PLANT SCIENCE TODAY
Vol 13(sp2): 1–15
https://doi.org/10.14719/pst.12515

eISSN 2348-1900

RESEARCH ARTICLE

Biosorption of heavy metal using *Aspergillus niger*: An eco-friendly approach for lead remediation

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Received: 30 October 2025; Accepted: 31 March 2026; Available online: Version 1.0: 15 June 2026

Cite this article: Ritu R, Swapnil S, Visakha B, Anupriya R, Pradip K. Biosorption of heavy metal using *Aspergillus niger*: An eco-friendly approach for lead remediation. Plant Science Today. 2026; 13(sp2): 1-15. https://doi.org/10.14719/pst.12515

Abstract

Heavy metal contamination has emerged as a major environmental concern due to its persistence, toxicity and bioaccumulation in ecosystems. Among these, lead (Pb) poses serious environmental and public health risks due to industrialisation and urbanisation. These non-biodegradable metals persist in ecosystems, bioaccumulate through food chains causing carcinogenic and teratogenic effects. Conventional heavy metal removal methods are often costly and inefficient at low concentrations, necessitating alternative remediation approaches. This study investigated *in vitro* training of *Aspergillus niger* against lead sulphate and evaluated the biosorption capacity of trained fungal biomass for lead removal from aqueous solutions. Fungal strains isolated from Meerut district soils (Uttar Pradesh, India) were progressively trained on lead sulphate-supplemented media (1000–4000 ppm). Biosorption experiments were conducted at Chaudhary Charan Singh (CCS) University, Meerut (January - June 2016), using trained and untrained biomass (10–40 mg) in lead solutions (100 and 200 ppm) with 10 min contact time. The training regimen revealed concentration-dependent growth responses, with maximum radial growth stimulation of 68 % observed at 2500 ppm, while concentrations exceeding 3000 ppm exhibited growth inhibitory effects. At 100 ppm, untrained 10 mg biomass showed highest biosorption (68.39 %) with Q-value of 341.95 mg/g. Trained M1 biomass (1000 ppm) exhibited 61.63 % biosorption with Q-value of 308.15 mg/g. At 200 ppm, trained M1 biomass demonstrated superior performance (44.28 %) compared to control (41.40 %). Freundlich isotherm model (R^2 = 0.984 for control at 100 ppm) showed better fit than Langmuir model, suggesting multilayer adsorption and heterogeneous surface binding. Overall, *in vitro* metal training enhanced fungal tolerance while maintaining effective biosorption capacity, highlighting its potential for sustainable lead remediation from contaminated waters.

Keywords: *Aspergillus niger;* Freundlich isotherm; heavy-metal pollution; *in vitro* training; lead bioremediation

Introduction

Pollution refers to the introduction of contaminants into the natural environment, causing adverse changes to ecosystems, human health and quality of life. Among various pollutants, heavy metals are a major environmental concerndue to their non-biodegradable nature and tendency to bioaccumulate through food chains (1, 2). Although some metals like iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are essential micronutrients, excessive accumulation results in severe toxicity in plants and animals (3, 4). Heavy metals are generally defined as metallic elements with density greater than 5 g/cm^3 or 6 g/cm^3, possessing relatively high atomic mass and exhibiting toxicity even at low concentrations (5, 6). Pollution caused by heavy metals is one of the most significant environmental problems affecting soil and water quality globally. The rapid industrialization and modern technological advancements have led to the discharge of various toxic metals such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni) and mercury (Hg) into natural ecosystems (7). These metals interfere with metabolic processes, damage cellular components and inhibit enzymatic activity (8).

Lead (Pb) is recognised as one of the most toxic and hazardous heavy metals to the environment and living organisms. Lead pollution specifically originates from numerous point and non-point sources including automobile emissions, battery manufacturing facilities, metal processing plants, paint industries, cable production, pigment manufacturing, steel and alloy production, glass manufacturing and plastic industries (9). The health implications of lead exposure are profound and multifaceted, encompassing neurological disorders, renal damage, gastrointestinal dysfunction, reproductive system impairment, haemoglobin synthesis inhibition and irreversible neurological damage particularly affecting paediatric populations whose developing nervous systems demonstrate heightened vulnerability (10, 11). Conventional methodologies employed for heavy metal removal from contaminated water and wastewater streams include chemical precipitation, ion exchange processes, membrane separation technologies, filtration systems and evaporation techniques. However, these conventional approaches frequently encounter significant operational limitations including high capital and operational costs, generation of secondary toxic

sludge's requiring additional treatment, incomplete metal removal particularly at low concentrations and technological complexities in implementation and maintenance (12, 13).

Bioremediation, utilising microorganisms such as bacteria, fungi and algae for pollutant removal, has emerged as a promising alternative (14). Biosorption, defined as the passive sequestration of metal ions by biological materials, has emerged as a promising green technology for heavy metal removal. Microbial-mediated remediation shows appreciable potential for ecosystem restoration and sustainable practices (15). Fungal biomass offers distinct advantages as biosorbents due to high surface area-to-volume ratios, abundant binding sites on cell walls (carboxyl, phosphate, amino and sulfhydryl groups) and tolerance to extreme environmental conditions (16). Several fungal genera including *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* have exhibited substantial tolerance against various heavy metal concentrations (17–19). Among these fungal species, *Aspergillus niger* is a ubiquitous saprophytic fungus widely employed in biotechnology for enzyme production (amylases, cellulases, pectinases) and organic acid fermentation. Its well-characterised physiology, high surface-area-to-volume ratio and ability to grow on cheap substrates make it a model organism for biosorption research (20). *Aspergillus niger* has consistently exhibited remarkable tolerance across a broad spectrum of heavy metals, including Pb, Cu, Zn, Ni and Cd, with documented efficient uptake of metallic cations (Cu^{2+}, Zn^{2+}, Cd^{2+}) from contaminated industrial effluents, thereby positioning it as a highly promising organism for metal bioremediation studies (21). The mycelial growth architecture confers competitive advantages over unicellular organisms, particularly regarding colonisation of insoluble substrates and biosorption capacity (22). Fungal cell walls comprise diverse functional groups including carboxyl, hydroxyl, sulfhydryl, phosphate and amino groups, which serve as binding sites for metal cations through mechanisms including electrostatic attraction, ion exchange, complexation, chelation and microprecipitation (23).

In vitro training involves exposing microorganisms' especially fungal strains to progressively increasing metal concentrations to enhance tolerance through repeated sub-culturing and potentially improve biosorption capacity (24). This training approach operates on the principle that repeated exposure to sublethal metal concentrations induces physiological adaptations or selects for spontaneous mutants exhibiting enhanced metal tolerance (25). Such adaptations may increase expression of metal-binding proteins, enhanced efflux system activity, modified cell wall composition increasing binding site availability, or intracellular sequestration mechanisms (26). The potential for training to improve biosorption efficiency while simultaneously enhancing metal tolerance presents significant practical advantages for industrial applications where biosorbents must function effectively in environments containing high and variable metal concentrations (27). Adsorption equilibrium modelling makes the utilisation of Langmuir and Freundlich isotherms that provides critical insights into biosorption mechanisms (28). The Langmuir model assumes monolayer adsorption on homogeneous surfaces with equivalent binding sites (29), while the Freundlich model accommodates surface heterogeneity and multilayer adsorption (30).

Lead contamination represents a priority environmental pollutant requiring cost-effective, environmentally sustainable and technically efficient removal technologies. The present investigation examined the biosorption potential of *A. niger* for lead remediation through systematic training and characterisation. The specific objectives were to: (i) isolate and morphologically identify *A. niger* strain from environmental soil samples, (ii) train the isolated strain *in vitro* through progressive exposure to escalating lead sulphate concentrations, (iii) assess the biosorption capacity of untrained and variously trained biomasses against different lead concentrations and biomass dosages and (iv) evaluate Freundlich and Langmuir adsorption isotherm models for describing experimental biosorption equilibria.

Materials and Methods

Soil samples collection

Soil samples were aseptically collected from unpolluted areas Meerut district, Uttar Pradesh, India, following established standardised protocols for microbiological sampling. The samples were carefully transported to the Microbiology and Biotechnology Laboratory, Department of Biotechnology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India, where all experimental procedures and analyses were conducted. During January–June 2016, approximately 1 kg of soil was collected from each sampling location. Sampling procedure involved the removal of superficial soil layer using a sterile stainless-steel trowel to eliminate extraneous organic litter, plant debris and surface contaminants. Soil samples from depths of 5–15 cm below the surface were then aseptically collected using flame-sterilised trowels and transferred into fresh sterile polyethylene bags and stored at 4 °C until processing. The soil samples were analysed for indigenous mycobiota population using the serial dilution plate technique (31, 32).

Isolation of fungal species

Fungal isolation was performed using the serial dilution plate method on potato dextrose agar (PDA) medium (33). The PDA medium was prepared following standard protocols (34). All glassware was dry-sterilised in a hot-air oven at 160 °C for 3 hr before use.

Soil dilution series was prepared by transferring 20 g soil sample into 200 mL of sterile distilled water, creating an initial dilution of 1:10. This suspension was vigorously stirred using a magnetic stirrer for 30 min at room temperature. 10 mL of this primary suspension were aseptically transferred to 90 mL of sterilised distilled water, generating a 1:100 dilution. This procedure was continued to prepare serial dilutions of 1:1000 and 1:10000. From the 1:100 and 1:1000 dilutions, 1 mL aliquots were transferred to 3 replicate sets of three Petri dishes each (31, 32). 20 mLof cooled molten PDA medium maintained at approximately 45 °C and supplemented with 30 ppm streptomycin sulphate that was poured into each petri dish. The Petri dishes were gently rotated to ensure thorough mixing and uniform distribution. After solidification, plates were inverted and incubated at 25±1 °C for 4–5 days, with daily observations recorded from the 3rd day onwards.

Morphological identification of fungal isolates

Incubated Petri plates were observed from the 3rd day when fast-growing fungi appeared. Slow-growing fungi were transferred to separate plates using a flame-sterilised inoculation loops to prevent overgrowth by fast-growing species (32, 33, 35). The identification of fungal species to generic and specific levels was accomplished through detailed examination of morphological

characteristics including colony colour and texture, growth rate, sporulation structures, conidiophore morphology and spore characteristics (36, 37). A comprehensive record was maintained documenting all fungal species. Pure cultures were obtained through repeated single-spore isolation or hyphal tip transfer techniques (31). After identification pure culture of *A. niger* was maintained by continuously sub-culturing within a week on PDA medium and incubated at 28 ± 2 °C for active growth (Fig. 1).

Preparation of lead sulphate solutions

Stock solution (5000 ppm) of Pb metal in the form of lead sulphate ($PbSO_4$) was prepared to assess the metal tolerance and biosorption capacity of isolated fungal biomass. All glassware used for solution preparation and storage was acid-washed with dilute nitric acid followed by thorough rinsing with double-distilled water to prevent any metal contamination from previous uses. Stock solutions of $PbSO_4$ were prepared at concentrations of 100–4000 ppm at varying intervals (9). Solutions were prepared fresh before each experiment to prevent precipitation or degradation of the metal salt.

In vitro training for enhanced metal tolerance

Aspergillus niger was selected for *in vitro* training experiments based on preliminary screening results demonstrating superior growth rate and overall performance in metal-amended media. The training protocol was designed to progressively expose the fungal strain to incrementally increasing concentrations of lead sulphate through 9 sequential steps (38, 39). In step 1, PDA media containing 100, 200, 400 and 500 ppm $PbSO_4$ were prepared alongside control media (unamended PDA). Streptomycin (30 ppm) was added to prevent bacterial contamination of each medium type were prepared separately with 30 ppm streptomycin sulphate. Approximately 25 mL of each medium type were dispensed into separate sets of 3 Petri dishes (40). After solidification, actively growing mycelium of *A. niger* was transferred to the centre of each plate using a flame-sterilised cork borer. The treatment designations were M (control), M_1 (100 ppm), M_2 (200 ppm), M_3 (400 ppm) and M_4 (500 ppm). All plates were incubated at 25 ± 1 °C for 4 days, after which colony diameters were measured in two perpendicular directions to minimise the changes and averaged. Steps 2–9 followed systematic protocols with progressively increasing $PbSO_4$ concentrations of 1000, 1500, 2000, 2500, 3000, 3500 and 4000 ppm respectively. In each subsequent step, control medium plates were inoculated with mycelium from previous step control plates, while metal-amended plates were inoculated with mycelium from the highest concentration plates of the previous step. Each step consistently employed 6 Petri plates comprising 3 control and 3 metal-amended plates, with identical incubation conditions (22, 24). At each step, radial growth was recorded and percentage stimulation or inhibition was calculated relative to control.

Fungal biomass production

After *in vitro* training of *A. niger* through progressive lead exposure, biomass from the untrained control strain and each trained variant was cultured in liquid medium (Fig. 2). Malt-glucose-yeast-peptone (MGYP) broth medium was selected for biomass production (41). The medium composition consisted of 3 g malt extract, 10 g glucose, 3 g yeast extract and 5 g peptone dissolved in 1000 mL distilled water, with pH adjusted to 6.8. A total of 45 Erlenmeyer flasks (250 mLcapacity) were employed, with 5 flasks for untrained control and 40 flasks for trained biomasses. 100 mL of sterile broth were dispensed into each flask followed by autoclaving. After cooling, each flask was inoculated with actively growing mycelium. The flasks were incubated at 27 ± 1 °C on an orbital rotatory shaker at 130 rpm for 7 days. After incubation when dense mycelial mats had developed, fungal biomass was separated from culture filtrate through filtration and washed with distilled water. The biomass from 5 replicate flasks of each strain was pooled together and used in wet form for biosorption studies.

Biosorption experimental design

Biosorption studies were conducted using wet fungal biomass at four different amounts (10, 20, 30 and 40 mg) with $PbSO_4$ solutions at 2 concentrations (100 and 200 ppm). Each treatment used 50 mL solution in 250 mL flasks (42). The experimental design included: Control (untrained) biomass: M and trained biomass from different concentrations: M_1 (1000 ppm), M_2 (1500 ppm), M_3 (2000 ppm), M_4 (2500 ppm), M_5(3000 ppm), M_6 (3500 ppm) and M_7 (4000 ppm). All biomass containing flasks were placed on a rotatory shaker at moderate speed for 10 min contact time and contact time period was selected based on previous research demonstrating optimal metal uptake (43, 44). After 10 min,

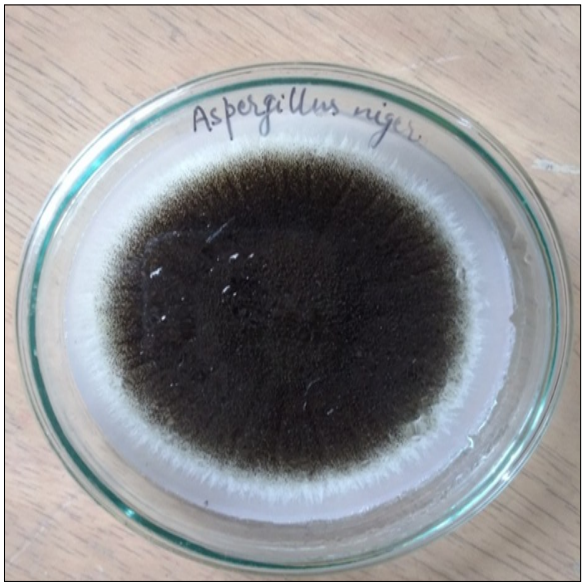


Fig. 1. Axenic culture of *Aspergillus niger* van Teighem.



Fig. 2. *Aspergilus niger* biomass in malt-glucose-yeast-peptone medium.

biomass was filtered through plastic sieves and the pooled filtrate from 3 replicates was analysed. Potassium iodide was added to form a lead-iodide complex (45) and residual Pb was quantified spectrophotometrically at 645–650 nm using standard calibration curves (41).

Data analysis

Metal uptake capacity

Specific metal uptake (Q, mg g^{-1}) and removal efficiency (R%) were calculated using standard equations (42, 43):

$$Q = \frac{V(C_i - Cf)}{m}$$

$$R = \frac{(C_i - Cf)}{c_i} \times 100$$

Where, V = solution volume (mL), C_i and Cf = initial and final concentrations (mg L^{-1}) and m = dry biomass (g).

The biosorptive efficiency of biomass was interpreted according to an established classification where Q-values from 0–10 indicate very poor biosorption capacity, 10–20 indicate poor capacity, 20–40 indicate moderate capacity, 40–60 indicate good capacity, 60–80 indicate very good capacity and 80–100 indicate excellent biosorption capacity.

Adsorption isotherm modelling

Experimental biosorption equilibrium data were analysed using Freundlich and Langmuir isotherm models, following recent approaches for biosorption characterisation (46). The Freundlich equation is expressed as (47):

$$q_e = K_F C_e^{1/n},$$

Where, q_e = metal adsorbed per unit weight at equilibrium (mg/g), K_F= Freundlich constant C_e= equilibrium concentration (mg/L) and n= adsorption intensity. The Langmuir equation is expressed as (29):

$$q_e = \frac{(q_{max} K_L C_e)}{(1 + K_L C_e)}$$

Where: q_{max} = maximum adsorption capacity (mg/g) and K_L = Langmuir constant (L/mg).

To evaluate model fitness and determine isotherm parameters, linear regression analysis was performed on the linearised forms of both Freundlich and Langmuir equations, with correlation coefficients (R^2) calculated to assess the goodness of fit (48, 49).

Results

Effect of *in vitro* training on fungal growth and metal tolerance

Aspergillus niger exhibited differential growth responses when trained on increasing $PbSO_4$ concentrations (Fig. 3, 4 and Table 1). At control condition, mean radial growth of fungal colonies measured 6.87 cm after 4 days of incubation at 25 °C. At 1000 ppm $PbSO_4$, mean radial growth increased to 7.60 cm, representing growth stimulation of 9.04 %. At 1500 ppm, the adapted strain exhibited mean radial growth of 8.34 cm, corresponding to 34.0 % stimulation. Further increase to 2000 ppm resulted in mean radial growth of 8.04 cm with 39.0 % stimulation. Maximum growth stimulation of 68.0 % was observed at 2500 ppm. However, beyond this concentration, growth inhibition occurred. At 3000 ppm, mean radial growth decreased to 5.64 cm (29.5 % reduction). At 3500 ppm, growth measured 5.30 cm (35.13 % reduction). At 4000 ppm, severe growth inhibition of 76.0 % was observed, with complete inhibition at concentrations exceeding 4000 ppm.

Biosorption performance at 100 ppm lead concentration

At 100 ppm test concentration, biosorption efficiency varied significantly with biomass amount and training status (Table 2). Untrained biomass (10 mg) achieved the highest biosorption (68.39 %), corresponding to a Q-value of 341.95 mg/g. Among trained biomasses, M_1 (trained at 1000 ppm) exhibited maximum removal (61.63 %, Q-value = 308.15 mg/g), followed by M_6 (48.06 %), M_3 (43.29 %), M_5 (41.66 %) and M_7 (36.58 %). M_2 and M_4 showed lower biosorption (7.21 % and 33.94 % respectively). As biomass quantity increased from 10 to 40 mg, biosorption percentage declined across all treatments, likely due to aggregation of binding sites, reduced surface area-to-volume ratio and inadequate mixing efficiency at higher biomass concentrations. For 20 mg biomass, untrained material removed 56.55 % Pb, while M_1 showed 46.23 % removal. At 30 mg, both $M_3$and M_7 showed similar removal efficiency (42.93 %), while at 40 mg, M_2 demonstrated peak performance (46.23 %) (Fig. 5

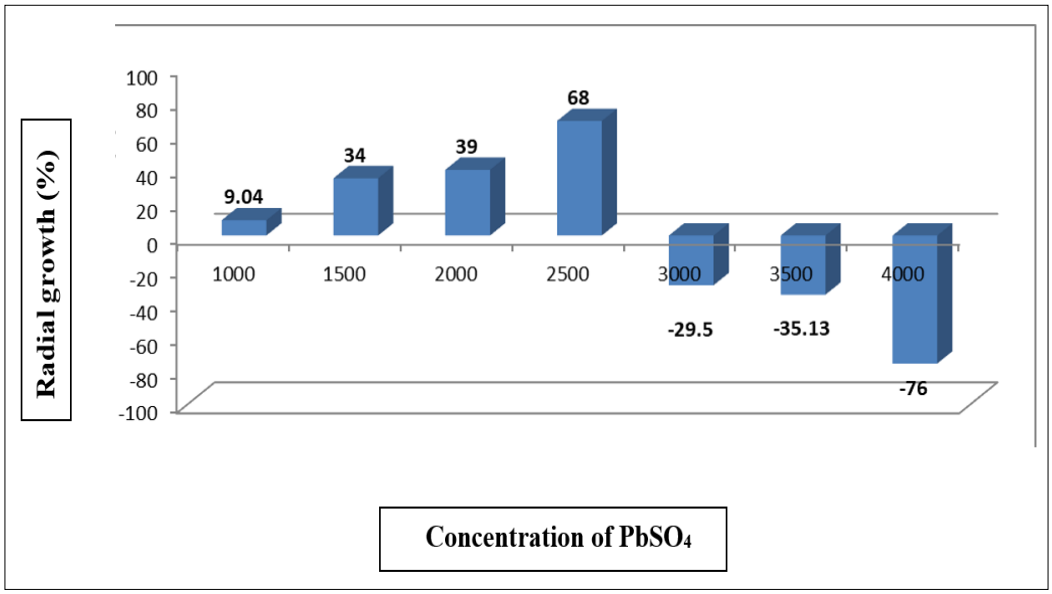
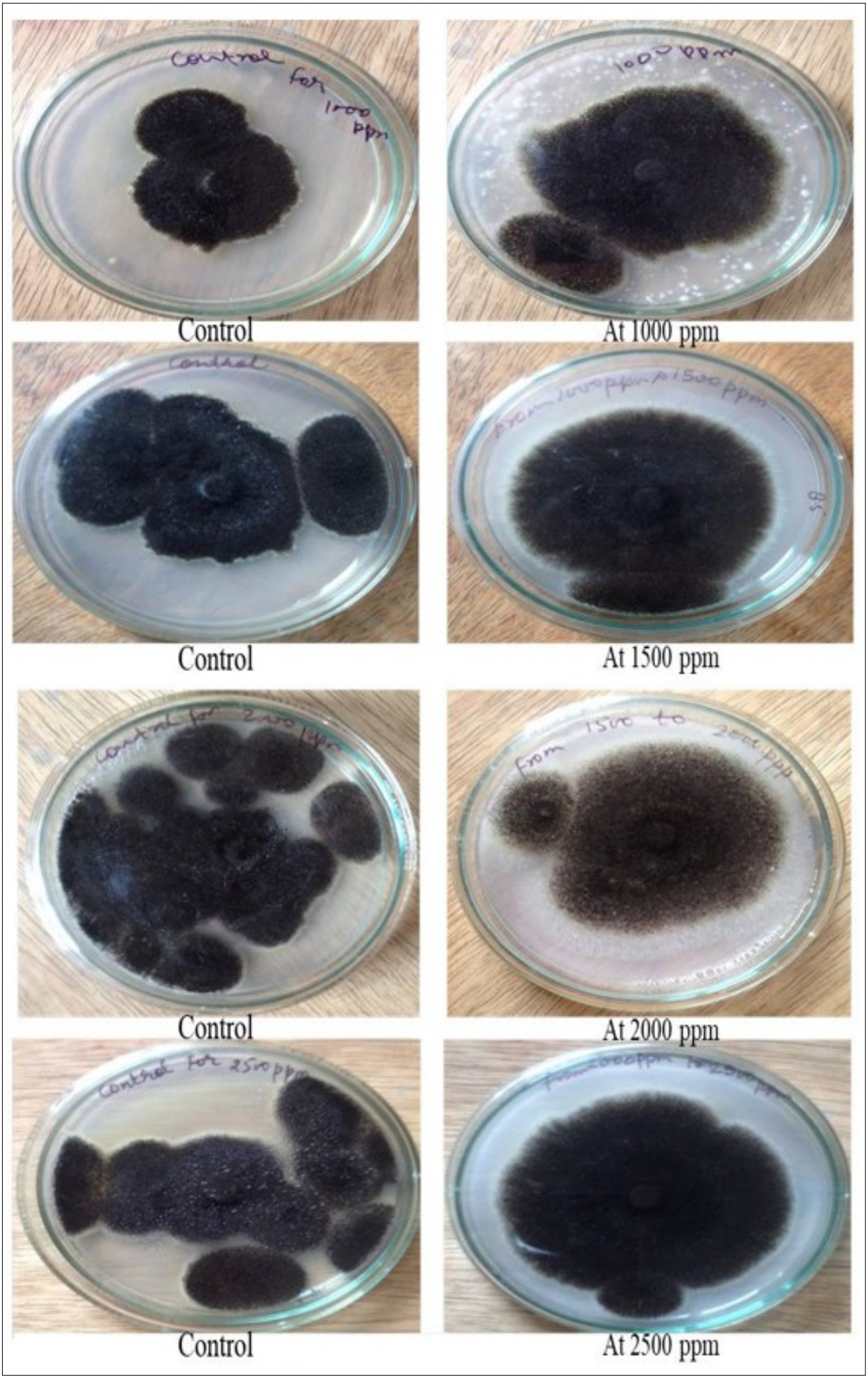


Fig. 3. Radial growth of *Aspergillus niger* in control and medium amended with different concentrations of lead sulphate ($PbSO_4$).


Control
At 1000 ppm
Control
At 1500 ppm
Control
At 2000 ppm
Control
At 2500 ppm

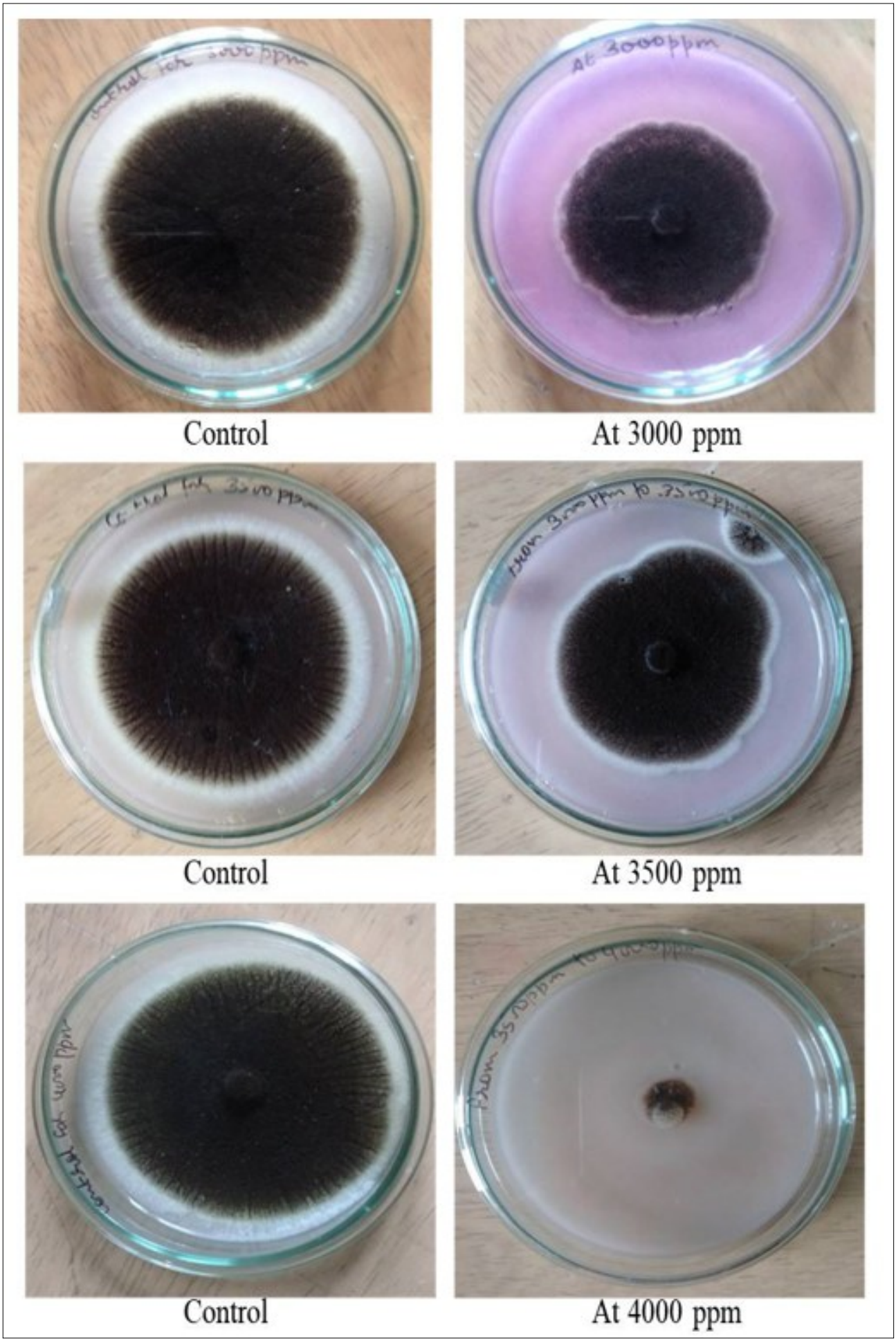


Fig. 4. Training of *Aspergillus niger* on different concentrations of lead sulphate ($PbSO_4$).

Table 1. Radial growth of *Aspergillus niger* in the control and medium amended with different concentrations of lead sulphate

Metal	Metal concentration (ppm)	Radial growth (cm)	% reduced (-)/ % induced (+)
	Control	6.87 ± 0.70	-
	1000	7.6 ± 1.82	+9.04
	1500	8.34 ± 0.20	+34.0
	2000	8.04 ± 0.20	+39.0
	2500	5.64 ± 0.76	+68.0
Lead	3000	5.3 ± 1.3	-29.5
	3500	5.64 ± 0.76	-35.13
	4000	-	-76.0

Table 2. Biosorption of lead from aqueous solutions of 100 ppm by different strains of wet biomass of *Aspergillus niger*

Type of trained biomass	Amount of biomass (mg)	Initial concentration of the metal in the solution (ppm)	Final concentration of the metal in the solution (ppm)	Amount of metal biosorbed (ppm)	Percentage of metal biosorbed	Q-value
Control	10	100	31.61	68.39	68.39 %	341.95
M_1			38.37	61.63	61.63 %	308.15
M_2			92.79	7.21	7.21 %	36.05
M_3			56.71	43.29	43.29 %	216.45
M_4			66.06	33.94	33.94 %	169.7
M_5			58.34	41.66	41.66 %	208.3
M_6			51.94	48.06	48.06 %	240.3
M_7			63.42	36.58	36.58 %	182.9
Control	20	100	43.45	56.55	56.55 %	141.37
M_1			53.77	46.23	46.23 %	115.57
M_2			6I.39	38.61	38.61%	96.52
M_3			55.04	44.96	44.96 %	12.4
M_4			59.25	40.75	40.75 %	101.87
M_5			59.86	40.14	40.14 %	100.35
M_6			61.39	38.61	38.61 %	96.52
M_7			63.78	36.22	36.22 %	90.55
Control	30	100	67.79	32.21	32.21 %	53.46
M_1			66.67	33.33	33.33 %	55.32
M_2			59.86	40.14	40.14 %	66.63
M_3			57.07	42.93	42.93 %	71.26
M_4			58.74	41.26	41.26 %	68.49
M_5			63.17	36.83	36.83 %	61.13
M_6			61.64	38.36	38.36 %	63.67
M_7			57.07	42.93	42.93 %	71.26
Control	40	100	67.43	32.57	32.57 %	40.71
M_1			56.86	43.14	43.14 %	53.92
M_2			53.77	46.23	46.23 %	57.78
M_3			61.69	38.31	38.31 %	47.88
M_4			63.42	36.58	36.58 %	45.72
M_5			62.81	37.19	37.19 %	46.48
M_6			62.91	37.09	37.09 %	46.36
M_7			64.23	35.77	35.77 %	44.71

M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

and 6). The superior performance of untrained biomass at 100 ppm suggests that training-induced modifications may reduce binding site accessibility at lower metal concentrations, possibly through conformational changes in cell wall components or occupation of binding sites by residual Pb from training.

Biosorption performance at 200 ppm lead concentration

At 200 ppm, biosorption dynamics exhibited a reversal in trend (Table 3). For 10 mg biomass, M_1 trained biomass achieved maximum removal (44.28 %, Q-value = 442.80 mg/g), slightly exceeding untrained biomass (41.40 %, Q-value = 414.00 mg/g). This reversal suggests that training confers advantages at higher metal concentrations by enhancing binding site density or affinity. Increasing biomass from 10 to 40 mg generally enhanced biosorption efficiency at 200 ppm contrasting with observations at 100 ppm. At 40 mg, control biomass reached 45 % removal, while M_1 achieved 42.48 % (Fig. 7 and 8). This concentration-dependent effect indicates optimal biosorbent dosage varies with initial metal concentration, requiring careful optimisation for practical applications.

Adsorption isotherm modelling

Freundlich isotherm and Langmuir isotherm at 100 ppm

The experimental biosorption data were fitted to Freundlich and Langmuir isotherm models to elucidate the mechanism of Pb biosorption by *A. niger* biomass (Tables 4–7). The comparison of correlation coefficients (R^2) revealed that model applicability varied with initial metal concentration and biomass type. At 100 ppm Pb concentration, the Freundlich isotherm model demonstrated superior fit for most biomass variants compared to the Langmuir model. The untrained control biomass exhibited excellent correlation with the Freundlich model (R^2 = 0.984) compared to Langmuir (R^2 = 0.870), suggesting multilayer adsorption on heterogeneous surface sites. Similarly, trained

Table 3. Biosorption of lead sulphate from aqueous solutions by different strains of wet biomass of *Aspergillus niger* at 200 ppm

Type of trained biomass	Amount of biomass (mg)	Initial concentration of the metal in the solution (ppm)	Final concentration of the metal in the solution (ppm)	Amount of metal biosorbed (ppm)	Percentage of metal biosorbed	Q-value
Control	10	200	117.20	82.80	41.40	414.00
M_1			111.44	88.56	44.28	442.80
M_2			121.88	78.12	39.06	390.60
M_3			119.00	81.00	40.50	405.00
M_4			118.10	81.90	40.95	409.50
M_5			140.24	59.76	29.88	298.80
M_6			127.82	72.18	36.09	360.90
M_7			131.06	68.94	34.47	344.70
Control	20	200	120.18	79.82	39.91	199.55
M_1			111.62	88.38	44.19	220.95
M_2			119.54	80.46	40.99	201.15
M_3			119.54	80.46	40.23	201.15
M_4			122.16	77.84	38.92	194.60
M_5			135.74	64.26	32.13	160.65
M_6			126.02	73.98	36.99	184.95
M_7			126.12	73.88	36.94	184.70
Control	30	200	111.44	88.56	44.28	147.00
M_1			112.34	87.66	43.83	145.57
M_2			117.20	82.80	41.40	137.44
M_3			121.34	78.66	39.33	130.57
M_4			123.14	76.86	38.43	127.58
M_5			140.96	59.04	29.50	98.00
M_6			126.92	73.08	36.54	121.31
M_7			136.10	63.90	31.95	106.07
Control	40	200	110.00	90.00	45.00	112.50
M_1			115.04	84.96	42.48	106.20
M_2			188.56	81.44	40.72	101.80
M_3			122.24	77.76	38.88	97.20
M_4			125.66	74.34	37.17	92.92
M_5			139.08	60.92	30.46	76.15
M_6			130.98	69.02	34.51	75.02
M_7			138.44	61.56	30.78	76.95

M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

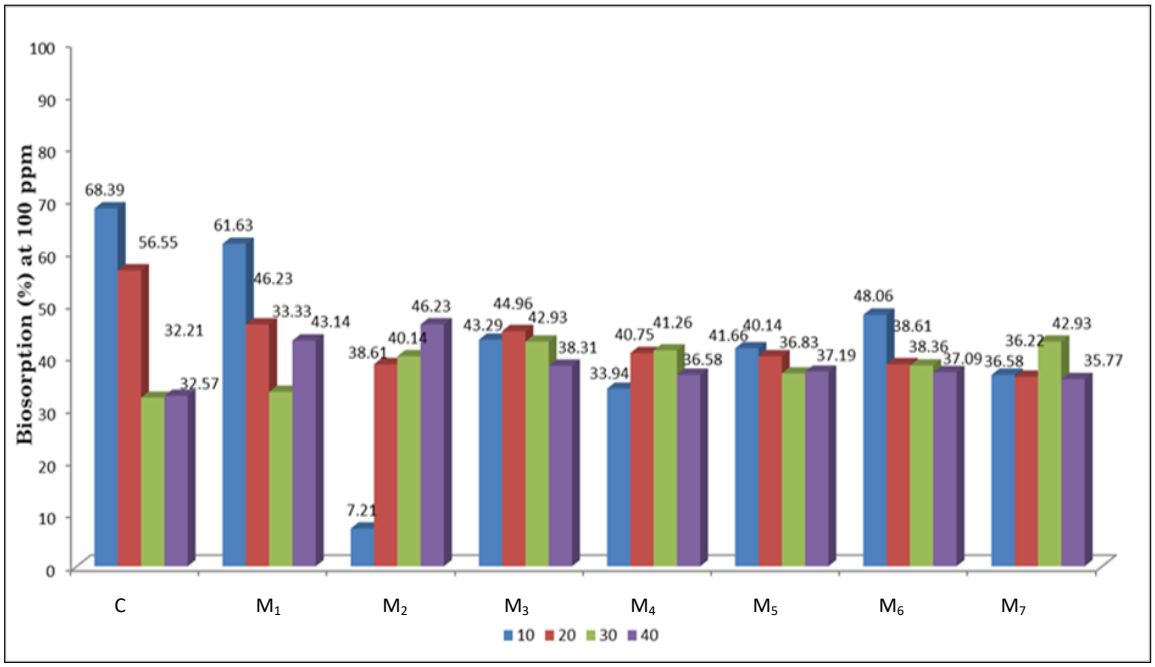


Fig. 5. Lead sorption profile of adsorption by *Aspergillus niger* at 100 ppm lead sulphate ($PbSO_4$). C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

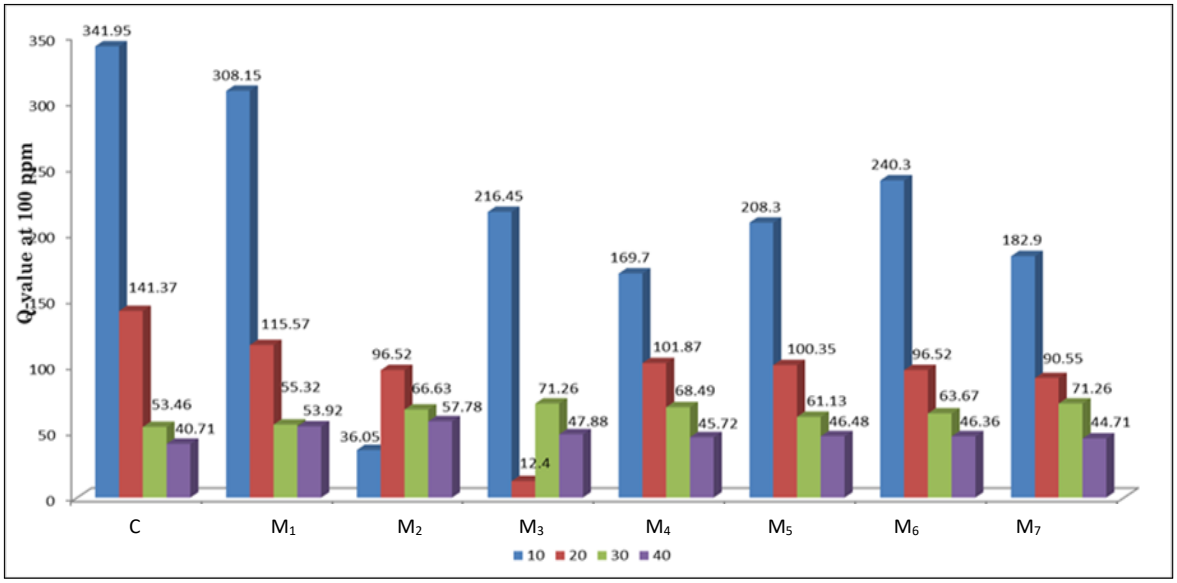


Fig. 6. Lead sorption profile of Q-value by *Aspergillus niger* at 100 ppm lead sulphate ($PbSO_4$). C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

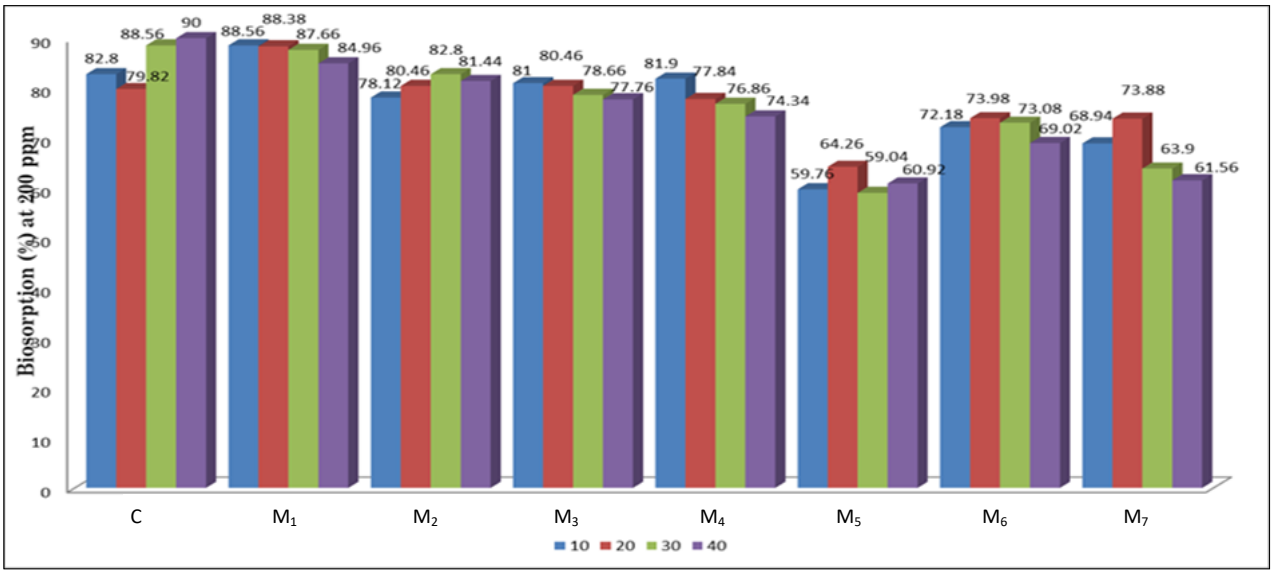


Fig. 7. Lead sorption profile of adsorption by *Aspergillus niger* at 200 ppm lead sulphate ($PbSO_4$). C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

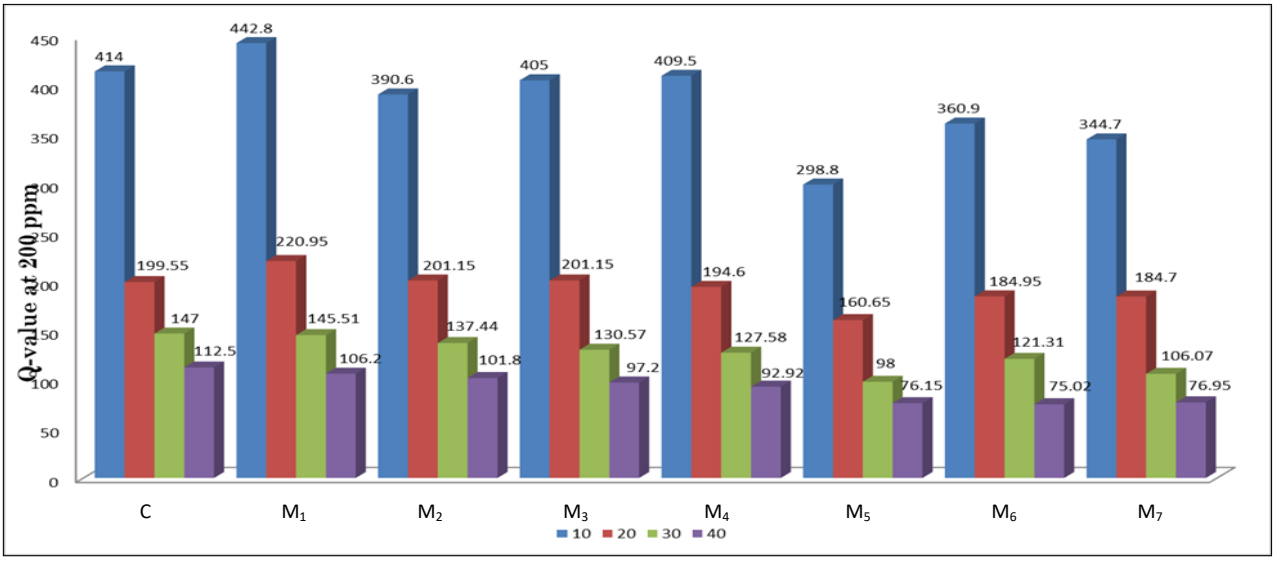


Fig. 8. Lead sorption profile of Q-value by *Aspergillus niger* at 200 ppm lead sulphate ($PbSO_4$). C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm.

Table 4. Freundlich isotherm: sorption profile of *Aspergillus niger* for lead metal as lead sulphate ($PbSO_4$) at 100 ppm

Type of biomass	N	1/n	K_F	R^2
Control	2.6455	0.378	287.4359	0.984
M_1	3.7453	0.267	182.1809	0.892
M_2	2.3310	0.429	387.2227	0.618
M_3	19.6078	0.051	72.7479	0.47
M_4	28.5714	0.035	54.3801	0.125
M_5	18.1818	0.055	78.1789	0.909
M_6	0.4655	2.148	3017683	0.428
M_7	100	0.010	59.1454	0.012

K_F: Freundlich constant; n= adsorption intensity; R: correlation coefficients; N: adsorption intensity; 1/n: surface heterogeneity, K_f: Freundlich constant; R^2: coefficient of determination.

Table 5. Freundlich isotherm: sorption profile of *Aspergillus niger* for lead metal as lead sulphate ($PbSO_4$) at 200 ppm

Type of biomass	N	1/n	K_f	R^2
Control	19.6078	0.051	87.6191	0.473
M_1	52.6315	0.019	124.5865	0.649
M_2	4.1322	0.242	472.4822	0.378
M_3	52.6315	0.019	133.0866	0.893
M_4	25.6410	0.039	150.0547	0.976
M_5	1000	0.001	140.0501	0.003
M_6	76.9230	0.013	137.1397	0.305
M_7	22.7272	0.044	166.002	0.5

K_F: Freundlich constant; n= adsorption intensity; R: correlation coefficients; N: adsorption intensity; 1/n: surface heterogeneity; K_f: Freundlich constant.

Table 6. Langmuir's isotherm: sorption profile of *Aspergillus niger* for lead metal as lead sulphate ($PbSO_4$) at 100 ppm

Type of biomass	a	b	1/ab	R^2
Control	0.747	1.031486	1.2978	0.870
M_1	0.562	1.026341	1.7336	0.782
M_2	0.355	1.018163	2.7667	0.604
M_3	0.097	1.018163	10.1253	0.689
M_4	0.020	1.161834	43.0354	0.023
M_5	0.081	1.017145	12.1375	0.881
M_6	2.467	1.088717	0.3723	0.063
M_7	0.850	1.044982	1.1258	0.005

a: Maximum adsorption capacity of lead on the surface of biomass; b: Langmuir affinity constant; R^2: coefficient of determination.

Table 7. Langmuir's isotherm: sorption profile of *Aspergillus niger* for lead metal as lead sulphate ($PbSO_4$) at 200 ppm

Type of biomass	a	b	1/ab	R^2
Control	7.63101	0.103	1.008032	0.616
M_1	0.03826	21.48	1.216527	0.821
M_2	2.75289	0.360	1.009041	0.531
M_3	33.0678	0.030	1.008032	0.966
M_4	13.5894	0.073	1.008032	0.857
M_5	248.2501	0.004	1.007025	0.026
M_6	42.2396	0.021	1.008032	0.537
M_7	17.748	0.056	1.008032	0.631

a: Maximum adsorption capacity of lead on the surface of biomass; b: Langmuir affinity constant; R^2: Coefficient of determination.

biomasses showed better Freundlich correlation: M_1 (R^2 = 0.892 vs. 0.782), M_5 (R^2 = 0.909 vs. 0.881) and M_2 (R^2 = 0.618 vs. 0.604). The consistently higher Freundlich correlation coefficients indicate that biosorption occurred predominantly through multilayer adsorption on heterogeneous binding sites with varying affinities (Fig. 9 and 10).

At 200 ppm Pb concentration, Freundlich model continued to provide better fit for most trained biomass variants. The M4 biomass showed excellent Freundlich correlation (R^2 = 0.976) compared to Langmuir (R^2 = 0.857), while M_3 demonstrated R^2 = 0.893 (Freundlich) versus 0.966 (Langmuir). The M_1 biomass exhibited R^2 = 0.649 (Freundlich) versus 0.821 (Langmuir) (Fig. 11 and 12). These results confirm that the Freundlich isotherm model better describes the biosorption behaviour of trained *A. niger* biomass across the studied concentration range, indicating that the biosorption process involves heterogeneous adsorption with multiple binding sites rather than homogeneous monolayer coverage.

Discussion

The progressive *in vitro* training of *A. niger* through stepwise exposure to escalating $PbSO_4$ concentrations revealed distinct concentration-dependent biphasic growth responses characteristic of hormesis, where intermediate concentrations stimulated growth while higher concentrations caused inhibition (26, 50). The remarkable 68 % growth stimulation observed at 2500 ppm indicates successful development of highly efficient metal tolerance mechanisms that not only prevented toxicity, but enhanced growth compared to control conditions. This finding corroborates recent research demonstrated that *A. niger* achieved complete Pb, Cu and Cd removal from industrial wastewater within 24 hr, attributing superior performance to mycelial cohesiveness and large mass facilitating surface area expansion upon wastewater contact (51). Similarly, early researchers confirmed that fungi employ two-step biosorption and bioaccumulation processes for heavy metal removal, with *Aspergillus* species demonstrating remarkable adaptability in contaminated environments (52).

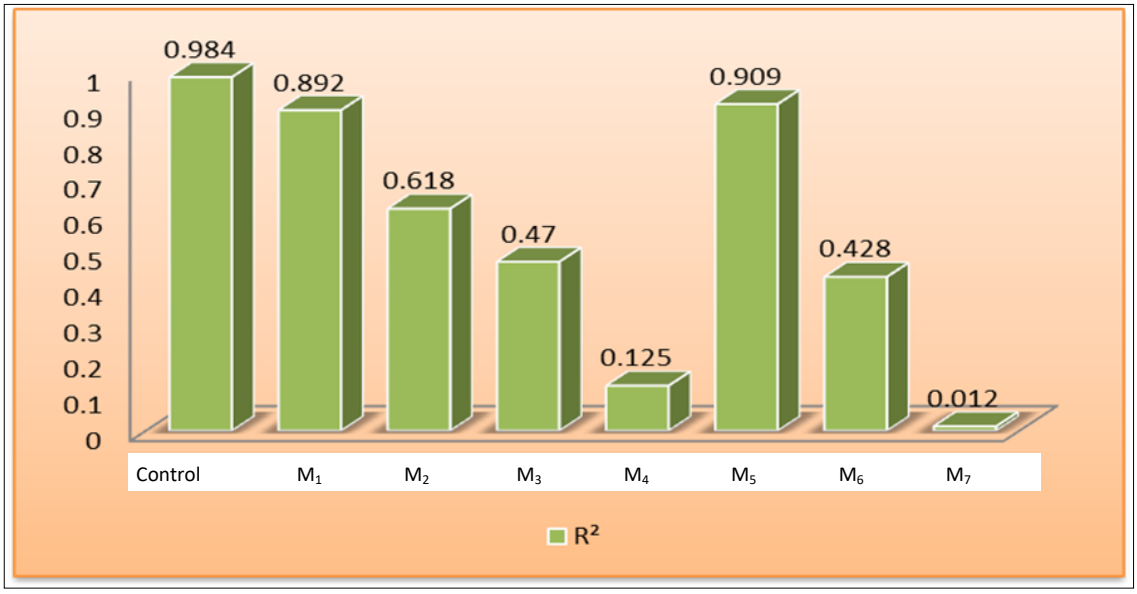


Fig. 9. Freundlich isotherm correlation coefficients (R^2) for lead biosorption by untrained (Control) and *in vitro* trained (M_1–M_7) *Aspergillus niger* biomass at 100 ppm lead sulphate ($PbSO_4$) concentration. C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm; R^2: Coefficient of determination



Fig. 10. Correlation coefficients (R^2) of Langmuir isotherm model for lead biosorption by untrained (control) and *in vitro* trained (M_1–M_7) *Aspergillus niger* biomass at 100 ppm lead sulphate ($PbSO_4$) concentration. C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained

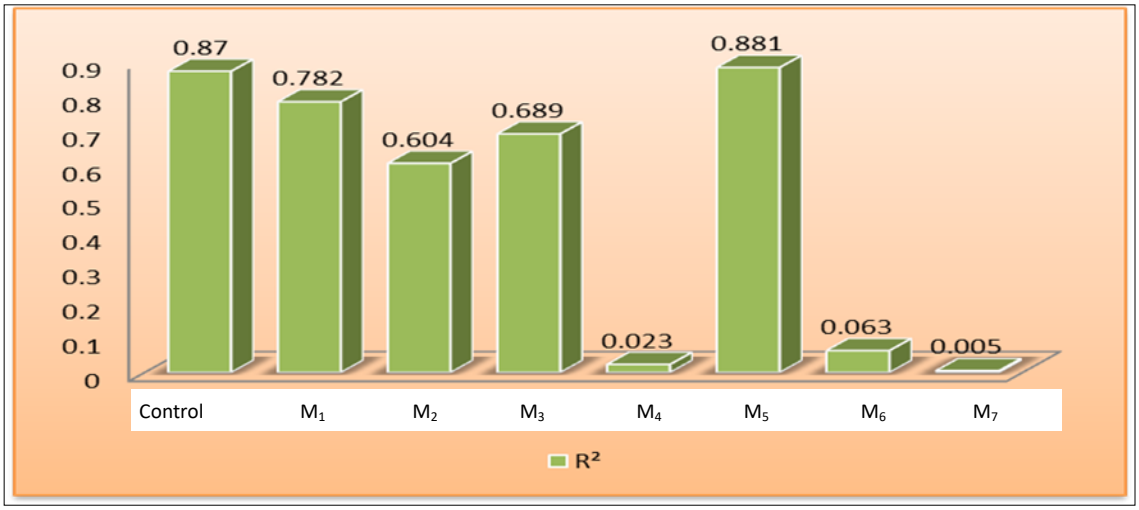


Fig. 11. Freundlich isotherm correlation coefficients (R^2) for Pb biosorption by untrained (control) and *in vitro* trained (M_1–M_7) *Aspergillus niger* biomass at 200 ppm lead sulphate ($PbSO_4$) concentration. C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm; R^2: coefficient of determination.

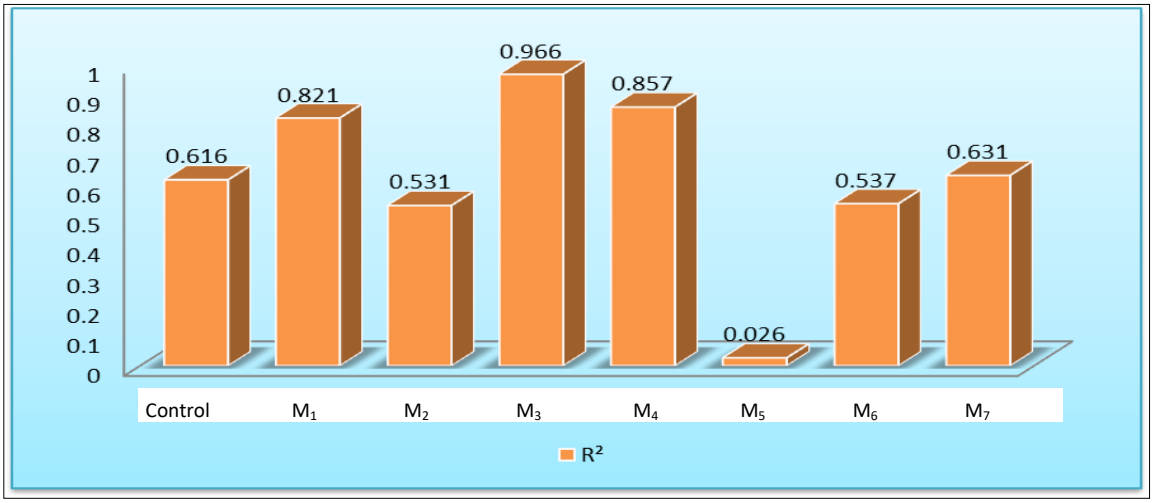


Fig. 12. Correlation coefficients (R^2) of Langmuir isotherm model for lead biosorption by untrained (control) and *in vitro* trained (M_1–M_7) *Aspergillus niger* biomass at 200 ppm lead sulphate ($PbSO_4$) concentration. C: untrained *Aspergillus niger* biomass; M_1: biomass trained at 1000 ppm; M_2: biomass trained at 1500 ppm; M_3: biomass trained at 2000 ppm; M_4: biomass trained at 2500 ppm; M_5: biomass trained at 3000 ppm; M_6: biomass trained at 3500 ppm; M_7: biomass trained at 4000 ppm; R^2: coefficient of determination.

Recently the metal tolerance involve phenotypic adaptation and selection mechanisms, supporting our hypothesis that the training protocol successfully enhanced both phenotypic adaptation and potentially selected for more tolerant fungal variants (53, 54). Molecular adaptations to metal toxicity in fungi include transcriptional activation of metallothionein genes and amplified expression of membrane-bound metal efflux systems (55). The physiological basis for enhanced tolerance likely involves multiple simultaneous mechanisms including increased expression of metal-binding proteins such as metallothioneins sequestering metal ions in non-toxic forms, enhanced metal efflux system activity pumping ions out before cellular damage occurs, modifications in cell wall composition altering metal binding characteristics, increased production of metal-chelating compounds such as organic acids complexing with metal ions and development of more efficient DNA repair systems counteracting metal-induced genetic damage (56).

Gadd demonstrated that filamentous fungal exposure to heavy metals leads to physiological adaptation or mutant selection potentially associated with increased metal adsorption capacity (57). Similarly, significant heavy metal reduction by *A. niger* and *Aspergillus flavus* in effluent compared to industrial-treated effluent, with maximum accumulation at 25 ℃, pH 5.0 and 120 hr growth period (58). 4 mg/mL Pb concentration inhibited *A. niger* strain growth, aligning with our observation of severe growth inhibition at 4000 ppm (59). The viable Pb removal using *Aspergillus versicolor* biomass with maximum adsorption capacity of 45 mg Pb(II)/g dry weight, considerably lower than the 341.95 mg/g achieved in our study (18).

In our study, exceptional biosorption performance demonstrated by *A. niger* biomass, particularly 68.39 % removal efficiency and 341.95 mg/g Q-value achieved by 10 mg control biomass at 100 ppm Pb concentration, positions this fungal species among the most effective biosorbents for Pb remediation (60). This finding gains particular significance when compared to recent studies. Modelling and biosorption potential of *Aspergillus heteromorphus* was reported for Pb-contaminated wastewater treatment, demonstrating continuing relevance of fungal biosorption research (61). The stability of Pb immobilisation investigated by *A. niger* and Fluor apatite, showing that the combination demonstrates great potential in lead remediation at pH 3.5–5.5, suitable for fungal growth and Pb mineral stability (62). Similarly, the scalability and economic feasibility confirmed the fungal biosorption systems for industrial applications (63, 64). This recent finding provides important context for understanding operational parameters optimising *A. niger* biosorption performance (65, 66). Additionally, in this research an inverse relationship observed between biosorbent dosage and specific uptake Q-value at 100 ppm concentration has been reported by multiple investigators (67, 68). The lower uptake at higher biomass concentrations can be attributed to electrostatic interactions at cell surfaces, where cells in higher suspension concentrations attach to each other and lowering surface area in contact with solution. This phenomenon, known as particle aggregation, reduces effective binding site availability (41). The biosorbent dosage optimisation is critical for maximising removal efficiency while maintaining economic viability (15). These collective findings suggest that optimising biosorbent dosage represents a critical parameter for maximising biosorption efficiency in practical applications (69, 70).

Comparative studies provide valuable context for biosorption performance across different species and conditions (60, 71, 72). The biosorption capacity of *A. flavus* ranging from 20.75–93.65 mg/g for Cu with initial concentration 200–1400 ppm, while *A. niger* for Pb ranged from 3.25–172.25 mg/g at the same concentration range (73). The maximum value of 172.25 mg/g for *A. niger* reported by investigators is still substantially lower than the 341.95 mg/g observed in our study, highlighting exceptional performance achieved through our experimental protocol (22, 74). The trained M_1 biomass exposed to 1000 ppm Pb demonstrated biosorption efficiency of 61.63 % at 10 mg dosage, only slightly lower than the 68.39 % efficiency of untrained control. This relatively minor reduction in biosorption capacity (approximately 10 % decrease) coupled with dramatically enhanced metal tolerance (ability to survive at 4000 ppm) represents an advantageous trade-off for practical applications. Recent studies have confirmed that trained microbial strains maintain functional capabilities while exhibiting enhanced stress tolerance, validating the training approach for industrial applications (53, 54).

The superior fit of Freundlich isotherm model compared to Langmuir model for describing lead biosorption by *A. niger* biomass has important mechanistic implications consistent with recent biosorption research (48, 49, 65). The Freundlich model assumes heterogeneous adsorption surfaces with binding sites

exhibiting range of affinities for adsorbate and multilayer adsorption where adsorbate molecules can bind on top of previously adsorbed molecules (28). The high correlation coefficients obtained with Freundlich model, particularly $R^2 = 0.979$ for M_6 biomass, indicate that these assumptions accurately describe the biosorption process (46, 75). In contrast, the Langmuir model assumes homogeneous surfaces with equivalent binding sites and monolayer adsorption, assumptions that apparently do not hold for the fungal biosorption system studied here (29). The heterogeneity of fungal cell wall surfaces reflects their complex composition comprising diverse structural components. Fungal cell walls contain chitin, a polymer of N-acetylglucosamine residues providing structural rigidity and containing amino groups capable of metal binding (23). Glucans including β-1,3-glucan and β-1,6-glucan represent major structural polysaccharides providing additional hydroxyl groups for metal coordination. Mannoproteins consisting of mannose oligosaccharides attached to protein backbones provide both hydroxyl groups from mannose residues and carboxyl, amino and sulfhydryl groups from amino acid residues (16). This compositional diversity necessarily creates binding site heterogeneity with different functional groups exhibiting different affinities for Pb ions (66). In our study, high correlation coefficient of 0.984 for control biomass using Freundlich isotherm confirms multilayer adsorption mechanism. *Aspergillus niger* employs both cell surface adsorption and chelating metals with cell-secreted compounds, supporting the multilayer and multi-mechanism biosorption model suggested by Freundlich isotherm applicability (76).

Conclusion

This comprehensive investigation on *in vitro* training and biosorption potential of *Aspergillus niger* for lead (Pb) removal has demonstrated significant findings with important implications for bioremediation technology development. The *A. niger* biomass exhibits strong biosorbent properties for Pb metal as lead sulphate ($PbSO_4$) with a high Q-value of 341.95 mg/g, indicating superior adsorption performance compared with several previously reported biosorbents. *In vitro* trained strains maintain good biosorbent capacity while demonstrating enhanced metal tolerance, with trained M1 biomass showing 61.63 % biosorption efficiency compared to 68.39 % for untrained control, representing only minor reduction in performance while conferring ability to survive and function at metal concentrations up to 4000 ppm. This dual enhancement of tolerance and maintenance of biosorption capacity represents significant advancement for treating industrial effluents with variable and high metal concentrations.

The training protocol involving progressive exposure through nine sequential steps with incrementally increasing metal concentrations successfully adapted the fungal strain to extreme metal stress conditions while preserving functional biosorption capacity, an approach that could be extended to develop multi-metal tolerant strains capable of treating complex industrial wastewaters containing mixtures of heavy metals. Equilibrium modelling analysis revealed that Freundlich isotherm model demonstrated superior correlation coefficients ranging from 0.875 to 0.979 compared to Langmuir isotherm model, confirming that biosorption involves multiple binding sites with varying affinities and multilayer adsorption mechanisms consistent with the complex composition of fungal cell walls comprising diverse functional groups. The Pb metal adsorption process represents a feasible and non-spontaneous chemisorption process, indicating stable and energy-requiring metal-biomass interactions, suggesting that desorbed metal could potentially be recovered in concentrated form through appropriate elution procedures, enabling both biosorbent regeneration and metal reclamation. The biosorption process exhibits strong dependence on experimental parameters including pH, temperature, biomass dosage, contact time, initial metal concentration and agitation speed, highlighting the importance of parameter optimisation for successful industrial implementation. *Aspergillus niger* biomass represents a major component for developing biotrap devices and can serve as principal component for managing heavy metal pollution in point-source treatment applications, continuous flow systems and emergency response situations. While conventional technologies for heavy metal cleanup from industrial effluents are time-consuming and less effective, biosorption offers an inexpensive, fast and promising alternative for bio-detoxification of heavy metal pollution from wastewaters, representing a sustainable approach aligned with green chemistry principles and circular economy concepts that could transform industrial wastewater treatment practices globally.

Acknowledgements

The authors express their sincere gratitude to the Department of Biotechnology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India, for providing the necessary facilities and financial support for this research.

Authors' contributions

RR and PK drafted the manuscript and designed the methodology. RR performed statistical analysis. SS, AR and VB also design methodology and assisted in compiling the data. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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