratio, with 5 kg in each polybag. The medium was then sterilized with 5% formalin at 2.5 mL/kg, covered for seven days, and then air-dried for another seven days (Mevianti et al., 2021). The sterilized media in each polybag was then added with Pb at a concentration of 100 ppm.

Bacillus sp. suspension was prepared by suspending isolates from slanted agar in 10 ml of distilled water and harvesting the cells with an inoculating needle. The suspension was then transferred into 100 ml of Nutrient Broth (NB) and incubated on a shaker at 150 rpm for 48 hours. Bacterial density was adjusted to 10^8 CFU/mL using serial dilution. A total of 100 ml of suspension was mixed with 900 ml of distilled water, and 100 ml was applied per polybag (5 kg soil) by pouring and mixing into Pb-contaminated soil (Esringü et al., 2014).

Plant Control and observation

Plant control includes manual weeding by pulling weeds directly. Plants are watered once a day in the morning as needed. Mechanical pest control, which involves removing pests directly, is carried out if pests are found on *Zinnia elegans* plants. Plant growth was observed based on plant height, number of leaves, and biomass (dry and wet weight). Plants were placed in a semi-open greenhouse with natural ventilation, the temperature during the study ranged between 28–33°C, with relative humidity between 65–85%, and natural light intensity around 10,000–40,000 lux during the day. Plant height and leaf number were measured every seven days, specifically on days 7, 14, 21, 28, 35, 42, 49, and 56.

Analysis of lead (Pb) concentration in soil

The soil was analyzed for lead (Pb) content at the beginning of the experiment, which was before and after the application of heavy metal Pb, and at the end after harvesting. Soil samples were analyzed to detect heavy metal lead content using the Atomic Absorption Spectrometry (AAS) method (Anggraeni and Triajie, 2021)

HASIL DAN PEMBAHASAN

Bacillus sp. Range Finding Test

The RFT resistance test results show that *Bacillus* sp. are able to grow at all tested lead (Pb) concentrations, namely 40 ppm, 60 ppm, 80 ppm, and 100 ppm (Figure 1.). The results of the total colony count in the RFT test showed that the highest number of colonies was found at a heavy metal Pb concentration of 60 ppm (1.99×10^{10} CFU/mL). The growth of bacteria in media containing Pb may indicate the presence of a resistance mechanism to metals. *Bacillus* sp. bacteria can still live and reproduce well under Pb heavy metal stress up to a concentration of 60 ppm (Table 1)., then the number of colonies decreases with increasing Pb heavy metal concentration, with the lowest number found at a concentration of 100 ppm (1.34×10^{10} CFU/mL). (Miranti et al., 2021) stated that higher metal concentrations cause greater damage to bacterial cells, resulting in fewer bacterial colonies. *Bacillus* sp. is resistant to lead (Pb) within a certain concentration range. However, the reduction in colony numbers occurs because lead ions enter bacterial cells through transport pathways that are normally used for essential metals. Lead exerts toxic effects by altering the structure of nucleic acids and proteins, inhibiting enzyme activity, impairing cell membrane function, disrupting energy production, and causing osmotic imbalance within bacterial cells (Fashola et al., 2016).

Table 1. Colony Counts in the Range Finding Test at Various Heavy Metal Concentrations

Pb Concentration (ppm)	Total colonies (CFU/mL)
40	1.95×10^{10}
60	1.99×10^{10}
80	1.54×10^{10}
100	1.34×10^{10}

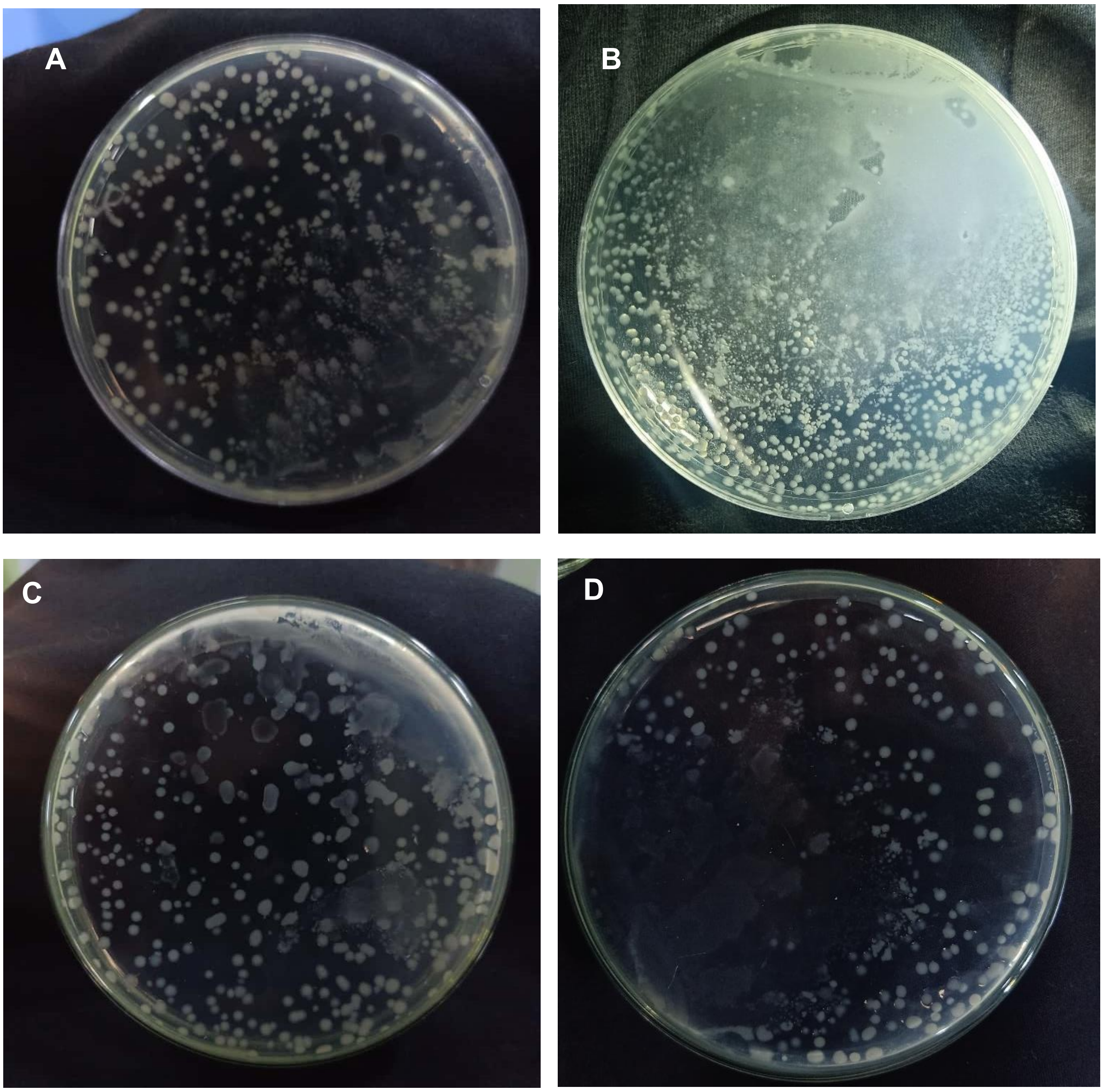

Figure 1. Results of bacterial colony count (Total Plate Count) on media with treatment concentrations of (a) 40 ppm, (b) 60 ppm, (c) 80 ppm, and (d) 100 ppm.

Analysis of lead (Pb) concentration in soil

The results of the ANOVA on lead (Pb) concentrations in soil before and after treatment showed significant differences among treatments. The initial Pb concentration before treatment in Pb-contaminated media at 100 ppm was highest in P1 (95.36 ppm) and lowest in P2 (40.79 ppm). Meanwhile, treatments P4 and P5 which served as controls without Pb addition were still detected to contain Pb at 5.79 ppm and 5.41 ppm,

Based on the results of Pb concentration analysis after treatment, a reduction was observed in all treatments. The highest reduction occurred in P3 (7.80 ppm) with a decrease of 62.31 ppm, while the lowest reduction was found in P2 (12.75 ppm) with a decrease of 28.04 ppm. The higher reduction in P3 compared to P2 and P1 indicates that *Bacillus* sp. can synergize with *Z. elegans* in the remediation of Pb in soil. This finding is consistent with Niu et al., (2021), who stated that *Bacillus* sp. can enhance Pb remediation by transforming soil Pb into more soluble and available forms, making it easier for roots to absorb and helping the accumulation of Pb from the root part of the plant to the upper part of the plant so that the plant can absorb more metl concentrations.

Bacillus sp. itself in this study was proven to reduce heavy metals Pb in soil, as seen from the results of the decrease in metal concentration in P2. The decrease in Pb metal concentration by *Bacillus* sp. is associated with the mechanisms of biosorption and bioaccumulation. Biosorption occurs passively without depending on cell metabolism and is related to the tendency of heavy metals to attach to the surface of bacterial cell walls (Rocco et al., 2024). Meanwhile, bioaccumulation occurs actively and requires transporters to bring lead into the cell, or through simple diffusion from high to low concentrations. *Bacillus* sp. exposed to lead will produce proteins in the cytoplasm, such as heat shock protein (HSP), metallothionein (MT), glutathione S-transferase (GST), and ubiquitin, which help bind and retain lead in the cell so that heavy metals do not harm other cells (Sevak et al., 2021).

The *Zinnia elegans* plant from the metal concentration test results (Table 4.2) showed a significant decrease in treatment P1 of 54.74 ppm (from 95.36 ppm to 40.62 ppm). This indicates that *Z. elegans* plants themselves have the potential to become phytoremediation agents that reduce the concentration of heavy metals (Pb) in the soil. (Amri and Adifa., 2025) explain that phytoextraction begins when metal ions start to dissolve with root exudates (organic acids and phytosiderophores) and are released from the soil. These ions are then absorbed by the roots, transported through the xylem to the upper parts of the plant, and stored in vacuoles or bound to cell walls. This mechanism allows heavy metals to be removed from the soil through the harvesting of plant biomass in the form of leaves.

Table 2. Lead (Pb) Concentration in Soil Before and After Treatment

Treatment	Pb Concentration (ppm)		Pb Reduction (ppm)
	Before Treatment	After Treatment	
P0	71.25 c	70.56 d	0.69
P1	95.36 d	40.62 c	54.74
P2	40.79 b	12.75 b	28.04
P3	70.11 c	7.80 b	62.31
P4	5.79 a	0.01 a	5.78
P5	5.41 a	0.39 a	5.02

Note: Numbers followed by the same letter indicate no significant difference according to the HSD test at the 5% level

Growth of *Zinnia elegans* Plants

The results of the ANOVA on plant height (Table 3.) showed no significant differences among treatments. The presence of Pb and the application of *Bacillus* sp. did not have a significant effect on the height growth of *Z. elegans* during the observation period. At week 8, *Z. elegans* in treatment P1 (Pb-contaminated soil + *Z. elegans*) reached an average height of 36.16 cm, which was almost the same as treatment P5 (uncontaminated soil + *Z. elegans*) with an average height of 37.5 cm. This indicates that *Z. elegans* is tolerant to Pb contamination in the growing medium under the conditions of this study.

Table 3. Plant height of *Zinnia elegans*

Treatment	Plant Height (cm) Week-							
	1	2	3	4	5	6	7	8
P1	18.50 a	21.67 a	26.00 a	30.16 a	31.67 a	32.67 a	33.83 a	36.16 a
P3	17.00 a	19.00 a	27.67 a	30.83 a	34.67 a	37.33 a	39.83 a	42.67 a
P4	20.00 a	26.33 a	31.67 a	34.83 a	38.00 a	40.33 a	42.00 a	44.50 a
P5	17.83 a	22.33 a	25.83 a	28.16 a	31.00 a	33.50 a	35.33 a	37.50 a

Note: Numbers followed by the same letter indicate no significant difference according to the HSD test at the 5% level

Table 4. *Number of Leaves on Zinnia elegans Plants*

Treatment	Number of Leaves Week-							
	1	2	3	4	5	6	7	8
P1	6.67 a	8.00 a	6.67 a	7.67 a	7.00 a	7.33 a	10.00 a	9.67 a
P3	6.67 a	7.33 a	8.67 a	9.00 a	10.33 a	12.00 b	12.67 a	12.33 a
P4	6.67 a	8.00 a	8.33 a	9.33a	10.00 a	12.67 b	13.33 a	14.00 a
P5	6.67 a	7.33 a	6.00 a	7.00 a	8.00 a	9.00 ab	12.00 a	10.33 a

Note: Numbers followed by the same letter indicate no significant difference according to the HSD test at the 5% level

The results of the ANOVA test of the observation data on the number of leaves (Table 4.) show significant differences between treatments in the sixth week. The average number of leaves in P3 (12 leaves) and P4 (12.67 leaves) was significantly different from treatment P1 (7.33 leaves). Treatments involving *Bacillus* sp. (P3 and P4) affected the increase in leaf number in the sixth week based on the 5% HSD test. This indicates that the presence of bacteria influenced leaf formation through the PGPR (Plant Growth-Promoting Rhizobacteria) mechanism, as *Bacillus* sp. is capable of producing phytohormones such as auxins and gibberellins that stimulate vegetative plant growth (Choliq et al., 2020).

The average number of leaves in the first to fifth weeks of the four treatments showed no significant differences. In week 8, P4 had the highest average number of leaves, namely 14 leaves, followed by P3 with 11.67 leaves, P5 with 10.33 leaves, and the lowest in treatment P1 with 9.67 leaves. The higher leaf number and consistent increase in P4 occurred because the plants were not exposed to heavy metal stress and benefited from the presence of *Bacillus* sp., which supported leaf development. Treatments P1 and P3 showed the lowest average leaf numbers due to the toxic effects of Pb. Although the average leaf number in P3 showed a consistent increase in the previous weeks due to the role of *Bacillus* sp. in leaf formation, by the eighth week a decrease was observed as a result of heavy metal accumulation. This is consistent with Zhou et al., (2018), who reported that one of the toxic effects of Pb ions is the reduction in leaf number due to disruption of the photosynthesis process.

Wet weight and dry weight are important parameters for determining the accumulation of biomass produced from the physiological process of plant growth. The wet weight of plants indicates the total mass of plant tissue that still contains water, while the dry weight of plants indicates the total plant biomass formed from photosynthesis and dense plant tissue. The results of the ANOVA test of wet weight and dry weight data indicate that there is no significant difference between wet weight and dry weight data. Based on the data (Table 5.), the highest wet weight was obtained in treatment P4 at 4.14 g, followed by P5 (3.56 g), P3 (3.05 g), and the lowest value was found in treatment P1 at 2.60 g. Although not significantly different, the wet weight values of P3 and P1 tended to be lower than those of P4 and P5. This indicates the effect of Pb on the growth of *Z. elegans* plants, where the uncontaminated growing medium in P4 and P5 allowed the plants to grow slightly better than in treatments P1 and P3, where growth was slightly inhibited by the presence of Pb heavy metal. These results are in line with the statement by Zulfiqar et al., (2019) where high Pb concentrations in the roots cause root growth to be disrupted, loss of apical dominance, and a reduction in wet weight of up to 10%. The highest dry weight based on (Table 5.) was the dry weight in treatment P4, which was 0.85 g, and the lowest was in treatment P1, which was 0.57 g. This is in line with the wet weight of the plants, where treatment P4 with *Bacillus* sp. and without heavy metal Pb contamination showed the most optimal growth, while treatment P1 exposed to heavy metals without *Bacillus* sp. treatment was the lowest due to slight growth inhibition. Plants that grow well will have a higher dry weight (Afifudin et al., 2022)

Table 5. Wet Weight and Dry Weight Results of *Zinnia elegans* Plants

Treatment	Wet weight (g)	Dry weight (g)
P1	2.60 a	0.57 a
P3	3.05 a	0.63 a
P4	4.14 a	0.85 a
P5	3.56 a	0.68 a

Note: Numbers followed by the same letter indicate no significant difference according to the HSD test at the 5% level

CONCLUSION

The results of this study demonstrated that *Bacillus sp.* was able to grow at all Pb concentrations tested (40–100 ppm), with the highest colony count observed at 60 ppm. Analysis of Pb concentration in soil showed a reduction in all treatments, with the greatest decrease occurring in the combination of *Bacillus sp.* and *Zinnia elegans*. This indicates a synergistic role of bacteria and plants in Pb remediation. Growth data showed that *Z. elegans* is tolerant to Pb, while the presence of *Bacillus sp.* supported leaf formation through PGPR activity. Although wet and dry weights were not significantly different, the highest values were obtained in treatments without Pb and with bacterial inoculation. In conclusion, the combination of *Bacillus sp.* and *Z. elegans* has potential as an effective agent for Pb bioremediation in soil.

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