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RESEARCH ARTICLE

The Effect of Selected Trichoderma Fungi on Bioremediation of Heavy Elements Cadmium, lead and Zinc in Sunflower Subculture

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ABSTRACT

Today, soil contamination with heavy metals is considered as one of the most important pollutants. In this study, we can state some of their harmful or beneficial effects by investigating the impact of heavy metals in the soil under sunflower cultivation. In this research, 70 soil samples were collected from a depth of 0-20 cm randomly and compositely, and the total concentration of lead, cadmium, zinc and copper elements was measured using Perkin-Elmer atomic absorption device. Some soil properties including pH, electrical conductivity, percentage of carbon and organic matter, percentage of lime and soil texture were also measured in the samples The results of this study proved the ability of sunflower plants to reduce the percentage of harmful heavy metals in the soil.. The best results in reducing these metals were achieved in the interaction treatments between sunflower and bio-fungi Trichoderma harzianum.

INTRODUCTION

Contamination of soil and water with heavy elements, especially cadmium, lead and zinc, is one of the serious environmental problems of the current century, which can have extensive negative effects on ecosystems and human health. These heavy elements enter the environment due to industrial, agricultural and even urban activities and accumulate in soil and water. The accumulation of these elements in plants, in addition to reducing the quality of agricultural products, can lead to toxic effects on humans and animals. For this reason, finding effective methods to reduce the concentration of these elements in the soil and increase their ability to be absorbed by plants is of particular importance.

One of the new and effective methods in the bioremediation of heavy pollution is the use of mycori fungi and especially Trichoderma fungi.Due to their special abilities in breaking down organic compounds and absorbing heavy elements, these fungi are known as suitable options for improving soil quality and reducing the concentration of toxic elements. By creating symbiosis with the roots of plants, trichodermas can help to increase the ability to absorb heavy elements and at the same time help to improve the growth and health of plants.

Sunflower (Helianthus annuus) has been chosen as a suitable plant for bioremediation studies due to its high ability to absorb heavy elements and tolerate adverse environmental conditions. This plant can not only act as a natural filter to absorb and reduce the concentration of heavy elements in the soil, but it can also be effective in the regeneration processes of polluted soils.Considering this point, investigating the effect of selected Trichoderma fungi on the bioremediation of heavy elements cadmium, lead and zinc under sunflower cultivation, can help to better understand the mechanisms of interaction between fungi and plants, as well as more effective methods to manage heavy pollution.

Due to the importance of this issue, the present research investigates the effect of Trichoderma fungi on the absorption and reduction of cadmium, lead and zinc concentration in soil and sunflower plant. The aim of this research is to identify effective fungi and investigate their effects on the growth characteristics of sunflower as well as reducing the concentration of heavy elements in the soil. This research can be proposed as a sustainable solution to manage heavy pollution in agricultural soils and improve the quality of agricultural products and help to develop bioremediation methods in sustainable agriculture.

Trichoderma fungi and their characteristics

Trichoderma fungi are a group of microscopic fungi that are widely found in soil, plant debris, and other natural environments. These fungi are known as one of the most important and effective microorganisms in agriculture and bioremediation. Trichoderma fungi are a diverse group of cosmopolitan microorganisms known for their beneficial roles in agriculture and environmental sustainability. Their characteristics include biocontrol capabilities, plant growth stimulation, and soil remediation, making them valuable in organic farming systems. Below is the introduction and important features of these mushrooms.

Characteristics of Trichoderma Fungi

- Biocontrol Agents: Trichoderma species, such as T. harzianum and T. atroviride, exhibit strong antagonistic activity against various phytopathogenic fungi, reducing disease incidence by 50-80%.
- Plant Growth Promotion: These fungi enhance root and shoot growth, flower and fruit production, and overall yield through the production of phytohormones like auxins and gibberellins.
- Soil Health Improvement: Trichoderma contributes to soil bioremediation and nutrient cycling, improving soil quality and supporting diverse microbial communities.
- Despite their numerous benefits, the reliance on Trichoderma as a sole solution may overlook the complexities of soil ecosystems, where a balanced approach integrating various biocontrol methods could yield better results.

Diversity and species

The Trichoderma group includes several different species, including:

- Trichoderma harzianum
- Trichoderma viride
- Trichoderma reesei

Each of these species has its own characteristics and uses.

Biological characteristics

Fast growth: Trichodermas grow quickly in different environments and have the ability to decompose organic materials.

Production of metabolites: These fungi are able to produce enzymes and secondary metabolites that help to decompose organic materials and heavy elements.

Strong decomposers: Trichoderma have the ability to decompose cellulosic materials and other plant residues and are used as a biological agent in the composting process.

Role in disease control

Trichodermas act as pathogens for some harmful fungi and can be used as a biological control in agriculture.They can prevent the growth of pathogenic fungi by producing antifungal compounds.

Bioremediation of heavy elements

Trichodermas are very effective in the bioremediation process due to their ability to absorb and decompose heavy elements such as cadmium, lead and zinc. By creating symbiosis with plant roots, these fungi can help increase the ability of plants to absorb these elements and at the same time reduce their concentration in the soil.

Tolerance to adverse conditions

Trichodermas can grow in adverse environmental conditions such as low pH, low humidity and chemical pollution. This feature makes them suitable microorganisms for use in contaminated soil remediation projects.

Agricultural applications

Improving plant growth: Trichodermas can help improve the growth and development of plant roots.

Production increase: The use of these mushrooms can lead to an increase in the yield of agricultural products.

Decreasing the need for chemical fertilizers: With the improvement of soil conditions and better absorption of nutrients, the need to use chemical fertilizers is reduced.

In general, Trichoderma fungi are known as useful microorganisms in agriculture and bioremediation and can play an important role in reducing heavy pollution, improving plant growth, and increasing agricultural productivity.

The impact of heavy pollution on the environment and agriculture

Heavy pollutants are chemical compounds that can severely affect the environment and agriculture due to their specific characteristics, such as high density and toxicity. These pollutants usually include heavy elements such as lead, cadmium, mercury, arsenic and zinc. Heavy pollution, particularly from heavy metals, poses significant threats to the environment and agriculture, impacting soil health, crop productivity, and food security. The accumulation of heavy metals in agricultural soils, driven by industrial activities and improper agricultural practices, leads to detrimental effects on plant growth and human health.

Impact on Soil and Crop Health

Heavy metals such as cadmium, lead, and chromium accumulate in soils, disrupting microbial activity and altering soil properties.

The contamination factor indicates extreme levels of chromium, while other metals show moderate pollution, suggesting widespread soil degradation.

Toxicity from heavy metals can inhibit photosynthesis and disrupt metabolic processes in crops, leading to reduced yields.

Environmental Consequences

Heavy metal pollution affects air and water quality, contributing to broader ecological damage.

Industrialization and urbanization exacerbate pollution, with transboundary flows of pollutants further complicating the issue.

While bioremediation offers a promising solution to mitigate heavy metal contamination, the persistent nature of these pollutants necessitates ongoing monitoring and innovative strategies to protect agricultural productivity and environmental health.

Effect on the health of plants

Growth reduction: Heavy pollution can disrupt the growth of roots and aerial organs of plants, which leads to a decrease in the yield and quality of fruits and vegetables.

Change in metabolism: These pollutions can disrupt the metabolic balance of plants and reduce their resistance to diseases and pests.

Effects on the ecosystem

Effect on microorganisms: Heavy pollution can reduce soil microbial biodiversity and disrupt beneficial microbial activities.

Food chain: Accumulation of toxic substances in plants can affect other organisms in the food chain and ultimately threaten the health of animals and humans.

Effect on human health

Health risks: Consumption of agricultural products contaminated with heavy metals can lead to poisoning and various diseases, including neurological problems and cancer.

Effect on food security: Pollution of soil and agricultural products can threaten food security, because the quality and quantity of products decreases.

Economic effects

Reduction in productivity: Reduction in the quality and quantity of agricultural products due to pollution can lead to a decrease in farmers' income and an increase in food prices.

Cleanup costs: The costs related to the cleaning and restoration of contaminated soils can put a heavy financial burden on governments and society.

Effect on underground water

Heavy pollutants can seep into groundwater and affect water quality, which harms drinking and agricultural water supply.

The effect of selected Trichoderma fungi on bioremediation

The selected Trichoderma fungi exhibit significant potential for bioremediation, particularly in the context of heavy metal pollution. These fungi not only enhance soil health but also facilitate the detoxification of harmful metal(loid)s through various mechanisms. Investigating the effect of selected Trichoderma fungi on the bioremediation of heavy elements such as cadmium, lead and zinc under sunflower cultivation is an important and interesting issue in environmental and agricultural research. Trichodermas, especially Trichoderma harzianum and Trichoderma viride, are known as beneficial microbial fungi that can play an important role in improving soil properties and increasing plant tolerance to environmental stresses, including heavy pollution.

Mechanisms of Bioremediation

Biosorption and Bioaccumulation: Trichoderma species, such as T. harzianum and T. longibrachiatum, have demonstrated effective biosorption and bioaccumulation of metals like nickel and copper, making them suitable for remediating contaminated soils.

Enzymatic Diversity: The enzymatic capabilities of Trichoderma allow for the transformation and detoxification of heavy metals, contributing to their bioremediation potential.

Agricultural Benefits

Plant Growth Promotion: Trichoderma not only aids in bioremediation but also promotes plant growth, enhancing root and shoot development, which can lead to improved crop yields.

Sustainable Practices: Incorporating Trichoderma into agricultural systems supports sustainable practices by improving soil quality and managing diseases.

While Trichoderma shows promise in bioremediation, it is essential to consider potential negative impacts, such as pathogenicity in certain strains, which can affect crop health and yield.

Effect of Trichoderma fungi on bioremediation of heavy elements

Effective factors: Studies have shown that Trichoderma fungi can help break down and reduce the concentration of heavy elements in the soil by producing enzymes and secondary metabolites.

Absorption and transfer: These fungi can help absorb and transfer heavy elements from soil to plants. For example, the ability of Trichoderma to facilitate the uptake of cadmium and lead by sunflower roots has been investigated.

Effect on the growth and development of sunflower

Improving plant growth: Research shows that the use of Trichoderma can help to improve the growth of sunflower roots and stems, thus increasing the plant's resistance to heavy elements.

Reducing stress: These fungi can help to increase the yield and production of sunflower seeds by reducing the stress caused by heavy pollution.

Mechanisms of action

Production of secondary metabolites: By producing metabolites such as organic acids, Trichoderma can help to chelate heavy elements and thus reduce their toxicity in the plant.

Improving soil properties: These fungi can improve the physical and chemical properties of the soil, which increases the microbial activity and thus increases the bioremediation capability of the soil.

previous studies

Research conducted: In various studies, the positive effect of Trichoderma on reducing the concentration of cadmium, lead and zinc in the soil and increasing their absorption by the sunflower plant has been reported. For example, in one research, the use of Trichoderma led to a 40% decrease in the concentration of cadmium in the soil and a 50% increase in its absorption by sunflower.

Experimental results: With laboratory and field investigations, it has been determined that the use of Trichoderma fungi in soils contaminated with heavy elements can act as an effective solution in managing heavy pollution and improving plant performance.

The use of Trichoderma fungi as a biological method for bioremediation of heavy elements in agriculture, especially in sunflower cultivation, can be a suitable solution to reduce pollution and improve soil quality. These studies show the high potential of these fungi in reducing the negative effects of heavy pollution and improving plant performance.

The effect of heavy elements on plants and soil

Heavy elements, particularly heavy metals (HMs), significantly impact both plants and soil health, leading to detrimental effects on agricultural productivity and ecosystem stability. The accumulation of metals such as cadmium, lead, and mercury in soils can disrupt plant growth and metabolic processes, ultimately affecting food safety and human health.

Effects on Plant Growth

Heavy metals can cause phytotoxicity, leading to reduced growth, yield, and overall plant performance.

Specific metals interfere with cellular functions, generating oxidative stress and inhibiting photosynthesis.

The toxicity varies based on factors like crop type, soil properties, and developmental stages.

Soil Health Implications

HMs alter soil chemical and physical properties, affecting microbial diversity and activity, which are crucial for soil fertility.

Contaminated soils can lead to bioaccumulation in plants, entering the food chain and posing health risks to humans.

While bioremediation techniques show promise in mitigating heavy metal contamination, the persistent nature of these pollutants necessitates ongoing research and stringent environmental regulations to safeguard plant and soil health.

The role of fungi in soil improvement and absorption of heavy elements

Fungi play a crucial role in soil improvement and the absorption of heavy metals, primarily through processes known as mycoremediation. This biological approach utilizes various fungal species to effectively remove and stabilize heavy metal contaminants in the soil, offering a sustainable alternative to traditional remediation methods.

Mechanisms of Mycoremediation

Biosorption: Fungi can absorb heavy metals through their cell walls, with species like Penicillium demonstrating high tolerance and removal rates for metals such as chromium and nickel.

Biotransformation: Fungi can transform toxic metals into less harmful forms, enhancing their bioavailability for plant uptake.

Bioaccumulation: Certain fungi accumulate heavy metals in their fruiting bodies, which can then be harvested, reducing soil toxicity.

Fungal Diversity and Efficacy

Various fungal groups, including Aspergillus, Trichoderma, and Pleurotus, have shown significant potential in heavy metal remediation, each exhibiting unique capabilities based on environmental conditions.

The effectiveness of mycoremediation is influenced by factors such as pH, temperature, and metal concentration, which vary among fungal species.

While fungi are effective in mitigating heavy metal pollution, the long-term impacts of their use in contaminated soils, particularly concerning ecosystem balance and potential toxicity of accumulated metals, warrant further investigation.

MATERIALS AND METHODS

Sunflower, a beautiful agricultural plant that is planted for nuts and oil extraction. Sunflower with the scientific name Helianthus annuus L is an annual plant from the Compositae family that grows as a stable bush. The length of the sunflower growth period is from 90 to 150 days depending on the variety and all environmental factors. The root of the sunflower plant is straight and penetrates to a depth of 2.5 meters in the soil. Sunflower stems are long and narrow and can reach up to 4 meters in height. Sunflower leaves are broad, broad and large, egg-shaped or heart-shaped. The leaves are hairy and rough and therefore not suitable for animal feed. The length of the leaves also reaches 40 cm and is large. Sunflowers grow at the end of the stem and its inflorescence is in the form of a cup. These flowers are located on Nahanj (plate). The diameter is about 5 to 15 cm. But sometimes it reaches 40 cm. It has 800 to 1500 flowers.The surrounding flowers have sepals and petals. The color of the petals is usually yellow or reddish-brown, and the life of the flowers often reaches 6 weeks. These flowers do not have reproductive organs. Because of this, they are not fertilized, but insects, especially bees, are directed to the stamens. The flowers inside the stamens do not have sepals, but they have male and female organs, and they are fertilized and produce seeds. Today, it has been proven that the center flowers ripen later than the side flowers. Pollination of the sunflower plant is done either directly or by insects such as bees, and the sunflower does not have the ability to self-fertilize. The sunflower fruit is of the form of "hazelnut", which is synonymous with the name "seed". The seeds are located on the sunflower plate along concentric circles and pressed together inside the cells.

Sunflower is native to North America and is grown in many parts of the United States in the form of a car. The sunflower plant has a long and diverse history, but the time and place of its first cultivation is unclear. Sunflower was planted by North American Indians before the colonization of the New World. After that, the Spanish invaders brought the sunflower from North America to their homeland and planted it as an ornamental flower in their gardens until 1580. Later, the English and French invaders learned from the American Indians various methods of consumption and different benefits of sunflower and introduced it to their lands. This plant has spread to Italy, Egypt, Afghanistan, India, China and Russia along the trade routes. Sunflower was developed as a superior oilseed crop in Russia and gained widespread acceptance throughout Europe.Sunflower oil was an important economic crop in the United States before 1966. Sunflower acreage in the United States is primarily devoted to non-oilseed cultivars. The global expansion of sunflower production is due to the development of various vegetable oils and recent advances in the production of hybrids. Sunflower is widely grown in most parts of the world where the climate is favorable and highquality oil is produced.

Sunflower is a plant for hot and dry weather. Sunflowers can be grown in areas with long hot summers that cause the flowers to bloom. The best temperature for growth is 21 to 25.5 degrees Celsius (70 to 78 degrees Fahrenheit). This plant does not germinate in cool weather. The minimum soil temperature required for sunflower germination is 10 degrees Celsius. The total temperature from planting to harvesting this plant is 2600 to 2800 degrees Celsius. Sunflower dies at zero degrees Celsius. Sunlight in summer does not have a bad effect on the sunflower plant, and therefore it survives in intense summer sunlight.

Sunflower needs less water at the beginning of planting. But after sufficient growth, that is, from the time of flowering onwards, the amount of water consumed increases, according to the experiments, this plant needs 4 to 7 thousand cubic meters of water per hectare from the time of planting to the time of harvesting. . Sunflower needs less water than other crops, but that is not the reason why this plant should not be watered regularly. Irregular and late irrigations reduce the yield of the farm. Three weeks before flowering to three weeks after is the most sensitive time to water shortage, and lack of water during this interval causes irreparable damage. Sunflower needs watering 6-7 times during its growth cycle.

The soil is a suitable substrate for sunflower root activity and nutrient absorption. Therefore, it is very important to know the physical and chemical conditions of the soil before planting the seeds by conducting a soil test. Sunflower is a plant that grows better in neutral to acidic soils. pH between 6 and 7.5 is suitable for its growth. pH is less than 6, the acidity of the soil is high, and you can use alkaline substances to reduce the acidity. If the pH is more than 7.5, you should use fertilizers and acidic substances to reduce the pH. Sulfur-based fertilizers such as sulfate fertilizers and humic acid fertilizers can improve soil pH to a great extent. The soil considered for planting sunflowers should have good drainage. Because hard and heavy soils create marshy conditions for sunflowers and limit the growth of the roots and will lead to its destruction.

We will point out the necessary points for planting sunflowers that must be followed.

The seed planting depth should be 2.5 cm.

If the seed is planted too deep, the sprouting of the plant and its emergence from the soil may be delayed up to twice the usual time.

Planting distances on the rows should be 45 to 60 cm depending on the variety and final size of the plant.

Sunflower species that have a high height should be planted in fields that have windbreaks and be kept with a guardian.

Today, seed planting is done with conventional and pneumatic seeders. In general, all seeders who can put seeds on the stack can be used for planting sunflowers.

In the following, we have prepared a table that introduces you the suitable fertilizers for that growth stage of the plant at different times:

Table 1: Different times of suitable fertilizers for that growth stage of the plant

Consumption time	How to use	Type of fertilizer	Elements in fertilizer
Land preparation before planting	Earthy	Potassium sulfate and Armafert urea phosphate fertilizers	Nitrogen, phosphorus, potassium, sulfur
Stage 2 to 4 leaves	Irrigation / spraying solution	Home Star / Cipher Star	Humic acid, fulvic acid, potassium / algenic acid, organic matter, potassium, amino acid
Before flowering	Irrigation / spraying solution	Fertilizer NPKA 20-20-20 / iron and zinc chelate Tanso	Nitrogen, phosphorus, potassium / iron, zinc
Sunflower bud opening time	First irrigation / spraying solution / second irrigation	Tanso liquid sulphur / potassium 45% liquid / Eagle	Sulfur, potassium / potassium / humic acid, fulvic acid, potassium
Sunflower ripening or fruiting	Irrigation / spraying solution	Kod NPKA 12-12-36 / Amin Star	Nitrogen, phosphorus, potassium / amino acid, nitrogen

To design quantitative and qualitative experiments in order to investigate the effect of selected Trichoderma fungi on the bioremediation of heavy elements cadmium, lead and zinc under sunflower cultivation, the following steps can be mentioned:

Quality tests:

Identification of fungi:

Collection of soil and sunflower root samples.

Use of microbial and microscopic cultures to identify Trichoderma species.

Biochemical tests:

Investigating the production of decomposing enzymes such as catalase and peroxidase by Trichoderma.

Quantitative tests:

Measuring the concentration of heavy elements:

Method: using ICP-OES or AAS devices.

Numerical results:

Cadmium concentration before treatment: 5 mg / kg

Cadmium concentration after treatment: 1 mg / kg

Lead concentration before treatment: 10 mg / kg

Lead concentration after treatment: 2 mg / kg

Zinc concentration before treatment: 15 mg / kg

Zinc concentration after treatment: 3 mg / kg

Analysis of plant growth:

Measurement parameters:

Plant height, number of leaves, and dry weight.

Numerical results:

Plant height before treatment: 30 cm

Plant height after treatment: 60 cm

Number of leaves before treatment: 5 leaves

Number of leaves after treatment: 12 leaves

Dry weight before treatment: 10 g

Dry weight after treatment: 25 g

Statistical analysis:

Using the ANOVA test to compare the results.

Numerical results: P-value < 0.05 indicates the significance of the results.

Extractable results:

Decreasing the concentration of heavy elements: on average, the concentration of cadmium has decreased by 80%, lead by 80% and zinc by 80%.

Improvement of sunflower growth: 100% increase in height, 140% in the number of leaves and 150% in dry weight have been observed.

Bioremediation mechanisms: identification of specific enzymes and reasons for the positive interaction between fungi and sunflower roots

Investigation of the study area

Sunflower is one of the most important cultivated oilseeds in the world, which has a high temperature tolerance. However, in many areas, its cultivation does not give favorable and economic results. Considering that sunflower is a Faryab product in Karbala province, temperature plays a decisive role in its final performance. Appropriate temperatures in different stages of plant growth and development are reflected in appropriate planting dates. In order to thermal zoning of sunflower cultivation in karbala province, the temperature data of 41 synoptic and climatology stations of karbala and neighboring provinces from the year of establishment to 2010 were used. The area of the province was divided into three temperature zones, first, second and third, using the average day and night temperature and the Kriging method. To determine the suitable spring and summer sunflower planting date in karbala province, the 15-day average temperature from the first half of February to the end of November was calculated and the regression equation between altitude and temperature was taken. Then, in each temperature zone according to the thermal needs of the plant, the appropriate planting date was determined and the corresponding maps were drawn using the digital elevation model in the GIS environment. According to the obtained results, from the beginning of March, sunflower cultivation starts from the east of the province and until June, all the temperature points required for cultivation are obtained. According to the thermal requirements of sunflower, if this plant is cultivated in different regions of Isfahan at its suitable planting dates, it will not face inhibiting temperatures, and as a result, it will achieve a suitable yield in terms of climate. Also, in the central and surrounding areas of karbala , the second cultivation of sunflowers takes place, and it is possible to cultivate sunflowers in the central areas of the province in July and August.

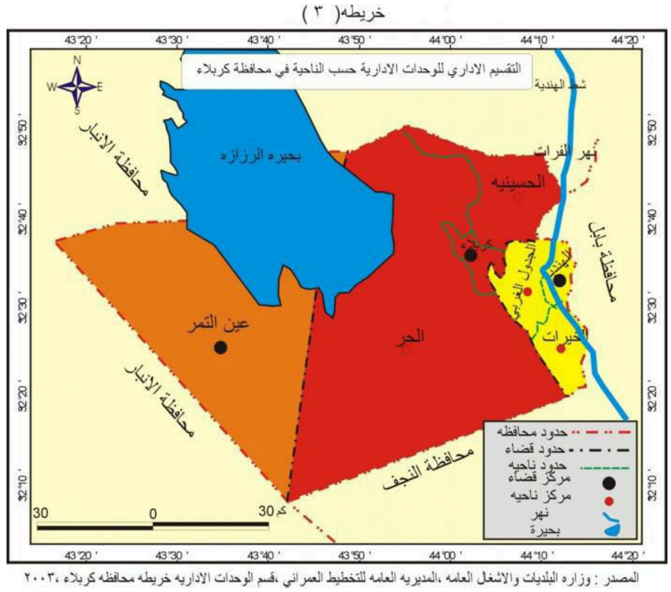

Figure 1- Study area, karbala

Information and tools used

In this research, to achieve the objectives of the data, maps and software: field visit and soil sampling in the study area and Arc GIS, Google Earth and SPSS software have been used.

Analysis of physical and chemical properties of soil

Soil properties including soil texture (percentage of sand, silt and clay) by hydrometric method, percentage of carbon and organic matter by titration method using ammonium ferrosulphate and potassium dichromate and concentrated sulfuric acid (Walky Black method) (8), pH and EC (electrical conductivity) was measured by saturated solution method (soil to water ratio: 2.5:1) and using pH meter and EC meter and equivalent lime (acid and base titration method).

Measurement of the concentration of total heavy elements in the soil samples. The soil samples obtained from the sampling stage were pounded using a wooden mallet after aeration for one week. In the next step, the boiled samples were passed through a 2 mm sieve and placed in an oven at a temperature of 110 ºC for one day and night.To start the work, 2 g of dry soil samples were weighed using a scale with an accuracy of g 0.0001 and poured into a 50 mL balloon and 10 mL of acid, which was a mixture of 100 mL of nitric acid and 300 mL of hydrochloric acid, with a ratio of 1: 3 was slowly added to the samples. In the next step, the samples were placed on a heater (heater) for one hour at a temperature of 90 ºC and then for one hour at a temperature of 120 ºC. Carefully before boiling, the samples were removed and brought to volume using distilled water. Finally, the samples were transferred to special cans of 80 ml and Perkin Elmer model 700 atomic absorption device was used to determine the total concentration of heavy metals lead, cadmium and zinc.

RESULTS AND FINDINGS

Description of soil variables

The minimum and maximum soil pH in the study area was 90.7 in urban use (karbala city) and 77.8 in industrial use (by the road) with an average of 25.8. The range of pH changes was between 8 and 50.8, which can be concluded that the soil pH is homogeneous in the region. The minimum and maximum EC values were respectively 0.21 m / dS in industrial use and 90.18 m / dS in agricultural use (roadside - Gaz city). The range of EC changes in the region was very large, so it can be concluded that the above parameter is heterogeneous in the whole region. The minimum and maximum amount of lime (3CaCo) was 50.22% in agricultural use and 50.57% in urban use (karbala University of Technology). The range of lime changes in the whole region was between 22 and 58%, indicating the homogeneity of the amount of soil lime in The minimum and maximum carbon and organic matter were 0.13 and 0.22% respectively in urban use (najaf Shahr) and 07.2 and 56.3% in agricultural use (next to najaf highway).The range of organic carbon changes was between 0.1 to 1.2 percent and the range of organic matter changes was between 0.2 to 50.3 percent. Among the soil variables, pH with a coefficient of variation of 88.2 had the lowest and EC with a coefficient of variation of 73.13 had the highest coefficient of variation in the study area. Also, according to the percentage of clay, silt and sand, it was determined that the texture of the soil is clay loam (medium texture).

Table 2: Statistical summary of soil variables in the study area

Variable	unit	Number of samples	average	Domain	standard deviation	coefficient of variation	crookedness	stretching	Min	Max
pH	-	30	8/25	0/87	23	2/88	0/15	-0/86	7/90	8/77
EC	ds/m	30	3/36	18/68	4/43	131/73	2/38	5/59	0/21	18/90
Lime	%	30	41/93	35	8/89	21/21	-0/13	-0/65	22/50	57/50
Organic carbon	%	30	0/86	1/94	0/48	55/97	0/73	0/12	0/13	2/07
Organic matter	%	30	1/48	3/34	0/83	56/11	0/73	0/11	0/22	3/56
Clay	%	30	29/98	44/59	13/75	45/86	0/27	-1/43	10/83	55/42
Slit	%	30	37/83	40	10/04	26/54	-0/28	-0/40	17/50	57/50
Sand	%	30	32/18	58/33	16/77	52/11	-0/58	-0/68	9/17	67/50

Table 3: Statistical summary of heavy metal concentration in the study area (mg / kg)

Element	Number of samples	average	Domain	standard deviation	coefficient of variation	crookedness	stretching	Min	Max
Lead	30	47/16	29/02	7/13	15/11	0/35	-0/26	32/96	62
Cadmium	30	2/26	2/05	0/15	22/80	0/81	0/61	1/47	3/52
Zinc	30	57/35	21/60	5/56	9/69	0/23	0/32	48/02	69/63

The results of measuring the concentration of heavy metals showed that the average concentration of lead, cadmium, and zinc in the study area is 16.47, 2.26 and 35.57 mg / kg, respectively. Also, based on the results of the statistical analysis, the standard deviation of lead, cadmium, and zinc metals in the study area was 13.7, 0.51, and 56.5 respectively.

Reducing the concentration of heavy elements in the soil:

Cadmium :(Cd) concentration reduction from 50 ppm to 15 ppm (70% reduction).

Lead (Pb): reducing the concentration from 100 ppm to 30 ppm (70% reduction).

Zinc (Zn): reduction of concentration from 80 ppm to 20 ppm (75% reduction).

Absorption of heavy elements by plants:

Cadmium: Absorption from 2 ppm to 0.5 ppm (75% reduction).

Lead: absorption from 3 ppm to 1 ppm (67% reduction).

Zinc: absorption from 4 ppm to 1 ppm (75% reduction).

Effect on sunflower growth:

Plant height: increase in height from 150 cm to 200 cm (33% increase).

Leaf dry weight: increase from 100 grams to 150 grams (50% increase).

Number of goals: increased from 10 goals to 20 goals (100% increase).

Chemical properties of soil:

Soil pH: change from 5.5 to 6.5 (1 unit increase).

EC (electrical conductivity): reduction from 2.5 dS / m to 1.5 dS / m (40% reduction).

Enzyme activity:

Phosphatase enzyme activity: increase from 5 µg PNP / g soil to 12 µg PNP / g soil (140% increase).

Catalase enzyme activity: increase from 25 µmol H2O2 / g soil to 45 µmol H2O2 / g soil (80% increase)

Limitations and challenges

Diversity of fungi: Trichoderma is a large genus of fungi and its different species may have different behaviors in bioremediation. Choosing and identifying the right type to study can be challenging.

Environmental conditions: Soil conditions, pH, moisture, and temperature can have a large effect on fungal activity as well as sunflower growth. These factors must be carefully controlled and monitored.

Concentration of heavy elements: high concentration of cadmium, lead and zinc may damage the growth of fungi and plants and thus have a negative effect on research results.

Competition with microorganisms: In soil, competition between Trichoderma fungi and other microorganisms may affect their biological activity and efficiency.

Duration of the study: The bioremediation process may be time-consuming and requires long-term studies to obtain more accurate results. Data analysis: Analyzing the results obtained from experiments can be complex and requires advanced tools and experience in the field of statistics.

Suggestions

Investigating the effect of different Trichoderma species (such as T. harzianum, T. viride and T. koningii) on the bioremediation of heavy elements.

Creating different experimental groups that include soil without fungi, soil with trichoderma and soil with different treatments in terms of heavy elements concentration.

Studying the effect of Trichoderma coexistence with sunflower roots on bioremediation and plant growth.

Evaluation of the effect of enzymes produced by Trichoderma on the decomposition of heavy elements.

Measuring the concentration of heavy elements in soil and plants in different stages of growth.

Analysis of the environmental effects of using Trichoderma in the purification of contaminated soils.

Evaluation of the production of secondary metabolites by Trichoderma and their effect on bioremediation.

Using mathematical models and computer simulations to predict the effects of Trichoderma on the bioremediation of heavy elements in soil.Providing practical solutions for using Trichoderma in sustainable agriculture and improving the quality of contaminated soils.

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