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Specific Impact Of Soil Microorganisms On Removing Heavy Metals From Contaminated Media

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ABSTRACT

The laboratory experiment was conducted in the lab. at Agriculture College of the / University of Basrah using soil taken from Abu Al-Khasib area alluvial clay soil (Silty clay). The study included several local isolates of the fungi and bacteria isolate to identify specific phosphate dissolving fungi (*Aspergillus niger*) using potato dextrose agar media and bacteria (*Bacillus Polymyxa*) using pikovisky media.

isolated fungi and bacteria inoculated on proth media contaminated with heavy matele (cadimun and lead) speartly cadmium at levels (0,5,10,20,50) ppm and lead at levels (0,5,10,25)ppm and incubated for different periods (7,14,21,28) days.

The results showed the effects of different cadmium levels on the number of fungi colonies in different incubation periods and the best increase in the number of fungi in the Cd. level (20ppm) and incubation periods (21, 28) days. While a noticeable decrease in the (50ppm) in the same incubation periods (21,28)days.

The beast increase in the number of fungi colonies was in the pb level (25ppm) at the periods (21&28) days. Moreover, decrease in fungi colony numbers at the level (0,5,10)ppm in incubation periods (7,14,21). Cadmium caused an increase in the *Bacillus* colonies number at the (0ppm) level in all incubation periods. In contrast, there was a decline in the number of colonies (10,20,50)ppm. Effects of lead levels the highest increase in the number of colonies at the incubation periods (7) days and a noticeable decrease in the number of bacterial colonies at the (10,25) ppm at (7,28)days.

The highest Aspergillus niger tolerance index % was at (20 ppm) Cd levels and (21) days incubation. Most substantial tolerance index % for *Aspergillus niger* at (25ppm) pb level and (14) days of incubation.

The best *Bacillus Polymyxa* bacterial tolerance index % at the (20ppm) to Cd. level at (28) days of incubation. And the beast tolerance index % for (5&10) ppm of lead levels in (21)days of incubation.

keywords: *heavy metals*, *Aspergillus niger*, *Bacillus Polymyxa*, *Tolerance index*

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Introduction*:*

One of the primary environmental pollution sources is heavy metals (HMs), and its contamination is generally caused by chemicals, fertilizers, mines, metallurgical processes. These activities increase metals' level due to atmospheric and industrial pollution accumulates in soil with are markable influences the ecosystem nearby (zouboulis. *et al.*, 2004).

An increase in heavy metals concentration also influences the soil microbial communities, especially their respiration and enzymatic activity, by blocking the essential functions as displacing essential metal ions or modifying the active biological molecules that serve as a good indicator of metal pollution (Doelman. *et al.*,1994).

Several studies have shown the negative relation between heavy metal concentrations and microbial activity. However, at relatively low levels, some heavy metal ions as Cd and Pb are essential for microorganism's growth (as bacteria and fungi) since they provide vital cofactors for Metalloproteins and enzymes (Eiland,1981). Many microorganisms have developed various resistance to the toxic metal ions (Nies,1999); these mechanisms include enzymatic detoxification of metal to the lowest toxicity effect active transport of HMs away from cell organisms (Bruins *et al*.,2000).

This study aimed to determine the efficacy of each dissolving fungi (*Aspergillus niger*) and bacteria (*Bacillus Polymyxa*) on removing heavy elements pollution in the liquid culture media and which one of them more effectiveness

Method and Material:-

Soil samples were collected from various Basrah provinces to obtain different isolates of dissolved phosphate fungi by using potato dextrose agar media (Razak et al., 1999). after incubation for seven days

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at 28±1Cº, the fungi colonies showed a transparent zone. The fungi isolate identified in the lab at the soil science and water resource department in the college of agriculture refers to *Aspergillus niger* according to macroscopic characteristics such as shape, diameter, morphology, appearance, and texture of colonies, and microscopic factors such as the presence of reproductive structures (spores), presence of sterile mycelium, septation in hyphae, conidia shape and color of hyphae

Bacteria dissolving phosphate isolate also isolated by using pikovisky media modified by Nabilah (1977) at the soil Microbiology, soil, and water resources department. Microbial efficiency for dissolving phosphate in media was noticed by clearing zones diameters according to Raper and Fenell (1965) at *Aspergillus niger* and Nabilah (1977) at Bacillus SP. Both microbes (fungi and bacteria) were incubated at various periods 7,14,21,28 days, then calculated some parameters as pH- degree and electrical conductivity as according to page, *et al.* (1982) moreover, soluble cadmium and lead as following standard procedure described by Lindsay and Norvell,(1978) number of microbes colonies (fungi and bacteria)as CFU g^{-1} as following the standard method described by black,(1965).

The Tolerance Index (TI%) was used to express the tolerance results (Fomina *et al*., 2005).

TI = D treated / D untreated*100

Statistical analysis

Fungus and bacterial numbers of colonies and tolerance index % (Ti) data were subjected to variance (ANOVA) using a randomized complete plot design with three replicants by using SPSS ver.11.0, and means compared using Revised Least Significant Differences (R.L.S.D) test at a significant level of 0.05. AL-Rawwi and Khalaf-Allah (2000).

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Result and discussion

According to the incubation periods when exposed to different cadmium concentrations, the finding result showed a noticeable decrease in ***Aspergillus Niger*** numbers of colonies.

Figure (1) shows that increasing the number of colonies of fungi by the effect of adding different concentrations of the contaminant element with incubation periods, as the highest increase was at the concentration (20ppm) of cadmium during (21,28) days and then As for concentration (50ppm), decreased the number of colonies during the same incubation period (21,28). This decrease is due to the increase in the degree of toxic effect, which depends on the concentrations of pollutants and their availability to the fungal population and the sensitivity of microorganisms exposed to heavy metal' influence (Ahmed, 2007).

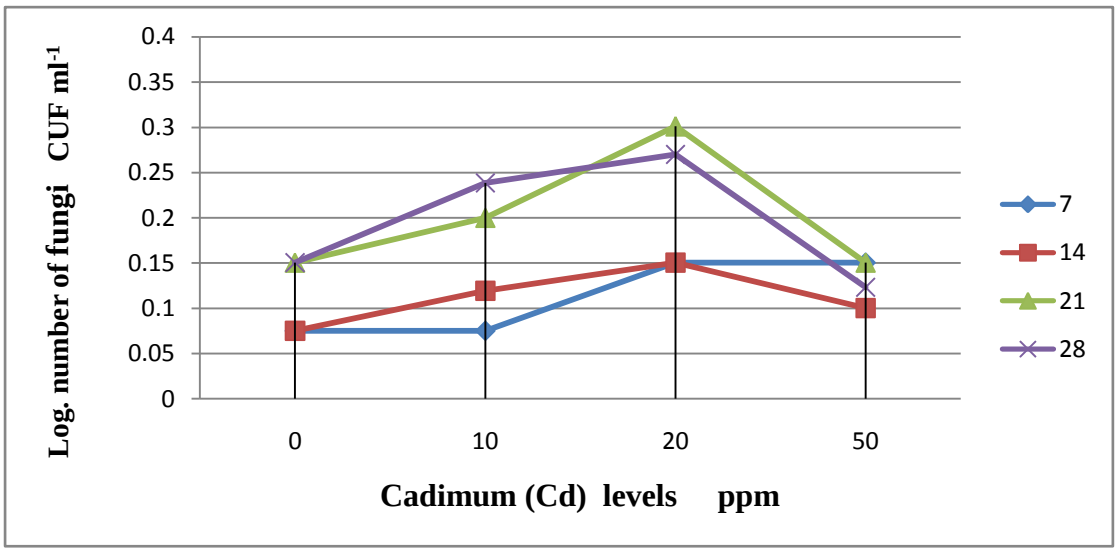

Fig. (1) Effect of different Cd levels (ppm) on the number of colonies (CUF ml^{-1}) of *Aspergillus niger* at different periods (days) of inoculation in PDA media

Figure (2) shows a noticeable increase in the number of colonies at Pb levels (0,5,10) ppm and (7,14,21) days. In contrast, (28) days at Pb level (10ppm), there was decreased in the fungi the number of colonies, on the other hand at (25)ppm lead level; there were decreases in fungi the

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number of colonies during (7,14,21 days) and an increase in 28 days of incubation.

The increase in the fungal number of colonies is due to fungi active defense mechanisms limiting the toxicity of minerals through their ability to metabolize or chelation by certain compounds inside and outside living cells. As in many fungal groups, heavy metal chelate through low molecular weight peptide compounds inside living cells. The reason for the superiority of the fungi *Aspergillus niger* is its high ability to represent heavy metal complexes, their aggregation, and sedimentation on the cell wall (Gomes *et al*.,1999).

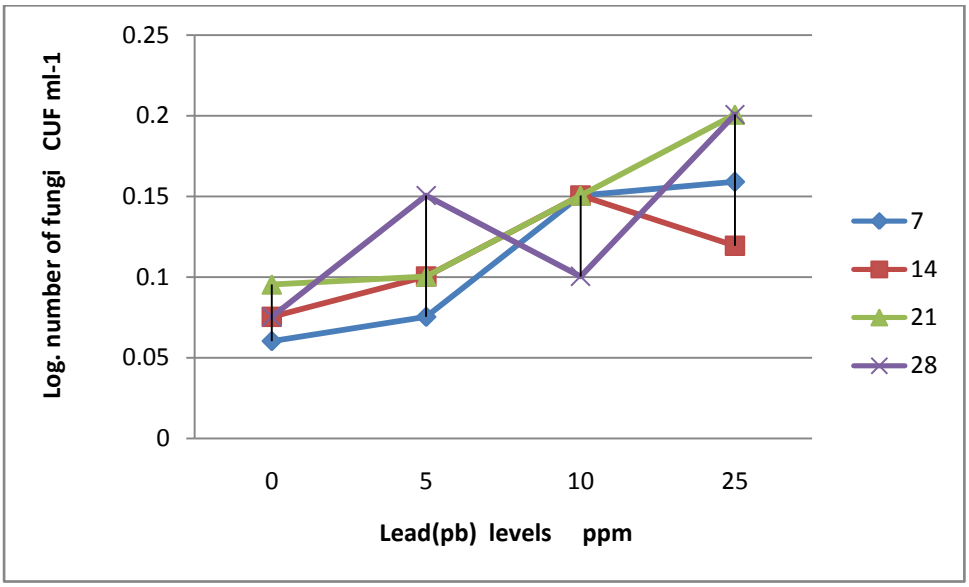

Fig. (2) Effect of different Pb levels (ppm) on the number of colonies (CUF ml^{-1}) of *Aspergillus niger* at different periods (days) of inoculation in PDA media

Figure (3) shows the effect of different cadmium levels on the bacterial number of colonies. There was an increased number of colonies in concentration (0ppm) in all incubation periods.

While the levels (10, 20 & 50) ppm, the number of colonies decreases in (7, 14, 21, and 28) days of the incubation where the contamination at the highest levels lead to inhibition of bacterial the

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number of colonies due to the effect of heavy metal on the natural bacterial groups, by inhibiting their growth and vital activity (Kim,1985). Toxicity of heavy metal to bacteria by reducing their complex formation with organic matter and biological transport of components (Al-Hrkane,2018).

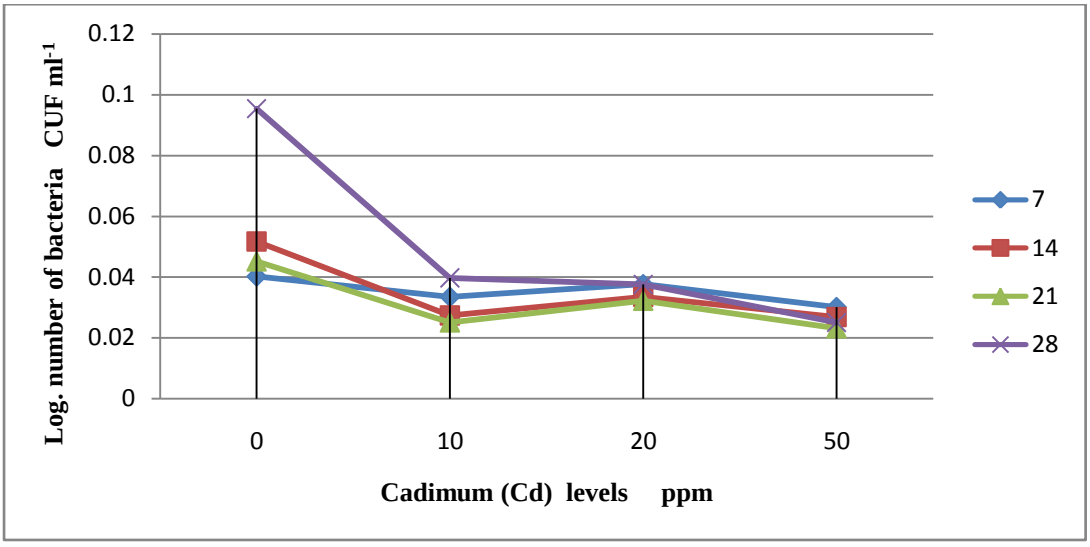

Fig. (3) Effect of different Cd levels (ppm) on the number of colonies (CUF ml^{-1}) of *Bacillus Polymyxa* at different periods (days) of inoculation in Pikovaysky media

Figure (4) showed that *Bacillus Polymyxa* the number of colonies a noticeable increase gradually at the incubation periods (7) days at a concentration (5) ppm of lead. There was a significant decrease, bacterial the number of colonies at (7 & 28) days, while an increase at (14 & 21) days incubation period, at the (10 ppm) concentration.

At the level (25) ppm at the (14 & 21) days, the bacterial number of colonies increased and then decreased (28) days. The optimal period at different concentrations in which the bacterial number of colonies persists was (21) days due to the depletion of the microorganisms of nutrients and stimulating growth within three weeks, which led to the emergence of the negative impact of heavy metal on bacteria and reduced their ability to grow (Al-Hrkane,2018).

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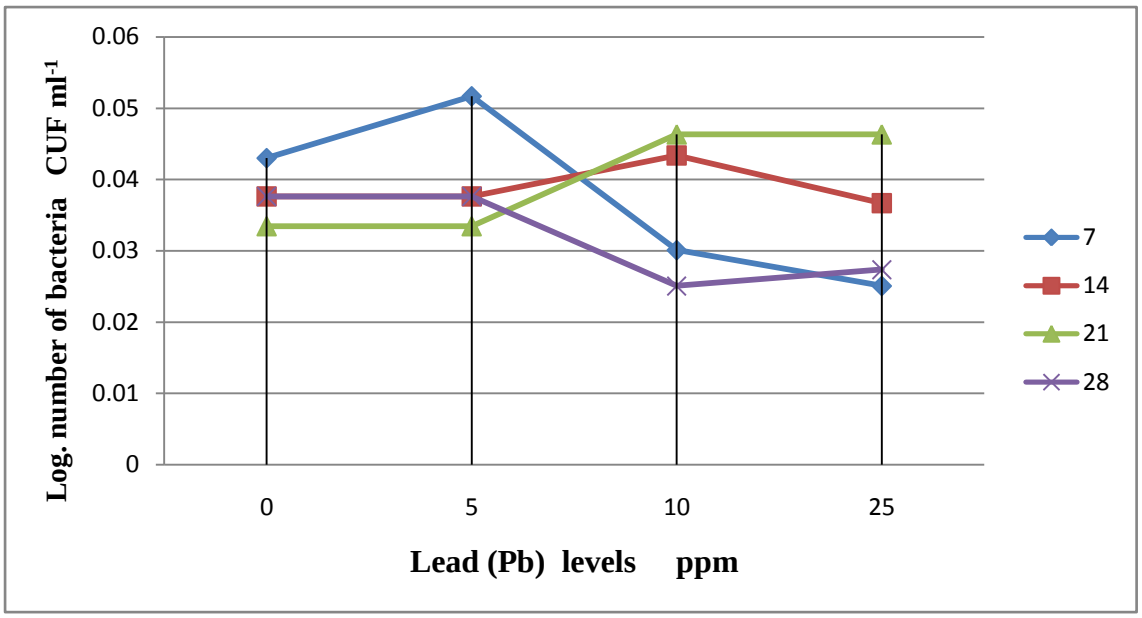

Fig.(4) Effect of different Pb levels (ppm) on the number of colonies (CUF ml^{-1}) of *Bacillus Polymyxa* at different periods (days) of inoculation in Pikovaysky media

Tolerance Index (TI)(%) to microorganisms for incubation periods with different concentrations of heavy metals

Table (1) shows a significant effect for inoculations and cadmium concentration on *Aspergillus niger* fungi tolerance index **(%).**

Aspergillus niger showed the highest tolerance index percentage at (20 ppm) concentration and (21) days of the incubation, this elevation of fungal (TI). At this level (20) ppm of cadmium led to an increase in the toxicity effects on the cell membrane by restricts its structural composition, thus hindering the exchange of necessary ions and organic substances for life, like proteins or sugars, or by preventing them from transporting through the membrane (Abdel Moneim and Al-Turki, 2012).

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Table(1) Tolerance Index(%)for *Aspergillus niger* exposed to Cd- levels(ppm) at different periods inocubation in vitro

Periods / Cd- levels	7	14	21	28	Mean
0	0	0	0	0	0
10	50.00 ±2.00	50.00 ±3.00	200.00 ±5.00	200.00 ±3.00	125.00 ±3.25
20	100.00 ±2.00	79.00 ±1.00	233.00 ±1.00	200.00 ±6.00	153.00 ±2.5
50	75.00± ±1.00	50.00 ±5.00	200.00 ±5.00	100.00 ±3.00	106.25 ±3.50
RLSD0.05					21.00

Table 2: referred to Aspergillus niger tolerance index percentage observed at a concentration of (5ppm) rise at a (7) days of incubation, after which a decrease in the two incubation periods (14 and 21) days while it increased during the (28) day, Whereas at (10 ppm) the most substantial tolerance recorded was in the (14) days of incubation, while at (25ppm) the highest tolerance index was during the (21) days of incubation. When comparing different concentrations with different incubation periods, the best tolerance index was at a concentration of (25ppm) and (21) days of incubation; this due to Lead cause inhibits catalytic and antibiotic reactions and reduces the organisms' protein building rate (Cole,1976). also interacts with sulfur groups (-SH) in the protein and inhibits cell wall functions, oxidative phosphorylation. (Bruins et al.,2000).

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Table (2):Tolerance Index(%)for *Aspergillus niger* exposed to Pb-levels (ppm) at different periods inocubation in vitro

Periods / pb- levels	7	14	21	28	Mean
0	0	0	0	0	0
5	200.00 ±1.00	150.00 ±5.00	150.00 ±3.00	200.00 ±1.00	175.00 ±2.5
10	233.00 ±2.00	316.00 ±1.00	300.00 ±2.00	150.00 ±5.00	249.75 ±2.50
25	250.00 ±5.00	200.00 ±1.00	383.00 ±3.00	200.00 ±3.00	258.25 ±3.00
RLSD0.05					23.333

Table(3) shows of bacteria *Bacillus polynyxa* highest tolerance index at (10ppm) was during at (14) days incubation period. In contrast, the concentration of (20ppm) was highest (TI)% at a (28) days incubation period, and the concentration of (50ppm) was the best (TI)% (7 and 28). days. When compared to the tolerance index for different concentrations during one incubation period, it found that during (28) days, the best (TI)% was for a concentration of (20ppm).

The reason is that high concentrations of heavy metal can ultimately impede biological groups by inhibiting their various vital activities, such as changing the nature of the protein, preventing cell division, and disturbing the functioning of the cell wall. (Deborah and Raj., 2016).

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Table (3): Tolerance Index(%) for *Bacillus polynyxa* exposed to Cd-levels(ppm) at different periods of incubation in vitro

Periods / cd- levels	7	14	21	28	Mean
0	0	0	0	0	0
10	108.67 ±7.57	122.00 ±3.00	112.00 ±2.00	112.00 ±1.00	113.67 ±3.39
20	125.00 ±2.00	144.00 ±1.00	121.00 ±2.00	150.00 ±5.00	135.00 ±2.50
50	95.00 ±1.00	92.00 ±3.00	90.00 ±4.00	95.00 ±5.00	93.00 ±3.25
$RLSD_{0.05}$					6.00

Table(4) shows that the best tolerance index (TI) % for (5and 10ppm) was at (21day) incubation period when comparing the three concentrations to different incubation periods, while at (25ppm), the incubation period was (7) days was the highest (TI)% in comparison with other incubation periods

Bacteria have a high ability to represent heavy metals and encourage their movement, which occurs through the vital metabolism processes (Wyszkowska *et al.*,2013).

Table (4):Tolerance Index(%) for *Bacillus polynyxa* exposed to pb-levels(ppm) at different periods incubation in vitro

Periods / Pb - levels	7	14	21	28	Mean
0	0	0	0	0	0
5	142.00 ±2.00	216.00 ±3.00	288.00 ±2.00	158.00 ±5.00	201.00 ±3.00
10	171.00 ±1.00	258.00 ±1.00	288.00 ±3.00	137.00 ± 3.00	213.50 ±2.00
25	120.00 ±1.00	100.00 ±4.00	100.00 ±6.00	100.00 ±3.00	105 ±3.50
$RLSD_{0.05}$					5.00

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Conclusion:

The finding result showed a decrease in the number of fungi and bacteria phosphate- dissolving in liquid culture media during incubation periods by increasing the concentrations of heavy elements cadmium and lead. Both ***Aspergillus niger*** fungi and ***Bacillus polynyxa*** varied in their ability to increase the number of colonies and their ability to tolerate pollutants at different concentrations. The results show a higher ability of ***Aspergillus niger*** fungi to remove heavy elements rather than bacteria ***Bacillus polynyxa.***

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