Determine The efficiency of *Pseudomonas aeruginosa* in bioremediation of some heavy metals

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Summary

Bioremediation by *Pse*udomonas *aeruginosa* was studied for copper in a concentrate of 85, 75, and 95 mg/L of water samples (28.9, 32.4 and 26.4%) and (50, 50 and 44%) of fish muscles. Chrome concentrations were 12, 11 and 10 mg/L for water samples and 19.8, 32.6 and 27% for fish muscles. Values for zinc were 47, 70 and 61%. The bioremediation results revealed there were significant ($P<0.05$) differences in fish samples, as they were 27.7, 28.0 and 27.9% for concentration 5, 6 and 7 mg/L for copper, those of the concentration 3, 4 and 5 mg/L were 50, 30 and 80% for accumulated in fish muscles. The treated percentages of the dissolve in water were 22.4, 32.6 and 22.5%.

Introduction

The heavy metal contamination is a global problem has two main sources, first :is natural as a result of rock, forest fires, volcanic activity and the second is anthropogenic result of the growing industry, mining and other industries that lead to put untreated waste into the water (Guo etal., 2010).

Some studies have indicated that the dangerous heavy metal toxicity due to their toxicity and lack of usability on the decomposition of non-biodegradability and bio-accumulation in biological systems lead to amplified biomagnifications within the food chain (Authman, 2008).

Some studies classified heavy metals into two classes, the first is a necessary such as copper, Cu and Mn and Fe and Zn but only when safely exposed to them, whereas, the increase in concentration from the normal level become toxic. The other class the is non-essential, such as lead Pb, cadmium Cd, mercury Hg and aluminum Al, chromium Cr, which do not have any vital function which is toxic even in low concentrations (Nkwelle et al., 2012).

Body need nearly 70 heavy metals, but there are only 12 toxic metals as Pb, Hg, Cd, Cr or Ni. They are interact with some metabolic and enzymes activities in the body (Ekpo et al, 2008). Among these toxic effects of the damage, which appears on the DNA, and also from the toxic effects of other such as contagious that appear as a result of long-term exposure to lead an anemia, cancer and interference with vitamin D, which causes coma and death when increase its concentration in the blood (Naik et.al., 2012).

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The use of bioindicaters become useful tools in understanding the complex interactions between the response of the organism to environmental stimuli and resistance to the effects of the deadly for many of them, and that the interaction cellular elements reflected on the internal processes that occur with the processes of accumulation, which gives a clear picture of the toxicity of the element, and that many of the neighborhoods waters can be used as an indicator to the quality of the water.

There are traditional methods to remove heavy metals from contaminated water, including chemical precipitation, ion exchange, reverse osmosis and demodulation using solvents are most commonly used to remove metal ions from solution (Singh and Gadi, 2012). Modern methods of using bacteria to remove heavy metals and reduce the toxicity and treatment called bioremediation (Safahieh etal., 2011), bioremediation known as a biotechnology that use microorganisms to remove contaminants technical, can generally be divided into treatment in their native habitat in situ and the outside environment (Vijanand etal., 2012). This technique is used to remove contaminants or convert them into less toxic compounds, movement and soluble (Vidali, 2001). Many researchers pointed to the bacteria with the ability to remove contaminants (trace metals) such as Pseudomonas sp., Aeromonas sp., Bacillus sp., Micrococcus sp., Microbacterium sp. And other (Kannan et al., 2012).

The present study aims to determine the efficiency of the bacterium Pseudomonas aeruginosa in the bioremediation of metals by raising survival rates and increase the average killing time and reduce the half time killing concentration.

Materials and Methods

Preparation airborne bacterial

Vaccinated test tubes which contain 5 ml of physiological saline solution 150 ml of bacterial culture grown on nutrient broth liquid at the age of 18 hours, then mix well, to prepare an approximation 10^8 cells / ml, and after comparing turbid growth formed with turbid solution turbid standard fixed in advance and hold the record count of the living cells in the solution.

Collection fish samples

The samples were collected from small Common Carp *Cyprinus carpio* of private farms. A total of 70 fish at a weight of ± 102 g and length of 8 ± 3 cm each experiment. The fish were in large container of a capacity of 50 liters filled with water from the environment until delivery to the laboratory and start experiments.

Acclimation

Fish were put in glass ponds length 70 cm, width 50 cm and 50 cm high. A 20 L of Tap water added, the water used was store 24 hours in advance in order to accumlate the fish to labrotary envirmonets such as temperature, light and oxygen was avalible by using air pump in each pond. Ten fishes were put in each pond. Commercial ration was used with adaptation period of seven days (Narayanan and Vinodhino, 2008).

Experiment Design and Bioremediation

The study concentrated on the effect of Pseudomonas aeruginosa in bioremediation of heavy metals and to decrease their toxicty through an increase in vaibility rate of young normal carps, decrease the particabation of heavy metals in some fish tissue and increase metal particabation in water. The focus was on the use of the four elements of trace metals and different concentrations of each of the elements of each of the ions as they lead concentrations in the initial experiments (15,25,35 mg / L) and copper (1,2,3 mg / L) and zinc (1,2.8 0.5 mg / L) and chromium (5,6,7) mg / L, While lead concentrations were changed to become (45,55,65,75,85,95) mg / L and copper (3,4,5) mg / l and zinc (5,6,7) mg / l and chromium (10.11 0.12 mg / L) on each unit for a period of 96 hours for each item. The concentrations of the last three are adopted as a result of the onset of the effects on fish and through the initial experiments, as shown in Table (1).

Table (1) Concentrations of heavy metals used in the study

Element	Concentration (mg/L)								
Pb	15	25	35	45	55	65	75	85	95
Cr	5	6	7	7	8	9	10	11	12
Zn	1	2.8	5	6	7				
Cu	1	2	3	4	5				

The experiment included 7 glass ponds of a capacity of 40 liters of water, was put 20 liters of water stored in advance and put 10 fish in each basin, allocated a basin to control by three replicates for each experiment, and different concentrations of each element and each concentration as it represents the first focus treatment of fish while refined metals for this focus is the treatment of metal fish in addition to the bacteria in order to know the role of bacteria *P. aerugenosa*, in bioremediation. The accumulations of metals were calculated in fish muscles and water. All concentrations were estimated by Atomic Absorption Spectrophotometer .

The average time of mortality and average lethal concentration and focus by Vinodhini and Narayanan (2008) by calculating the average survival rates for each element were cut nutrition during the trial period, amounting to 96 hours to reduce the effects of outputs metabolic The results were recorded every 24 hours. Samples of fish muscle were taken after the end of the experiment period of 96 hours. Values of bioaccumulation of heavy metals, either in water sample drawn from the basin itself were compared with those of treatment basins to measure precipitated from dissolved heavy metals. The standard solutions were attending temporarily during the experiment to begin with washing glassware well with distilled water.

Estimation of accumulated metals in tissues Muscles were used to obtain the chemical analysis. Part of muscles (0.10-0.15 gm) were minced after separation and dried by electrical oven for 24 hrs. at 105 c. The weighted tissue was put in glass basin with HNO3 and HCL for digestion (Al-Ali, 1999). The level of accumulated elements were estimated by using a flame atomic absorption spectroscopy by calibration curve, and the application of the law of estimating the concentration of heavy metals in tissues containing:

Statistical Analysis

The results were statistically analyzed by using the statistical packages of social sciences (SPSS, 2006) under a probability of 5% (Sorile, 1995).

Results and Discussion

After 96 hours on treating the fish under study by P. aeruginosa bacteria and heavy metals (Pb, Cr, Zn, Cu). Water sample and fish muscles were analyzed to determine rates and quantity of metals accumulated in the muscles and the extent of the role of bacteria in the removal of toxicity as shown in Table (2). Which refers to the comparison between the amount of mineral in water and fish muscle prior to treatment was compared with the efficiency of the bacterium P. aeruginosa on the basis of the remaining water in the basins minerals and fish without the addition of bacteria after 96 hours at the start of the experiment, as well as bioaccumulation of fish with tubs of treatment to see the role of bacteria in metals reduction.

Table (2) Values of heavy metals in water samples and fish muscles in control group

Control	Cr	Cu	Pb	Zn
Water	٠.٠٤٢± ٠.٠٠٢	٠.٠٠٩٧±٠.٠٠١٨	٠.٠٤٦±٠.٠٠٤	٠.٠٠٢٢±٠.٠٠١١
Fish	.٠٠٠٨±٠.٠٠٠٦	٠.٠٠١١±٠.٠٠٠٤	٠.٠١١±٠.٠٠٥	٠.٠٩٢±٠.٠٠١٥

Bioaccumulation and bio-processing component of the Lead

The results indicate in the table (3) to the bioaccumulation of lead element using the muscles of the common carp as evidence vital bio indicators and bioremediation using a bacterium P. aeruginosa after 96 hours. The result showed the accumulation in muscles was highest when the concentration was 95 mg/L, whereas, the lowest was shown by the concentrates 75 and 85 mg/L (0.00006). The rate of accumulation correlated positively with concentration.

As for the treatment of accumulated in fish muscle ratio decreased with increasing concentration with negative correlation. It decreased from 50% at the concentrations of 75 mg / L for to 44% when the concentration was 95 mg / L. Bacteria of P. aeruginosa was able to reduce the accumulated fish muscle of 0.0006 ppm before treatment to 0.0003 ppm after treatment with 75 and 85 mg / l. The concentration 95 mg / l was treated at a rate of 44%, the decline in the value of accumulated pre-treatment was 0.0009 to 0.0005 after treatment. As for the efficiency of the bacteria to remove the lead element of the total deposited and remaining in the water concentration 85 mg / l recorded the highest removal rate of 32.22%, while the proportion of treatment for concentration 95 mg / L 26.44% a difference of 6.22% from the earlier concentration.

The results of the statistical analysis indicated the existence of significant differences at the level of significance 0.05 ($P < 0.05$) for the treatment of water samples and that for the fish did not show any significant differences for all concentrations ($P > 0.05$).

Table (3) Accumulation of Pb in water samples and fish muscles before and after treatment with P. aeruginosa

Concentration (mg/kg)	Before 96 hrs. of treatments		After 96 hrs. of treatments	
	water	**fish**	**Water**	**fish**
75	٠.٥±١٠.٦٦٠ ab	٠.٠٠٠٤±٠.٠٠٠٦ a	٠.٩٨±٧.٥٧٤٠ Ab	٠.٠٠٠٢±٠.٠٠٠٣ A
85	٠.٠٨±١١.٧٦٤ ab	٠.٠٠٠٥±٠.٠٠٠٦ b	١.٠٠٧±٧.٩٤٦٠ Ab	٠.٠٠٠٢±٠.٠٠٠٣ A
95	١.٠٥±١٢.١٤٠ ab	٠.٠٠٠٤±٠.٠٠٠٩ c	٠.٩±٨.٩٣٠٠ Ab	٠.٠٠٠٤±٠.٠٠٠٥ B

Different letters indicate significant differences among means ($P<0.05$).

Bioaccumulation and bioremediation of Cd element

All present results in tables (4-13) indicated that bioaccumulation and bioremediation by bacteria of C. carpio (L) muscles during the period of 96 that The results of the current study in Table (4-13) to each of the bioaccumulation and Bioremediation using bacteria in the muscles of the common carp C. carpio (L) exposed for a period of 96 hours to different concentrations of chromium element availability of a positive correlation between the concentration, biological treatment. These results agreed with that of Safahieh et al., (2011). The efficiency of the Pseudomonas sp. increases in bioremediation with the increase of concentration (Oh et al 2009).

The efficiency of bacteria was the lowest concentration 0 mg, 47% are less efficient than the highest concentration of 12 mg, 61.3%. The decrease of accumulation in the muscles of fish from 0.0044 to 0.0017 before treatment after treatment respectively was observed. While bioremediation of water samples showed bacteria as the Pseudomonas sp. The percentage reduction of the concentrations of 10, 11 and 12 mg / L ratio of 19.8 and 32.6 treatment and 27%, respectively, where the largest proportion of the concentration of 11 mg / L as well as this percentage is the highest in the muscles of fish. The results of the statistical analysis showed significant differences at the 0.05 level of significance.

Table (4). The accumulation value of Cr in water and fish muscles exposed to this element before and after treated with P. aeruginosa

Concentration (mg/kg)	Before 96 hrs. of treatments		After 96 hrs. of treatments	
	water	fish	water	fish
10	٠.٠٠١±٠.٥٠٩٠ ab	٠.٠٠٠٦±٠.٠٠١٧ a	٠.٠٠٦±٠.٤٠٨٠ c	٠.٠٠٠٥±٠.٠٠٠٩ A
11	٠.٠٤٧±٠.٦٥٢٠ b	٠.٠٠٠٨±٠.٠٠٢٠ c	٠.٠٨±٠.٤٣٩٠ ab	٠.٠٠٠٥±٠.٠٠٠٦ C
12	٠.٠٠٩±٠.٦٨٣٠ a	٠.٠٠٠٩±٠.٠٠٤٤ b	٠.٠٠٩±٠.٤٩٥٠ ab	٠.٠٠٠٩±٠.٠٠١٧ B

Different letters indicate significant differences among means (P<0.05).

Bioaccumulation and Bioremediation of zinc element

The table shows (5) the results of bioaccumulation and the results of the bioremediation of the bacterium P. aeruginosa heavy metals of muscles fish exposed to different concentrations of Zn for a period of 96 hours, as the results showed that the rate of accumulation of the concentrations of the three concentrations were almost close. While the highest concentration 7 mg / l recorded higher proportion of treatment of accumulated element in the muscles of fish C. carpio amounted to 27% of the sum of all of the water and fish. The treatment of accumulated was the highest value for the concentration of which is 13% for fish muscle (Table 5). The remaining in the water was the highest concentration of the 6 mg / l to reach 28.2% in a difference of 1% from the first and third concentrations (7-5 mg / L). The results of the statistical analysis have shown the existence of significant differences for the three concentrations at the 0.05 level of significance before and after treatment.

Table (5). Accumulation of Zn in water and Fish muscles before and after treatment with P. aeruginosa

Concentration (mg/l)	**Before 96 hrs. of treatments**		**After 96 hrs. of treatments**	
	Water	**fish**	**water**	**fish**
5	**0.0020±0.0005 a**	٠.٠٠٦٠±٠.٠٧٨٠ **A**	٠.٠٠٠٥±٠.٠٠٢٠ **a**	٠.٠٠١±٠.٠٥٦٤ **Ab**
6	٠.٠٠٠٩±٠.٠٠٢٠ **ab**	٠.٠٠٨٠±٠.٠٩٢٠ **B**	٠.٠٠١٢±٠.٠٠٢٠ **ab**	٠.٠٠٨٧±٠.٠٦٦٠ **C**
7	٠.٠٠١٠±٠.٠٠٢٣ **a**	٠.٠١٠٠±٠.٠٩٥٧ **B**	٠.٠٠١٥±٠.٠٠٢٠ **c**	٠.٠٠٧٥±٠.٠٦٩٠ **Ab**

Different letters indicate significant differences among means (P<0.05).

Bioaccumulation and Bioremediation of Copper element

The table (6) shows results of bioaccumulation of heavy metals in the muscles of young common carp Cyprinus carpio (L) exposed to different concentrations of copper as it turns out that the link was directly proportional between concentration and bioaccumulation and exposure time, however, the results of bioremediation came different. The second concentrate recorded a 4 mg / l higher value processing 32-45%, while the highest concentration recorded the highest value accumulation in the muscles of fish and the highest value in the treatment of fish muscle, reaching about 87% of the total accumulation in fish muscle, but for vital treatment the college has recorded a higher concentration 5 mg / L ratio of less treatment after 96 hours at the start of the experiment by 21.5% difference of 1% for smaller concentration 3 mg / l.

This result was consistent with the study conducted by Oh et al (2009) that bio sorption inversely proportional to the concentration. Hussein et al (2004) indicated that P. pseudomonas was able to remove a maximum of Chrome 88% and copper was about 93% and this contrasts with the current study, it showed that the efficiency of the bacteria in the treatment of Cr compared to the Cu or a difference of 1%. The current study contrasted with Bhatnagar and Hakeem (2010) that Pseudomonas sp able to get rid of more than 93% of Cu. Kumar et al (2010) recorded that it can reduce 68% of Cu in the media. The result showed significant differences (P<0.05).

Table (6) Accumulation values of Cu in water and fish muscles before and after treatments with P. aeruginosa

Concentration mg/L	Before treatment (96 hrs.)		After treatment (96 hrs.)	
	water	fish	water	fish
3	٠.٠٢٠٠±٠.١٢٩٠ A	٠.٠٠٠٣±٠.٠٠٠٢ A	٠.١٠٠٠±٠.٠٩٣٦ c	±٠.٠٠٠١ ٠.٠٠٠١ A
4	٠.٠٠٤٠±٠.١٤١٠ ab	٠.٠٠٠١±٠.٠٠٠٣ B	٠.٠٠٥٦±٠.٠٩٥٠ a	±٠.٠٠٠٢ ٠.٠٠٠١ B
5	٠.٠٠٤٠±٠.١٤٢٠ b	٠.٠٠٠٨±٠.٠٠١٦ C	٠.٠٠٣٠٠±٠.١١٠٠ a	±٠.٠٠٠٢ ٠,٠٠٠١ Ab

Different letters indicate significant differences among means (P<0.05).

The study of Kumaran et al (2011) showed that removing of Pb were 87.9% and 49.8%

for Cu, it means that htis bacteria is more efficient in the treatment of lead from copper in a large margin. While the current study revealed almost similar efficient which may be because live in aquatic environments contaminated by industrial waste, and in the current study isolated bacteria from natural waters away from industrial pollution. Al.Weher (2008) indicated in his study on the accumulation of metals in various tissues of fish C. carpio that the Cu had the ability to accumulate in the muscles of fish more than zn, and this is in contrary to the current study. Mol et al (2010) found that the accumulation of zinc and Copper in fish muscles were (17.40 - 0.350) mg / kg of dry weight. Ashraf (2006) found that Cr accumulation in Tuna by about 40 mg / kg.

The study of Ozuni et al (2010) revealed that M. barbatus showed an accumulation of 0.40 mg/kg from Cr and M. merluciusm 0.13 mg/kg. Study of Zhang et al (2007) covered 19 species of fish included C. capro, the results showed that accumulation in intestine was greater than that of muscles and accumulation increased towards low parts. The results explained Pb, Cu and Zn accumulation. Eneji et al (2011) compared Zn concentration in different organ of Tilapia. It accumulated 7.15, 5.66 and 5.24 mg/kg in gills, intestine and tissue respectively. Those values were 7.05, 6.86 and 3.85 in Clarias fish. The study also included Pd which recorded 1.00, 0.678 and 0.801 mg/kg in Tilapia, those of Cu were 2.98, 5.36 and 1.56 mg/kg and 2.07, 2.26 and 1.56 mg/kg respectively in other species.

Shah (2005) conducted a study on fish Tincatinca that the accumulation of the lead after 96 hour exposure period was in (gills 1.86, 1.47 muscle, liver 2.55) mg / kg. While Ashraf (2006) studied the Tuna fish, the lead concentration was 40 mg / kg, the copper accumulation was mg / kg. Ozuni (2010) that the rate of accumulation in the muscles of fish C. carpio of chromium, zinc, copper was (0.031, 4.9, 0.212) mg / kg respectively. The results of the current study showed that the accumulation of zinc was 0.04%, of the concentration of 5 mg / l and the least was of lead with a concentration of 75 mg / l within 96 hours. This result was compatible with that of Nongbri and Syiem (2012) and Ozuni et al (2010) as the accumulation of lead was very little. The bioremediation was the largest percentage of copper, P. aeruginosa was able to remove approximately 87% of copper in fish muscle, 67.7% of the cumulative component of the Cr, 50% of lead and the least was Zn (0.13%) of the highest concentration of 7 mg / l.

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تحديد كفاءة بكتريا *Pseudomonas aeruginosa* في المعالجة الحيوية Bioremediation لبعض المعادن الثقيلة

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الخلاصة

درست المعالجة الحيوية بإستخدام بكتريا *Pseudomonas aeruginosa* لعنصر الرصاص بتركيز (٧٥،٨٥، ٩٥) ملغم/ لتر لعينات الماء (٢٨.٩%،٣٢.٤%،٢٦.٤%) و(٥٠%،٥٠%،٤٤%) لعضلات الأسماك، أما تراكيز عنصر الكروم فكانت (١٠،١١،١٢) ملغم/ لتر لعينات الماء (١٩.٨%،٣٢.٦%،٢٧%) ولعضلات الأسماك (٤٧%،٧٠%،٦١%) والخارصين، اظهرت نتائج المعالجة الحيوية فروقا معنوية (P<0.05) على عينات الأسماك إذ كانت للتراكيز (٥،٦،٧) ملغم/ لتر (٢٧.٧%،٢٨%،٢٧.٩%) ولعنصر النحاس وللتراكيز (٣،٤،٥) ملغم/ لتر (٥٠%،٣٠%،٨٠%) للمتركم في عضلات الأسماك أما الذائب في الماء فكانت نسبة المعالجة (٢٢.٤%،٣٢.٦%،٢٢.٥%).