University of Tehran Press

Pollution

Print ISSN: 2383-451X
Online ISSN: 2383-4501

https://jpoll.ut.ac.ir/

Trichoderma tomentosum Ts141 as a Potential Candidate for Bioremediation of Cadmium, Lead, and Nickel Ions

Samira Hoseinzadeh[3] | Ali Asghar Aliloo[1]✉ | Saleh Shahabivand[2] | Mohammad Ghaderi[1]

1. Department of Production Engineering and Plant Genetics, Faculty of Agriculture, University of Maragheh, Iran, P.O. Box 55181-83111, Iran
2. Department of Biology, Faculty of Science, University of Maragheh, Maragheh, Iran P.O. Box 55181-83111, Iran
3. Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran

Article Info

Article type:
Research Article

Article history:
Received: 21 Oct 2022
Revised: 24 Jan 2023
Accepted: 23 Jun 2023

Keywords:
Bioremediation
Fungi
Heavy metals
Pollution

ABSTRACT

Fungi are successful microorganisms in the bioremediation of environmental pollution. So, this study aimed to determine the potential of *Trichoderma tomentosum* to remediate cadmium, lead, and nickel contaminations from potato dextrose agar (PDA) and potato dextrose broth (PDB) media. Growth rates, toxicity tolerance sporulation, bio-sorption capacity, and bio-sorption efficiency of the fungus were evaluated under different concentrations of $CdCl_2$, $Pb(NO_3)_2$, and $NiCl_2$. The findings demonstrated that the growth rate of the fungus differed depending on concentration, metal type, and medium. More metals in PDA medium induced more inhibition on fungus growth rates; however, the rate was independent from the heavy metals concentrations in PDB medium. Cadmium was the most toxic metal tested against *T. tomentosum*, with a 72h LC50 of 37 ppm. It was about 3.16 and 4.24 times as toxic as nickel and lead, respectively. In the control condition, sporulation of the fungus began at 72 hours, but under the heavy metals, it began at 168, 168, and 192 hours, respectively, for Pb, Ni, and Cd. Both the bio-sorption capacity and efficacy of the fungus were significantly enhanced by an increase in metal content and the highest values were obtained at 200 ppm of the salts. The heavy metals total bio-sorption capacity order was Ni < Cd < Pb in the aqueous medium. The conclusion was that *T. tomentosum* has a greater potential for the biosorption of heavy metals; hence, the fungus may be employed for the bioremediation of heavy metals from polluted sites, particularly wastewater and industrial influents.

Cite this article: Hoseinzadeh, S., Asghar Aliloo, A., Shahabivand, S., and ghaderi, M. (2023). Trichoderma tomentosum Ts141 as a Potential Candidate for Bioremediation of Cadmium, Lead, and Nickel Ions. *Pollution*, 9 (3), 1128-1139. https://doi.org/10.22059/poll.2023.350178.1671

 Publisher: University of Tehran Press.
DOI: https://doi.org/10.22059/poll.2023.350178.1671

INTRODUCTION

The importance of the environment to human health is inevitable; therefore, scientists and environmentalists consider its sustainability. There are many factors that cause disorder in the environment functions; one of these factors is the occurrence of pollution substances such as metals, semi-metals, and organic contaminants in the environment that have harmful effects on the organisms (Shi et al., 2022; Mohsenzadeh and Shahrokhi, 2014; Siddiquee et al., 2013). During economic transitions, industrial activities and technological advancements have significantly increased the levels of heavy metals in the environment, making heavy metal contamination a serious environmental threat, so, they accumulated to the extent of toxicity in the tissues of organisms (Yaashikaa et al., 2022). They easily interfere with plant physiological functions, soil microbial community, and soil physicochemical characteristics. They are long-

*Corresponding Author Email: *aliasghar.aliloo@gmail.com*

term persistent in the environment, so eliminating them is essential for public health (Iram et al., 2015; Singh et al., 2012; Kumar et al., 2006 ;Yang et al., 2005). Metal plating, paint industry, metallurgy, mining, surface finishing, energy and fossil fuel production, fertilizers, washing pesticides, and natural rock erosion are the primary sources of heavy metals released into the environment (Alengebawy et al., 2021; Mohsenzadeh and Shahrokhi, 2014; Siddiquee et al., 2013; Iskandar et al., 2011). The spread of these contaminations is to such an extent that even the reports show the sediments of water reservoirs are a source of accumulation by heavy metals (Mohammadi et al., 2018). Due to the easy mobilization and accumulation of metals in animal and human bodies through the food chain the threat is significant. For instance, the findings of Shokri et al.(2022) demonstrated that all onion samples contained heavy metals, particularly, Cd concentration in the province of West Azerbaijan, Iran was 10 fold more than the standard value. They bind to biomolecules such as proteins and act as enzyme inhibitors, preventing biochemical processes, DNA construction, and cell membrane integrity. Due to of their mutagenic ability, they also exhibit carcinogenic properties (Rao et al., 2010; Yang et al., 2005).

The remediation methods used to remove heavy metals from the environment are divided into two basic categories: non-biological and biological. The earlier includes physical (precipitation/ neutralization) and chemical (ion exchange, membrane separation, electrodialysis, and activated carbon adsorption) methods (Sahu et al., 2012; Rao et al., 2010). For example, studies of Dehghani et al., (2018) showed that poly urea-formaldehyde can be efficiently remove highly concentrated cadmium ions from aqueous solutions. The downsides of these technologies include partial metal removal, high cost, limited specificity, high energy needs, labor intensiveness, environmental disruption, and the production of harmful chemicals (Idris et. al., 2023). Therefore, researchers focus increasingly on biological approaches (Bioremediation) that are eco-friendly with higher performance and without the mentioned disadvantages for non-biological methods (Lin et al., 2022; Iram et al., 2015; Selen et al., 2014; Faedda et al., 2012; Sahu et al., 2012). Bioremediation uses biological agents like plants, algae, bacteria, fungi, and yeasts for remediation of heavy metals from contaminated sites; indeed, they act as biosorption agents (Yaashikaa et al., 2022; Mohsenzadeh and Shahrokhi, 2014; Mohsenzadeh et al., 2012; Kumar et al., 2006; Yang et al., 2005). Macro- and micro-organisms alone or in combination with other synthesized substances can play a role in the absorption of heavy metals. For example, absorption of chromium by Fe3O4 loaded on activated carbon prepared from alga has been reported (Afshin et al., 2021). The type of selected bioagents for the bioremediation of heavy metals is critical. They must be able to efficiently work in a polluted environment and adapt to the conditions. One of the most effective bioremediation groups is fungi. Their biodiversity and adaptability are remarkable. So, they are frequently used to reduce contaminated sites (Kaur et al., 2023; Liu et al., 2017; Dugal and Gangavane, 2012; Kumar et al., 2012; Mohsenzadeh et al., 2010). The cell wall compositions of fungi have a high affinity to binding with metals; thus, they are more efficient in the sorption of heavy metals even without physiological activity. Fungi can adapt and grow under various conditions and detoxify heavy metals through valence transformation, extra and intracellular precipitation, and active uptake (Siddiquee et al., 2013).

Trichoderma species belonging to the Hypocreales order of the Ascomycota division are naturally common, soil-inhabiting, teleomorphic, genetically very diverse, and act as biocontrol agents. They are saprophytic fungi that grow in the soil, rhizosphere, and bark, and it has been shown that they can adapt to many environmental conditions (Tyśkiewicz et al., 2022). *Trichoderma* spp. have various functions in agriculture; There are reports of their use to remove heavy metal contamination from soil and water (Malkoc et al., 2021; Zin and Badaluddin, 2020; Khan et al., 2020; Zhang et al., 2015; Tripathi et al., 2013; Kacprzak et al., 2014; Faedda et al., 2012; Fu et al., 2012; Druzhinina et al., 2011). For instance, Siddquee et al. (2013) studied the tolerance of *Trichoderma* species (*T. virense, T. harzianum,* and *T. auroviride*) to remediate

lead and nickel contamination. They found that *T. virense* had the maximum tolerance to heavy metals. Dixit et al. (2011) used *T. virense* ability to increase endurance and refine heavy metals in tobacco. Wang and Wang (2013) found copper removal from acid wastewater by *T. viride*. Yazdani et al. (2010) studied the uptake capacity of Zn by *T. atroviride*; the results showed that the uptake capacity of *T. atroviride* ranged from 18.1 to 26.7 $mg.g^{-1}$. Kumar et al. (2011) have studied the sorption of heavy metals ions immobilization by *T. viride* biomass at a packed-bed column. The research of Puglisi et al. (2012) on *T. harzianum* tolerance in mercury and cadmium polluted conditions identified genes expressions differences in response to mercury and cadmium that had a significant role in their tolerance mechanism.

Based on our litterateurs, the studies on *Trichoderma tomentosum* potential to remediate heavy metals pollution are rare; therefore, in this research, we tried to answer the following two important questions. a) Does this fungus could tolerate heavy metals of Cd, Pb, and Ni and what is the concentration of their toxicity? b) Is there a difference in bioremediation of this fungus in aqueous and semi-solid environments? To our knowledge this is the first report on *T. tomentosum* potential to bioremediation of the heavy metals.

MATERIAL AND METHODS

Preparation species of fungi

Trichoderma tomentosum was obtained from the Institute of Genetics and agricultural biotechnology, Sari University of Agricultural and Natural Resources Sciences, Iran. The fungus was incubated in sterilized potato dextrose agar (medium at 29 °C for 7 days until to complete growth). After incubation and the full growth of the fungus, plates were stored in the refrigerator at 4°C for use in future experiments.

Preparation of solid medium and determine radial growth and sporulation

After the initial incubation of species of fungi, PDA solid medium containing concentrations of 0, 25, 50, 100, and 200 ppm of $CdCl_2$, $Pb(NO_3)_2$, and $NiCl_2$ was prepared. The mediums were inoculated with 5mm diameter of agar disc cut from the edge side of 7-day actively growing cultivations of *Trichoderma tomentosum*. The discs were placed in the center of the petri dishes and incubated at 29° C. Cultures were observed daily, and their radial growth were measured until the radial growth of treatment (in more than 50% replications) reached the edge of the medium. The treatments were checked for the initial phase of sporulation every day. For each treatment, the growth inhibition percentage was obtained by equilibrium (1). The toxicity factor (TF) of treatments was calculated by equilibrium (2). Five replicates were performed for each treatment as subsamples, and the experiments were repeated five times.

$$IP = (1 - \frac{r_i}{R}) \times 100 \quad (1)$$

Where IP= the growth inhibition percentage (%); r_i =radial of final growth (mm); R = radial of plate (mm)

$$TF = \frac{72\ h\ Lc50\ value\ of\ other\ metals}{72\ h\ Lc50\ value\ of\ most\ toxic\ metal} \quad (2)$$

Where LC_{50}= the median lethal concentration at 72th hour

Preparation of broth medium

PDB (potato dextrose broth) liquid medium was autoclaved at 121°C, pressure 25 for 15

minutes; then the mediums were inoculated with 5mm diameter of agar disc cut from the edge side of 7-day actively growing fungus. The flasks were agitated on a rotary shaker for 8 days at 150 rpm and at 29°C. All the experiments were repeated 3 times.

Determination of fungal growth at different concentrations of heavy metals

After 8 days of incubation, flasks containing fungal biomass were filtered through Whatman No. 42 filter paper. The supernatant was stored for further testing. The fresh weight of biomass was measured, and then samples were left in an oven at 60°C for 24 to lose all the moisture content and achieved a constant dry weight.

Removal of cadmium, nickel and lead at different concentrations by the fungus

The remaining metals that exist in the supernatant were determined by an atomic absorption spectrophotometer (Shimadzu, AA-6300, Japan). The bioaccumulation capacity of the fungus about any of the metals was calculated by the following equilibrium (3).

$$Q = \left(\frac{C_i - C_f}{m}\right)V \quad (3)$$

Where Q=mg of metal uptake per gram biomass ($mg.g^{-1}$); C_i=initial concentration of the metal ($mg.l^{-1}$); C_f= final concentration of metal ($mg.l^{-1}$); m= dry weight of the biomass (g); and V= volume of reaction media (ml).

Determining the efficiency of metal uptake

The following equilibrium (4) was used for determining of metal bioaccumulation efficiency by fungus.

$$E = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \quad (4)$$

In this equilibrium, E= percentage of metal uptake by the fungal biomass; Ci= initial concentration of the metal (ppm); C_f= final concentration of metal (ppm) in the experimental media.

Statistical analysis and Dose-Response analysis

The statistical analyses of variance were analyzed by SAS Ver. 9.1 software, included ANOVA and Duncan's multiple range test ($p<0.05$) procedures. Toxicological dose-response data involving quantal response (inhibition percentage) were analyzed by probit analysis (Finney, 1971) based on IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSION

The heavy metals and growth of Trichoderma sp on PDA

Petri dish assays showed that *T. tomentosum* could grow on PDA contaminated with 100 ppm of $CdCl_2$, 200 ppm $NiCl_2$ and 300ppm $Pb(NO_3)_2$. With the increasing in the concentration of the metals, the radial growth was decreased significantly ($p \leq 0.05$). According to the Table 1, the inhibition percentage (IP) was increased with the increasing of metals concentrations. For $CdCl_2$, with increasing concentrations of the metal from 0 ppm to 100 ppm, the IP was significantly increased; thereafter; the growth was stopped completely. The result for cadmium revealed a severe inhibition by passing from 25 to 50 ppm, so that, the rate reached up to 80 percent at 50 ppm concentration (Table 1). The similar trend was found for $Pb(NO_3)_2$, however,

Table 1. Inhibition percentage of different concentration of $CdCl_2$, $Pb(NO_3)_2$ and $NiCL_2$ on growth of *T. tomentosum*

Concentration of salts (ppm)	Inhibition percentage (%)		
	$CdCl_2$	$Pb(NO_3)_2$	$NiCl_2$
0	00.00 ± 0.0 [e]	00.00 ± 0.00 [e]	00.00 ± 0.00 [e]
25	15.87 ± 3.9 [c]	00.00 ± 0.00 [e]	00.00 ± 0.00 [e]
50	82.00 ± 1.2 [a]	7.12 ± 4.5 [d]	00.00 ± 0.00 [e]
100	95.62 ± 0.5 [a]	35.08 ± 11.5 [b]	35.62 ± 16.1 [b]
200	100.0 ± 0.0 [a]	45.86 ± 7.3 [b]	92.12 ± 0.83 [a]
300	100.0 ± 0.0 [a]	88.87 ± 0.6 [a]	100.0 ± 0.00 [a]

Different letters have significantly differences at p≤0.05; Means has been shown by 'means ±SE'

Table 2. Lethal concentration (LC_{50}) of $CdCl_2$, $Pb(NO_3)_2$ and $NiCl_2$ for *Trichoderma tomentosum* after 72 h growth in PDA solid medium

Elements	The regression line equation	LC_{50} (ppm), 95% Confidence (Lower Bound-Upper Bound)	TF
Cd	Y= 4.954x – 7.776	37 (27 – 48)	1
Ni	Y= 6.614x – 13.675	117 (108 – 125)	3.16
Pb	Y= 3.038x – 6.674	157 (93 – 320)	4.24

LC_{50}, The median lethal concentration at 72[th] hour by probit ; PDA, Potato dextrose agar; TF, toxicity factor

the given concentrations did not stop the growth of *T. tomentosum* even at 300 ppm (Table 1). Also, the inhibition rates among their concentrations were almost increased in a regular maneuver than cadmium. Based on bioassay for $NiCl_2$, at two first concentrations, T. tomentosum grows without any limitation but at 100 ppm their growth was significantly decreased and 300 ppm of $NiCl_2$ halt the growth of the fungus. Nevertheless of increasing in IP percentage, fungus performance under 200 and 300 ppm of $NiCl_2$ had not significantly differences ($p \leq 0.05$).

Relative acute toxicity test of the heavy metals tested against Trichoderma tomentosum

The cumulative percentage of inhibition in *Trichoderma tomentosum* exposed to different concentrations of the heavy metals was analyzed by probit regression with log concentration in ppm (Table 2). The LC_{50} for Cd, Ni, and Pb individuals was 37, 117, and 157 ppm, respectively. Cadmium, with a 72-hour LC_{50} of 37 ppm, was the most toxic metal tested against *T. tomentosum*, followed by Ni and Pb in descending order of toxicity (Table 2). Cadmium was significantly (no overlaps in 95% confidence of the 72-hour LC_{50} values) more toxic than the metallic compounds tested against *T. tomentosum*. Computed toxicity factors (72 h LC_{50} ratios) showed that cadmium was about 3.16× and 4.24 × more toxic than Ni and Pb, respectively (table 2). Due to overlaps at 95% confidence levels, there were no significant differences between Ni and Pb; however, according to low LC_{50} records for Ni in comparison with Pb, this metal was more toxic than Pb (Table 2).

Biomass of the fungus under the metals effect in suspension medium

According to the results measured in the suspension medium for Cd and Pb, the concentrations used for both metals did not significantly (p≤0.05) change the fresh and dry weights of the fungus (Tables 3, 4). Interestingly for Ni, results found a stimulus influence of Ni on fresh weight with adding the metal to the medium that had significant differences with the control. A similar result was found at 50 ppm Ni for dry weights (Tables 3 and 4).

Table 3. Fresh weigh of *T. tomentosum* biomass under different concentration of $CdCl_2$, $Pb(NO_3)_2$ and $NiCL_2$

Concentration of Salts (ppm)	Fresh Weighs ($gr.L^{-1}$)		
	$CdCl_2$	$Pb(NO_3)_2$	$NiCL_2$
0	1.61 ± 0.04^{e}	1.61 ± 0.04^{e}	1.61 ± 0.04^{e}
25	2.35 ± 0.28^{cde}	3.60 ± 0.46^{abcd}	4.40 ± 0.41^{ab}
50	2.24 ± 0.51^{de}	2.98 ± 0.53^{bcde}	4.08 ± 0.49^{ab}
100	2.36 ± 0.44^{cde}	3.73 ± 0.55^{abc}	4.02 ± 0.67^{ab}
200	1.87 ± 0.45^{e}	2.60 ± 0.10^{cde}	4.96 ± 0.14^{a}

Different letters have significantly differences at $p \leq 0.05$, Means has been shown by 'means ±SE'

Table 4. dry weigh of *T. tomentosum* biomass under different concentration of $CdCl_2$, $Pb(NO_3)_2$ and $NiCL_2$

Concentration of Salts (ppm)	Dry weighs ($g.L^{-1}$)		
	$CdCl_2$	$Pb(NO_3)_2$	$NiCL_2$
0	0.63 ± 0.002^{b}	0.63 ± 0.002^{b}	0.63 ± 0.004^{b}
25	0.67 ± 0.013^{b}	0.67 ± 0.012^{b}	0.68 ± 0.103^{b}
50	0.66 ± 0.009^{b}	0.65 ± 0.000^{b}	0.81 ± 0.009^{a}
100	0.63 ± 0.007^{b}	0.68 ± 0.001^{b}	0.69 ± 0.001^{b}
200	0.64 ± 0.014^{b}	0.64 ± 0.008^{b}	0.71 ± 0.002^{b}

Different letters have significantly differences at $p \leq 0.05$, Means has been shown by 'means ±SE'

Bio-sorption capacity of T. tomentosum in removing of Cd, Pb and Ni from aqueous medium

The biosorption ability of the *T. tomentosum* was studied on different concentrations of Cd, Pb, and Ni. Results revealed that the metal bioaccumulation capacity of the fungus was significantly affected by both metal type and metal concentrations in the media (Table 5 and Fig. 1). With increasing concentration from 25 to 200 ppm, bio-sorption capacity increased significantly ($p \leq 0.05$) as follows; for Cd, it was increased from 1.91 to 23.81 $mg.g^{-1}DW$, for Pb increased from 4.1 to 48.38 $mg.g^{-1}DW$ and for Ni increased from 2.05 to 13.64 $mg.g^{-1}DW$ (Table 5). There were no significant differences at 25 ppm concentration among metals in terms of bio-sorption capacity; however, at 50 ppm concentration, the value for Pb was noticeably higher than the Cd and Ni. The highest bio-sorption capacity was also obtained by Pb at 200 ppm (Table 5 and Fig 1). The total bio-sorption capacity order of heavy metals was Ni < Cd < Pb. Based on the regression equation that was plotted between biosorption capacity and metals, the slope of the lines varied, and a high slope coefficient was recorded for Pb.

Bio-sorption efficiency of T. tomentosum in removing of Cd, Pb and Ni from aqueous medium

Analysis of variance for bio-sorption efficiency data revealed significant differences ($p \leq 0.01$) among heavy metals and their concentrations. With increasing the heavy metal concentrations, the bio-sorption efficiency of the fungus was increased significantly and reached maximum rates at 200 ppm (Table 6 and fig 2). Also, the biosorption efficiency of *T. tomentosum* to remove the metals was different. The highest efficiency was recorded for Cd, followed by Pb and Ni, respectively (Table 6 and fig 2). The result showed high efficacy in removing Pb and Cd at low concentrations than Ni. For the element, the fungus efficiency started from ~39% at 25 ppm and reached ~72 at 200 ppm concentration (Table 6, and fig 2). The results of fitting a polynomial regression model to each element against the concentrations are shown in Fig. 2. The predictors in the model are x and x2, where x2 is x2. Note the sign for x2 in each of the models. The negative sign of x2 shows that the curve is concave. So, at higher concentrations,

Table 5. bio-sorption capacity of *T. tomentosum* under different concentration of $CdCl_2$, $Pb(NO_3)_2$ and $NiCL_2$

Concentration of Salts (ppm)	Bio-sorption capacity ($mg_E.g^{-1}_{DW}$)		
	Cd	Pb	Ni
25	1.919 ± 0.14 ^{e}	4.101 ± 0.37 ^{e}	2.054 ± 0.08 ^{e}
50	4.870 ± 0.28 ^{e}	10.427 ± 0.15 ^{d}	4.082 ± 1.00 ^{e}
100	12.77 ± 0.73 ^{d}	18.342 ± 0.41 ^{c}	10.195 ± 0.38 ^{d}
200	26.12 ± 2.80 ^{b}	48.388 ± 3.65 ^{a}	13.643 ± 0.10 ^{d}

Different letters have significantly differences at p≤0.05; Means has been shown by 'means ±SE'

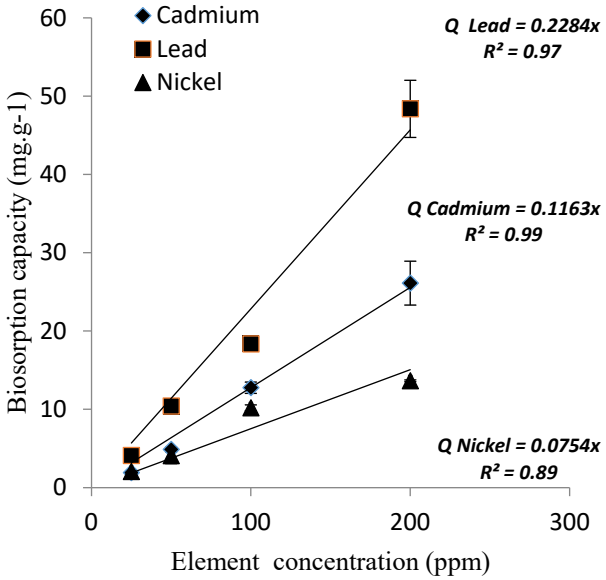

Fig. 1. Biosorption capacity of T. tomentosum in the presence of different Cd, Pb and Ni concentrations

Table 6. bio-sorption efficiency of *T. tomentosum* under different concentration of $CdCl_2$, $Pb(NO_3)_2$ and $NiCL_2$

Concentration of Salts (ppm)	Bio-sorption efficiency (%)		
	Cd	Pb	Ni
25	66.53 ± 0.53 ^{e}	70.00 ± 1.97 ^{d}	38.94 ± 1.64 ^{g}
50	79.43 ± 0.09 ^{c}	80.90 ± 0.97 ^{c}	54.85 ± 0.17 ^{f}
100	87.54 ± 11.6 ^{b}	84.63 ± 1.34 ^{b}	64.87 ± 0.72 ^{e}
200	92.71 ± 7.06 ^{a}	88.23 ± 2.47 ^{b}	71.74 ± 0.02 ^{d}

Different letters have significantly differences at p≤0.05; Means has been shown by 'means ±SE'

the efficacy will decline in comparison with low concentrations (Fig. 2). This mode is more evident for Ni and Cd curves, respectively. Moreover, the high R-squared values recorded for Ni and Pb (0.97 and 0.91) show that the models have fitted the data with high accuracy.

Besides other methods, the use of fungi to remove or degrade pollutants from contaminated sites is going to become the most practical method due to their diversity and applicability. In this study, we examined the growth tolerance of *Trichoderma tomentosum* in contaminated mediums with Cd, Pb and Ni. Results revealed that the growth of the fungus in the contaminated PDA medium varied among the metals. Cadmium started its inhibition from 25 ppm and was determined at 100 ppm. Lead initiated its prevention at 50 ppm; however, it did not stop fungal growth even at 300 ppm concentration. For nickel, the threshold of inhibition was 100 ppm, and

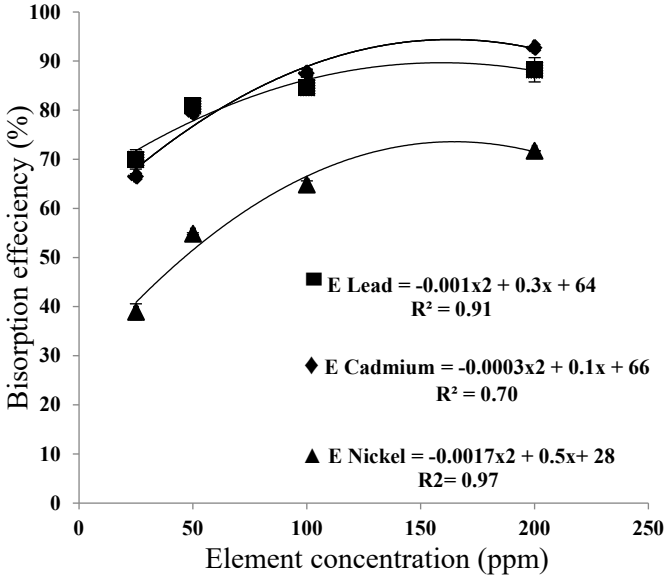

Fig. 2. Biosorption effeciency of T. tomentosum in the presence of different Cd, Pb and Ni concentrations

that 300 ppm concentration suppressed *T. tomentosum* growth completely (Table 1). Further information came out of probit analysis that proved the high toxicity of cadmium to the fungus growth on PDA with low LC_{50} (37 ppm), which was 3.16 and 4.24 folds more toxic than nickel and lead, respectively (Table 2). Oxidative stress and ionic homeostasis are common upsets of heavy metals in a living organism that lead to fast cellular damage and cell death. The generation of reactive oxygen species as a result of heavy metals exposure may result in systematic depletion or inhibition of the cell's antioxidant defenses. So, the accumulation of harmful free radicals damage biomembranes (especially phospholipids), enzymes, proteins and DNA. Disruptions or inhibitions of the scavenging capacity of the antioxidant system in living organisms by Cd, Pb and Ni have been reported by researchers (Xu et al., 2016; Sytar et al., 2013; Nagajyoti, et al., 2010; Yadav, 2010). Differences in the level of toxicity among heavy metals to living organisms are determined by many factors, and exposure-related, biological and chemical properties are the main regulating factors in absorption, metabolism and elimination of the toxicants. In the present investigation, Cd was found to be the most significantly fungi toxic metal in PDA medium (Table 2). Distribution and mobilization of heavy metals play a critical role in the growth rate of organisms. It has been reported that cadmium is easily distributed through *Sinapis alba* plants (Fargašová, 2004). The same phenomenon was maybe occurred in the cell wall or through the fungus hypha; therefore, the toxicity of cadmium was raised in comparison with lead and nickel. Also, cadmium interferes with the S-H groups of proteins (amino acid cysteine), iron and copper of cytoplasmic and membrane proteins like ferritin, which leads to the dysfunction of related enzymes.

There is evidence that reported inhibition of spore germination of VAM fungi by heavy metal ions. The study confirmed the phenomenon; however, sporulation time of the fungus was only affect. The results revealed that the sporulation of *Trichoderma tomentosum* in the control condition was initiated after 72 h while under the heavy metals, it was started later at 168,168 and 192 h for Pb, Ni and Cd, respectively. According to the finding, the toxicity ranking order of the heavy metals was again seen at sporulation time. The result is in agreement with the finding of Azevedo and Cássio (2010), who reported inhibitory effects of Zn, Cu, Ni, and Cd on the growth and sporulation of aquatic fungi. Also, they found that cadmium was more toxic than the other metals.

The growth of *Trichoderma tomentosum* in liquid medium (PDB) was quite different from that in solid medium (PDA). Given concentrations of Cd and Pb, slightly improved fresh weight of the fungus, especially at low and middle concentrations; however, for nickel the value increased remarkably (Table 3). For dry weight, the metals enhanced the rate but not significantly compared to the control, except for nickel at 50 ppm. In the cells, vacuoles are the center of heavy metals homeostasis. Their capability to compartmentalize heavy metals enables cells stay active under the presence of high amounts of environmental contaminations. It has been reported that both plants and fungi are capable of in heavy metal compartmentation (Gonzalez-Guerrero et al., 2008; Sharma et al., 2016). This active transport of inorganic ions to inside vacuoles produces osmotic pressure to maintain water influx. So, increasing fresh weight and stability of dry weight of *Trichoderma tomentosum* in PDB medium may be due to osmoregulation processes. The adaptive behavior of fungi has been studied by Valix and Loon (2003) and Anahid et al., (2010). They reported that in some strains of fungi, with an increase in ion concentration, the growth of fungi could be increased due to the development of fungi tolerance. In the present study, the finding in dry weight supported that theory.

The results revealed a high potential biosorption capacity and biosorption efficiency of the fungus against cadmium, lead, and nickel contaminations in the PDB medium (Tables 5 and 6). The rates follow up on metal concentrations and metal types. Increasing the metal concentrations, increased both of bio-sorption capacity and efficiency of the fungus, so that, the highest values were obtained at 200 ppm of the salts. Heavy metals accumulation in fungi includes passive and active mechanisms. The mostly purposed approaches are binding of the metal to proteins, and polymers, compartmentation in vacuoles and formation of insoluble metal-S components.

The living cells of *T. tomentosum* biosorbed the metal ions Cd, Ni, and Pb. The findings are in agreement with the effects of *Trichoderma* sp. on heavy metals removal from contaminated mediums. Removal of chromium and cadmium from contaminated soil by *Trichoderma viride* has been reported by Singh et al. (2012), and the tolerance of *T. viride* was up to 200 ppm for the metals; this concentration is very close to the result of the present study for *T. tomentosum*. Again, the growth and bioaccumulation capacity of *Trichoderma viride* under different levels of Pb^{2+} and Cd^{2+} have shown a more positive result for accumulation of Pb than Cd (Sahu et al., 2012). These results are consistent with our findings. We found that the order of bio-sorption capacity was Ni < Cd < Pb which lead was about biosorbed two fold than cadmium by the fungus. The most recent result of Teng, et al. (2015) showed that *Trichoderma reesei FS10-C* possessed high Cd resistance (up to 300 mg L^{-1}). Also, the effectiveness of *Trichoderma* sp. fungi for Pb, Ni and Cd removal has been verified (Ezeonuegbu et al., 2015). Nickel biosorption capacity of *Aspergillus niger*, *A. terreus*, *A. flavus*, *Rhizopus arrhizus*, *Cunninghamella echinulata*, *Alternaria alternata* and *Trichoderma harzianum* revealed the maximum biosorption capacity for *T. harzianum* (11.77 mg. g^{-1}_{DW}) (Shoaib et al., 2012). Similar results came out from this study that the result for *T. tomentosum* biosorption capacity of Ni was 13.64 mg. g^{-1}_{DW}. Moreover, it has been purposed that the dried biomasses of *Trichoderma* sp. are able to remove total contamination of Cd, Cr and Ni in the groundwater during 48 h and a greater affinity to the biomass has been reported for Cd (Chew et al., 2012). According to the results, the biosorption capacity was increased at high concentrations. It could be due to the existence of unsaturated charge sites (binding sites) on cell the wall of hypha under low concentrations of heavy metals. So, the chance of binding with free sites has increased with the increase of ions in the medium. Furthermore, the adaptive behavior of *T. tomentosum* under high concentration could be more helped to increase biosorption capacity.

CONCLUSION

Heavy metals as contaminants in the environment cause very severe harm to the growth and development of organisms. The results of the experiments presented in this paper allow the establishment of preliminary lethal concentrations (in PDA medium) associated with 50%

fungus inhibition after 72 hours (LC_{50}-72hr) for the heavy metals which were 37, 117 and 157 ppm for Cd, Ni and Pb, respectively. Also*, Trichoderma tomentosum* showed a high potential for biosorption of the heavy metals in the following order; Ni < Cd < Pb at PDB medium. We suggest using the ability of the *Trichoderma* to reduce heavy metals from water environments, especially waste and sewage from factories before releasing them into the environment. Because it is easier to manage biological agents in such environments than in urban and natural environments. Also, recycling of these metals from biological substances should be emphasized in research works.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Afshin, S., Rashtbari, Y., Vosough, M., Dargahi, A., Fazlzadeh, M., Behzad, A. & Yousefi, M. (2021). Application of Box–Behnken design for optimizing parameters of hexavalent chromium removal from aqueous solutions using Fe3O4 loaded on activated carbon prepared from alga: kinetics & equilibrium study. JWPE, 42;102113.

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. & Wang, M.Q. (2021). Heavy metals & pesticides toxicity in agricultural soil & plants: Ecological risks & human health implications. Toxics, 9(3);42.

Anahid, S., Yaghmaei, S. & Ghobadinejad, Z. (2011). Heavy metal tolerance of fungi. Sci. Iran., 18(3);502-508.

Azevedo, M.M. & Cássio, F. (2010). Effects of metals on growth & sporulation of aquatic fungi. Drug Chem. Toxicol., 33(3);269-278.

Chew, A.W., Rahman, N.N., Kadir, M.O. & Chen, C.C. (2012), Dried & wet *Trichoderma* sp, biomass adsorption capacity on Ni, Cd & Cr in contaminated groundwater, IPCBEE, 10-11.

Dehghani, M.H., Tajik, S., Panahi, A., Khezri, M., Zarei, A., Heidarinejad, Z. & Yousefi, M. (2018). Adsorptive removal of noxious cadmium from aqueous solutions using poly urea-formaldehyde: a novel polymer adsorbent. MethodsX, 5;1148-1155.

Dixit, P., Mukherjee, P.K., Sherkhane, P.D., Kale, S.P. & Eapen, S. (2011), Enhanced tolerance & remediation of anthracene by transgenic tobacco plants expressing a fungal glutathione transferase gene, J. Hazard. Mater., 192(1);270-276.

Dugal, S. & Gangawane, M. (2012). Metal tolerance & potential of penicillium species for use in mycoremediation. Int. J. Chem. Pharm. Res., 4(5);2362-2366.

Ezeonuegbu, B.A., Machido, D.A. & Yakubu, S.E. (2015), Capacity of fungal genera isolated from refinery effluents to remove & bioaccumulate lead, nickel & cadmium from refinery waste, Int. j. sci. technol., 3(6);47.

Faedda, R., Puglisi, I., Sanzaro, V., Petrone, G. & Cacciola, S.O. (2012), Expression of genes of *Trichoderma harzianum* in response to the presence of cadmium in the substrate, Nat. Prod. Res., 26(24);2301-2308,

Fargasova, A. (2004). Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young

seedlings of Sinapis alba L. Plant, Soil Environ., 50(1);33-38.
Fu, K., Fan, L., Li, Y., Gao, S. & Chen, J. (2012), Tmac1, a transcription factor which regulated high affinity copper transport in *Trichoderma reesei*, Microbiol. Res., 167(9);536-543.
Gonzalez-Guerrero, M., Melville, L.H., Ferrol, N., Lott, J.N., Azcon-Aguilar, C. & Peterson, R.L. (2008), Ultrastructural localization of heavy metals in the extraradical mycelium & spores of the arbuscular mycorrhizal fungus Glomus intraradices, Can. J. Microbiol., 54(2);103-110.
Idris, M. O., Yaqoob, A. A., Ibrahim, M. N. M., Ahmad, A., & Alshammari, M. B. (2023). Introduction of adsorption techniques for heavy metals remediation. In Emerging Techniques for Treatment of Toxic Metals from Wastewater (pp. 1-18). Elsevier.
Iram, S., Shabbir, R., Zafar, H. & Javaid, M. (2015). Biosorption & bioaccumulation of copper & lead by heavy metal-resistant fungal isolates. Arabian J. Sci. Eng., 40(7);1867-1873.
Iskandar, N.L., Zainudin, N.A.I.M., & Tan, S.G. (2011). Tolerance & biosorption of copper (Cu) & lead (Pb) by filamentous fungi isolated from a freshwater ecosystem. Res. J. Environ. Sci., 23(5);824-830.
Kacprzak, M.J., Rosikon, K., Fijalkowski, K., & Grobelak, A. (2014), The effect of Trichoderma on heavy metal mobility & uptake by Miscanthus giganteus, Salix sp, Phalaris arundinacea, & Panicum virgatum, Appl. Environ. Soil Sci.
Kaur, S., Midha, T., Verma, H., Muduli, R. R., Dutta, O., Saini, O., ... & Dhiman, M. (2023). Bioremediation: A favorable perspective to eliminate heavy metals from polluted soil. In Metagenomics to Bioremediation (pp. 209-230). Academic Press.
Khan, R.A.A., Najeeb, S., Hussain, S., Xie, B., & Li, Y. (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms, 8(6);817.
Kumar, R., Bhatia, D., Singh, R., Rani, S., & Bishnoi, N. R., (2011), Sorption of heavy metals from electroplating effluent using immobilized biomass *Trichoderma viride* in a continuous packed-bed column, Int. Biodeterior. Biodegrad., 65(8);1133-1139.
Kumar, Y.P., King, P. & Prasad, V.S.R.K. (2006). Equilibrium & kinetic studies for the biosorption system of copper (II) ion from aqueous solution using Tectona grandis Lf leaves powder. J. Hazard. Mater., 137(2);1211-1217.
Kumara, R.R., Leeb, J.T. & Chob, J.Y. (2012), Toxic cadmium ions removal by isolated fungal strain from e-waste recycling facility, J. Environ. Health Sci. Eng., 23(19);8745.
Lin, H., Wang, Z., Liu, C., & Dong, Y. (2022). Technologies for removing heavy metal from contaminated soils on farmland: A review. Chemosphere, 305;135457.
Liu, S.H., Zeng, G.M., Niu, Q.Y., Liu, Y., Zhou, L., Jiang, L.H., Tan, X.F., Xu, P., Zhang, C. & Cheng, M. (2017). Bioremediation mechanisms of combined pollution of PAHs & heavy metals by bacteria & fungi: A mini review. Bioresour. Technol., 224;25-33.
Malkoc, S., Kurt, H., Ozbayer, C. & Yagci, E. (2021). Mycoremediation of *Trichoderma harzianum* & *Penicillium chrysogenum* to Pb Exposure: Effect on Metal Bioaccumulation, Oxidative Stress & Antioxidant System. CRPASE, 7(2);1-4.
Mohammadi, A.A., Yousefi, M., Soltani, J., Ahangar, A.G. & Javan, S. (2018). Using the combined model of gamma test & neuro-fuzzy system for modeling & estimating lead bonds in reservoir sediments. ESPR, 25(30);30315-30324.
Mohsenzadeh, F. & Shahrokhi, F. (2014). Biological removing of Cadmium from contaminated media by fungal biomass of *Trichoderma species*. J. Environ. Health Sci. Eng., 12(1);1-7.
Mohsenzadeh, F., Chehregani Rad, A. & Akbari, M. (2012), Evaluation of oil removal efficiency & enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils, Iran. J. Environ. Health Sci. Eng., 9(1);1-8.
Mohsenzadeh, F., Nasseri, S., Mesdaghinia, A., Nabizadeh, R., Zafari, D., Khodakaramian, G. & Chehregani, A. (2010), Phytoremediation of petroleum-polluted soils: Application of Polygonum aviculare & its root-associated (penetrated) fungal strains for bioremediation of petroleum-polluted soils, Ecotoxicol. Environ. Saf., 73(4);613-619.
Nagajyoti, P. C., Lee, K. D., & Sreekanth, T.V.M. (2010), Heavy metals, occurrence & toxicity for plants: a review, Environ. Chem. Lett., 8(3);199-216.
Puglisi, I., Faedda, R., Sanzaro, V., Piero, A.R.L., Petrone,G. & Cacciola, S.O. (2012), Identification of differentially expressed genes in response to mercury I & II stress in *Trichoderma harzianum*, Gene., 506(2);325-330.
Rao, M.A., Scelza, R., Scotti, R. & Gianfreda, L. (2010). Role of enzymes in the remediation of polluted environments. J. Soil Sci. Plant Nutr., 10(3);333-353.

Sahu, A., Mandal, A., Thakur, J., Manna, M.C. & Rao, A.S. (2012). Exploring bioaccumulation efficacy of *Trichoderma viride*: an alternative bioremediation of cadmium & lead. Natl. Acad. Sci. Lett. (India), 35(4);299-302.

Selen, V. Özer, D. & Özer, A. (2014). A study on the removal of Cr (VI) ions by sesame (Sesamum indicum) stems dehydrated with sulfuric acid. Arabian J. Sci. Eng., 39(8);5895-5904.

Sharma, S.S., Dietz, K.J. & Mimura, T. (2016). Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant, Cell Environ., 39(5);1112-1126.

Shi, C., He, H., Xia, Z., Gan, H., Xue, Q., Cui, Z. & Chen, J. (2022). Heavy metals & Pb isotopes in a marine sediment core record environmental changes & anthropogenic activities in the Pearl River Delta over a century. Sci. Total Environ., 814;151934.

Shoaib, A., Naureen, A., Tanveer, F. & Aslam, N. (2012), Removal of Ni (II) Ions from Substrate using Filamentous Fungi, Int. J. Agric. Biol., 14(5).

Shokri, S., Abdoli, N., Sadighara, P., Mahvi, A.H., Esrafili, A., Gholami, M., Jannat, B. & Yousefi, M. (2022). Risk assessment of heavy metals consumption through onion on human health in Iran. Food Chemistry.,14;100283.

Siddiquee, S., Aishah, S.N., Azad, S.A., Shafawati, S.N. & Naher, L. (2013). Tolerance & biosorption capacity of Zn^{2+}, Pb^{2+}, Ni^{3+} & Cu^{2+} by filamentous fungi (*Trichoderma harzianum*, *T. aureoviride* & *T. virens*), SCIRP.,4(4);1-14

Singh, G., Nema, R., Khare, S., Singh, D., Jain, P., Pradhan, A., Gupta, A. & Naidu, S. (2012), Tolerance & biodegradation capacity of *Trichoderma viride* with special reference to heavy metals (Cr, Cd), Indo Am. J. Pharm. Res., 2(10);1007-1014.

Sytar, O., Kumar, A., Latowski, D., Kuczynska, P., Strzałka, K. & Prasad, M.N.V. (2013), Heavy metal-induced oxidative damage, defense reactions, & detoxification mechanisms in plants, Acta Physiol. Plant., 35(4);985-999.

Teng, Y., Luo, Y., Ma, W., Zhu, L., Ren, W., Luo, Y., Christie, P. & Li, Z. (2015), *Trichoderma reesei* FS10-C enhances phytoremediation of Cd-contaminated soil by Sedum plumbizincicola & associated soil microbial activities, Front. Plant Sci., 9;220.

Tripathi, P., Singh, P.C., Mishra, A., Chauhan, P.S., Dwivedi, S., Bais, R.T. & Tripathi, R.D. (2013), *Trichoderma*: a potential bioremediator for environmental clean up, Clean Technol. Environ. Policy., 15(4);541-550.

Tyśkiewicz, R., Nowak, A., Ozimek, E., & Jaroszuk-Ściseł, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens & stimulation of plant growth. Int. J. Mol. Sci., *23*(4), 2329.

Valix, M. & Loon, L.O. (2003). Adaptive tolerance behaviour of fungi in heavy metals. Miner. Eng., 16(3);193-198.

Verena, S.S., Alfredo, H.E., Enrique, M. & Susanne, Z. (2011). *Trichoderma*: the genomics of opportunistic success. Nat. Rev. Microbiol., 9(10).

Wang, B. & Wang, K. (2013). Removal of copper from acid wastewater of bioleaching by adsorption onto ramie residue & uptake by *Trichoderma viride*. Bioresour. Technol., 136;244-250.

Xu, P., Zeng, G., Huang, D., Liu, L., Zhao, M., Lai, C., Li, N., Wei, Z., Huang, C. & Zhang, C. (2016), Metal bioaccumulation, oxidative stress & antioxidant defenses in Phanerochaete chrysosporium response to Cd exposure, Ecol. Eng., 87;150-156.

Yaashikaa, P.R., Kumar, P.S., Jeevanantham, S. & Saravanan, R. (2022). A review on bioremediation approach for heavy metal detoxification & accumulation in plants. Environ. Pollut., 119035.

Yadav, S.K. (2010). Heavy metals toxicity in plants: an overview on the role of glutathione & phytochelatins in heavy metal stress tolerance of plants. S. Afr. J. Bot., 76(2);167-179.

Yang, X., Feng, Y., He, Z. & Stoffella, P.J. (2005). Molecular mechanisms of heavy metal hyperaccumulation & phytoremediation. J. Trace Elem. Med. Biol., 18(4);339-353.

Yazdani, M., Yap, C.K., Abdullah, F. & Tan, S.G. (2010). An in vitro study on the adsorption, absorption & uptake capacity of Zn by the bioremediator *Trichoderma atroviride*. Environ. Asia., 3(1);53-59.

Zhang, T., Tang, J., Sun, J., Yu, C., Liu, Z. & Chen, J. (2015), Hex1-related transcriptome of *Trichoderma atroviride* reveals expression patterns of ABC transporters associated with tolerance to dichlorvos, Biotechnol. Lett., 37(7);1421-1429.

Zin, N.A. & Badaluddin, N.A. (2020). Biological functions of *Trichoderma* spp. for agriculture applications. Ann. Agric. Sci., 65(2);168-178.