Assessment the ability of *Trichoderma harzianum* Fungi in Bioremediation of some of Heavy Metals in Waste Water

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Abstract:

The study included the use of *Trichoderma harzianum* for biodegradation heavy metals in a wastewater treatment plant and their ability to decompose these minerals through a series of tests. The first test involved the use of two *Trichoderma harzianum* isolates for biodegradation through a filtration action. In the second test, the comparison was used in pre and post-treatment in Petri dishes, The result showed a clear green growth of fungi and their use of heavy metals as an energy source. Scanning Electronic Microscope SEM) was used to compare the vegetative growth of fungi before and after treatment on P.D.A media, which showed clear and normal growth with a short growth period of the Petri dish after treatment and compared it to the control of the Petri dish with different magnification powers (6738x, 5988x, 2713x, 2509x), . 6453x, 5708x, 2598x, 2400x). A third test used a Atomic Absorption technique to quantify heavy metals for the site 2 after 28 days of bioremediation. The results showed the highest removal rate of Cd was 98.63% and Mn was94.35% for (T.h1) and the lowest biodegradation of Zn and Fe (39.77% and 47.43%,) respectively. The nutrient had the highest removal rate of PO₄ was 69.87% and the lowest percentage of NO₃ was 8.91%.The results also showed the highest removal rate for (T.h2) Mn and Cd with rates of 86.41% and 81.73%, the lowest percentag of decomposition of Zn was 52.27%, and the highest percentage of decomposition of PO₄ was 70.74%. Lowest removal rate of NO₃ was 36.49%. A Atomic Absorption technique was used to quantify heavy metals after 28 days of biological treatment for the site1 . The results showed the highest biodegradation percentage (T.h1) for manganese and Pb 83.27% and 84.50%, respectively, and the lowest biodegradation percentage was Zn and Fe (33.67%, 38.09%,) respectively, and the nutrient removal rate was the highest, NO₃ was 85.27 %, The lowest rate is 27.74% of SO₄. The results showed the highest removal rate (T.h2) for the elemental fungus Mn (87.32%) and Pb (84.01 %) and the lowest percentage for Fe was (36.90%).The results of the experiment showed that the fungi largest biomass are the most efficient in the bioremediation process and the removal of pollutants from wastewater.

Keywords: Heavy Metals, Nutrients, Bioremediation, Wastewater, *Trichoderma harzianum*.

1. Introduction

Heavy metals, also known as trace metals, are one of the most persistent pollutants in wastewater. Untreated or inadequately treated heavy metal contaminated wastewater effluents cause a variety of health and environmental impacts when released into receiving water bodies. In aquatic ecosystems, heavy metals greatly reduce the number of living organisms. The negative effects of heavy metals on aquatic ecosystems include the death of aquatic life, impacts on plants such as reduced seed germination, decreased enzyme activity, and inhibition of photosynthesis (Gardea-Torresdey et al., 2005), algal blooms, habitat destruction from sediments, debris, increased water flow, and other short and long term toxicity of chemical pollutants and can cause serious disturbances in biological wastewater treatment plants. The presence of heavy metal pollutants represents a major threat to the soil and the plants growing in such soils, with these plants being consumed by animals and humans.
due to their entry into the food chain through biomagnification and bioaccumulation, leading to severe adverse effects (Al-Saeedi, 2010). It is reported that the intake of toxic minerals in vegetables and corn products accumulates in the kidneys, which leads to their malfunction. Some reports have linked structural damage (osteoporosis) in humans to heavy metals, such as high levels of selenium (Abdullahi, 2013). The nature of effluents contaminated with heavy metals to humans may be toxic (acute, chronic, or semi-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Duruibe et al., 2007). Although individual metals have been reported to show specific signs of their toxicity, the signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminum are gastrointestinal disturbances, diarrhea, stomatitis, tremors, ataxia, paralysis, and vomiting, and convulsion, depression and pneumonia when volatile vapors are inhaled (Duruibe). et al., 2007). Although heavy metals are natural components of the earth's crust that cannot be degradable, they are only toxic when they are not metabolized and synthesized by the body and when accumulated in the soft tissue of the body. As an example, in aquatic ecosystems, the concentration and availability of lead can lead to decreased dissolved oxygen, which may make young aquatic organisms, such as young fishes vulnerable to lead than adult fish. The presence of lead may also cause blackening of the tail region and spiral deformity to young fishes (European Commission DG ENV, 2002).

Sources of heavy metals in liquid wastewater The two main natural and human sources, it is known that the presence of these heavy metals in water bodies leads to a significant deterioration in the quality of these waters. It is noteworthy that many rocks and volcanic materials are responsible for the presence of minerals in soil and water. This is because the diffusion of acidic volcanic gases through permeable rocks contributes to the transport of hydrological materials into volcanic layers. Activities from volcanoes have been reported to be responsible for releasing minerals such as arsenic, mercury, aluminum, rubidium, lead, magnesium, copper, zinc, and a host of others into the water. (Amaral et al., 2006). During runoff due to erosion, heavy metals can be captured and distributed to the environment. In some cases, during rains, some heavy metal wastes are washed into poor drainage systems and subsequently into nearby rivers (Taiwo et al., 2011). Some of the human sources of heavy metals in wastewater are metal finishing, electroplating, mining and extraction operations, textile activities, and nuclear energy. Metal finishing and electroplating involve the deposition of thin protective layers into the metallic surfaces of the metal using electrochemical processes. When this happens, toxic metals can be released into the wastewater. Wastewater treatment can generate large quantities of wet sludge that contains high levels of toxic metals (cuchne,1985).

In addition, wastewater contains many nutrients, such as nitrates and phosphates are essential nutrients for the growth of plants, wildlife and humans. However, high concentrations of nitrates and phosphates in surface and groundwater are a serious global concern with devastating impacts on ecosystems (Olesen.al.et., 2019). Public sources of nitrate and phosphate pollutants in water bodies usually arise from human activities waste such as Discharges from industrial practices and agricultural uses such as inorganic fertilizers, compost, and wastewater treatment (Hakeem.al.et., 2017). Phosphates generally arise from an element called phosphorous, and it affects water quality through the disproportionate development of algae. The phosphate ions the amount of dissolved oxygen in waterways from streams and ponds (Nijboer and Verdonschot., 2004). Likewise, excessive levels of nitrates are a major harmful waste to ground and surface waters, and pose a serious threat to the survival of aquatic life. According to the World Health Organization, the acceptable limits for nitrate and phosphate ions in drinking water are 40 mg / L and about 0.1 mg / L respectively (Rice.al.et., 2012). The presence of sulfates in water greater than 250 mg can cause sulfate. / L in the occurrence of diarrhea, dehydration in humans and corrosion in pipeline systems, and thus their removal becomes very important (Belkada.al.et., 2018), sulphates are the most common form of sulfur in wastewater.
Although sulfates are harmless to the environment. However, the activities of bacteria that reduce the sulfur present in wastewater sources lead to the production of sulfide, which is characterized by its high toxicity, corrosion, and unpleasant odor, as well as harmful to human health. Therefore, the efficient and effective removal of sulfates from wastewater becomes important. The nutrients and heavy metals present in wastewater can be removed by physical, chemical and biological means. It includes three methods, chemical precipitation is the use of chemicals to precipitate heavy elements, which is an expensive method and is used in small basins, as for ion exchange, is the use of filters that contain the heat of expensive ion exchanges, and the third method is reverse osmosis, meaning the use of a semi-permeable membrane that allows water to pass through without Particles and plankton cannot be used to reclaim rivers and streams contaminated with heavy elements (Pawak et al., 2005). Biological removal using sulfur-reducing bacteria has the disadvantage of releasing hydrogen sulfide into the atmosphere, which may be difficult to maintain in anaerobic conditions as required. For this method, removal of heavy metals from wastewater is very expensive and does not remove heavy metals from wastewater to the desired extent. Recently, therefore, the option of using microorganisms to remove heavy metals, phosphate ions and nitrates, which are microbes that act as biological adsorbents to remove heavy metals and ions from wastewater in a low cost and environmentally friendly manner, has been proposed and studied (Bai, Abrahim, 2003). Most of the previous research has focused on exploring the mechanisms and processes associated with metal resistance through microbes. It happens that bio-absorption is more promising than bioaccumulation. Living organisms require nutrients for their growth and development and thus absorb oxygen and promote a higher level of chemical demand for oxygen (Hussain et al. 2005). Cost-effective for effective removal into the wastewater stream prior to discharging to recipient bodies or for reuse. (Ebbers.al.et., 2015). And that is through bioremediation.

Bioremediation is a technique of alternative processing for removing the heavy metals ions from the wastewater. that offers the possibility to destroy or render contaminants using natural biological activity in the ecosystem (Siddiquee et al., 2015). Bioremediation is considered naturally living organisms to reduce environmental pollutants into less toxic forms, by bacteria and fungi or plants to degrade or detoxify hazardous ingredients to human health or the environment (Qazilbash, 2004). Defines bioremediation as a process by which organic or inorganic waste biologically degraded or transformed usually into innocuous materials. The process can function naturally or can be enhanced by adding an electron acceptor, nutrient, or other factors. As such, it uses relatively low-cost technology, which generally has a high public acceptance and can often be carried out on-site (Su, 2014). For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them into harmless products. (Su, 2014). Bioremediation is one of the economical methods compared to another method useful and low-cost method to check the best biodegradation conditions by utilizing the microorganism to heavy metals degradation which is the source of carbon and energy for the microorganisms (Iranzo et al., 2001). It is a better technology for treating and removing pollutants to minimal serious based on the metabolic ability of the microorganism to mineralization or converting organic contaminants to low serious and harmless substances which are then entering to natural biogeochemical cycle Bioremediation has been utilized in the most polluted region around the world (Sihag et al., 2014). It is known that the heavy elements are biodegradable but can be converted into complex organic compounds. (Garbisu and Alkorla, 2001) Therefore, the microorganisms developed their ability to protect themselves from the toxicity of the heavy elements by possessing several mechanisms such as adsorption, absorption, oxidation and reduction, methylation. (Fernandez et al., 2012) Because of the cost of economic high methods of physical and chemical treatment for the purpose of solving the problem of pollution with heavy metals and toxic found an alternative with the low-cost methods and efficient in the removal of toxic elements of a biological
method using biomass (Biomass), which have the ability to remove heavy metals in aqueous solutions through several mechanisms (Aravindhan et al., 2004).

**Bioremediation mechanisms of the heavy metal-contaminated include:-**

Biosorption is the group of all processes, during which alive or dead biomass removes heavy metals or other pollutants from solutions. Biosorption occurring with the participation of microorganisms may be conducted by surface adsorption concerning the gathering of metals on the cell surface and linking them with extracellular polymers. The other method relies on metal infiltration to the middle of the cell (this term is close by meaning to intracellular accumulation). It is often when biosorption occurs as the first phase of the following intracellular accumulation and the process of surface adsorption occurring very fast – during several minutes may have a dominant role in metal linking or may lead to high metal accumulation in the middle of the cell in a longer time (Kisielowska et al., 2010). Bioaccumulation takes place when the absorption rate of the contaminant is higher than the rate of losing it. Thus, the contaminant remains contained and accumulates inside the organism (Chojnacka, 2010).

Bioaccumulation is a toxicokinetic process that affects the sensitivity of living organisms to chemicals. Organisms can normally resist concentrations of chemicals up to certain levels, beyond which these chemicals become toxic and endanger the organism. The sensitivity of organisms to chemicals is highly variable depending on the types of organisms and chemicals involved (Mishra & Malik, 2013).

Bioremediation: Microbiological transformations of heavy metals are reactions of oxidation, reduction, methylation, and demethylation. The enzymatic systems of microorganisms take part in reactions. Practically useful may be reactions of significantly toxic or valuable metal reduction, like bacteria Gram-positive isolated from tannery sewers, caused the reduction of highly toxic chromium (VI) to less toxic chromium (III), which may be removed from the environment (Kisielowska et al., 2010). Any bacteria, microscopic fungi, may conduct a reduction of metal ions (particularly valuable as gold or silver) to metallic form. This reaction may occur in vacuoles, on the cell surface, and in the extracellular environment, which is important from the point of view of this metal recovery (Sklodowska, 2000).

Biotransformation: Microbiological transformations of heavy metals are reactions of oxidation, reduction, methylation, and demethylation. The enzymatic systems of microorganisms take part in reactions. Practically useful may be reactions of significantly toxic or valuable metal reduction, like bacteria Gram-positive isolated from tannery sewers, caused the reduction of highly toxic chromium (VI) to less toxic chromium (III), which may be removed from the environment (Kisielowska et al., 2010). Any bacteria, microscopic fungi, may conduct a reduction of metal ions (particularly valuable as gold or silver) to metallic form. This reaction may occur in vacuoles, on the cell surface, and in the extracellular environment, which is important from the point of view of this metal recovery (Sklodowska, 2000).

Bioprecipitation and biocrystallization as a result of microorganism’s activity, the precipitation or crystallization of heavy metal compounds may occur, which causes the transformation of metal into form sparingly, which lowers their toxicity at the same time. Some precipitation and biocrystallization processes take part in biogeochemical cycles, like forming microfossils, depositing of iron and manganese, and mineralization of silver and manganese. Precipitation of metals on the surface or inside of the cell may be the result of not only the direct activity of enzymes but also the result of the galactosis of secondary metabolites (Sklodowska, 2000).

Bioleaching of metals: Bioleaching based on the application of microorganisms, bacteria, and fungi their metabolism products to transfer the metal contained in mineral to solution in relation to sulfide materials has become a known industrial technology. The basis of this process is based on the transformation of compounds of metals present in the environment in the form of sparingly soluble substances (most often sulfides) into forms easily soluble, where removal of metals is an easy task (Kisielowska et al., 2010). The ability of fungi to bleach, the mobilization of metals from indigent ores, and industrial waste is connected mainly with two processes: the creation of various organic acids in the living environment (citric acid, gluconic acid, oxalic acid) and secretion of complexion agents. For such fungi, the following types may be included: Aspergillus sp., Penicillium sp., Rhizopus sp., Mucor sp. in metal leaching because of their biochemical abilities and relatively high
resistance to bad factors such as pH and temperature. It is used mainly when it is not possible to apply classical methods of chemical leaching (Blaszczyk, 2007).

2. Materials and Methods:

2.1. Water Samples Collection:

Samples of wastewater were collected from two sites in Al Baraka wastewater treatment plant for the purpose of conducting laboratory tests for treatment as well as isolating and diagnosing fungi from wastewater, using polyethylene containers capacity (5 liter) after being washed with dilute hydrochloric acid (10%) and then rinsed with distilled water (Nollet, 2007).

2.2 The Used Media:

2.2.1 Potato dextrose Agar (PDA):

PDA is attended according to Himedia company that was used for fungal isolation, by adding (39) gm of ready-made potato powder and dissolved in 1 liters of distilled water in a conical flask with a capacity of (1) liters, then sterilized with an autoclave at a temperature of 121 °C and 15 psi for 20 min, the medium left to cooling until 45 °C. Then add 250mg of chloramphenicol to prevent bacterial growth and to obtain a distinct growth of the fungi, then pouring the medium in plastic petri dishes.

2.2.2 Potato Dextrose Broth (PDB):

The PDB was prepared with a weight of 200 gm of potato, where it was washed with water to remove dirt and extra impurities, peeled and cut it with a clean and sterile knife into small pieces, then put 500 ml of distilled water in a metal container and boiled 20 min then filtered with a cloth and the final volume is completed to 1 liters, then add 20 gm of sucrose sugar, then add 25mg of chloramphenicol and sterilized in autoclave (Diwan and Al-Hussaini, 2009).

2.3. Estimation of heavy metals in wastewater samples

Take 100 ml of the sample and put it in a digestion flask, add 6 ml of nitric acid, concentrated HNO to it, and put it in a water bath at a temperature of (70 °C) for 30 minutes, after which the samples were left to cool down and 6 ml of concentrated nitric acid was added to it, and placed on a hot plate At a temperature of 70 °C until it reaches a stage before dehydration, then 2 ml of concentrated HCL hydrochloric acid and (50 ml of distilled water) were added to it, then separated using a centrifuge on (3600 minutes) for a period of one minute, and after the completion of the separation process, The filtrate was taken and 100 ml of distilled water was added to it, and heavy metals and nutrients (lead, zinc, iron, manganese, cadmium, nickel, nitrates, phosphates, and sulfates) were taken before and after treatment, with the Automic Absorption device in the central laboratory of the Faculty of Pharmacy, University of Kufa. (Schnitzer and Hoffman, 1976). The number of wastewater treatments was, by three replications, for the purpose of statistical analysis.

2.4 Fungal Bioremediation for heavy metal in wastewater

2.4.1 Preparing fungi filtrates

The fungi filtrates used in the study were prepared by using P.D.B. from wastewater Where the medium was distributed in glass perforated bottles from the bottom by an electric perforated and clogged with a rubber seal to facilitate the entry of medical syringes and withdraw the filtrate from them and according to the required quantity without withdrawing the mycelium that is usually on the top surface of the liquid which facilitates the process of filtering and getting the filtrate as quickly as
possible without contamination of the remaining filtrate In the original bottles and sterile in the same way as stated in the solid medium and after sterilization, the bottles were inoculated with a diameter of 1 cm per 100 ml of fungi growing at 7 days of nominee, where the filters incubated for a period of (28) days at a temperature of 25 ± 2 ° C taking into account shaking every 3-4 Days to determine the effectiveness of the filter in the heavy metal decomposition using a spectrophotometer (Diwan and Al-Hussaini, 2009).

2.5. Scanning electron microscopy (SEM):
A PDA medium was prepared from two samples of wastewater for two different locations, then sterilized with an autoclave at a temperature of 121°C and 15 psi / for 20 min, and petri dishes were poured and left until the medium solidified, then one tablet of T. harzianum was placed in the center of the petri dish at a rate of three replicates per concentration with a comparison treatment with a medium of distilled water (control) and incubated at A temperature of 27 ° C for a period of (5-7) days to observe the effect of fungus on heavy metals, and the results were documented using a scanning electron microscope. / FEI is manufactured in the Netherlands which was used to fight fungi. Fungi is a biological sample containing water, so the monitoring method uses low vacuum, low acceleration voltage, small spot size, and short time without chemical fixation. Double sided tape was used to attach the fungus to the sample basin. (Goldstein et al., 2017)

3. Results and Discussion:
3.1 Concentration and Removal Percentages of Heavy Metals before and after addition of T.harzianum Isolates:
The results in Figure (2-1) for site 1 showed that the highest removal rate was for manganese and lead, as the manganese concentration decreased. From (2.13 ppm) to (0.33 ppm) with a removal rate of 84.50% for T.h1 , and the concentration of the element lead decreased from (2.69 ppm) to (0.45 ppm) with a removal rate of 83.27%. The lowest removal rate was Zn, Fe, with a removal rate of 33.67% and 38.09% respectively for T.h1, as in Figure (2-13). In Figure (2-1) for site 1 showed that the highest removal rate was for manganese and lead, as the Mn concentration decreased. From (2.13 ppm) to (0.27 ppm) with a removal rate of 87.32% for T.h2 and the concentration of the Fe decreased from (2.69 ppm) to (0.43 ppm) with a removal rate of 84.31% for T.h2. The lowest removal rate was for Zn, Fe, with a removal rate of 39.79% and 36.90%, respectively for T.h2, as in Figure (2-14).

The results in Figure (2-2), for site 2 of the study showed that the highest removal rate was for cadmium and manganese, the cadmium element decreased concentration from (2.19 ppm) to (0.03 ppm) with removal rate 98.63% for T.h1 and the Mn concentration decreased from (2.48 ppm) to (0.09 ppm) with a removal rate of 94.35% for T.h1. The lowest removal rate was for Zn, with a removal rate of 39.77% for T.h1, as in Figure (2-15). In the Figure (2-2), for site 2 of the study showed that the highest removal rate was for manganese and cadmium, the Mg element concentration decreased from (2.43 ppm) to (0.33 ppm) with a removal rate of 86.41% for T.h2 and the concentration of the Cd decreased from (2.19 ppm) to (0.40 ppm) with a removal rate of 81.73% for T.h2. The lowest removal rate was for Zn, Ni with a removal rate of 52.27% and 69.07% for T.h2, as in Figure (2-16).

The results in Figure (2-3), for site 1 of the study showed that the highest removal rate was for the NO3 concentration at site 1 from (1.63 ppm) to (0.24 ppm) with a removal rate of 85.27% for T.h1. The lowest rate was SO4 concentration from (236.10 ppm) to (170.59 ppm) as in with removal rate of 27.74% for T.h1 as in Figure (2-17). And Figure (2-3), for site 1 of the study showed that the highest removal rate was for the nitrate concentration at (1.63 ppm) to (0.23 ppm) with a removal rate of 85.88% for T.h1. The lowest rate was PO4 concentration from (79.09 ppm) to (42.09 ppm) with a removal rate of 46.78% for T.h2 as in figure (2-18).

The results in Figure (2-4), for site 2 of the study showed that the highest removal rate was for the PO4 concentration decreased from (126.55 ppm) to (38.12 ppm) with a removal rate of 69.87% for T.h1. The lowest rate was NO3 concentration from (1.57 ppm) to (1.43 ppm) with a removal rate of 8.91% for
As in figure (2-19), also the figure (2-12) for site 2 showed that the highest removal rate was for the PO4 concentration decreased from (126.55 ppm) to (37.02 ppm) with a removal rate of 70.74% for T.h2. The lowest rate was NO3 concentration from (1.57 ppm) to (0.99 ppm) with a removal rate of 36.94% for T.h2 as in figure (2-20).

**Figure (2-1)** the concentration of heavy metals (PPM) in (Site1) before treatment

**Figure (2-2)** the concentration of heavy metals (PPM) in (Site2) before treatment

**Figure (2-3)** the concentration of nutrients (ppm) in (Site1) before treatment

**Figure (2-4)** the concentration of nutrients (ppm) in (Site2) before treatment

**Figure (2-3)** the concentration of nutrients (ppm) in (Site1) before treatment

**Figure (2-4)** the concentration of nutrients (ppm) in (Site2) before treatment
**Figure (2-5)** the concentration of heavy metals (ppm) in (Site1) After treatment with T.h1

**Figure (2-6)** the concentration of heavy metals (ppm) in (Site1) After treatment with T.h2

**Figure (2-7)** the concentration of heavy metals (ppm) in (Site2) After treatment with T.h1

**Figure (2-8)** the concentration of heavy metals (ppm) in (Site2) After treatment with T.h2

**Figure (2-9)** the concentration of nutrients (ppm) (Site1) after treatment with T.h1

**Figure (2-10)** the concentration of nutrients (ppm) (Site1) after treatment with T.h2

**Figure (2-11)** the concentration of nutrients (ppm) (Site2) after treatment with T.h1

**Figure (2-12)** the concentration of nutrients (ppm) (Site2) after treatment with T.h2
3.2 Removal percentages:

**Figure (2-13)** Removal percentages of heavy metals(%) in (site1) after treatment with T.h1

**Figure (2-14)** Removal percentages of heavy metals(%) in (site1) after treatment with T.h2

**Figure (2-15)** Removal percentages of heavy metals(%) in (site2) after treatment with T.h1

**Figure (2-16)** Removal percentages of heavy metals(%) in (site2) after treatment with T.h2

**Figure (2-17)** Removal percentages of nutrients (%) in (site1) after treatment with T.h1

**Figure (2-18)** Removal percentages of nutrients (%) in (site1) after treatment with T.h2
Comparison of electron microscopy images of fungi before and after treating heavy metals in wastewater:

The SEM tests for *Trichoderma harzianum* were performed five days after the growth of a petri dish containing a medium prepared from the water of the first site and in another dish containing the fungal growth planted on a medium from the water of the second site, where the microscopic images of all dishes were taken by scanning an electron microscope with different magnification power.

Image (5-1) represents a micrograph of *T. harzianum* isolate before treatment (control). Magnifying power (Mag) was (6738x), working distance (WD) (7.0mm) and acceleration voltage (hv 15.00 kV). The Image (5-2) represents the growth of *T. harzianum* after treatment for the first site. On power zoom (5988x), WD (6.4mm) and high elevation (15.00 kV).

Image (5-3) represents a micrograph of isolate *T. harzianum* before treatment (control) where the magnification power was (2713x), WD (7.0 mm), high voltage (15.00 kV). The Image (5-4) represents the growth of *T. harzianum* after treatment for the first site. On power zoom (2509x), WD (6.4 mm) and high elevation (15.00 kV).

Where the dense growth gives an important picture of the fungi’s ability to biological decomposition, especially since the increase in the surface area of the fungi filaments formed by the fungi means increased contact between heavy elements and the cells of the fungi, which speeds up the process of drawing heavy metals into the organism for analysis, as well as increasing the enzymes secreted by the fungus from the cells. Biosafety level (Johnsen et al., 2005 and al-Fair, 2006).

Image (5-5) reproduces a micrograph of *T. harzianum* isolate before pretreatment (control). It has zoom power (6453 x), WD (7.1 mm), and hv (15.00 kV). Image (5-6) reproduces a micrograph of isolate *T. harzianum* after processing for the second site with magnification power (5708x), WD (6.7 mm) and high voltage (15.00 kV). Gardina et al., (2007) indicated that the high susceptibility of these fungi to break down heavy metals may be due to the nature of the enzymatic system they adopt to break down pollutants. Some fungi use more than one enzyme system, so their ability to break down these minerals increases, so it is now demonstrated that many fungi can secrete extracellular enzymes for use in chemical decomposition, including heavy metals.

Image (4-7) is a micrograph of *T. harzianum* isolate before treatment (control) with magnification power (2598x) and WD (7.1 mm). High (15.00 kV). Also, Image (4-8) is a micrograph of *T. harzianum* isolate after treatment with magnification (6754x) and WD (6.7 mm). High (15.00 kV). Bennett et al. (2002) stated that the rapid growth of these fungi and their high consumption of heavy metals in

![Figure (2-19) Removal percentages of nutrients](image1)

![Figure (2-20) Removal percentages of nutrients](image2)
their circles indicates that these isolates possess a wonderful nature of these minerals so that they can break a wide range of them.

**Image (5-1):** T. harzianum before treatment (control)  

**Image (5-2):** T. harzianum after treatment (site1)

**Image (5-3):** T. h isolate before treatment (control)  

**Image (5-4):** T. h isolate after treatment (site1)
References:

Abdullahi, MS (2013). Toxic effects of lead in humans: an overview. Global Advanced Journal of Environmental Science and Toxicology, 2(6): 157-162

Ahalya, N., Ramachandra, T.V and Kanamadi, R.D (2004). Biosorption of Heavy Metals. Energy/water/paper/biosorption/biosorption .htm.

Al-Saeedi, M.J., Al-Khayat, B., Al Enezi, D.R., Aslan, A., Luzardo, J.P. and Carrera, C.A., 2010, January. Successful HPHT application of potassium formate/manganese tetra-oxide fluid helps improve drilling characteristics and imaging log quality. In SPE Deep Gas Conference and Exhibition. Society of Petroleum Engineers.

Amaral, A, Cruz, JV, Cunha, RT and Rodrigues, A (2006). Baseline levels of metals in volcanic soils
of the Azores (Portugal). Journal on Soil & Sediment Contamination, 15:123 –130

Aravindhan, R., Madhan, B., Rao, J.R., Nair, B.U and Ramasami, T. (2004). Bioaccumulation of chromium from tannery wastewater: An approach from chrome recovery and reuse. Environ. Sci. Technol. 38:300-306.

Bai SR, Abrahim TE (2003) Studies on chromium (VI) Adsorption-desorption using immobilized fungal biomass. Bioresour Technol 87:17–26

Belkada, F. D., Kitous, O., Drouiche, N., Aoudj, S., Bouchelaghem, O., Abdi, N., ... & Mameri, N. (2018). Electrolysis for fluoride and nitrate removal from synthesized photovoltaic industry wastewater. Separation and purification technology, 204, 108-115.

Blaszczyk, M. K. (2007). Mikroorganizmy w ochronie środowiska [Micro-organisms in environmental protection]. Wydawnictwo Naukowe PWN.

Chojnacka, K. (2010). Biosorption and bioaccumulation – the prospects for practical applications. Environment International, 36(3), 299–307. https://doi.org/10.1016/j.envint.2009.12.001

Cushnie, GC (1985). Electroplating Wastewater Pollution Control Technology. Noyes Publication: New Jersey: pp. 375–377.

Duruibe JO, Ogwuegbu MOC and Egwurugwu JN (2007). Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences. 2(5): 112-118.

Ebbers, B., Ottosen, L. M., & Jensen, P. E. (2015). Electrodialytic treatment of municipal wastewater and sludge for the removal of heavy metals and recovery of phosphorus. Electrochimica Acta, 181, 90-99.

European Commission DG ENV. E3. (2002). Heavy metals in waste final report. Project Environment, COWI A/S, Denmark, Europe, 1-86.

Fernandez PM, Martorell MM, Farina, J.I. and Figueroa LIC. Removal Efficiency of Cr6+ by Indigenous Pichia sp. Isolated from Textile Factory Effluent. The Scientific World Journal. 2012, Article ID 708213, 6 pages doi:10.1100//708213.

Gardea-Torresdey, J. R., Peralta-Videa, J. R., Rosa, G.D and Parsons, J.G (2005). Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy, 249(17-18): 1797-1810.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J., & Joy, D. C. (2017). Scanning electron microscopy and X-ray microanalysis. Springer.

Hakeem, K. R., Sabir, M., Ozturk, M., Akhtar, M. S., & Ibrahim, F. H. (2017). Erratum to: Nitrate and Nitrogen Oxides: Sources, Health Effects and Their Remediation. Reviews of Environmental Contamination and Toxicology Volume 242, E1-E1.

Hussein H, Farag S, Kandil K, Moawad H (2005) Resistance and of heavy metals by pseudomonas. Process Biochem 40:955–961.

INECAR (2000). Position paper against mining in Rapu-Rapu. (Institute of Environmental Conservation and Research (INECAR). Available at: www.adnu.edu.ph/Institutes/inecar/postpaper1.asp

Iranzo, M., Sainz-Patro, I., Boluda, R., Sanchez, J., & Mormeneo, S. (2001). The use of microorganisms in environmental engineering. Annals of Microbiology, 51,135-143.

Kisielowska, E., Hołda, A., & Niedoba, T. (2010). Removal of heavy metals from coal medium with application of biotechnological methods. Górnicztwo Geoinżynieria, 34, 93–104.

Mishra, A., & Mallick, A. (2013). Recent advances in microbial metal bioaccumulation. Critical Reviews in Environmental Science and Technology, 43(11), 1162–1222. https://doi.org/10.1080/10994292.2011.562704.
Nijboer, R. C., & Verdonschot, P. F. (2004). Variable selection for modelling effects of eutrophication on stream and river ecosystems. *Ecological Modelling*, 177(1-2), 17-39.

Olesen, J. E., Bærgesen, C. D., Hashemi, F., Jabloun, M., Bar-Michalczuk, D., Wachniew, P., ... & Refsgaard, J. C. (2019). Nitrate leaching losses from two Baltic Sea catchments under scenarios of changes in land use, land management and climate. *Ambio*, 48(11), 1252-1263.

Pawlak, Z., Zak, S. and Zablocki, L. (2005). Removal of Hazardous Metals from groundwater by reverse osmosis, J. Environmental Studies. 579-583.

Qazilbash, A. A. (2004). Isolation and characterization of heavy metal tolerant biota from industrially pol-luted soils and their role in bioremediation [Doctoral dissertation]. Quaid-i-Azam University Islamabad.

Salam Hussein Ewaid et al 2020 J. Phys.: Conf. Ser. 1664 012143.

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). Standard Methods Fort he Examination of Water and Wastewater. Part, 2540, 2-66.

Salam Hussein Ewaid et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 722 012008

Saidi, M (2010). Experimental studies on effect of heavy metals presence in industrial wastewater on biological treatment. *International Journal of Environmental Sciences*, 1(4): 666-676.

Siddiquee, S., Yusof, N. A., Salleh, A. B., Tan, G. S., Bakar, F. A., Yap, C. K., Yusuf, U. K., Ismail, A., Tan, S. G., Naher, L., Ho, C. L., & Yusuf, U. K. (2011). Assessment of surface water quality in the Malaysian coastal waters by using multivariate analyses. *Sains Malaysiana*, 40(10), 1053–1064.

Sihag, S., Pathak, H., & Jaroli, D. P. (2014). Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. *International Journal of Pure & Applied Bioscience*, 2(3), 185-202.

Skłodowska, A. (2000). Biologiczne metody lugowania metali ciezkich-biohydrometalurgia. *Postepy Mikrobiologii*, 39(1), 73–89.

Su, C. (2014). A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. *Environmental Skeptics and Critics*, 3(2), 24.

Taiwo, AM, Adeogun, AO, Olatunde, KA, Adegbite, K I (2011). Analysis of groundwater quality of hand-dug wells in peri-urban areas of Obantoko, Abeokuta, Nigeria for selected physico-chemical parameters. *Pacific Journal of Science and Technology*, 12(1): 527-534.

Ghazay, A., Mayar Hezam, A., Alkhuzae, M., & Obayes, I. S. (2020). Study the effect of different temperatures on the biofilm production in Proteus mirabilis isolated from urinary tract infection patients. Al-Qadisiyah Journal Of Pure.

Salah, A. (2020). The New Combination of Semi-Analytical Iterative Method and Elzaki Transform for Solving Some Korteweg-de Vries Equations. Al-Qadisiyah Journal Of Pure Science, 25(1), Math. 23-26.

Ali , W., & R.Anon, M. (2020). Biological Effective of organic solvent extracts of Mirabilis jalapa Leaves in the Non-cumulative for mortality of Immature stages Culex quinquefasciatus Say ( Diptera : Culicidae ). Al-Qadisiyah Journal Of Pure Science, 25(1), Bio 1-6.

Sami Abd ali , mohammed, Shaker Hussein, A., & mohammed hadi, H. (2020). Study The Current Density-Voltage (J-V) Characteristics of α-Fe2O3 Thin Film Prepared by Spray Pyrolysis Technique. Al-Qadisiyah Journal Of Pure Science, 25 (1), Phys 1-7.