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Compost Biostimulation for Improved Soil Bioremediation using Endophytic *Bacillus* Sp.

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The use of bacterial endophytes in soil bioremediation is limited by nutrient availability in the soil. In this study, garden compost was used as a biostimulant during the bioremediation of lead contaminated soil with a bacterial endophyte. The experimental data showed that the cell wall of the *Bacillus* sp. strain MHSD_36 resulted in 36% lead biosorption. The addition of glucose and peptone in the growth media, enhanced biomass growth and lead biosorption, achieving a maximum of 76% lead removal. The use of compost as a cheaper carbon and nitrogen source improved the biosorption capacity of strain MHSD_36 during the bioremediation of lead contaminated soil. Compost biostimulation resulted in a residual lead of 250 mg/Kg, from an initial concentration of 300 mg/Kg, compared to 282 mg/Kg without biostimulation. The endophytic bacteria *Bacillus* sp. strain MHSD_36 is a potential lead biosorbent. Moreover, compost is an organic biostimulator with potential application in soil decontamination.

1. Introduction

The fast growth nature of bacteria and ability to adapt their growth strategies in harsh environments has accelerated their interest and use in bioremediation (Pande et al., 2020). Bacteria possess an array of mechanisms for the detoxification of hydrocarbons and heavy metals because they carry a diverse set of catabolic genes and enzymes (Pal et al., 2022). Bacteria synthesize and secrete surface active compounds such as siderophores and biosurfactants and use efflux pumps to remove toxic compounds from the cytoplasm to the periplasm and environment (Presentato et al., 2020). Bacteria can modify their cell membrane to maintain biological function and survive in contaminated environments (Dell'Anno et al., 2023). Furthermore, bacteria can adsorb heavy toxic metals, such as Pb, Hg, Zn, Cd and Cu, on their cell wall (Priyadarshanee and Das 2021).
Bacterial biosorption is regarded as the most reliable and efficient method for the bioremediation of toxic heavy metals (Rizvi et al., 2020). Bacterial biosorption is an effective method because of its specificity for certain metal ions and potential application at low concentrations (Tarekegn et al., 2020). Moreover, biosorption is a passive process facilitated by the presence of active chemisorption sites on the cell wall or extracellular polysaccharides (Mosa et al., 2016). The cell wall functional groups are responsible for the physico-chemical interactions with metal ions. The physical interaction mechanisms involve electrostatic interactions while chemical interaction involve ion exchange displacement of metal cations.
Biosorption can be constrained by low nutrient availability in the soil, and this consequently affects the bioremediation efficiency of bacteria (Qin et al., 2013). Biostimulation is the addition of limiting nutrients to promote sufficient biomass growth and optimal biosorption (Priyadarshanee and Das 2021). Biostimulation, using organic solids or synthetic fertiliser, enhances the bioremediation efficiency and accelerates the process (Roy et al., 2011). The use of organic fertilizer and agricultural manure can, however, result in environmental pollution through nutrient runoff into the ecosystem and water bodies (Litskas et al., 2023).
Bacterial biosorption is an attractive option for soil bioremediation however, the use of synthetic fertilizer and manure to support biomass growth is economically and environmentally unsustainable. This necessitates the prospection of alternative organic and renewable biostimulants for application in the bioremediation of contaminated soil. Garden waste is an abundant renewable resource and can be valorised into organic compost which is a rich source of carbon and nitrogen.

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This approach will alleviate pressure from landfills and does not pose threat to food and energy security. Therefore, this study investigated the feasibility of using organic compost, as a sustainable biostimulant, in the bioremediation of lead (Pb) contaminated soil using a bacterial endophyte, *Bacillus* sp. strain MHSD_36, isolated from the medicinal plan *Solanum nigrum*.

2. Materials and Method

2.1 Bacterial strains maintenance and growth

Routine culture maintenance for the *Bacillus* sp. strain MHSD_36, isolated *from Solanum nigrum*, was done by plating a 30% glycerol stock of the bacterial culture on nutrient agar and incubation for 24 h at 28°C. The bacterial culture was grown on nutrient broth (NB) at 30°C, 150 rpm for 24 h.

2.2 Pb biosorption

The Bacterial culture grown on NB was harvested and washed with distilled water by centrifugation at 2500g for 10 mins at 4ºC. The cells were resuspended in fresh media with Pb (10 mg/L) and without Pb and incubated for 24 h and subsequently centrifuged at 2500g and 4ºC for 15 min. The cells were washed twice with 0.03 mol/L Tris buffer containing 2.5 X 10^{-3} mol/L EDTA, pH 8.0 and resuspended in the buffer. Lysozyme was added to a final concentration of 200 mg/mL and incubated for 30 min at 25ºC for the spheroplast preparation. This was followed with centrifugation at 3500 rpm for 15 min and resuspension in 0.03 mol/L Tris buffer containing 3 x 10^{-3} mol/L EDTA, pH 8, for the spheroplasts collection. The periplasmic fluid was contained in the supernatant. The spheroplasts were disrupted through the vibronic ultrasonic processor, followed by centrifugation at 3000 rpm for 15 min to enable the removal of debris and unbroken cells. The resulting supernatant consisting of membrane and cytoplasmic fractions was centrifuged at 3500 rpm for 30 min. The pellet consisted of both outer and inner membrane envelopes. The obtained fractions were used for the Pb determination using Inductively coupled plasma optical emission spectroscopy (ICP-OES) after centrifugation at 4000 rpm for 10 min and filter sterilization with a 0.45 µm syringe filter. The % Pb recovered from the different fractions was calculated according to equation 1;

$$\%\ Pb\ recovery = \frac{Pb\ (\frac{mg}{L})}{Inital\ Pb\ (\frac{mg}{L})}\ x\ 100 \quad (1)$$

2.3 Determining the impacts of carbon and nitrogen source on Pb biosorption

Batch experiments were conducted in 50 ml Erlenmeyer flasks containing 20 mL of nutrients (glucose or peptone) prepared according to the central composite design (CCD). The experiments were carried out with 150 mg/L of Pb, inoculated with 1% v/v cells grown to late exponential phase, and incubated for 48 h at 30ºC with shaking at 150 rpm. The culture broth was subsequently centrifuged at 4000 rpm for 10 min, filter sterilized with a 0.45 µm syringe filter and the residual Pb determined using ICP-OES. The % Pb removal was calculated according to equation 2;

$$\%\ Pb\ removal = \frac{Residual\ Pb\ (\frac{mg}{L})}{Inital\ Pb\ (\frac{mg}{L})}\ x\ 100 \quad (2)$$

2.4 Soil remediation with compost biostimulation

Plastic pots containing 1kg of soil contaminated with Pb (300 mg/Kg) were used for the soil remediation experiments. Compost (40% w/w), obtained from a local nursery, was added as a biostimulant. A compost control was included in the experimental set-up. 100 ml of bacterial culture suspension with a concentration of 1 x 10^6 cfu/ml was added to the pots. The pots were left standing at room temperature, water was sprayed every second day to keep the soil moist and the soil mixed weekly to ensure sufficient aeration. The experiments were performed in triplicates and terminated after 30 days and the residual Pb in the soil determined with mass spectrometry.

2.5 Experimental design

A five-coded levels central composite design (CCD) was used to determine the impact of the carbon (glucose) and nitrogen (peptone) on Pb biosorption. The experiment had 13 runs including 5 center points. The factors were used with the following range, glucose (0.68, 1.0, 1.75, 2.5, 2.81 g/L), and peptone (0.17, 1.0, 3.0, 5.0, 5.8 g/L). The general formula for the response is shown in the equation below:

$$y_i = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j + \epsilon \quad (3)$$

where y_i is the i^{th} response variable, x_i is the i^{th} input parameter, n is the number of input parameters and β_0, β_i, β_{ii}, β_{ij} are the fixed response, linear, quadratic, and cross products coefficients, respectively.

Design–Expert (Stat-Ease Inc. Minneapolis, MN, USA) was used for the experimental design and statistical analysis at 5% level of significance.

2.6 Statistical analysis

All the experiments were performed in triplicates and results were presented in the form of mean ± SD. ANOVA was performed at 5% level of significance to check the significance of the findings.

3. Results and discussion

3.1 Biosorption of Pb by *Bacillus* sp. strain MHSD_36 and distribution in biomass

Cell wall biosorption is one of the main mechanisms through which bacteria remove toxic heavy metals from the environment. The experimental data illustrated that approximately 44% of Pb was removed from the broth, thus the residual Pb was approximately 56%. A significant amount of the Pb removed from the broth was recovered from the cell wall fraction (Figure 1) of strain MHSD_36. The Pb recovery from the cell wall was equivalent to 36% of Pb from the initial concentration. Furthermore, approximately 5% was recovered from the cell membrane (Figure 1). The 5% Pb recovery from the cytoplasmic fraction illustrates the ability of strain MHSD_36 to actively remove toxic heavy metals from the cells to the periplasm. The greater bacterial cell wall Pb biosorption capacity can be attributed the presence of lipids, proteins and polysaccharides which are significantly composed of metal binding groups such as phosphates, sulphates, amino and carboxyl groups (Javanbakht et al., 2014).

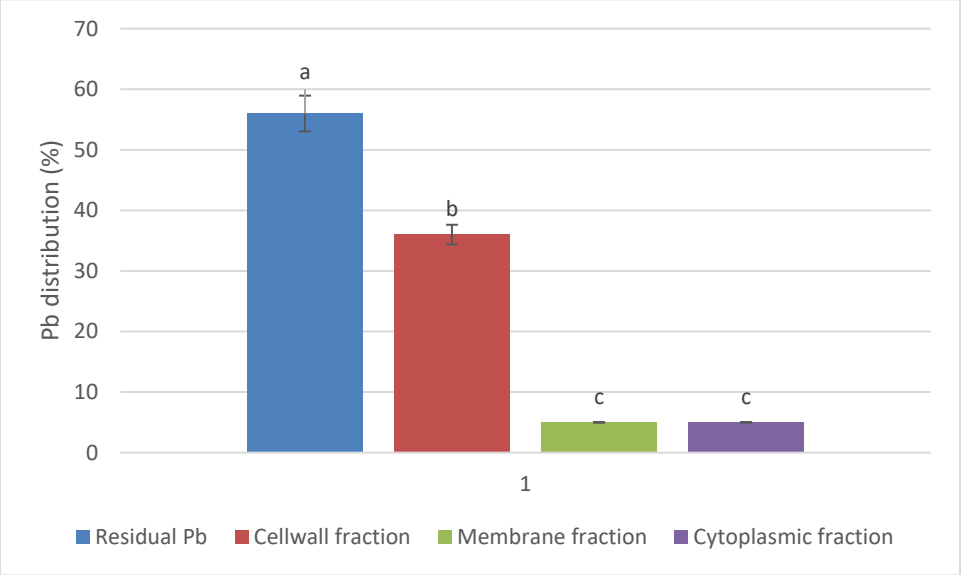

Figure 1: Biosorption and distribution of Pb by Bacillus sp. strain MSHD_36 strain. The percentage (%) distribution of Pb from the cell wall, cell membrane and cytoplasm are based on the total amount (%) of the initial Pb in the contaminated water. The data is represented as mean values ± standard deviation of the three replicates. The different letters represent significant differences ($p < 0.05$)

3.2 The impact of nitrogen and carbon on Pb biosorption by the cell wall

Biostimulation provides bacteria with important nutrients and micronutrients for biomass growth to support biosorption. A statistical design was applied to determine the impact of glucose and peptone on the biosorption capacity of strain MHSD_36. The ANOVA data (Table 1) showed that carbon and nitrogen were important for both biomass growth and efficient biosorption of the heavy metal Pb. Moreover, the adjusted R^2 was 0.8081 and 0.8749, respectively. Thereby 80.81% variation in biomass growth and 87.49% variation in the % biosorption of Pb, by strain MHSD_36, can be attributed to the glucose and peptone concentrations.

The impact of glucose and peptone on biomass growth and Pb biosorption is shown in Figure 2. Biomass growth increased with an increase in the concentration of glucose and peptone (Figure 2a). Pb biosorption followed a similar trend however, the biosorption reached a peak as the nutrient concentration increased further (Figure 2b). The maximum attained Pb removal was 76%. Excessively high biomass concentration results in biomass aggregation and consequently a reduction in bacterial contact surface area and metal binding sites, thereby negatively affecting the biosorption efficiency (Limcharoensuk et al., 2015).

Table 1: Analysis of Variance for biomass growth and Pb biosorption

Source	d.f.	Biomass growth			Pb biosorption		
		Co-efficient	Sum of squares	p-value	Co-efficient	Sum of squares	p-value
Linear							
Glucose (A)	1	1.76	24.75	0.0103	-6.07	294.77	0.0400
Peptone (B)	1	2.86	65.55	0.0008	2.35	44.22	0.3622
Quadratic							
A^2	1	1.25	16.98	0.0236	-14.53	1467.83	0.0008
B^2	1	-1.56	0.0272	0.9115	-18.73	2441.50	0.0003
Interaction							
AB	1	1.25	6.25	0.1241	9.02	325.34	0.0333
Regression	12		128.00			4464.82	
Residual error	7		14.33			325.89	
Lack of fit	3		4.33	0.6602		186.21	0.2940
Pure error	4		10.00			139.67	
Model	5		113.67	0.0032		4138.93	0.0007
R^2		0.8881			0.9270		
Adjusted R^2		0.8081			0.8749		
Intercept	1	10			87.43		

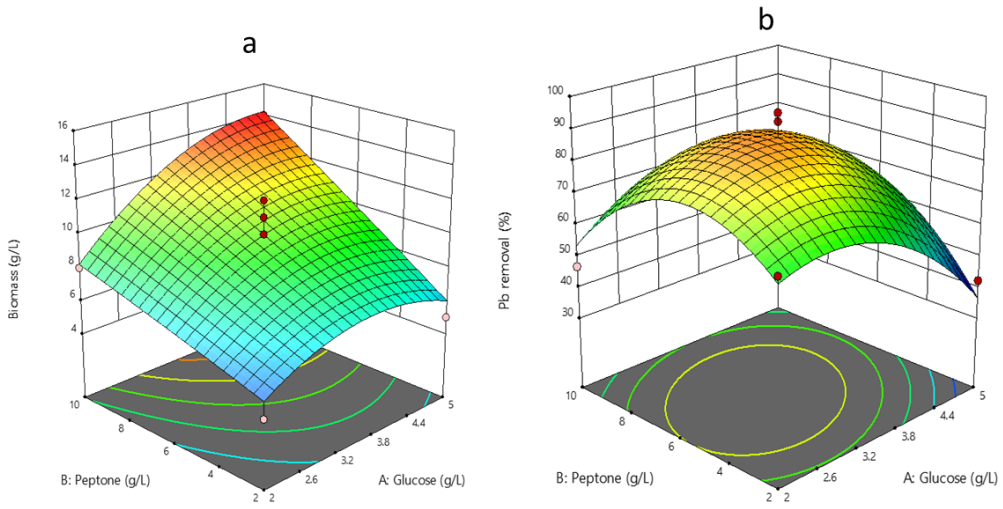

Figure 2: Response surface plot showing the interactive effects of glucose (A) and peptone (B) on biomass growth (a) and Pb biosorption (b).

3.3 Soil remediation with compost biostimulation

Compost, a rich source of carbon and nitrogen, can be applied as a biostimulant in bioremediation to enhance the soil environment and stimulate bacterial growth. Organic compost can be used to alleviate nutrient limitation in the soil and improve the water holding capacity of the soil thereby improving the bioremediation efficiency. Biostimulation studies were carried out in pot plants, with an initial Pb of 300 mg/Kg, over a period of 30 days. The residual Pb from contaminated soil is represented in Figure 3. The data shows that biostimulation with organic compost significantly improves the biosorption capacity of strain MHSD_36. Compost is a rich source of carbon and nitrogen as well as other important micronutrients required for bacterial growth (Mladenov, 2018).

The residual Pb was 250 mg/Kg when the soil was treated with compost-biostimulated strain MHSD_36 compared to 282 mg/Kg when the soil was treated with strain MHSD_36 only (Figure 3). The addition of compost, without the bacterial culture, resulted in a residual Pb of 265 mg/Kg from the contaminated soil. The residual Pb was however, lower than the compost-MHSD_36 treatment. Thus, compost has the capacity to act as biosorbent of the heavy metal Pb in addition to biostimulating biomass growth and enhancing the biosorption efficiency of strain MHSD_36.

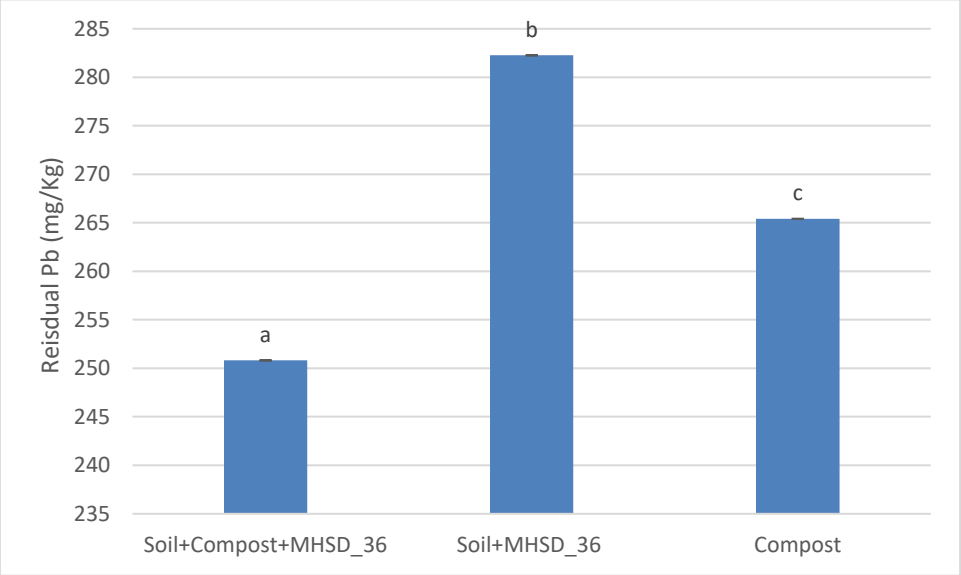

Figure 3: Residual Pb from contaminated soil treated Bacillus sp. strain MSHD_36 strain. The data is represented as mean values ± standard deviation of the three replicates. The different letters represent significant differences ($p < 0.05$)

3. Conclusions

The potential of organic compost as a biostimulant for the bioremediation of Pb contaminated soil using a bacterial endophyte, *Bacilllus* sp. strain MHSD_36, was demonstrated. Strain MHSD_36 demonstrated a great capacity for application as a biological biosorbent for the heavy metal Pb bioremediation. Moreover, the addition of compost, during soil remediation, enhanced the biosorption capacity of the strain. The strain resulted in residual Pb of 250 mg/Kg compared to 282 mg/Kg without compost biostimulation. Interestingly, compost is also a potential biosorbent and thereby can be used as a both a biostimulant and biosorbent in heavy metal bioremediation. The use of compost for biostimulation offers an inexpensive, organic, and renewable carbon and nitrogen source for application in soil bioremediation.

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