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Removal of Lead from Aqueous Solution by *Fusarium oxysporum*: Equilibrium and Phytotoxicity Studies

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KEYWORDS

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ABSTRACT

Lead is a toxic metal of public health concern. The applicability of *Fusarium oxysporum* biomass as a biosorbent for the removal of lead ions from wastewater is assessed in the present investigation. Batch experiments were conducted under different experimental conditions for analysis of the lead biosorption capacity of live and dead biomass of *Fusarium oxysporum.* Lead ions were significantly absorbed at pH 5 with a 2g adsorbent amount at 30^0C. Equilibrium results were analyzed by Langmuir and Freundlich isotherms and found that Langmuir isotherm is the best fit under this condition. A phytotoxicity study revealed that the growth parameters of wheat seeds were significantly increased in the lead solution treated with dead biomass as compared to the live biomass of *F. oxysporum*. Further, dead *F. oxysporum* significantly removed lead within 3 hours whereas live fungal biomass took two days for the complete removal of lead. Therefore, the results of the study suggested that live and dead biomass of *F. oxysporum* can be used as an effective, safe, and economically feasible sorbent for the removal of lead present in industrial effluent or wastewater systems.

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1 Introduction

Increased urbanization, anthropogenic activities, industrialization, and careless management practices have shown adverse effects on the environment by dumping wastes containing heavy metals. Heavy metal pollution is one of the most serious environmental problems due to the long-term persistence, toxicity, and non-degradable nature of metals which impose adverse effects on human health after entering into the food chain (Trikkaliotis et al. 2022). Heavy metals belong to a group of elements characterized by high atomic weight and density of more than 5 g/cm^3 (Ali and Khan 2018). They have been categorized into a group of pollutants known as emerging contaminants (Lodeiro et al. 2019). Heavy metals are toxic, non-biodegradable with a long half-life and easily react with organic substances and form toxic metal-organic complexes and trigger long-term implications on human beings, animals, and environmental health (Hong et al. 2020). Heavy metals cannot be easily eliminated after entering the human body and tend to accumulate which may cause changes in biochemical processes and show chronic effects (Abdus-Salam and Adekola 2018; Hong et al. 2020). Among heavy metals, lead (PbII) is one of the most hazardous pollutants in the environment and it is a serious ecological concern due to its impact on human health, animals, and plants (Wang et al. 2022). Lead is used in > 900 industries such as mining, metal plating, and finishing, battery manufacturing, smelting, fertilizers, pesticides, photographic materials, explosive, ceramic, and glass industries (Shao et al. 2020; Wang et al. 2021). As per the Institute for Health Metrics and Evaluation, the University of Washington, more than nine lakh death and twenty-one million disability cases have been reported worldwide due to lead exposure (Irawati et al. 2022).

As per guidelines of the Environmental Protection Agency (EPA) and World Health Organization (WHO), the permissible limit of lead in potable water is 15 and 50 µg/L, respectively (Mahmud et al. 2016). Lead is extremely toxic even at very low concentrations and causes anemia, hypertension, and headache (Alghamdi et al. 2019) and damage to the kidney, liver, nervous system, and reproductive and gastrointestinal system of human beings (Bouabidi et al. 2018; Kumara et al. 2019).

Different procedures like ion exchange, membrane separation, reverse osmosis, chemical reduction, and electrochemical treatment have been used for heavy metals elimination from industrial effluent. These methods are costly, not effective at large scale, and require expertise and sophisticated equipment. Therefore, obtaining material with desirable sorption properties is still a major challenge for researchers.

Biosorption is a physicochemical process that utilizes biomass for the removal of heavy metals from contaminated medium (Chauhan et al. 2020a). The surface of fungal biomass has high electronegativity which can attract and bind metal ions (Chauhan et al. 2020b). Dead microbial biomass can also be used for the recovery of metal ions as it binds metal ions more efficiently (Kapoor 2022). In comparison to conventional methods, biosorption has many edges like high efficiency, low cost, simple process, stability, high surface area, recovery of metals, and no sludge formation (Sarma et al. 2020).

The study of pertinent literature revealed that the effect of live and dead biomass of *Fusarium oxysporum* has not been studied for the removal of lead and the impact of the treated solution on the growth of wheat seeds. The present study was executed to determine the potential of live and dead biomass of *F. oxysporum* for lead removal from aqueous solution and its effect on the growth of wheat plants. Hence, the development of biosorbent from *F. oxysporum* can be a sustainable approach for lead removal from aqueous solution and environmental restoration.

2 Materials and Methods

Experiments were conducted at Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida.

2.1 Fungal biomass

F. oxysporum was isolated from soil samples and culture was maintained on Potato dextrose agar medium at 4^{0}C and sub-cultured within fifteen days.

2.2 Growth conditions

One disk of fungal biomass (4 mm diameter) was inoculated into Sabouraud Dextrose medium [Dextrose 40g, Peptone 10g, Distilled water 1 liter] at pH 5.8. Flasks were incubated for a week at 25±2^{0}C. After that, fungal biomass was separated by filtration, washed with deionized water, and dried properly with filter papers for wet biosorbent (Saad et al. 2016).

2.3 Dead fungal biomass preparation

Dead biomass was prepared by boiling *F. oxysporum* with 0.5N sodium hydroxide solution for fifteen minutes and then thoroughly rinsed with deionized water till eluent pH reached neutral. After proper washing, the biomass was dehydrated at 5^{0}C for 24 h and powdered. Dead fungal biomass was stored in a desiccator and used in experiments. Around four grams of live biomass of *F. oxysporum* was equivalent to 0.38 g of dead biomass.

2.4 Batch test for lead removal by *Fusarium oxysporum*

Lead solution (1000 ppm) was prepared by mixing lead nitrate in deionized water and desired concentrations were prepared. *F.*

oxysporum live or dead biomass was added to hundred milliliters of metal solution and lead solution without live or dead *F. oxysporum* was also incubated in the same manner called control. For maximum adsorption of lead, different experimental conditions such as time (1-96 hours), pH (1-6), initial lead concentration (20-140 mg/l), and temperature (10-50^{0}C) were used. Different amount of fungal biomass (0.5-3.5g live and dead biomass) was used for the absorption of lead ions and flasks were kept in a shaking incubator at 150 rpm as per the treatment. After filtration, fungal biomass was removed and the supernatant was analyzed for identification of residual metal (Saad 2015).

2.5 Lead biosorption capacity

The amount of lead ions absorbed at equilibrium q_e (mg/g) shows the metal uptake and it was assessed by the following equation:

$$q_e = (C_i\text{-}C_e)\ V / m$$

V = lead solution volume, C_i and C_e= initial and final lead metal concentrations, m= biosorbent mass.

2.6 Equilibrium Studies

Two isotherm models were applied for the analysis of sorption equilibrium. Different concentrations of lead metal solution were treated with different adsorbent doses of fungal biomasses.

2.6.1 Langmuir isotherm

Langmuir isotherm model predicts the monolayer mode of the adsorption procedure. It also explains that adsorption energy is consistent throughout adsorbed layer on the adsorbent surface at a constant temperature (Bharathi and Ramesh 2013). Langmuir equation can be denoted as:

$$C_e/q_e = 1/q_e\ K_1 + C_e/\ q_m$$

q_e (mg/g) = lead amount adsorbed at equilibrium, q_m (mg/g)= lead amount adsorbed, C_e (mg/l)= lead concentration at equilibrium, K_1 = Langmuir constant associated with a binding capacity of lead on fungal surface.

2.6.2 Freundlich isotherm

Freundlich isotherm reflects solute molecules distribution between aqueous and solid phases at equilibrium (Ng et al. 2002). The Freundlich equation can be reflected:

$$\text{Log } q_e = \log K_f + 1/n \log C_e$$

Freundlich constants like K_f = adsorption capacity, n = adsorption intensity, respectively. n shows nature of process as linear phenomenon (n=1), chemical phenomenon (n < 1), physical process(n > 1).

2.7 Phytotoxicity assessment

The impact of the lead solution was analyzed on the growth of wheat (*Triticum aestivum* var. UP2554) seeds before and after treatment with *F. oxysporum* live and dead biomasses.

2.7.1 Seed germination bioassay test

Fresh and healthy seeds of wheat (*Triticum aestivum* var. UP2554) were procured from the Seed agency of Noida. Seeds of wheat were properly rinsed with tap water to eliminate dirt for five minutes and sterilized with $HgCl_2$ (0.1% w/v) for five minutes to inhibit the activities of microbes and rinsed with deionized water five to six times. Wheat seeds were soaked in lead (Pb II) solution before and after the treatment with live and dead biomass of *F. oxysporum for* up to 4 hours respectively. Wheat seeds were transferred into Petri dishes and irrigated with distilled water or lead solution treated with live or dead *F. oxysporum* biomass as per the treatment. Petri dishes were kept in a seed germinator for 10 days under 75% relative humidity at 26±2^{0}C with 12 hours of photoperiod as per ISTA (2008).

Germination (%): Total number of wheat seeds germinated / wheat seeds taken for germination x 100

Length of the seedling and vigor index was determined in control and treatment after ten days of seedling growth by the following methods:

Seedling length: Length of the radicle and plumule were measured with a measuring scale and denoted in centimeters.

Vigour index: It was calculated by the following formula: Vigour index = Length (radicle and plumule) (mm) x germination (%).

2.8 Statistical analysis

Treatments were arranged as randomized block designs with three replications. Results were assessed by ANOVA in SPSS software. The standard error of the mean was calculated for presentation with tables and the treatment mean was analyzed by using DMRT at $P < 0.05$.

3 Results and Discussion

The optimum conditions for lead elimination were assessed by comparative analysis of live and dead biomass of *F. oxysporum* by changing different parameters such as pH, contact time, lead concentration, adsorbent dose, and temperature through the batch experiments.

3.1 Impact of pH

The effect of pH on lead removal by live and dead *F. oxysporum* is reflected in Figure 1. Results of the study revealed that pH plays a pivotal role in the lead metal biosorption process. For both live and dead biosorbents, Pb(II) uptake level was escalated with an increase in pH from 1 to 6 reaching the maximum sorption at pH 5. The highest 81 and 92% lead was removed with live and dead *F. oxysporum* at pH5 respectively. Sorption ability for both live and dead biosorbents decreased as the pH value was increased beyond 5. Protonation of the biosorbent surface was decreased with a rise in pH, formation of a negative charge showed electrostatic repulsion between biosorbent and lead, and reduced adsorption capacity.

3.2 Effect of the exposure period

The exposure period is an important parameter for the estimation of lead removal by live and dead biomass of *F. oxysporum*. Fungal biosorption capacity was increased by enhancing exposure time (Figures 2 & 3). Maximum lead removal was reported after 2 days with live biomass whereas 3 hours for dead biomass of *F. oxysporum*. Maximum 89 and 93% lead removal was recorded with live and dead biomass of *F. oxysporum*, respectively. However, after the optimum exposure period, the lead removal efficiency was reduced.

3.3 Effect of initial lead concentration

Results presented in figure 4 revealed the effect of initial lead metal concentration on the adsorption of Pb(II)by *F. oxysporum* live and dead biomass. Lead removal was increased by enhancing lead ions concentrations. The results reflected that the adsorption capacity of lead was increased with an increasing initial concentration of lead ions as 73 and 87% lead elimination was observed with lead concentration (100 mg/l) by live and dead *F. oxysporum* respectively. Lead ions generate driving pressure to promote mass transfer resistance of lead ions between solid and liquid sorbent. An increase in the initial lead ion concentration increased interaction between the lead ions in the liquid phase and surface of *Fusarium* and escalated lead absorption by *F. oxysporum*.

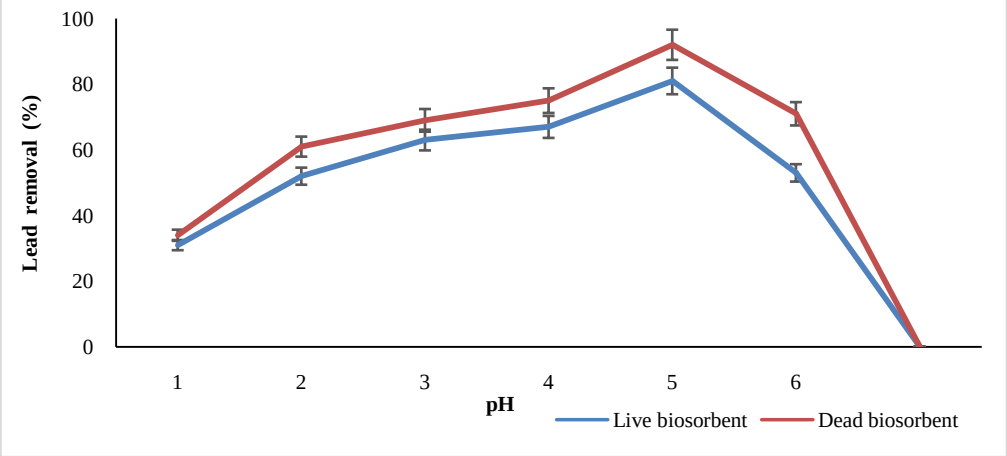

Figure 1 Biosorption of lead by *F. oxysporum* live and dead biomass at different pH; given values are mean ± SEM of three replicates.

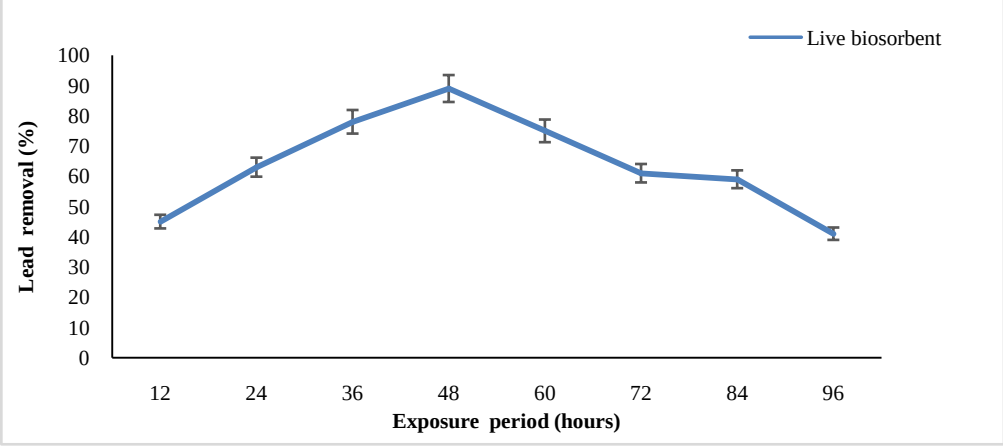

Figure 2 Effect of exposure period on lead removal by live *F. oxysporum* biomass; given values are mean ± SEM of three replicates.

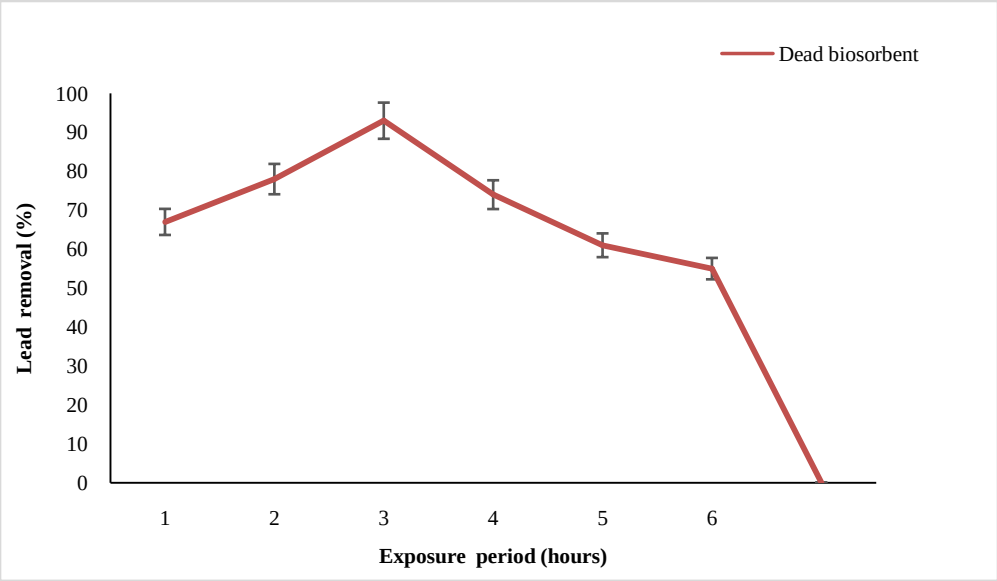

Figure 3 Effect of exposure period on lead removal by dead *F. oxysporum* biomass; given values are mean ± SEM of three replicates

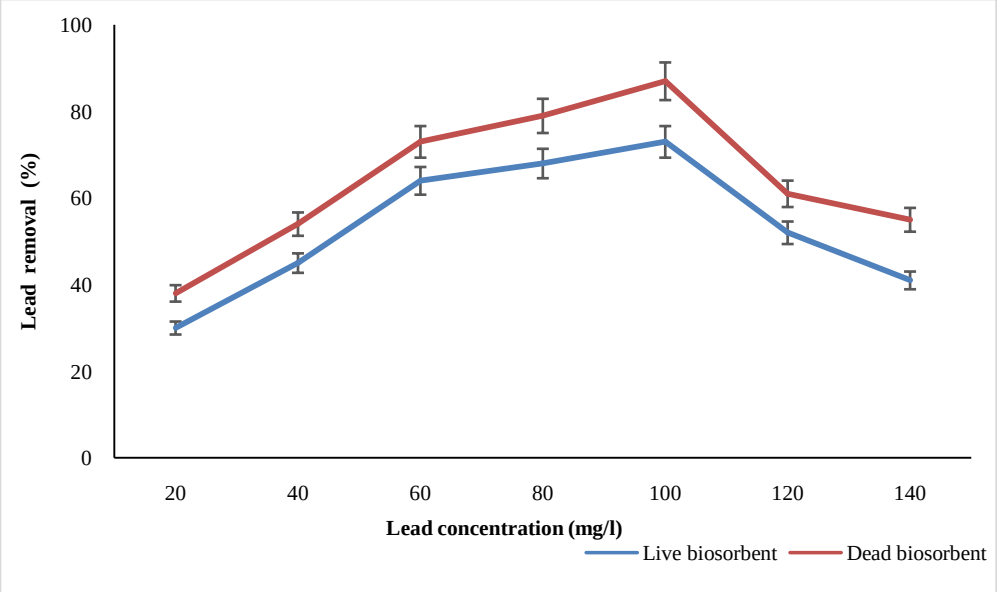

Figure 4 Effect of initial lead concentration on the removal of lead ions from aqueous solution; given values are mean ± SEM of three replicates

3.4 Effect of adsorbent dose

The biosorption rate of lead was escalated with an increase in the amount of *F. oxysporum* (Figure 5). Live and dead *F. oxysporum* absorption ability is reflected in the following trend: 2 > 2.5 > 3> 3.5> 1.5 > 1 > 0.5. The highest lead ions were absorbed with 2 g of both types of fungal biomass. Further, the results of the study revealed that dead biomass was more effective in lead removal as compared to the live biomass of *F. oxysporum* (Figure 5). It might be due to more surface area and availability of more functional groups on the dead biomass in comparison to live biomass. Maximum lead removal (90%) was recorded by 2 g dead biomass where's 81% sorption was observed by the same amount of live biomass of *F. oxysporum* but a further increase in adsorbent dose could alter the uptake of lead ions. This might be because of the non-availability of active sites on fungal biomass and equilibrium establishment between the lead on biosorbent and solution. Results are in agreement with the findings of Sarikaya (2019), who reported that an increase in biosorbent doses enhanced hexavalent chromium sorption by *Agaricus campestris.*

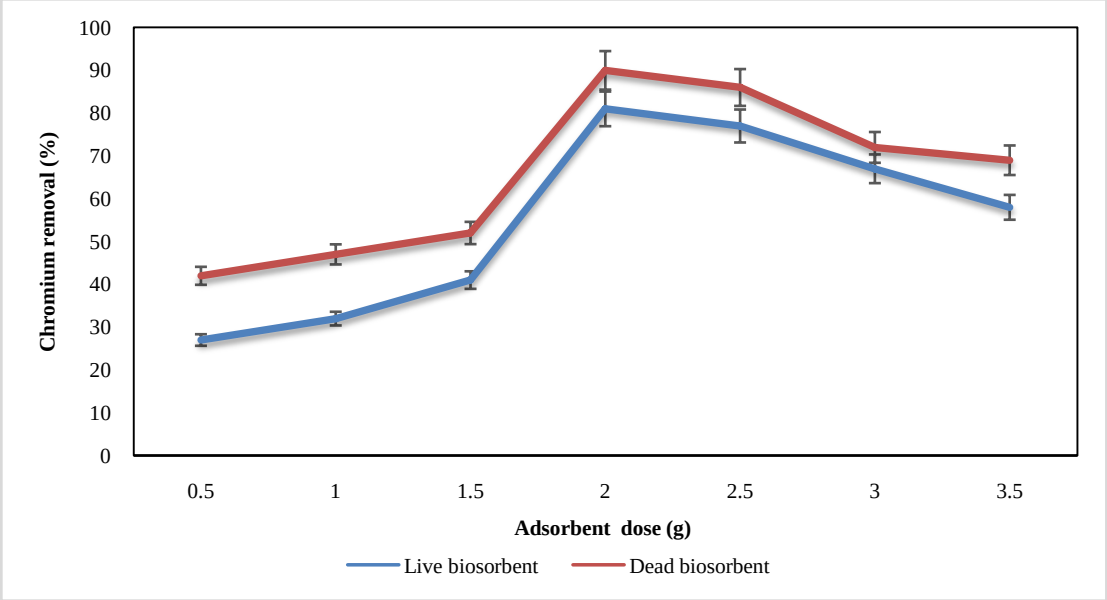

Figure 5 Effect of adsorbent dose on the lead removal; given values are mean ± SEM of three replicates

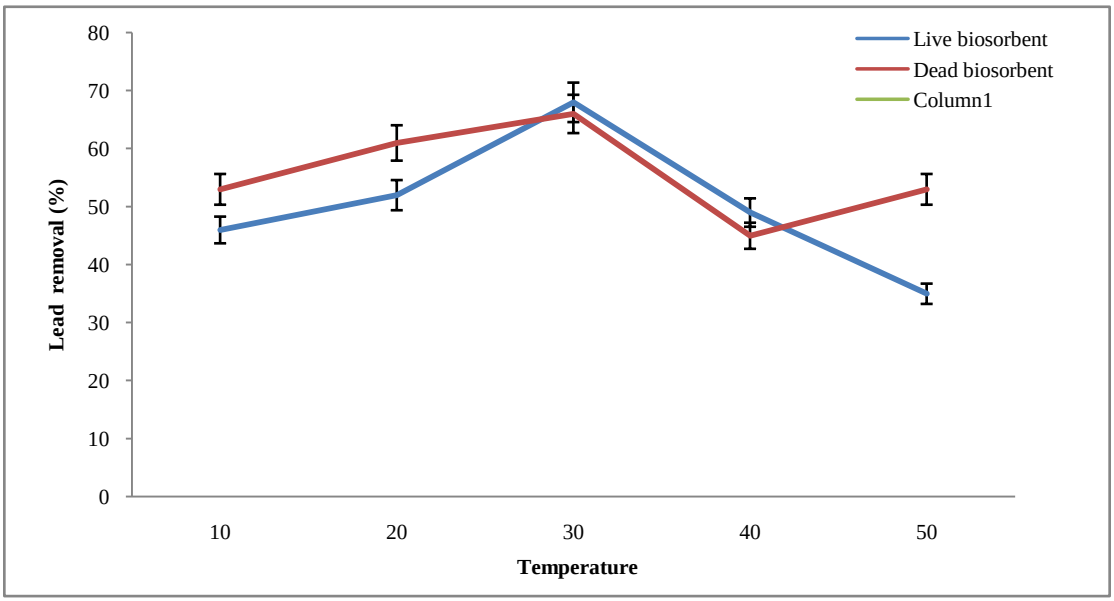

Figure 6 Effect of temperature on the lead removal by live and dead *F. oxysporum* biomass; given values are mean ± SEM of three replicates

3.5 Effect of temperature

Temperature also showed a pivotal role in the biosorption procedure. The biosorption process was enhanced with a rise in temperature as 68% was observed at 30^0 C with live *F. oxysporum* (Figure 6). With the increase in temperature, the biosorption ability of live sorbent declined. The dead biomass did not show any significant alterations in biosorption capacity with a change in temperature as they were resistant to temperature. Results of the study revealed that dead biomass did not modify the functional groups of the biosorbent with a rise in temperature.

3.6 Adsorption isotherm

The adsorption isotherm model explains the mechanism of sorption, surface property, and sorbent capacity. Langmuir isotherm defines the homogeneous distribution of active sites on the adsorbent surface, which adsorb a monolayer with no interaction between sorbed molecules whereas the Freundlich model applies to a heterogeneous system with the interaction between adsorbed molecules. It describes the rise of dye concentration also increases biosorbent, while the energy of sorption reduces on completion of sorption centers of an adsorbent.

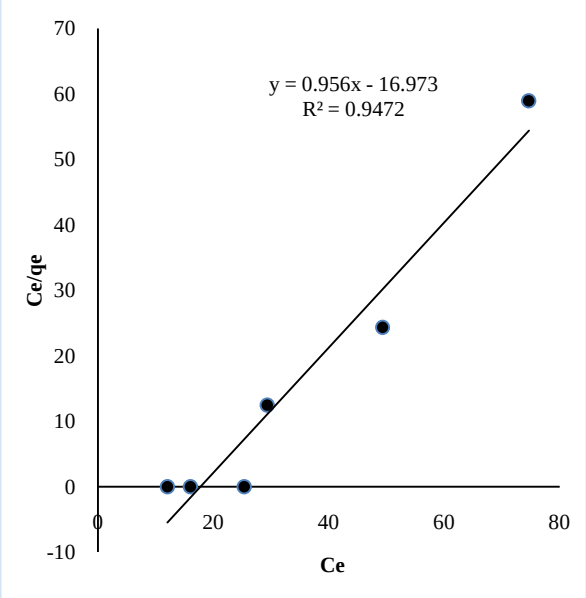

Figure 7(a) Langmuir isotherm for adsorption of lead by dead *F. oxysporum*

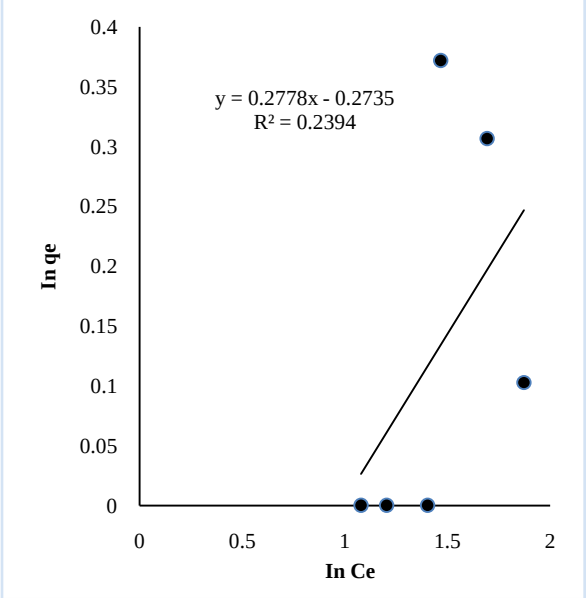

Figure 7(b) Freundlich isotherm for adsorption of lead by dead *F. oxysporum*

Table 1 Isotherm constants for lead sorption by dead *F. oxysporum*

Isotherm	Equation	Plot	Parameters	Value
Langmuir	$C_e/q_e = 1/q_e K_1$ `+ C_e/q_m	A plot Ce/qe versus Ce showed a straight line of slope 1/qm and an intercept of 1/(Ka qm)	qm (mg/g) K (l/mg) R^2	1.046 15.52 0.9472
Freundlich	Log q_e= log K_f +1/n log C_e	K_f and 1/n values were evaluated from the intercept and slope of the linear plot of lnqe versus lnCe, respectively	n K_f (mg/g) R^2	3.599 1.3146 0.2394

Therefore, isotherm results of lead adsorption on *F. oxysporum* was analyzed by Langmuir and Freundlich models (Table 1). Further, the Langmuir isotherm model showed a better fitting model as compared to Freundlich with a high correlation coefficient (R^2= 0.9472). Langmuir constants indicated values: q_m= 1.046 mg/g, k = 15.52 mg^{-1}, R^2 = 0.9472; Freundlich constants were K_f = 1.3146, n = 3.599, R^2 = 0.2394 (Figure 7a and b).

3.7 Phytotoxicity study

A phytotoxicity test was also conducted to compare the effect of lead ions and treated lead solution with live and dead biosorbents of *F. oxysporum* on the growth parameters of wheat seeds. Significant variations were observed under various studied treatments i.e. seed germination and growth characteristics of wheat seeds (Table 2). In control, 97% seed germination was observed whereas lead solution-treated wheat seeds reflected only 21% germination. Further, in the case of radicle and plumule length, it was reported 2.9 and 10.2 cms in control and these parameters decreased to 0.31 and 2.4 cms with lead solution treatment. Wheat seed germination was enhanced by 247.6 and 328.5% with live and dead *F. oxysporum* treatment respectively over the lead metal solution and the growth parameters were significantly enhanced with dead fungal biomass treated lead solution in comparison to living fungal biomass treatment. Seedling length and vigor index of wheat seeds indicated the following trend: Control > dead biosorbent treated lead solution > live biosorbent treated lead solution > lead solution.

Results of the study suggested that lead contamination affected various biochemical processes in plant cells. Lead toxicity reduced wheat seed germination, biomass, and other growth parameters. Lead showed disruption in minerals uptake in plants (Sharma and Dubey 2005). Lead produces reactive oxygen species in plants which reduces plant growth and photosynthesis (Ekmekci et al. 2009). Javaid et al. (2011) used *Aspergillus niger* after treatment with acid and sodium carbonate for the elimination of copper and nickel metals. Rao and Bhargavi (2013) observed that pretreated fungal biomass can be used as an adsorbent for the removal of lead and nickel from the single and binary metal system and found that Zinc metal ions were significantly removed by *Aspergillus flavus* (Mali et al. 2014). Further, Garcia et al. (2016) observed lead biosorption by *Bacillus* species and Mahish et al. (2018) reported a 90% lead adsorption ability of *Penicillium oxalicum.*

Table 2 Effect of live and dead biomass of *F. oxysporum* on germination and seedling length of *T. aestivum* var. UP2554.

Treatment	Seed germination (%)	Radicle length (cms)	Plumule length (cms)	Vigour index
Control	97 ± 0.79[a]	2.9± 0.21[a]	10.2 ± 0.68[a]	12707
Lead solution	21± 0.62[c]	0.31± 0.03[c]	2.4± 0.17[c]	569.1
Lead solution treated with live fungal biomass	73± 0.34[b]	1.2± 0.04[b]	3.5± 0.56[b]	3431
Lead solution treated with dead fungal biomass	90± 0.56[a]	1.7± 0.12[b]	7.9± 0.72[a]	8640

Results are mean ± sem of three replicates; different letters on values have shown significant differences at $P < 0.05$ among treatments as per ANOVA and DMRT

Table 3 Lead removal efficacy of various biosorbent

Metal	Biosorbent	Adsorption capacity (mg/g)	References
Lead	Watermelon-Fe_3O_4 composite	138	Adebowale et al. (2020)
	Polypyrrole-based activated carbon	50	Alghamdi et al. (2019)
	Hazelnut husk	13	Imamoglu and Tekir (2008)
	Apricot stone	21	Mouni et al. (2011)
	Juniperus procera	30	Ali et al. (2019)
	Sugarbeet pulp	71	Pehlivan et al. (2008)
	Sugarcane bagasse	23	Salihi et al. (2016; 2017)
	Fusarium oxysporum	1.046	This study

3.8 Performance of *F. oxysporum* with other sorbents

Results presented in table 3 showed the lead removal efficiency by *F. oxysporum* and other sorbents. Lead elimination ability is in agreement with earlier reports suggesting that lead can be sorbed on *F. oxysporum*. Results of the study revealed that fungal biomass is cost-effective and promising sorbent for lead elimination.

To the best of our information, very few studies have been conducted to check the ability of live and dead biosorbents for heavy metal removal from aqueous solution or wastewater system (Hu et al. 2020). The effect of live and dead biomass of *F. oxysporum* has not been observed previously. Results of the study suggested that dead biosorbents are better alternatives as compared to live sorbents due to several advantages like high efficiency, cost-effective nature, no requirement of nutrients or growth media, and no waste sludge production (Cheng et al. 2015). Further, Paul et al. (2012) suggested that bacterial biosorbent enhanced its heavy metal absorption capacity after heat treatment because of the degradation of cell walls and exposure of binding sites for metal ions. Live biosorbents can transport adsorbed heavy metals into cells and alters heavy metal ion's state into less toxic forms (Yin et al. 2018). Hlihor et al. (2017) reported that biosorbents can easily remove heavy metals even present at very less concentration.

Conclusion

Results of the present investigation can be concluded that lead can be significantly eliminated from aqueous solution with the help of *F. oxysporum*, which is a cost-effective and sustainable option for environmental protection. Further investigations are needed for its application at a large scale for the treatment of industrial effluents containing heavy metals and organic contaminants.

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Conflict of interest

The author declares that there is no conflict of interest.

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