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RESEARCH ARTICLE

Isolation and Molecular Identification of Some Isolates of Trichoderma SPP and Evaluation of Its Efficiency in the Bioremediation of Some Heavy Metals in Soil

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ABSTRACT

The aim of the current research is to isolate and identify molecularly some isolates of Trichoderma spp. The research method is experimental and includes soil sampling, separation and purification of fungal isolates and molecular identification of the isolates. To perform sequencing and molecular identification of the isolates, the PCR product was sequenced after quality confirmation. Then, the similarity of the obtained sequences with the sequences in the gene bank was checked using BLAST software and the isolates were identified. The test factors included microbial inoculation (fungal inoculation and no inoculation) and heavy metal contamination of cadmium, lead and zinc. This study concluded that fungal isolates Trichoderma spp can reduce the percentage of heavy elements in the Soil well,and the reduction percentage differed according to the type of targeted element ,SAS software was used for statistical analysis of data, including analysis of variance and mean comparison through Duncan's multi-range test at the probability level of 0.05. Graphs were also drawn using Excel software.

1. INTRODUCTION

Today, soil contamination with heavy metals is one of the leading problems in agricultural systems. Indiscriminate and unreasonable use of chemical fertilizers, the entry of effluents from urban and industrial sewage, as well as the entry of sludge from mining treatment plants that contain minerals are among the main sources of contamination of agricultural soils with heavy metals, which in addition to Serious environmental damage has a negative effect on the health of people in the community as well as the quantity and quality of crops (Vardhan et al, 2019).

Most of the heavy metals are not chemically or biologically decomposed, so they have a higher persistence capacity than organic pollutants in the soil. On the other hand, there are various methods to reduce the concentration and mobility of heavy metals in soils, which is considered as an effective and efficient approach to deal with environmental pollution. Today, there are many methods for controlling and cleaning heavy metals in soils contaminated with them, but choosing a suitable, inexpensive, relatively fast and environmentally friendly method is very important. One of the suitable methods for removing environmental pollutants is the use of bioremediation. This method means using soil microorganisms such as fungi, bacteria and algae. Li et al. (2016) reported that the use of some fungi reduces the metal toxicity of elements and increases plant growth.Lyli Mazlumi et al. (2015) also stated that the use of different species of Trichoderma fungi in reducing the toxic effects of metals can be considered as a significant factor. Trichoderma is a soil-borne, non-pathogenic and symbiotic fungus with plant roots that is abundantly found in most agricultural soils (Kaewchai et al., 2009). Some species of this mushroom also have the ability to survive in high concentrations of heavy metals, and therefore they can be used to clean soils contaminated with these elements (Anand et al., 2006).

Throughout history, humans have always been looking for higher quantity and quality forage plants to feed their livestock. Therefore, improving the yield and quality of fodder plants plays an important role in the health of livestock and then human health. In industrial areas, the increase of human activities has led to high contamination of soils with heavy metals, it is necessary to provide a suitable solution to deal with the destructive effects, the environment of industrial pollutants.

Research on the molecular detection of Trichoderma spp isolates and their efficiency in biological treatment of heavy metals in soil is necessary for various reasons. Heavy metal contamination in soil is an important environmental problem with destructive effects on the ecosystem and human health. Conventional remediation methods often have limitations and can be costly and environmentally disruptive. Therefore, there is a fundamental need to explore alternative, sustainable and environmentally friendly approaches such as bioremediation. Investigating the potential of Trichoderma species in bioremediation can help address this pressing environmental concern (Elkhateeb WA., et al. 2021).

As a result, conducting research on molecular detection of Trichoderma spp isolates and evaluating their efficiency in bioremediation for heavy metals in soil is necessary to address environmental concerns, develop sustainable remediation approaches, and improve our understanding of the molecular mechanisms involved. have practical applications in environmental management and help develop policies and guidelines for effective and environmentally friendly practices. Different soil environments differ in terms of heavy metal composition, pH, organic matter content and microbial communities. Therefore, it is very important to investigate the efficiency of Trichoderma spp strains in bioremediation for specific heavy metals and soil conditions. Research can help identify the most suitable Trichoderma strains for different infested sites and ensure a targeted and effective remediation approach. Understanding the molecular mechanisms of bioremediation of heavy metals by Trichoderma spp can enable researchers to optimize bioremediation strategies. By manipulating the expression of genes involved in metal absorption, accumulation and transformation, it may be possible to increase the bioremediation efficiency of Trichoderma species. This knowledge can lead to the development of appropriate approaches that maximize the removal or immobilization of certain heavy metals from contaminated soil (Ambiental EY., et al. 2010).

Risk assessment and management: Research on the efficiency of Trichoderma species in bioremediation can help in risk assessment and management strategies. By evaluating the fate and transport of heavy metals during bioremediation processes, researchers can assess the potential for metal leaching, runoff, or bioaccumulation in the environment. This information is critical for designing effective containment measures, minimizing potential risks, and ensuring the long-term sustainability of bioremediation projects. Bioremediation using Trichoderma spp can be integrated with other remediation techniques to achieve synergistic effects. For example, combining bioremediation with phytoremediation (using plants to remove pollutants) or chemical amendments can increase the overall efficiency of heavy metal removal from soil. Research can explore the compatibility and effectiveness of such integrated approaches, leading to the development of comprehensive and multifaceted remedial strategies. Advancement of knowledge and innovation: Research on the bioremediation of Trichoderma spp contributes to the advancement of scientific knowledge in the field of environmental microbiology and biotechnology. By examining the potential of natural microbial agents to solve environmental challenges, it strengthens innovation. The findings of such research can inspire further research and collaboration leading to the development of new and improved bioremediation techniques in the future. Global impact on the environment: Heavy metal pollution is a global issue that affects many regions around the world. By conducting research on the bioremediation of Trichoderma spp, scientists can help address this global environmental problem. The generated knowledge can be shared internationally and enable the transfer of expertise, cooperation between researchers and the adoption of sustainable bioremediation practices in different countries. Academic and Career Development: Research on the molecular diagnosis of Trichoderma spp and its biological application offers significant academic and career advancement opportunities. It allows researchers to expand their knowledge and expertise in environmental microbiology, molecular biology and biotechnology. In addition, findings can lead to publication in scientific journals, participation in conferences, and potential collaborations with other researchers and industry professionals (Anderson GR., et al. 2011).

By conducting research on molecular detection of Trichoderma spp isolates and evaluating their efficiency in bioremediation of heavy metals in soil, scientists can contribute to environmental sustainability, develop effective remediation strategies, advance scientific knowledge, and positively impact global environmental health. Therefore, the purpose of this research includes the following.

1) Isolation and molecular identification of Trichoderma isolates using molecular techniques such as DNA sequencing and phylogenetic analysis
2) Evaluation of heavy metal tolerance and accumulation capabilities of isolates of Trichoderma spp
3) Evaluation of bioremediation efficiency of Trichoderma species for heavy metals in soil
4) Investigating the potential and effect of Trichoderma spp potent strains in phytoremediation of heavy elements (Cd, Pb and Zn)

RESEARCH METHOD

This study includes three types of experiments that were conducted in Urmia University from 1401 to 1403.

- **The first test:** Isolation and identification of Trichoderma fungus isolates from soils contaminated with heavy metals and screening of superior isolates.

Soil sampling

In order to obtain isolates of Trichoderma fungi, sampling was done from areas contaminated with heavy metals in Karbala province in Iraq (70 soil samples). Sampling was done randomly from 0-15 cm soil depth. The geographical coordinates of the sampling areas (Table 1-3) are recorded by GPS device and the soil samples are transferred to the laboratory after entering the date, collection location and sample code inside the plastic bags for further studies.

Table 4-1: Sampling areas

	The name of the region	**Number of soil samples**	**The distance from the traffic routes / meters**	
			samples	**Comparative samples**
1	Western Table Area	7	50	5 km
		7	100	5 km
2	Al-Khairat Area	7	50	5 km
		7	100	5 km
3	Al-Hussainiya Area	7	50	5 km
		7	100	5 km
4	Al-Hur Area	7	50	5 km

		7	100	5 km
5	Al-Najaf Road Area	7	50	5 km
		7	100	5 km
	Total	70		The number of comparative samples

Isolation and purification of fungal isolates

Trichoderma mushrooms were purchased from Aria Keshavarz company. The target sample was dry ice. Isolates are isolated by serial dilution plate method and using selective culture medium (Davett, 1979). After the growth of Trichoderma mushroom colonies on the culture medium, they are transferred to the PDA culture medium. Since it is necessary for various laboratory investigations, including diagnostic morphology studies and biocontrol of genetic purity of isolates. All the isolates are purified by hyphal tip method, the purified isolates are transferred to test tubes containing PDA and after growing, they are stored in a refrigerator at a temperature of four degrees Celsius for further studies.

Molecular identification of isolates

Molecular identification of isolates includes preparation of mycelium and DNA extraction, evaluation of extracted DNA, amplification of part of tef gene, electrophoresis, purification and sequencing of PCR products and phylogenetic analyses.

From the primer pair? PCR method was used to amplify the DNA fragment per 1500 base pairs for molecular detection of this specific form. The isolate was extracted by CTAB method. PCR reaction in a volume of 25 microliters in a mixture containing (12.5 microliters 2X PCR Master Mix, 8.5 microliters injection distilled water, 1 microliter (10 pmol / ml) specific primer F, 1 microliter specific primer R, 20 ng of fungal DNA) in a thermocycler () Made in Singapore.The thermal program for the reaction consisted of one cycle of Augier annealing at 94°C for 10 min, followed by 36 cycles of annealing at 14°C for 1 min, annealing at 58°C for 1 min, and extension at 72 °C for 1 min with a final extension at 32 °C for 6 min. The PCR product was passed through 0.8% agarose gel in TBE buffer at 110 V for 60 minutes in the electrophoresis machine. DNA size indicator SM0323 was used in the gel, which showed fragments with size of 100 to 300 bp. Photography of the gel was done with UVITEC device made in England.

To perform sequencing and molecular identification of the isolates, the PCR product was sequenced after quality confirmation. Then, the similarity of the obtained sequences with the sequences in the gene bank was checked using BLAST software and the isolates were identified.

- **The second test:** measurement of tolerance and accumulation of heavy metals in isolated isolates:

The selected Trichoderma isolates were subjected to heavy metal (Cd, Pb and Zn) tolerance and accumulation tests. In this test, the isolates were exposed to different concentrations of heavy metals, including lead, cadmium and zinc, individually. Then, the amount of fungal growth on the solid medium of PDA with a concentration of 600 ppm was checked, or the growth of fungal biomass was evaluated, and the amount of metal absorption by the isolates was measured using atomic absorption. The fungi that show the most resistance to heavy metals were selected to perform other tests.

Determination of minimum inhibitory concentration (MIC)

The minimum concentration of inhibiting the growth of fungal isolates is the lowest concentration of metals that inhibits the growth of fungal isolates. First, PDA solid culture medium was prepared.

Concentrations of 0.5 to 5 mg / ml of heavy metals were added to the PDA culture medium. Then, the fungal isolates with the determined spore population (10 x 6 spores per ml) were inoculated in PDA solid culture medium. The prepared culture medium was kept in an incubator at a temperature of 28°C for 7-4 days. This experiment was performed in three replicates and the minimum concentration of growth inhibition was checked based on the growth of the isolates in the modified culture medium with metals (Iream et al., 2009).

Assessment of bio absorption and bioaccumulation:

In order to prepare the inoculum from the selected mushrooms, first the mushrooms were cultured in PDA solid culture medium. After the growth of the fungi, the population of the mushroom suspension was homogenized and 1 ml was added into 250 ml Erlenmeyer flasks containing 100 ml of heavy metal culture medium of zinc, cadmium and lead with concentrations of cd (0, 50, 100, 300, 500, 800 ppm), Pb (0, 50, 100, 300, 500, 800 ppm) and Zn (0, 100, 500, 750, 1500 ppm) were added. Erlens were kept for 5 days at a temperature of 28 degrees Celsius in a shaker-incubator. Finally, the concentration of elements was measured with an atomic absorption device. The amount of zinc, cadmium and nickel absorbed in equilibrium was calculated using the following equation (Sharma et al., 2020).

$Q = v (C0 - Cf)/M$

Q = element absorption (dry weight of mushroom biomass mg/kg)

C0 and Cf, the order of primary and secondary metal concentrations (mg/l)

M = weight of dried mushroom biomass (g)

Determining the removal rate of heavy metals by fungi

The removal percentage of zinc, cadmium and nickel metals was calculated using the following equation (Sharma et al., 2020).

$\% \text{ Removal} = (C0 - Cf)/Cf \times 100$

C0 and Cf, the order of primary and secondary metal concentrations (mg/l)

- **The third experiment:** investigating the effect of selected Trichoderma fungi on the bioremediation of heavy elements cadmium, lead and zinc under sunflower cultivation (greenhouse cultivation):

The bioremediation experiment was conducted to evaluate the efficiency of Trichoderma isolates in reducing the concentration of heavy metals in contaminated soil. Soil samples with specific levels of heavy metal pollution (concentrations from the results of previous tests) were prepared, and the selected isolates were added to the soil as a mixture using appropriate methods. Treated soil samples were monitored over time and heavy metal concentrations were measured using appropriate analytical techniques. Sunflower is often chosen for phytoremediation due to its ability to accumulate heavy metals including lead, zinc and cadmium in its tissues. It has a deep and extensive root system that enables it to effectively extract pollutants from the soil. In order to create heavy metal pollution in the soil, the elements were dissolved in a specific amount of distilled water and added to the soil uniformly. After applying heavy metal treatments in the pots and bringing its humidity to the FC level in order to achieve the balance of heavy metals with the soil of the pots, they were kept in incubation for one month. The nutrients needed by the plant were added to the pots based on the soil test results before planting. After that, the seeds were sterilized and transferred to pots. To prepare mushroom inoculum, a fresh culture was prepared and then a suspension was prepared from mushroom spores with a specific population and the seeds were inoculated with it. During the growth period, the pots were irrigated with distilled water using the weight method at 70-80% of the agricultural capacity.After 70 days of the vegetative period and at the beginning of the reproductive period, the plants were cut from the crown, placed in an oven at 70 degrees Celsius for 48 hours, and their dry weight was recorded. Measurement of nitrogen (N) in the extract obtained from the digestion of plants in root and stem by Kjeldahl method (Bremner and Breitenbeck, 1983), phosphorus concentration by colorimetric method at a wavelength of 470 nm through a spectrophotometer (Shimadzu UV3100), potassium concentration (K) was measured by a flame photometer, iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) by an atomic absorption spectrometer (Shimadzu 6300 AA) in the extract obtained from dry digestion of plants. Planquart et al., 1999). Then the samples were milled and the desired tests were performed on them. Also, in soil samples, electrical conductivity (ECe) and pH in the extract obtained from saturated mud (Nelson and Sommers, 1982), organic carbon by Walkly and Black (1934), nitrogen by Kjeldahl method, absorbable phosphorus (Olsen, 1954) et al.), usable potassium (Helmeke and Sparks, 1996) and micro elements were measured by DTPA extraction method (Lindsay and Norvell, 1978).

Methods and tools for collecting information

The experiment was conducted as a factorial in the form of a completely randomized design with 3 replications. The test factors included microbial inoculation (fungal inoculation and no inoculation) and heavy metal contamination of cadmium, lead and zinc. SAS software was used for statistical analysis of data, including analysis of variance and mean comparison through Duncan's multi-range test at the probability level of 0.05. Graphs were also drawn using Excel software.

Checks and separations:

In total, 80% of the species isolated from the collected samples include of T. harzianum. % 45 T. longibrachiatum, 35% T. virens, 12% T. brevicompactum , % 8

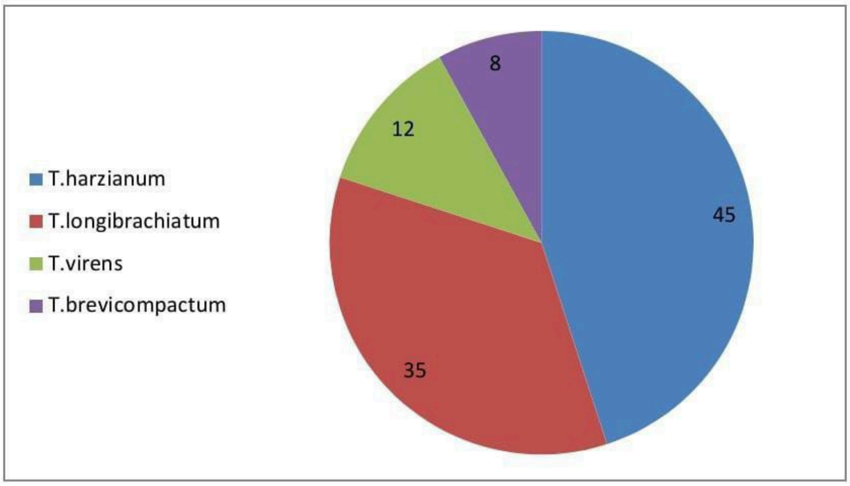


Figure 4-1: Frequency of trichoderma fungus isolates

Morphological identification was not sufficient for species identification, so to confirm species identity, sequence analysis of fifty isolates was performed, which was initially based solely on morphological parameters. Comparison of oligonucleotide fragments of rDNA sequences with reference sequences from public databases showed that they are very similar. Morphologically characterized isolates (T. virens, T. harzianum, T. brevicompactum, T. longibrachiatum) were amplified using universal primers of ITS1-ITS4 regions (Masero et al., 2020). An amplified product of 650 bp was observed in all amplified isolates, which confirmed their presence in gel electrophoresis (Figures 4-2). Purified samples were sequenced and identified using the BLAST tool at NCBI for homology with Determine the previously reported sequences available in the database.Bioinformatic analysis was performed to find the evolutionary relationship of Trichoderma species with previously reported species.

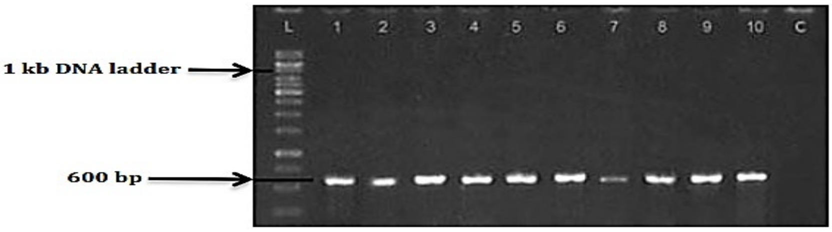


Figure 4-2: Two isolates from each Trichoderma group (T. virens, T. harzianum, T. brevicompactum, T. longibrachiatum) were amplified using ITS1-ITS4 universal primers

Amplification of the ITS-rDNA region was also successful in this species, using general primers ITS4 / ITS1F, and a band of 650 bp was amplified. isolate 54-1 Tl was identified as T. longibrachiatum (Figure 3-4).

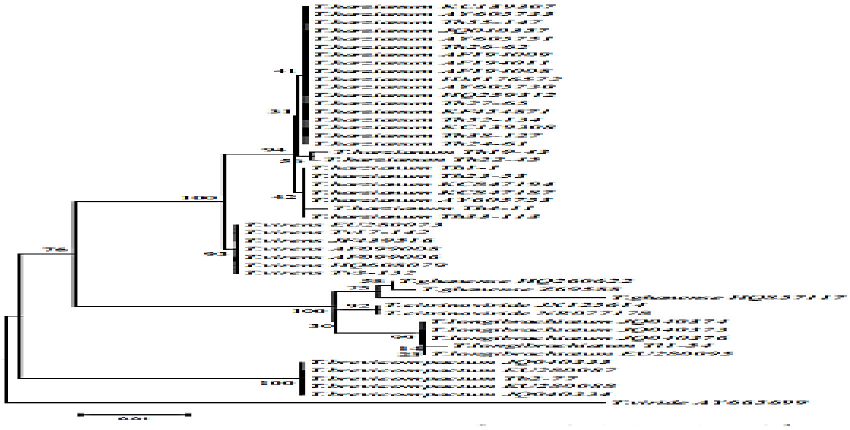


Figure 4-3: Evolutionary relationships of Pachybasium and Longibrachiatum species based on the Joining-Neighbor method. Validation test with 1000 repetitions along with branches.

Comparison of ITS region sequence of harzianum isolates. T and longibrachiatum. T also showed that there are 45 game differences between the two species in the ITS1 region and 17 game differences in the ITS2 region (Figure 4-4). The results show that the most game change between the species is related to the ITS1 region.

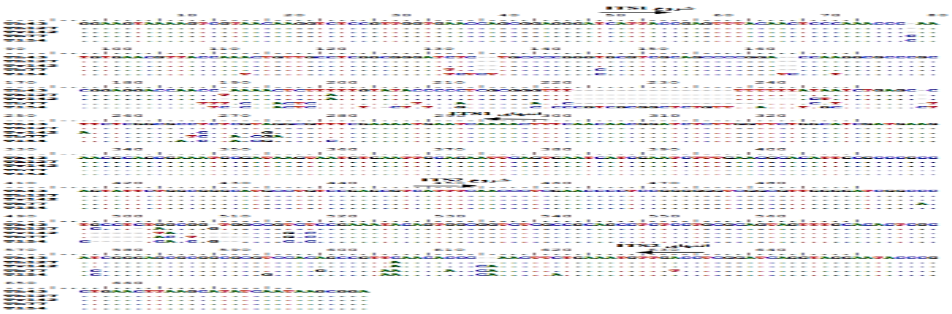
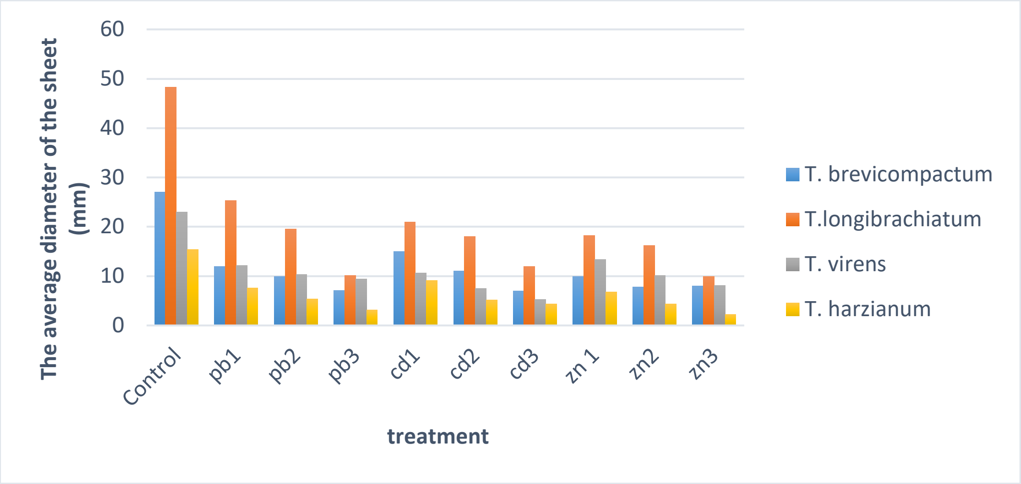


Figure 4-4: Comparison of rDNA-ITS region sequence of T. virens, T. harzianum, T. brevicompactum and T. longibrachiatum

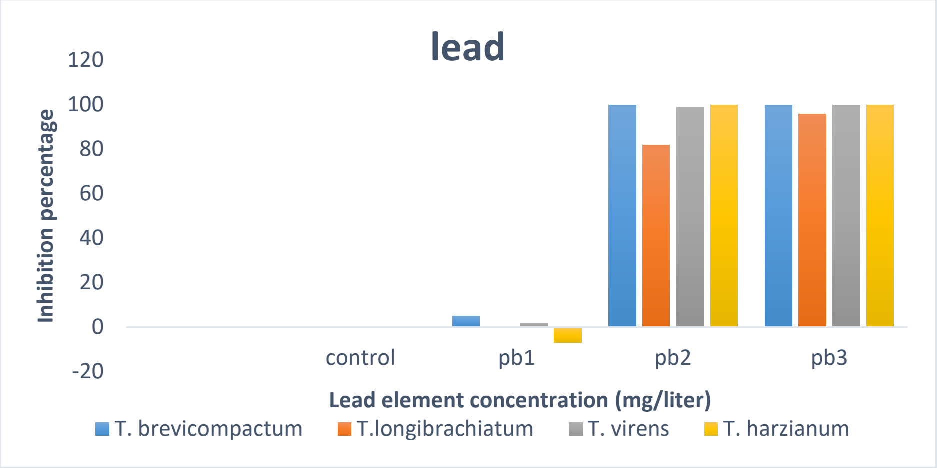
Effect of heavy metal concentration on the growth of fungal species

The results of the test of the effect of heavy metal concentration on the growth of fungal species are shown in Figures 4-5 (it should be noted that only the treatments with growth are given in the results). According to the graph, T.longibrachiatum species had the fastest growth among the three species tested and in the first 72 hours after planting the highest diameter of the diameter (3/48) was recorded in this species, while in the same period T. harzianum species among the three species tested. The aforementioned three species had the lowest pargana diameter (4/15) recorded. Among the treatments where fungal growth was observed, the 20ppm lead concentration had the least inhibitory effect (3/25 mm) on T. longibrachiatum species and the 60ppm zinc concentration had the most inhibitory effect on T. harzianum species.



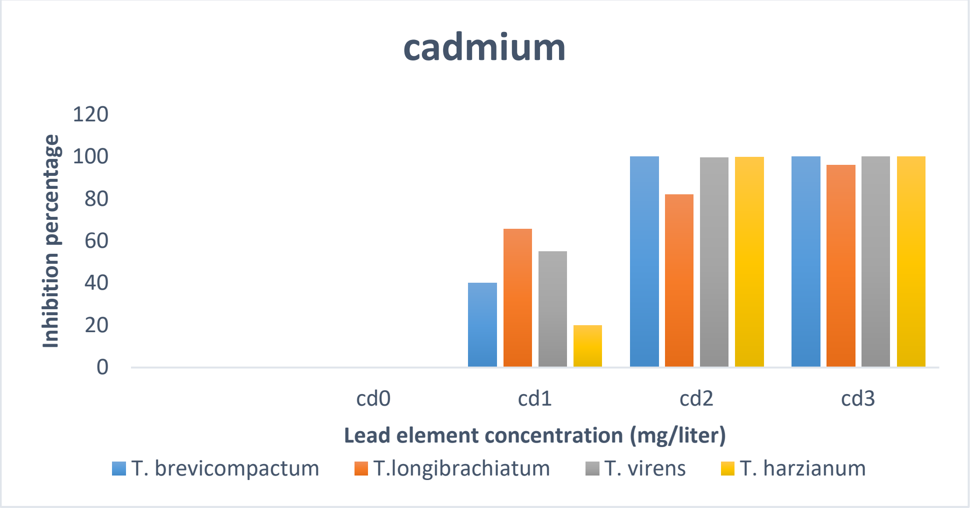
Graph 4-1: Comparison of the effect of different levels of copper, manganese and nickel on the growth of four species of Trichoderma fungus at 25 degrees Celsius, 72 hours after cultivation Pb1: 50 ppm, Pb2: 100 ppm, Pb3: 1500 ppm; cd1: ppm50, cd2: ppm100, cd3: pp

As can be seen in the graph (4-1), the concentration of 20 ppm of lead not only did not have an inhibitory effect on T. harzianum, but also increased its growth by seven percent. Also, this element with the above concentration had the highest inhibitory effect on T. brevicompactum species. From another point of view, the lead element in other tested concentrations has 100% inhibitory effect on all three fungal species (except for the concentration of 40 ppm in which T. longibrachiatum species is possible It grew by 18 percent.



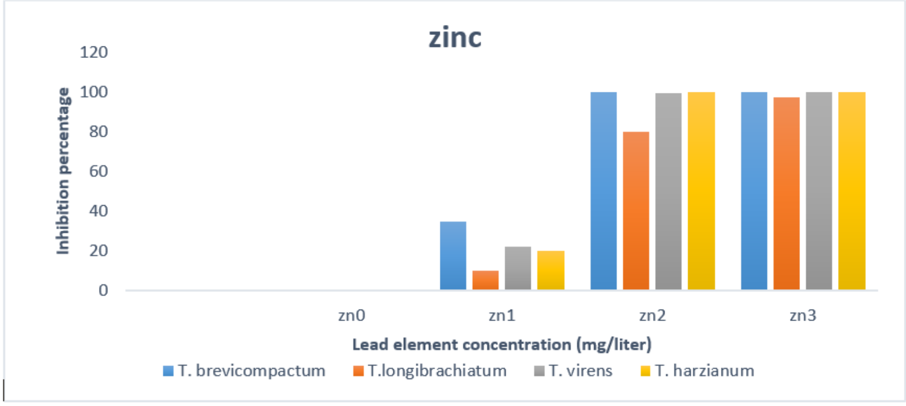
Graph 4-2: Inhibition percentage of different concentrations of lead element (Pb1: 50 ppm, Pb2: 100 ppm, Pb3: 1500 ppm) on the growth of four species of Trichoderma fungus in 72 hours after cultivation

According to graph (4-2) by comparing the inhibition rate of cadmium element on the tested species, it can be seen that the concentration of 20ppm cadmium had the highest percentage of inhibition (65.6%) in T. longibrachiatum, while the concentration of 40ppm had the lowest inhibition (82 %) was on the mentioned species among the tested species.



Graph 4-3: Graph of inhibition percentage of different concentrations of cadmium element (cd1: 50 ppm, cd2: 100 ppm, cd3: 1500 ppm) on the growth of four species of Trichoderma fungus 72 hours after cultivation

As can be seen in the graph (4-3), the zinc element in other tested concentrations has a 100% inhibitory effect on all three fungal species (except for the concentration of 40 ppm in which T. longibrachiatum had the possibility of 21% growth.



Graph 4-4: The inhibition percentage of different concentrations of zinc element (Zn1: 50 ppm, Zn2: 100 ppm, Zn3: 1500 ppm) on the growth of four species of Trichoderma fungus in 72 hours after cultivation.

Comparison of inhibition concentration (IC50) 50% of compounds containing lead, zinc and cadmium for the tested species

In this research (Table 1) it shows that the lowest value of this parameter (25.65 ppm) was for the element cadmium in T. longibrachiatum and the highest value (1100.22 ppm) was for the element zinc in T. harzianum. Also, it can be concluded from the above table that among the three tested species, the lowest difference in IC50 value was for cadmium element and the highest for zinc element.

Table 3-2: Table of 50% inhibition concentration of lead, zinc and cadmium elements for Trichoderma species (96 hours after cultivation)

element	separate	IC50
cadmium	T. longibrachiatum	25/65
	T. harzianum	28/34
	T. brevicompactum	32/22

	T. virens	30/21
lead	T. longibrachiatum	31/24
	T. harzianum	32
	T. brevicompactum	30/2
	T. virens	29/3
zinc	T. longibrachiatum	26/69
	T. harzianum	1100/21
	T. brevicompactum	34/25
	T. virens	34/33

Determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration (MIC) is presented in Table 2. For fungal species, Zn showed higher toxicity, showing growth inhibition at the lowest tested concentration of 200 mg/L for three fungal species. The highest MIC was observed at a concentration of 600 mg/L and for T. longibrachiatum species. Two strains of T. longibrachiatum and T. harzianum were equally sensitive to lead and zinc (Table 3-3).

Table 3-3: Table of the minimum inhibitory concentration of fungal species for cadmium, lead and zinc

element	separate	IC50
cadmium	T. longibrachiatum	600
	T. harzianum	400
	T. brevicompactum	300
	T. virens	500
lead	T. longibrachiatum	200
	T. harzianum	400
	T. brevicompactum	500
	T. virens	400
zinc	T. longibrachiatum	200
	T. harzianum	400
	T. brevicompactum	200
	T. virens	300

Assessment of bioabsorption and bioaccumulation:

The results of data variance analysis showed that the simple effects of bacteria, different levels of cadmium, lead and zinc, as well as the mutual effects between them on the index of bioaccumulation and bioabsorption were significant at the 1% level (Table 3-4).

Table 3-4: Variance analysis of bioaccumulation and biosorption of heavy metals by fungi

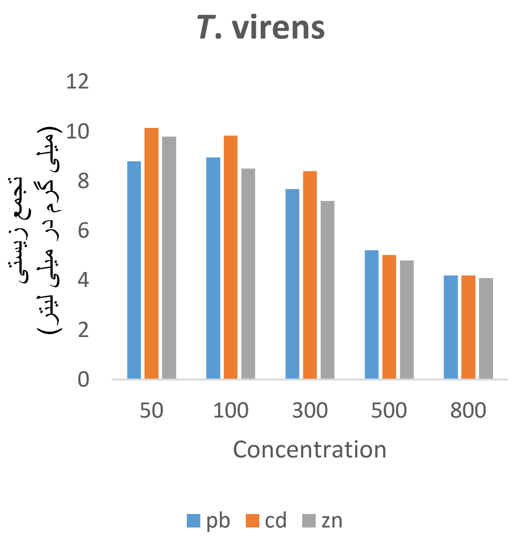
	degrees per	mean square	
		bio absorption	Bioaccumulation
mushroom	3	0/001**	0/1*
repetition	2	0/001	0/051
element	2	42/546**	34/5**
Pollution levels	4	2091/735**	206/421**
Fungus × contamination levels	12	0/004**	0/341*

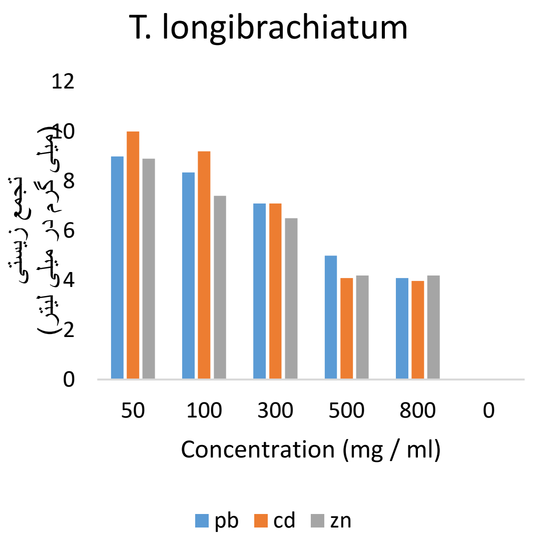
Element × pollution levels	8	3/751**	4/054**
Mushroom × element	6	0/004**	0/09*
Element × contamination levels × fungus	24	0/0021**	0/412**
experimental error		0/0049	0/0021
** Significance at 1% level, * Significance at 5% level			

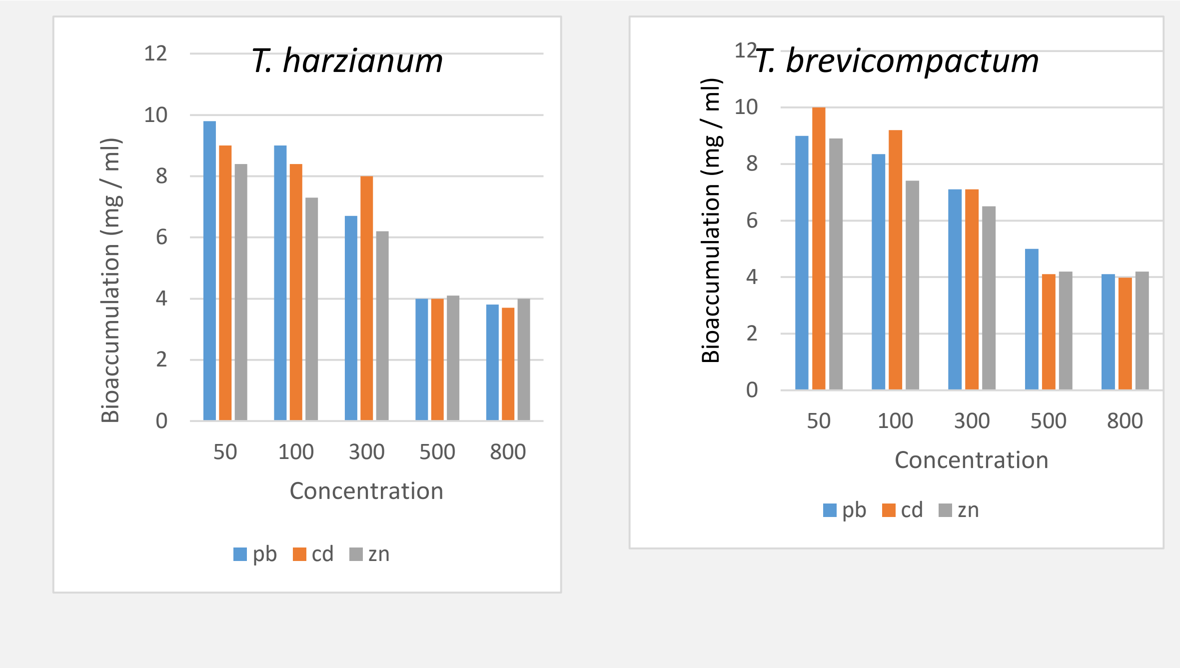
The results showed that in all fungal treatments, with the increase in the concentration of heavy metals, the amount of bioaccumulation also increased. The lowest and highest percentage of bioaccumulation of all three elements in all four mushrooms were observed at 50 ppm and 400 ppm pollution levels, respectively. Examining the bioaccumulation percentage of lead, cadmium and zinc by these fungi shows that T. longibrachiatum has more ability to accumulate lead, cadmium and zinc than the other three fungi. The bioaccumulation sequence of lead, cadmium and zinc by fungi was as follows:

1) T. longibrachiatum
2) T. brevicompactum
3) T. virens
4) T. harzianum

The highest percentage of accumulation of cadmium, lead and zinc in all four types of mushrooms occurred at the pollution level of 40 ppm, so that with the increase in the concentration of elements, despite the increase in the amount of bioaccumulation, the percentage of bioaccumulation was accompanied by a decrease (Graph 4-5).

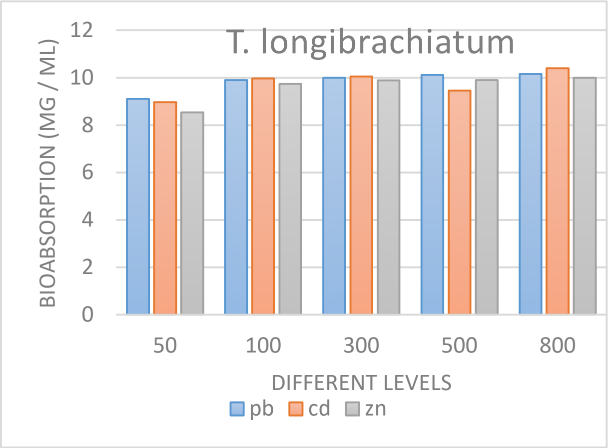

T. virens

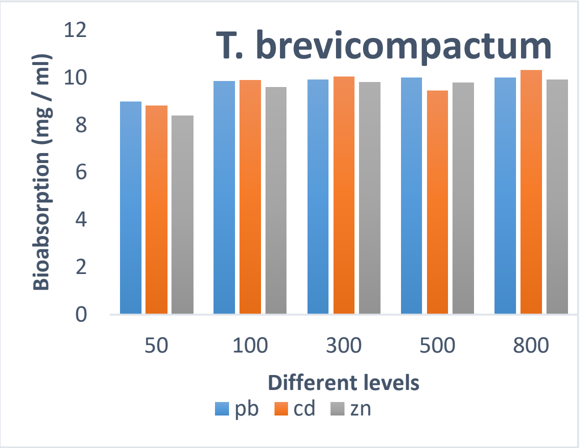

T. longibrachiatum

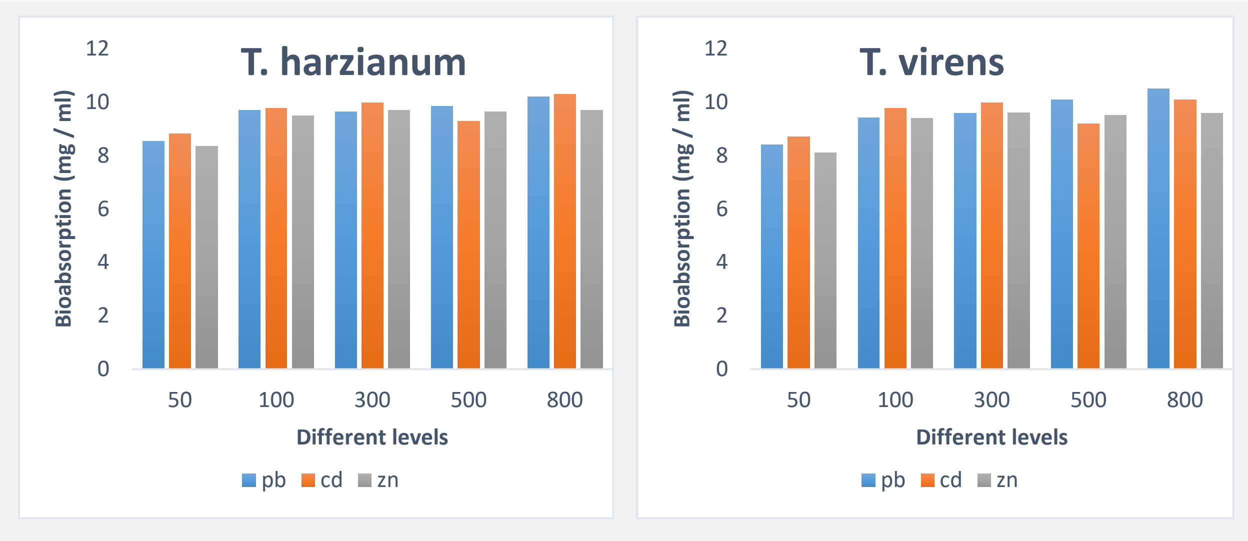


Graph 4-5: Bioaccumulation percentage of zinc, cadmium and lead in four levels of contamination by fungi

The results of the average data comparison showed that the percentage of bioabsorption increased with the increase of pollution levels, so that the highest bioabsorption was observed at 800 ppm levels and the lowest bioabsorption was observed at 50 ppm concentration. Also, the highest amount of bioabsorption was observed in the fungal species T. longibrachiatum. Also, the highest percentage of biological absorption was related to the element cadmium (Graph 4-6).



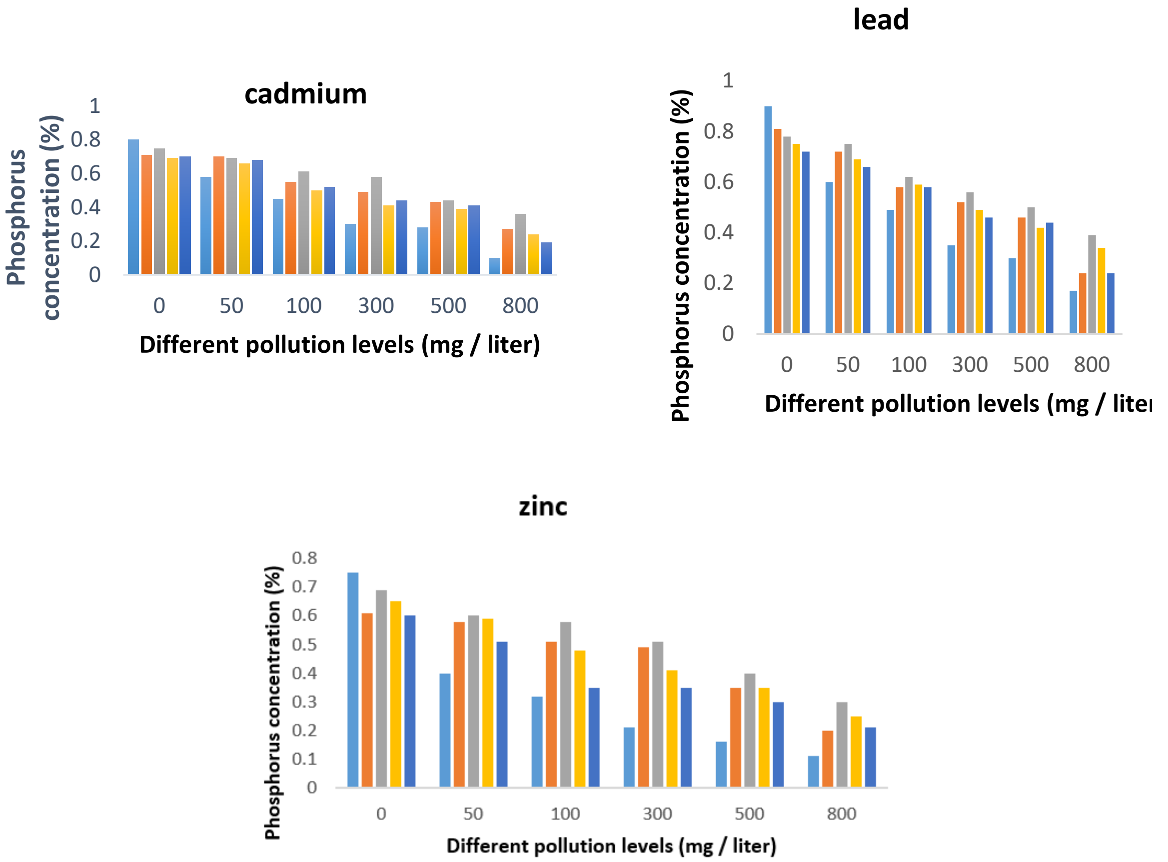




Graph 4-6: The percentage of bioabsorption of zinc, cadmium and lead in four levels of pollution by fungi, the effects of selected Trichoderma fungi in the bioremediation of heavy elements cadmium, lead and zinc under sunflower cultivation (greenhouse cultivation)

The amount of phosphorus

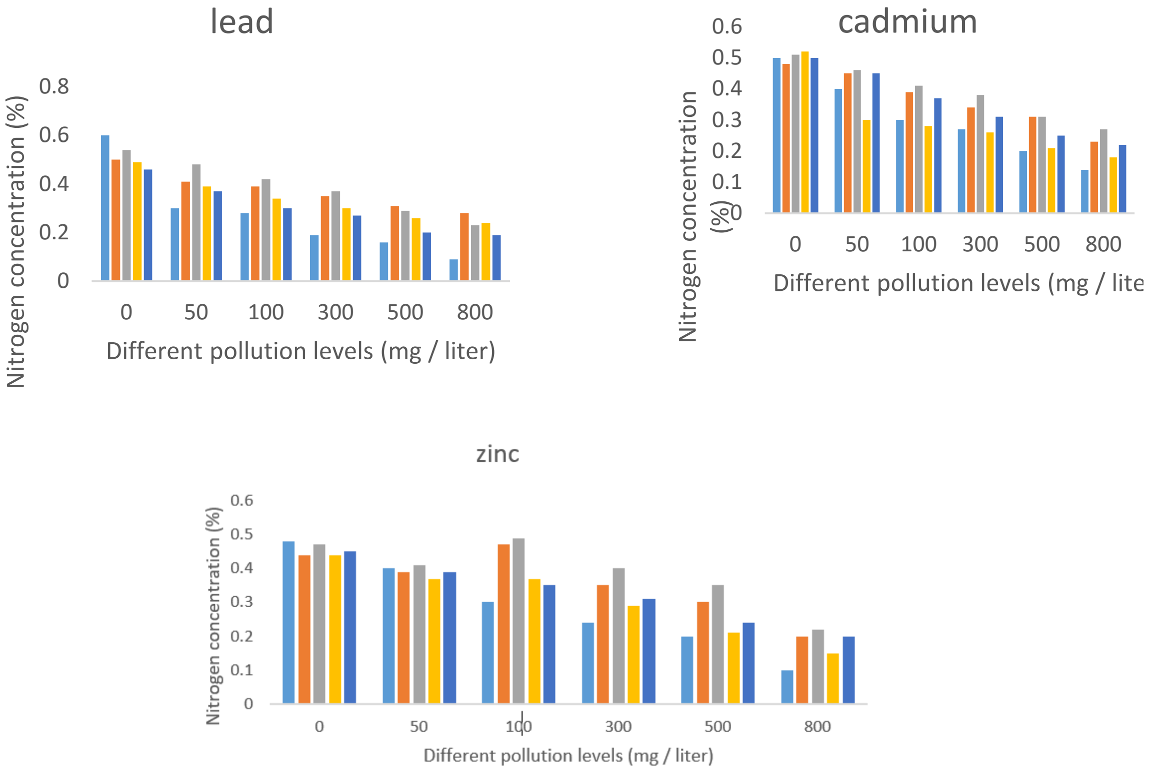
The results of comparing the averages showed a significant difference between different levels of pollution with cadmium, lead and zinc elements on the percentage of phosphorus obtained, so that the percentage of phosphorus decreased with the increase of pollution levels. Also, the results show that the highest and lowest absorption of phosphorus belongs to lead and zinc elements, respectively. As can be seen, the highest amount of phosphorus is related to T. longibrachiatum, followed by T. brevicompactum (Graph 7-4).



Graph 4-7: Phosphorus absorption percentage under different pollution levels by fungal species

Nitrogen content

The results of comparing the averages showed a significant difference between different levels of pollution with elements cadmium, lead and zinc on the amount of nitrogen percentage obtained, so that with the increase of pollution levels, the amount of nitrogen percentage decreased. Also, the results showed that the highest and lowest nitrogen absorption belonged to cadmium and zinc, respectively. As can be seen, the highest amount of nitrogen is related to T. longibrachiatum followed by T. harzianum (Chart 12-4).



Graph 4-8: Nitrogen absorption percentage under different levels of contamination by fungal species

The results obtained in this study with the results reported by

DISCUSSION

Different authors show that Trichoderma spp. It is a fungus found in many types of environments around the world (Rivera-Méndez et al., 2018). The classification of Trichoderma isolates has been determined based on changes in its structural and sporulation characteristics. Trichoderma species use different mechanisms to control the development and spread of harmful pathogens such as parasitism, competition and antibiosis. The present study was designed to isolate and identify molecular isolates of Trichoderma spp. Trichoderma species differ based on colony color, growth, mycelium, odor, conidium, conidiophore, and phialides and are classified into different morphological divisions (Shah et al., 2012). The characteristics of the fungal organisms observed in the micro and macroscopic studies correspond to the genus Trichoderma sp. Several scientists also observed similar features (Andrade-Hoyos et al., 2019; Chaverri et al., 2015; du Plessis et al., 2018; Jang et al., 2018; Nawaz et al., 2018). Variations between Trichoderma isolates may be influenced by variations in geographic area and soil type. Scientists worked on the identification of Trichoderma species. and distributed the isolates into different groups based on the branching pattern of conidiophores, short extravagant phialides with a plate, and small spores (conidia). T. asperellum has conidiophores that terminate in 2 or more phialides, and primary branches develop at about 90° to the main axis. T. longibrachiatum, conidiophores complex and progressively longer, often paired, secondarily branched. Phialides arise directly from secondary branches, usually not in whorls. Previous studies showed the presence of T. harzianum. T. hamatum and T. viride in these areas. Various researchers reported that the morphological characteristics usually fluctuated, making them unable to identify species.

The concept of phylogenetic species based on the concordance of multiple gene genealogies has revolutionized the classification of fungi and revealed weaknesses in traditional morphology-based identification. Morphologically identified Trichoderma species were precisely identified and confirmed using the internal transcribed region (ITS) that resulted in a 600 bp product in all isolates. The ITS is a conserved rDNA sequence that has been widely used to describe and perform phylogenetic analysis of fungal isolates (Skouboe et al., 1999).

In agricultural systems, pesticides containing metals and chemical fertilizers are increasingly used, and industrial wastewaters are released into the soil. However, some minerals have an important role on organisms and their growth and development, and it is possible through blocking, decomposition and inactivation of some important biological molecules such as proteins and enzymes, especially in the case of metals such as cadmium and mercury. which are without biological function, cause toxicity in higher concentrations (Ochia, 1987).

With the advancement of technology, the environment suffers from the harmful effects of industrial pollution, including heavy metals. Nowadays, biological methods to reduce or eliminate pollutants, due to their cheapness, compatibility with the environment and high efficiency, have been receiving increasing attention. Biological absorbents, including filamentous fungi, algae, bacteria, and yeasts, are able to absorb heavy metals even in very dilute environments. Trichoderma species are free-living fungi that exist in the soil and plant phyllosphere, and by producing various substances with antibiotic properties, they prevent the growth of pathogenic fungi and play a useful role in agriculture and ecosystem management. Therefore, the use of isolates of Trichoderma species that are resistant to heavy metals in soils contaminated with heavy metals can be effective against soil pathogenic fungi (Kredics et al., 2003).

One of the criteria that is very important in the process of biological absorption is the absorption index. This index indicates the process of plant extraction and indicates the transfer of cadmium from the soil to the roots and shoots and is known as the most efficient and appropriate method in the purification of soil from heavy metals (Babaeian et al., 2012; Mohammadi et al., 2011). The results of this study showed that the cadmium absorption index had an increasing trend with the increase in the pollution level. According to the obtained results, the highest absorption index was observed in the presence of T. longibrachiatum at a concentration of 50 mg / liter of cadmium, which had a 53% increase compared to the treatment without the use of mushrooms.

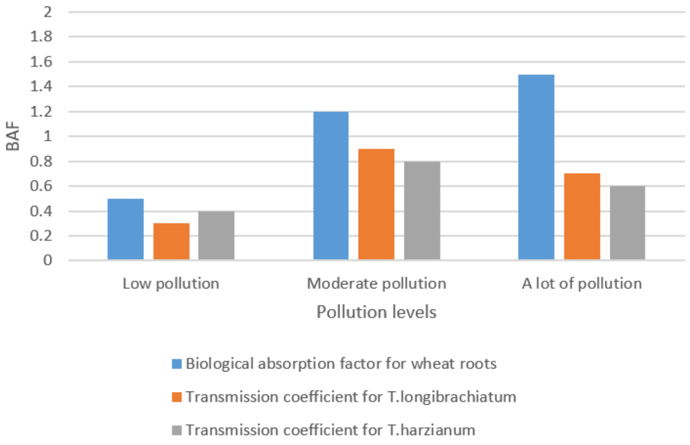
Natural low molecular weight organic acids (NLMWOA) such as citric acid, oxalic acid or malic acid and humic substances (HS) belong to natural chelating agents. These compounds play an important role in metal solubility indirectly through their effects on microbial activity, rhizosphere physical properties and root growth dynamics and directly through acidification, chelation, precipitation and oxidation reduction reactions in the rhizosphere (Evangelou et al., 2007). Also, many species of soil fungi, including Trichoderma, are also able to dissolve through the release of chelating compounds of organic acids. Organic acids released by the fungus cause acidification of the environment, which helps to increase the mobility of heavy metals (Barea et al., 2005). Our study confirms these reports. In addition, all three Trichoderma species entered the soil, which caused the mobilization of cadmium, lead and zinc at the beginning of the process and led to the increase of leaching of heavy metals into the soil solution.

Heavy metals are important pollutants that become available to plants due to high accumulation in the surface parts of the soil, and by being absorbed through the roots, they cause changes in some metabolic processes of the plant, disrupting the absorption of essential elements and their growth and development. to be Phosphorus is one of the essential components of energy metabolism, a part of nucleic acids and biological membranes. The main biochemical processes such as photosynthesis and respiration are activated by inorganic phosphate (Pi) or its organic derivatives. The results of the present study show that with the increase in the levels of cadmium and lead pollution, the percentage of phosphorus and nitrogen decreased. Research results have shown that cadmium is an effective factor in reducing phosphorus absorption by plants due to nutritional disorders. Cadmium toxicity may cause phosphorus deficiency or problems related to phosphorus transport in the plant (Kalah Kej and Enayati, 1400).

It has been reported that the use of Trichoderma increases the efficiency of nitrogen use in plants (Shoresh et al., 2010). They also stated that plants treated with Trichoderma reduced their nitrogen requirement by 30-50%. In a research, the use of T. harzianum significantly increased nitrogen, phosphorus, potassium, iron, calcium, magnesium, and manganese in chickpea seeds (Mohammadi et al., 2010). Considering the high potential of these fungi in the production of hydrolase enzymes such as protease, kinase, cellulase, etc., it can be said that the action of Trichoderma in increasing nitrogen absorption directly through the destruction of chitin and protein compounds in the rhizosphere environment and as a result increasing its availability to the plant is On the other hand, it can be said that the mentioned fungus has indirectly increased their availability for other nitrogen-fixing heterotrophic microorganisms by destroying and breaking down complex compounds and converting them into simpler compounds.Therefore, in this way, it increases nitrogen absorption in the plant (Elad et al., 1985).

CONCLUSION

The current research showed that in the soil contaminated with cadmium, lead and zinc, the biological absorption factor of the roots was greater than one, which indicates the ability of the wheat plant to accumulate cadmium in the roots. Since in the bioremediation method to measure the efficiency of a microorganism and its association with the plant to remove heavy element pollution from the soil, transfer coefficient and transfer factor are important indicators, therefore, in this research it was shown that the presence of T. longibrachiatum and after T. harzianum in medium to high levels of contamination with cadmium, lead and zinc decreased the transmission factor to less than one, which shows the positive effect of trichoderma in the accumulation of cadmium, lead and zinc in the roots and its non-transmission to the aerial parts. Therefore, as a biological absorbent, it can be a suitable option for purifying soils contaminated with cadmium, lead and zinc and producing healthy plants. However, a detailed understanding of the mechanisms involved in this process requires more research.



Graph 4-9: Investigating the effect of the presence of Trichoderma on different contamination levels and transmission coefficient in the roots of wheat plants

SUGGESTIONS:

1) According to the results, it is recommended to use the fungus T. longibrachiatum to remove heavy metals in the soil.
2) It is also suggested to perform this experiment on other agricultural plants

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