Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5096 - 5103
Received 15 May 2021; Accepted 20 May 2021.

The Use of the Fungi Penicillium and Rhizopus to Remove Some Heavy Metals from the Wastewater in Hospital in Mosul City

Bayda A. Yahya Author
Universiti of Mosul, College of Nursing, Mosul-Iraq
*Nursing@uomosul.edu.iq

ABSTRACT

The importance of using microorganisms to detoxify heavy metals lies in the huge quantities that are discarded daily to rivers from various sources, including hospitals wastewater, which leads to contamination and increased dangerous effects on humans and the environment. The study aimed to detoxify some heavy metals from the wastewater of Mosul hospital by using the fungi penicillium and Rhizopus.

The study has been conducted from the 1st of January to 30th of April 2020 and it included isolation and diagnosis of some fungi from the surrounding sewage and soils. The fungi penicillium and Rhizopus were selected for having the highest frequency and studying their efficiency in removing the toxicity of the elements lead, cadmium, and copper from the wastewater of Mosul hospital. The concentrations of Pb, Cd and Cu elements were measured before and after the fungi cultivation process using an atomic observation device for an incubation period of 0, 3, 5, 7 and 10 days to find out the percentage of precipitation of each element compared to the standard of curve that was previously prepared.The results showed superiority of penicillium in sedimentation of Pb and Cd, as it reached 61.25%, 78.18% on the tenth day of incubation compared to Rhizopus 53.75%, 72.73%. respectively, Whereas, the rate of copper precipitation of the fungus Rhizopus on the tenth day was 87.18% and it was higher than that of penicillium 73.07% for the same incubation period. The study demonstrated the efficiency of using the fungi penicillium and Rhizopus in removing lead, cadmium and copper and reducing it is toxicity in hospital wastewater.

Keywords: Fungi Penicillium And Rhizopus

Introduction

Hospital wastewater contains many infectious and dangerous compounds, including heavy metals with high toxicity such as mercury, silver, lead, chromium, cadmium, zinc and copper.(Izah, Inyang, Angaye, & Okowa, 2017; Vardhan, Kumar, & Panda, 2019) Hospitals may put out daily various dangerous chemical liquids resulting from the cleaning and sterilization processes for floors, equipment and tools used in surgical operations, large quantities of acids and alkalies and chemicals used in laboratory analyses, radiograph departments, pharmaceutical waste , dental services, paint residues dyes and oils in maintenance departments.(Carraro et al., 2016; Mathur, Patan, & Shobhawat, 2012; Taghipour & Mosaferi, 2009). Most hospitals do not have unit for treating this type of pollutants, which drives them to drain heavy water into the main sewage network and then reach the river, which leads to raising their water-polluting dangerous effects on humans and environment(Cruz-Morató et al., 2014; Khan et al., 2020). Studies have emphasized the importance of adopting biological treatment systems instead of chemical and physical treatments to remove heavy metals, as they are easy to apply and low-cost methods(Ahmaruzzaman & science, 2011; Dobson & Burgess, 2007; Kikuchi, Tanaka, & Technology, 2012). Bioremediation can be defined as choosing a microorganism that could remove some of the pollutants in the surrounding environment so that the product is harmless(Bhatnagar, Kumari, &Biology, 2013; A. Kumar, Bisht, Joshi, & Dhewa, 2011; V. Kumar, Shahi, & Singh, 2018). The use of fungi is an effective way to remove heavy elements from water, because microorganism need some of these elements(Abbas, Ismail, Mostafa, &

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Sulaymon, 2014; Srivastava & Majumder, 2008). Since the heavy elements are not degradable, they can be converted into complex organic compound (Wu, Cui, Li, & Sun, 2015). Therefore, microorganisms have developed their ability to protect themselves from toxicity of heavy elements by processing several mechanisms such as absorption, adsorption, oxidation and reduction and methylation(Dixit et al., 2015; Gavrilescu, 2004; Mohammed, Kapri, & Goel, 2011). Because of the high economic cost of physical and chemical treatments to detoxify heavy elements, alternative methods were found that are efficient and inexpensively, which is the biological method using biomass, which has the ability to adsorb heavy elements(Carolin, Kumar, Saravanan, Joshiba, & Naushad, 2017; Krstić, Urošević, & Pešovski, 2018).The removal of heavy elements by the living or dead biomass involves two paths. The first is the binding of ions to the cell surface and extracellular materials and it is called passive mode uptake without the need for metabolic activities. As for the second, it takes the elements through the cell membrane into the cell and is called an active uptake, and this depends on the metabolic activities inside the cell(Abbas et al., 2014; Chojnacka, 2010). The study aimed to isolate the diagnose fungi from sewage and surrounding soils and then study their efficiency and choose the best ones for removing lead, cadmium, and copper from the hospital wastewater.

Materials and Methods

1. Estimate the elements lead, cadmium, and copper in the sample: A sample of the discharged water was taking from a hospital in Mosul City after the filters with three replications, then 100ml were taken from it and filtration process was performed on it and 10ml of a mixture of nitric and hydrochloric acid was added to it in a ratio of 1:3 in a 250ml glass baker. The sample was placed in the hood and heated to a boil after covering it for two and a half hours, then remove the cap and heat the solution to dryness to get rid of the volatile organic matter. Then 10ml of 10% nitric acid was added to it and heated with stirring until the solution became clear and the concentrations off the elements lead, cadmium and copper were measured with an spectroscopy atomic absorption device using the standard curve prepared for each elements (Fig. 1,2,3)(Norval & Butler, 1973).
2. Fungi isolate: The fungi were isolated from the sewage water and the surrounding soil, the fungi penicillium and Rhizopus were selected because they have highest frequency (Fig. 4,5) and then study the efficiency of these fungi in removing lead, cadmium, and copper from hospital water.
3. Preparation of element solution (Pb, Cd and Cu) for standard curve: 1.5985 grams of lead nitrate Pb $(No3)_2$ were dissolved in 1.5ml of concentrated nitric acid and the volume was completed with distilled water to a liter. also 1.63 grams of cadmium chloride were dissolved, and the volume was completed with distilled water to liter. 2.512 grams of copper sulfate were dissolved, and the volume was completed with distilled water to liter. The weight of each element was calculated according to the following equation:

Molecular weight of the compound
Weight of compound required = ________________________________
per 1 gram of element Weight
The atomic weight of the element

The concentrations were made for each element using distilled water, which are 0, 2, 4, 6, 8 and 10 ppm. Before estimating heavy elements, the atomic absorber is calibrated by injecting the three concentrations from each element to obtain a standard curve for each element from in which

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the Pb, Cd and Cu concentration will be extracted in the measured samples, the sample injection is repeated twice to ensure that the device is set, and one of the standard solutions is passed, which is close to its concentration of the measured elements, with three replications for each sample.

4. Treating water samples with fungi: The water sample was distributed in glass bottles with an amount of 750ml and the pH was adjusted (6.8), then sterilized with autoclaves, after cooling, 30ml of the fungal vaccine penicillium and Rhizopus were added (three replicates for each fungus and then incubated at(25 C°).

5. Estimation of Pb, Cd and Cu in culture samples: 100ml of the sample was withdrawn from the culture at a time of 0, 3, 5, 7 and 10 days to estimate the concentration of studied elements and find the percentage of removal for each of them, sample were filtered and digested as previously prepared for measurement and calculation of the concentration of each element in comparison with previously prepared standard curves(Gopi et al., 2012) . The elements precipitation ratio was calculated by the following equation:

$$\text{The sedimentation ratio of the element} = \frac{\text{Concentration rate before implantation} - \text{Consideration rate after implantation}}{\text{Consideration before implantation}} \times 100$$

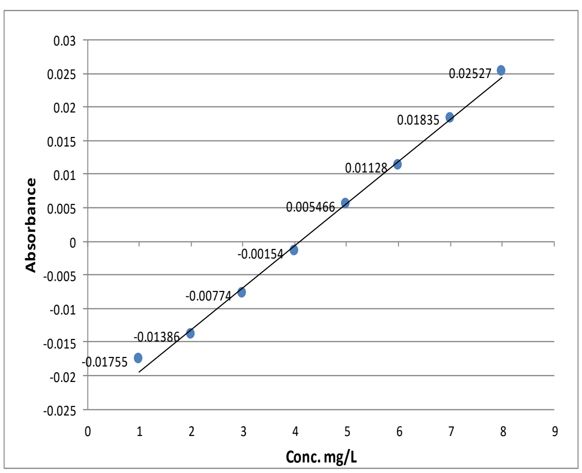

Figure1. Standard curve for Pb

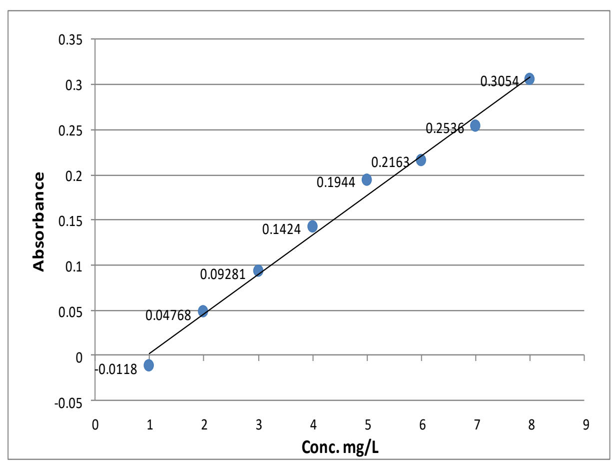

Figure2. Standard curve for Cd

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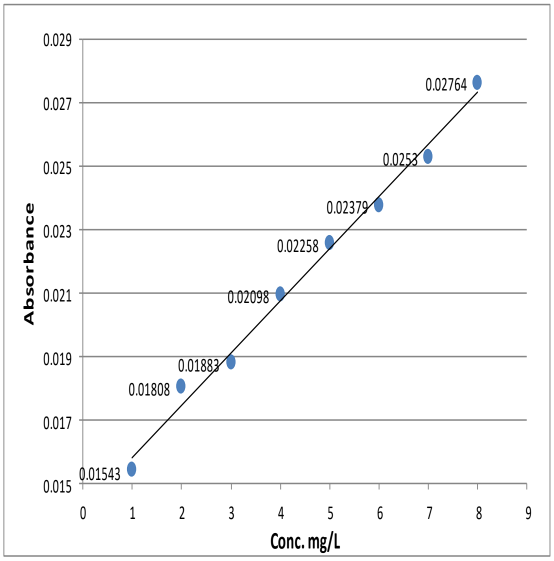

Figure 3. Standard curve for Cu

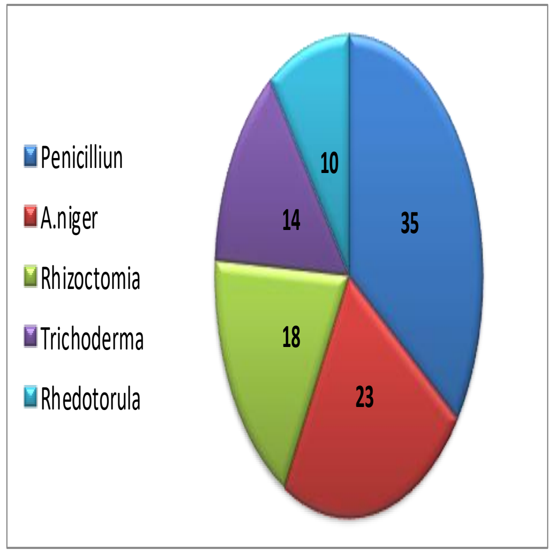

Figure 4. The percentage of frequency of fungi isolated from sewage.

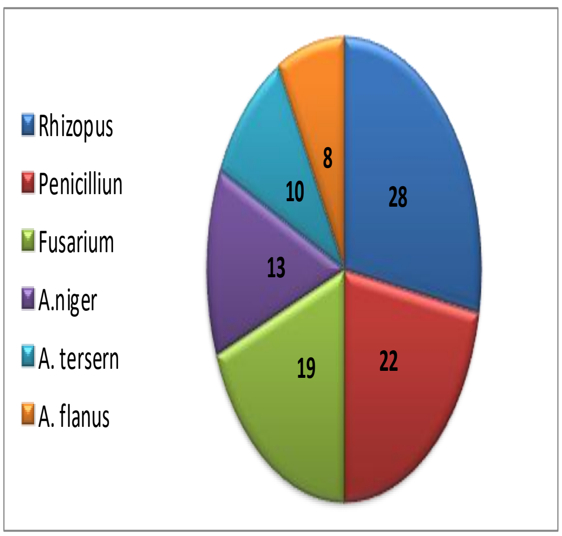

Figure 5. The percentage of frequency of fungi isolated from surrounding soil with sewage

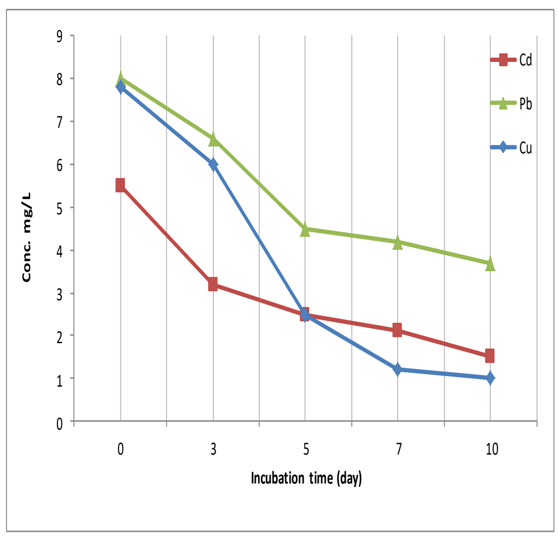

Figure 6.Concentration of Lead (Pb), Cadmium (Cd) and Copper (Cu) by using Rhizopus

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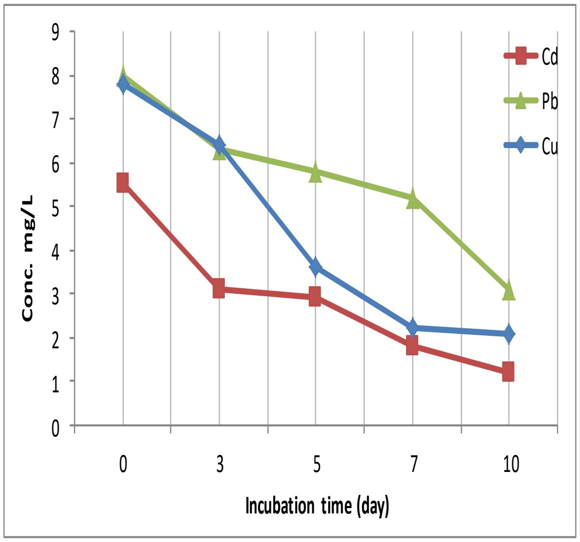

Figure 7. Concentration of Lead (Pb), Cadmium (Cd) and Copper (Cu) by using penicillium.

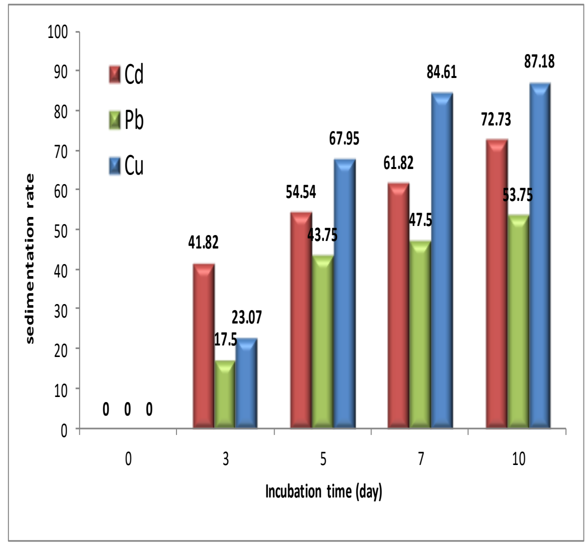

Figure 8. The sedimentation rate of Pb, Cd and Cu by using Rhizopus.

Discussions

The study included, firstly, isolating and diagnosing fungi from the surrounding and sewage and soils. The fungi penicillium and Rhizopus were selected because they obtained the highest frequency ratio of 35% and 28% respectively (Fig. 4,5). and secondly, to study the efficacy of these fungi in reducing concentration of lead, cadmium and copper elements in the water sample expelled from hospital. The results showed a decrease in the concentration of lead and cadmium during the incubation period. On the tenth day, lead reached 3.1mg/liter for the fungus penicillium and (3.7 mg/liter) for fungus Rhizopus, while it reached for cadmium at the same incubation period (1.2 mg/liter) and (1.5 mg/liter) for the same fungi respectively (Fig. 6,7). The reason for this decrease is due to the presence of receptors binding sites on the surface of fungal cells that adsorb the toxic metal element. Where multiple mechanisms are involved in the removal of heavy metals by fungi, such as metal attachment to the cell surface, its adsorption on surfaces, or mineral deposition when the necessary anions are available for its sedimentation and effective transport(Elderfield, Cooper, & Ganssen, 2000). Whereas Abou-Shanab et al. (Abou-Shanab, Ghozlan, Ghanem, Moawad, & Toxicology, 2007) mentioned that the fungi possess groups found on the cell wall, such as carbonyl, hydroxyl and amide, which have the ability to bind with toxic heavy elements during bio-absorption process. As well as the presence of amine, oxygen and nitrogen in proteins which work to bind with the toxic elements and remove their toxicity from the environment (McNeill & Edwards, 2001). In addition to the ability of the fungi to secrete external mucous substances (emulsifier) that bind these elements and prevent their entry into the cell and remain on the wall(McNeill & Edwards, 2001; Paraszkiewicz, Bernat, Długoński, & Biodegradation, 2009) . The results in (Fig.6,7) showed a decrease in the copper element concentration in the sample during the incubation period, and it reached on the tenth day (2.1 mg/liter) for penicillium, which is close to the seventh day of incubation (2.2 mg/liter), while is decrease to (1.0 mg/liter) for Rhizopus on the tenth day, which is also close to the seventh day of incubation (1.2 mg/liter). The reason for the decrease may be because the copper element is one of the trace nutrients needed by the fungi and has an active role in the work of the enzymes necessary for their metabolism, it also plays an important role in making the cell wall of fungi and formation of spores. Copper has an ionic state that works to for stable complexes with protein(Walker, 2004; White & Walker, 2011). Bio-absorption is an inexpensive and effective way to remove copper from wastewater. These results were consistent with Maysoon et al., study in evaluating the efficiency of some fungi in treating water contaminated with heavy elements. The figure (8,9) shows the superiority of penicillium in sedimentation of lead and cadmium, as it reached 61.25%, 78.18% on the tenth day of incubation compared to Rhizopus 53.75% , 72.73% respectively, whereas the rate of copper precipitation of the fungus Rhizopus on the tenth day was 87.18% and it was higher than that of penicillium 73.07% for the same incubation period, the reason of the high disposition rate may be due to the ability of these minerals to bind to the active groups on the surface of the fungal cells and that this association depends on the chemistry of the medium and the ionization of the mineral, and when the mineral attaches to the cell wall, the effective transport role comes by transporting it across the cell membrane of the microorganism (Volesky & Holan, 1995) .The results of this study were in agreement with the results of Raja et.al.(Raja, Anbazhagan, Selvam, & Biotechnology, 2006) study in eliminating the toxicity of lead by using Pseudomonas aeruginosa and with Tehrani et al., (Tehrani, Azar, Namin, & Dehaghi, 2013) study in removal of lead from wastewater using (EM) technology. While Siddiquee (Siddiquee et al., 2015)mentioned that filamentous fungi play an important role in removing heavy metals from contaminated media, as he found that the best removal efficiency was for lead, copper and zinc on the eighth day of incubation when using Aspergillus niger. The high concentration of these minerals in hospital discards is a dangerous sign must be dealt with great caution because it plays an important role in pollution of the water environment as it can easily enter the food chain and then reach the person who is the final consumer, in addition it is dumped into rivers causing damage to live stock and polluting drinking water(Wijewardena, Roudaki, & Society, 1997). This is confirmed by the study conducted by Riadh et al. (Jabar, Ali, & Kamel, 2013) which showed an increase in the elements (Cu, Cr, Zn, Ni, Co, Cd and Pb) in the waters of the Tigris river north of the city of Tikrit/Iraq as evidence of the pollution of Iraqi water with heavy elements.

Acknowledgement

we are thankful to the college of Environmental Science and Technologies, University of Mosul. and the hospital administration for their cooperation with us to complete this work.

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