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PHYTOREMEDIATION OF AGRICULTURAL SOILS CONTAMINATED WITH HEAVY METALS WITHIN THE CITY OF BAGHDAD USING THE *MEDICAGO SATIVA* INOCULATED WITH *GLOMUS MOSSEAE*

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Abstract

The study aim to rehabilitate agricultural soils adjacent to the Tigris River within the city of Baghdad, contaminated with heavy elements using Mycorrhiza fungus *Glomus mosseae* isolated from the same area and tested its efficiency in extracting cadmium, nickel and copper from the soil, use the *Medicago sativa* a host plant. After 60 days of agriculture, the results of the chemical analysis of the heavy metals of the plant after cultivation demonstrated the efficacy of isolated fungi. Of the study area to increase copper accumulation at a rate of 26.38 mg/kg in the shoot and 0.21 mg/kg in the root of the *Medicago* plant. The increase in nickel accumulation was at a rate of (50.52, 6.88) mg/kg in the shoot and root, respectively, compared with unvaccinated plants with fungal isolation, while the concentration of cadmium in non-pollination plants was higher compared to mycorrhizal plants. The results of soil analysis after cultivation have also demonstrated a clear reduction of copper and zinc in the soil of the mycorrhizal plants after cultivation compared to the soil of non-mycorrhizal plants. These results reflect the role of fungi *G. masseae* cohabiting with a *Medicago sativa* plant in the plant treatment of nickel-contaminated soils and copper.

Key words : *Medicago sativa*, *Glomus mosseae*, cadmium, chemical analysis, mycorrhizal plants.

Introduction

Pollution by heavy metals is an important and common environmental problem at the time, especially in industrial cities. Also, the spread of high air, water and soil concentrations leads to a defect in the ecosystem and threats the health of humans and other beings. It also has the ability to Bioaccumulate in the tissues and organs of organisms, whether plant or animal and even the person who feeds them and thus it was dangerous to remain in the soil for a long period of time without being decomposed (Saadi, 2006).

There are many chemical and physiological methods to reduce and remove the heavy metals in the soil, which are expensive and long . So many studies have tended to use the method Phytoremediation (A group of techniques that use the plant to remove environmental pollutants from heavy metals by planting directly in the soils contaminated with heavy metals is a safe and low-cost technology) (Bhalerao, 2013). Among these techniques are Mycorrhizoremediation, which uses plants that are fertilized with Arbuscular mycorrhizae (AM) fungi in the treatment of soils contaminated with heavy metals (Gomathy *et al*., 2011). AM fungi are widespread in agricultural environments and establish a symbiotic relationship with more than 80% of plants as beneficial to both partners (Plant and fungi). It provides the plant with the major and minor nutrients nutrients. The plant is protected from the pathogenic pathogenesis in the soil by increasing its plant resistance. Studies have also confirmed that they have a role in increasing hormonal and enzymatic activity for the plant vaccinated with Arbuscular mycorrhizae (AM) fungi (Smith and Read, 2008; Mahdi, 2010).

In general, AM fungi has the ability to withstand the high concentration of heavy elements in the soil; therefore, this kind of studies is useful in the field of plant treatment (Tahat *et al*., 2009).

The aim of this study is to use the inoculated plant with Arbuscular mycorrhizae (AM) fungi, which are

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isolated from an area contaminated with heavy elements in reducing and removing heavy elements from contaminated soil to make it safe for agriculture.

Materials and Methods

Plant seeds used in the experiment

Medicago sativa seeds were obtained from the local markets for the purpose of using the experiment and were diagnosed in the Faculty of Science for women, University of Baghdad.

Collecting the soil used in the experiment

Soil samples were collected from adjacent to the Tigris River and the nearby agricultural areas of the electronics industry saffron company south of Baghdad, as shown in the picture 1. Samples were taken at the rate of 25 samples and five sites within the study area and at a depth of 10 cm and they were placed in nylon clean bags and then transferred into a laboratory College of Science women, University of Baghdad after drying them under the sun's rays and filtering. Use of a 2mm diameter sieve to dispose of gravel, the soil was sterilized with autoarcliff.

Collection and preparation of fungal vaccine

Twenty samples of soil surrounding the roots of some plants were collected in the study area and from five different sites within the study area; they were put in clean nylon bags and then transferred to the laboratory for the purpose of isolating and diagnosing the fungal isolates. Using the method of wet sieving and decanting described by Utobo (2011) and following the diagnosis of the fungus *Glomus mosseae* and depending on the classification key of Oehl (2011) the insulated spinning and spinning of the sieves were loaded onto soil of 1: 3 with moths and soil mixtures sterile autoclave for use as fungal vaccine in the experience of the pots.

Physical and chemical analysis of soil used in the experiment

- **pH :** The pH of pots soil was measured by pH meter (McCauley et al, 2009).
- **Electrical Conductivity :** The electrical conductivity EC was measured in the saturated paste extract in a device using an electrical conductivity device.

Determination of total content of heavy elements

The concentration of heavy elements in soil was estimated by method (Achakzai, 2012) using the Flam Atomic Absorption Spectrophotometer (FAAS).

Pots culture experience in class house

In this experiment, the soil set from the study area was used and after sterilization, plastic pots of 1 kg sterile and three for each transaction, the soil was inoculated with fungal isolation and 50 grams per in the pad 2011. Al-Yahya *et al*. (2011) cm and after coverage with a thin layer of soil planted the seeds of the cured plant, which represents the host plant after the superficial sterilization of the seeds sodium hypochlorite, with 10 single seeds, and then covered with an appropriate layer of soil, The field capacity of the dye was measured and the germination was completed. Plants have been reduced to 5 plants for each plant. The plants have been followed and watered whenever the need arises to ensure that the soil remains moist. Plants for field circumstances have been a subject to a proper lighting and temperature.

Estimating the concentration of heavy elements in soil and *Medicago* plant

The concentration of heavy elements in soil and Alfalfa was estimated after 60 days from the date of the start of the experiment and after the tissue of plant samples (shoot, root) both individually and after drying they were digested with concentrated nitric acid. The soil sample were digested with nitric acid and perchloric acid . Metal concentrations in the plant and soil were determined by method (Achakzai, 2012) Using the Flam Atomic Absorption Spectrophotometer (FAAS).

Calculation of *G.mosseae* fungi infection rate

The ability of the percentage of the root parts of the pollinator to be inoculated with *G.mosseae* after cutting the plant's roots into small pieces (1 cm) and a 5-per-plant cut per single pot, and after Acid fuchsin dye (Kormonik *et al*., 1980), the pieces were loaded on a glass hanger for the purpose of scanning with photolysis. The rate of infection is calculated according to the following formula: (Giovannetti & Mosse, 1980).

$$\text{Fungial infection rate} = \frac{\text{Number of infected pieces}}{\text{Total number of pieces examined}} \times 100\%$$

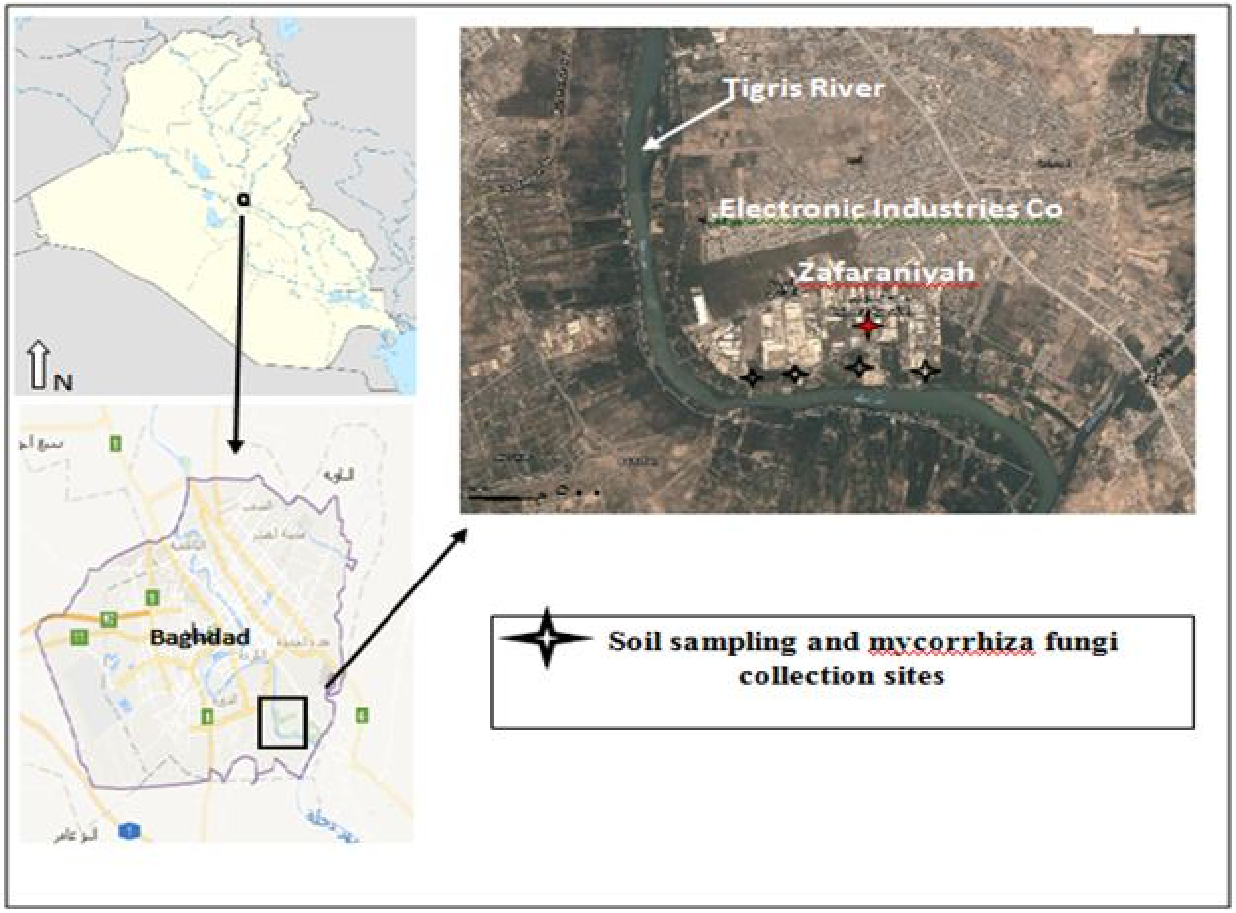
Statistical analysis

Statistical analysis System-SAS (2012) was used to analyse data to study the impact of fungal isolation on plants in the range of their accumulated heavy elements in plant tissue, the moral differences between the averages were compared with the lowest moral difference test (Least significant DIFFERENCE-LSD).

Result and Discussion

Diagnosis of fungi *Glomus mosseae*

Based on the taxonomic qualities reported by Oehl *et al*. (2011), it was found that isolated fungi from the



Picture 1 : The study area is located within the area south of Baghdad.

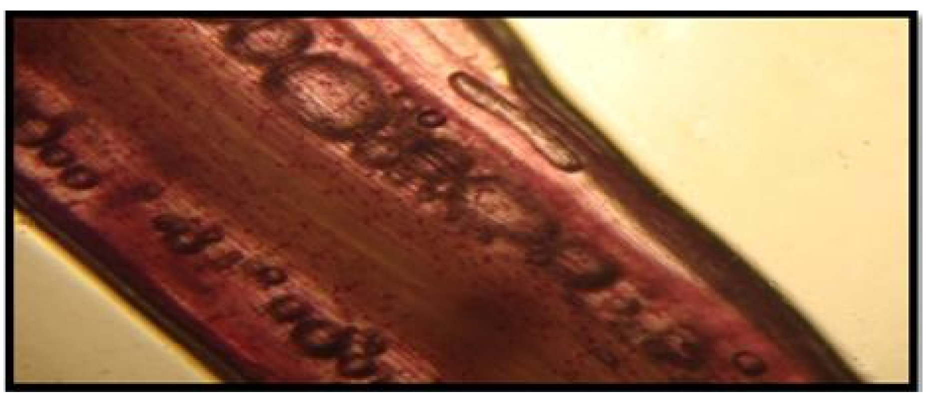


Image 2 :

study area belonged to the two genes *Glomus* spp. and *Giagaspora* spp. Despite the supremacy of genes *Glomus* spp in all samples group and of five sites within the study area and after genes diagnosis, it turns out to be of the type. *Glomus Mosseae* and this agrees with Alwan *et al.* (2010), the Al-Kartani *et al.* (2013) that referred to the supremacy of genes *G.mosseae* in Iraqi soil and directed the reason to adapting it to the Iraqi environment in terms of soil type, plant type and other environmental conditions.

Physical and chemical characteristics of the study soil

The results of the physical analysis showed that the ph of the soil was 8.920 and as shown in table 1. This is within the normal rate, as the ph affects the readiness of nutrients that have agood impact on the growth of plants (Al Nuaimi, 19990), as shown by the results of the chemical analysis of the concentrations, heavy elements in soil that cadmium is a record of 9.09 ppm, copper 45.04 ppm, and nickel 94.71 ppm and thus exceeded the

Table 1 : Illustrates the physical and chemical characteristics of the soil used in the experiment from the study area.

Soil characteristics	Mean	SD
pH	8.92	
Conductivity (sμ/ cm)	3.90	
Heavy metal in sample soil (PPM)		
Cd	9.09	±2.70
Ni	94.71	±36.18
Cu	45.04	±8.70

P ≤ 0 05.

succeeded in infecting the roots of the plant after the radical pieces were Acid fucin, 100% and all the pots after sampling under the optical microscope which appeared in Image (2).

Plant analysis

The results of the chemical analysis of heavy metals in the shoot of the *Medicago* plant at the end of the experiment generally, which showed in Table (2) significantly increased Ni concentration approximately 50.52 ppm in inoculated plants while the copper was approximately 26.38 ppm compared with non- inoculated

Table 2 : shows the elements of heavy metal in the shoot and root of *Medicago* plant.

Heavy metal (ppm)	Shoot		LSD	Root		LSD
	Inoculated plants	non-inoculated plants		inoculated plants	non-inoculated plants	
Cd	1.06	2.92	0.493 *	1.21	3.49	0.104 *
Cu	26.38	18.03	4.677 *	8.70	6.82	0.941 *
Ni	50.52	33.42	6.027 *	6.88	4.82	1.267 *

*LSD value at P<0.05.

Table 2 : shows the elements of heavy metal in the soil of *Medicago* plant.

Heavy metal (ppm)	Soil before culture	Soil after culture		Value LSD
		Inoculated plants	Non-inoculated plants	
Cd	9.09	8.70	7.90	0.644 *
Cu	45.04	26.24	30.31	5.702 *
Ni	94.71	62.51	70.05	11.79 *

* LSD value at P<0.05

allowable limit of the heavy elements in the soil by WHO and this is consistent with Habib *et al*. (2012).

This may be due to industrial activities, the use of insecticide-containing and fungal pesticides and the increase in soil organic matter and irrigation with water sanitation, and may be caused by fumes and fumes in the air emitted by heavy vehicle exhaust and traffic and burning Plastic materials as well as volatilized fumes from the factories scattered in the region, the most important of which is the electronics industry, as well as the nearby refinery, which is also borne by wind to the soil surface. (Mohamed, 2009; Habib *et al.*, 2012).

The plant used in the experiment

Use the *Medicago sativa* plant Planting pots to withstand heavy elements (Cajuste, 2008; Al-Rashdi & Sulaiman, 2013; it also maintains a relationship with Arbuscular mycorrhizal (AM) (Zaefarian *et al*. (2011).

Calculating the rate of Percentage arbuscular mycorrhizal fungal infection:

The results show that the *G. mosseae* fungus had

plants with *G.mosseae* The concentration of both copper and nickel (18.03, 33.42) ppm were respectively, and these results consistent with Davies *et al*. (2005), who referred that Mycorrhizal plants have more Zn and Cu concentration compared with non- Mycorrhizal plants, and agreed with Da-silva *et al.* (2006), who referred to the role of mycorrhiza is increasing the ability of plants to extract nickel from contaminated soils. These results are in Agree with Achakzai *et al*. (2012), which indicated that isolated mycorrhiza fungi from a contaminated area increased the plant's susceptibility to extracting heavy elements from contaminated soils.

While the results shows a table 2 a moral decline in the compilation of the Cd in the shoot and root of the inoculated plants approximately (1.06, 2.0) ppm, respectively, compared to non-inoculated plants with *G.mosseae* and these results correspond to Zaefarian *et al*. (2011), as the plants were found to be non- inoculated with *G.mosseae* fungi, the focus of the Cd is higher than the inoculated plants and this result agrees with Koltai and Yoram (2010), who indicated that isolated mycorrhiza fungus from a contaminated place reduces the absorption of a Cd in the plant tissue.

The results also show an increase in the accumulation of Ni and Cu in the root of inoculated plants more than in shoot, and these results came from the approval of Davamani *et al*. (2010), who pointed out that many of the heavy elements such as Cd, Pb, and Cu record their accumulation at the roots of the mycorrhizal plants more than their accumulation in shoots, the reason for the role of Fungi was directed mycorrhiza in increasing absorption and accumulation of heavy elements in root fungal compositions as well as accumulating in plant tissues and fungi restricting heavy elements in one of the free aggregates of the fungi wall, such as the group of secretary Amino, hydroxyl and carboxyl and other free Group.

The results also show an increase in cadmium in the radical total more than the total of the mycorrhiza, and these results correspond to the Zaefarian *et al*. (2011) as they found that the inoculated plants with G.mosseae fungi have a higher Cd in root concentration than the shoot in mycorrhizal plants and the Atabi (2007) mentioned that cadmium accumulates in the root and higher than the shoot of the sunflower plant fertilized with the AM and interpreted . This is the role of the mycorrhiza that stands in front of the transfer of cadmium to the total plant, and this behavior is one of the strategies of mycorrhiza in increasing the carrying of inoculated plants for heavy elements. These results are also consistent with what both Koltai and Yoram (2010) indicated that isolated fungi from contaminated place reduce cadmium in the shoot part of the *Medicago truncatula* plant.

Soil analysis after experience

The results of the chemical analysis of the heavy elements of soil after cultivation and when compared to the soil prior to agriculture show moral differences in the reduction of Cu concentration. Ni in the soil of the inoculated plants with G.mosseae compared to the soil of non-inoculated plants was at a rate of 94.71ppm and 26.24 ppm, respectively as shown in table 3.

These results reflect the role of *G.mosseae* fungi in lowering the concentration of Cu, Ni by increasing its taking fungal from a contaminated area increases the assembling in the Alfalfa plant, as confirmed by that results of the chemical analysis of the plant's heavy elements mentioned earlier, and these results correspond to Achakzai *et al*. (2012) and with Khani *et al*. (2011), who indicated that the inoculation of the plant with the isolated mycorrhiza efficiency of plant extraction of the elements the heavy of contaminated soil improves the growth of the host plant in general and increases its endurance to heavy elements.

The results of the experiment show a moral increase in the reduction of the focus of the Cd on the soil of non-inoculated plants compared to the inoculated plants and at the rate of 7.90 ppm. This indicates the role of mycorrhizal fungus in reducing the uptake of the Cd from contaminated soil and thus less toxic to the host plant and that's what the study has bean through. The results of the soil analysis are consistent with Javaid (2011).

In general, the results reflect the role of the *G.mosseae* and *Medicago* in the possible use of the plant treatment of contaminated soils by Ni, Cu (Kanwal *et al*., 2015; Zaefarian *et al*., (2011).

The Symbion *G. mosseae* with plants have a role in restricting and accumulating heavy elements either in the soil or in the host plant. This makes them suitable in the plant processing applications of contaminated soils, especially when using isolated fungi from contaminated soils (Khan *et al*., 2011).

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