Bioremediation of pollutant waters by *Trichoderma harzianum* using EDX and laboratory Examination

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Abstract

The results of water examinations for Al-Najaf sea by used energy dispersive x-ray and laboratory examination and it compare with river water were pH value was 5.5, Chlorides 950 mg/l, Sulfate 834 mg/l and intractability of water was 811 mg/l, for water samples from Al-Najaf sea. While were the results for the river samples of water in Al-Najaf city, it were pH value was 6.7, Chlorides 344 mg/l, Sulfate 290 mg/l and Intractability of water was 390 mg/l. The laboratory results showed the role of *Trichoderma harzianum* in reducing the clear pollution factors in the pollutant waters after detecting it with an electron microscope. Where the results of the biological treatment by *Trichoderma harzianum* showed that there clear significant differences between the initial tests for the pollutant waters of AL-Najaf sea and the sea waters that were treated as follows: pH value was 6.4, Chlorides 720 mg/l, Sulfate 400 mg/l and Intractability of water was 460 mg/l, for pollutant waters after treatment by *Trichoderma harzianum*.

Key words : Bioremediation, *Trichoderma harzianum*, energy dispersive x-ray.

Introduction :

The microorganisms since their appearance on the surface of earth and they have ability to live with high concentrations of minerals scattered in nature(1). Which were known as basic minerals such as iron, manganese, calcium, potassium and others, but today after
industrial development the accident and the frequent use of these materials, which led to an increase in their concentration in the environment and increased with it Harm to humans, animals and plants(2). Fungi are one of the fungal fungi that is caused by cystic fungi, which grow at a temperature of 25-30 ° C, and belongs to *Trichoderma* more species exist in different environments(3). Biological treatment was represented by making use of the metabolism of microorganisms to get rid of pollutants(4). The technologies used can be classified into technology on-site or off-site(5). Where it includes technology in fact used under name of biological treatment of polluted materials at the site, here it located (6).While it describes an out of reality technology used under name of biological treatment of pollutants in other places without the place where it was found(7). Also, getting rid of the environment and cleaning it from most pollutants and wastes requires an increase in our understanding of the relative importance of the various ways and regulatory networks for the flow of ions from mineral elements in different environments and for multiple compounds as well(8). In addition to that it will accelerate the development of technologies and methods of biological treatment and biotransformation processes(9). The study aimed to detect water pollutants in the water of Al-Najaf sea and the possibility of using biological methods to get rid of pollutants through effective metabolism processes.

**Material and methods :**

**Sample collection:** Ten samples of water from Al- Najaf sea and ten samples of river water were collected in the same city. Tests were performed by used an electron microscope (*energy dispersive x-ray*) to detect the concentrations of elements in water, with some laboratory tests were carried out, which were intractability testing for water samples and determinate the pH values.

**Biological treatment by *Trichoderma harzianum* :**

*Trichoderma harzianum* was obtained from the central laboratory in the College of Science, University of Kufa, for purpose of its development was on the agricultural medium designated for cultivation and used in biological treatment of polluted water in
the laboratory. In glass bottles (conical flask) to purpose of biological treatment of polluted waters.

**Prepare the culture medium:**

The medium of potato and dextrose was prepared by dissolving 39 g of ready-made powdered media from the Indian company Himedia, adjusting the pH to 6.5, then adding 50 ppm of streptomycin. It is placed in a conical flask and sterilized with a sterilizer under pressure of 1 atmosphere and at a temperature of 121 °C and then distributed in Petri dishes (10).

**Electron microscopy examinations (energy dispersive x-ray):**

The process of examining the water samples in the center of the electronic microscope in College of Science / University of Kufa.

**Result and discussion:**

The results showed in table (1) a set of measurements for water samples from Al-Najaf sea and river, that have been compared with the natural limits of the World Health Organization recommended in terms of water viability for human and animal uses. The tests were pH value was 5.5, Chlorides 950 mg/l, Sulfate 834 mg/l and Intractability of water was 811 mg/l, for water samples from Al-Najaf sea. While were the results for the river samples of water in Al-Najaf city, it were pH value was 6.7, Chlorides 344 mg/l, Sulfate 290 mg/l and Intractability of water was 390 mg/l, the results showed significant differences between sea and river water in Al-Najaf city and this pollution in the waters for Al-Najaf sea exceeded the permissible limits, therefore it was considered unfit for human and animal uses, this result agree with(11).

Table (1) Electronic microscopy and laboratory examinations for water samples from Al-Najaf sea and River.

| No. | Water samples | pH | Chlorides mg/l | Sulfate mg/l | Intractability in water mg/l |
|-----|---------------|----|----------------|--------------|-----------------------------|
|     |               | Val | Natural | Value | Natural | Val | Natural |



It was noticed that there was no change in pH values, due to the high salinity and lack of presence of organic materials needed for growth of microorganisms, and thus the small amount of carbon dioxide released from these organisms and thus small amount of carbonate resulting from dissolved this gas(12). While intractability of water, which were caused by the nature of the soil and high salt and which dissolved due to drift in addition to the operations of industrial waste and sewage(13). Whereas, with regard to chloride and Sulfate rates were high and outside the permissible proportions according to the recommendations of World Health Organization(14). We conclude from this study
that waters of Al-Najaf sea of were environmentally polluted due to high levels of salts as well as intractability in addition to the main ions of water that were detected using an electron microscope, thus, this water was not suitable for human and animal uses. Bioremediation was an effective treatment to eliminate water pollution (15). Therefore, the fungus of *Trichoderma harzianum* was used to reduce pollution in water, the most important of which the ions from mineral elements (16). The laboratory results showed the role of *Trichoderma harzianum* in reducing the clear pollution factors in the pollutant waters after detecting it with an electron microscope. While the results showed of biological treatment by *Trichoderma harzianum* that there clear significant differences between the initial tests for the pollutant waters of AL-Najaf sea and the sea waters that were treated as follows: pH value was 6.4, Chlorides 720 mg/l, Sulfate 400 mg/l and Intractability of water was 460 mg/l, for pollutant waters after treatment by *Trichoderma harzianum*.

Table (2) Electronic microscopy and laboratory examinations for pollutant waters from Al-Najaf sea after treatment with *Trichoderma harzianum*.

| No. | Water samples                                      | pH    | Chlorides mg/l | Sulfate mg/l | Intractability in water mg/l |
|-----|---------------------------------------------------|-------|----------------|--------------|-----------------------------|
|     |                                                   | Value | Natural Value  | Natural Value| Value Natural Value         |
| 1   | Treatment waters with *Trichoderma harzianum*    | 6.4   | 6.5-8.5        | 720          | 200-800                     | 400          | 200-400                     | 460          | 300-500                     |
| 2   | Al-Najaf sea                                      | 5.5   | 6.5-8.5        | 950          | 200-800                     | 834          | 200-400                     | 811          | 300-500                     |
|     | L.S.D.                                            | 0.9   | -              | 1.7          | -                          | 5.1          | -                          | 5.9          | -                          |

*Trichoderma harzianum* worked to metabolize the compounds by their active enzymes and to eliminate their presence in pollutant waters (17). It was important the safety and non-seriousness of *Trichoderma harzianum* in biological treatment and the fear not to spread further, but within the limits of control and within the scope of biological
treatment(18). The bioremediation method was inexpensive, easy to apply, and has guaranteed safety. *Trichoderma harzianum* showed a high biological effectiveness in getting rid of pollutants in the water, with a maximum of four days. *Trichoderma harzianum* possess a high ability to grow in high environments in concentrations of contaminated elements, including heavy elements and the possibility of treatment and disposal.

![Figure (2) Electronic microscopy and laboratory examinations for pollutant waters from Al-Najaf sea and compare between of pollutant waters after treatment with *Trichoderma harzianum*.](image)

The results were consistent with (19), where he indicated the great role of microorganisms in treating pollutants in water.

References:

1. Shailaja, M.S.; Parameswaran, P.S. and Singh, S.K. (2006). Removal of Polycyclic Aromatic Hydrocarbons from Aqueous Media by Themarine Fungus NIOCC312: Involvement of LigninDegrading Enzymes and Exopoly-saccharides. Indian Journal of Geo-Marine Sciences (IJMS). 35:373-379.
2. April, T.M.; Foght, J. M. and Currah, R.S. (2000). Hydrocarbon degrading filamentous fungi isolated from flare pit soils in Northern and Western Canada. Canadian Journal of Microbiology, 46(1), 38-49.
3. George-Okafor, U.; Tasie, F. and Muotoe-Okafor, F. (2009). Hydrocarbon Degradation Potentials of Indigenous Fungal Isolates from Petroleum Contaminated Soils. J. Physical and Natural Sciences. 3(1): 1-6.
4. Evans, C.S. and Hedger J.N. (2001). Degradation of Plant Cell Wall Polymers. En: Fungi in bioremediation. (G.M. Gadd, ed). Cambridge University Press, Cambridge, pp. 1-26.
5. APHA(2005). Standard method for water examination.
6. Arthur, L. (2007). Evaluation of Philodina (Rotifers) as a Bioassay organism for heavy metals. Journal of the American water resources association.
7. Arthur, L.; Buikema, Jr.; John Cairn, Jr. (1971). Rotifers as monitors of heavy metal pollution in water. Bulletin 71.
8. Bjorkman, T.; Blanchar, L. M.; Harman, G. E. (1998). Growth enhancement of shrunken sweet corn by Trichoderma harzianum 1295-22: Effect of environmental stress. Amer. Society for Horticult. Sc., 123(1), 40-42.
9. Harman, G. E. (2006). Overview of mechanisms and uses of Trichoderma spp. Phytopathology. 96, 190-194.
10. Verma, M.; Brar, S. K.; Tyagi, R. D.; Surampalli, R. Y.; Valero, J. R. (2007). Antagonistic fungi, Trichoderma spp.: Panoply of biological control. Biochem. Engineer. J., 37, 1-20.
11. Brown, M.; Depledge, M., (1991). Determination of trace metal concentration in marine organisms. Aquatic Environ. p.185-217.
12. AL-Imarah, F.J. and Manther, E.A. (1993). Pollution of Arabian Gulf Conference Marin Science Centre, Basrah University, Basrah, 28-30 March, 1993.
13. Hynes, H.B. (1991). The Biology of Polluted Water. Liverpool Univ. Liverpool, UK.
14. World Health Organization (WHO), (1980). International Standards for Drinking Water. 3rd Ed., Geneva.
15. Environment Protection Agency (EPA) (1971). Office Programs of Water. Washington, D.C. USA.
16. El-Ashry, M. A., El-Sayed, H.M., Fadel, M., Metwally, H.M. and Khorshed, M. M. (2002). Effect of chemical and biological treatments of some crop residues and their nutritive value: 2. Effect of biological treatments on chemical composition and in-vitro disappearance. Egyptian J. Nutrition and Feeds, 5: 43-54.
17. D’Annibale A., Rosetto F., Leonardi V., Federici F. and Petruccioli M. (2006). Role of Autochthonous Filamentous Fungi in Bioremediation of Soil Historically Contaminated with Aromatic Hydrocarbons. Appl. Environ. Microb. 72, 28-36.
18. Eun, J.S, Beauchemin, K.A, Hong, S.H. and Bauer, M.W. (2006). Exogenous enzymes added to untreated or ammoniated rice straw: Effect on in vitro fermentation characteristics and degradability. Anim. Feed Sci. Technol, 131:86-101.
19. Tortella G.R., Diez M.C. and Durán N. (2005). Fungal Diversity and Use in Decomposition of Environmental Pollutants. Crit. Rev. Microbiol. 31,197-212.

20. Hazim A.Walli. Determination of PAHs in Surface Water of AL-Dalmaj Marsh, AL-Diwayia Province, Iraq, 5(2015), 234-238.