

Full Length Paper.

Adsorption of Zinc Ion by different bacterial species

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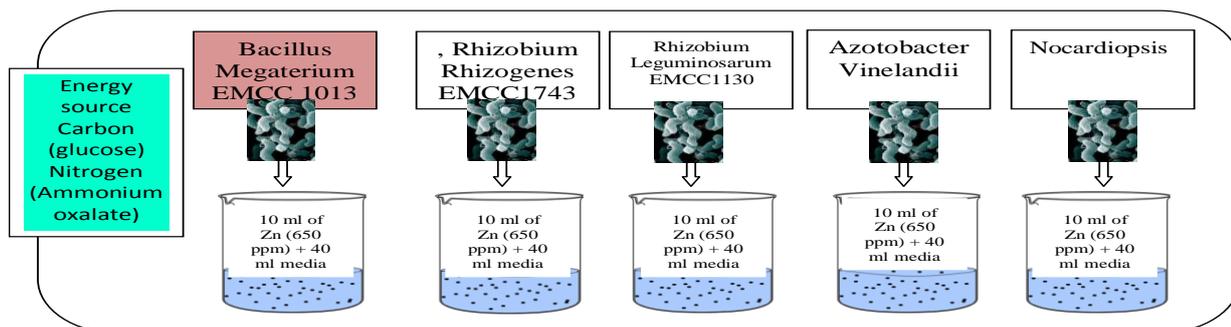
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Heavy metals are generally toxic to microorganisms, especially if they exist at high concentrations. Environmental pollution particularly in soil with heavy metals can stem from industrial activities or sewage discharges. In this study, Five different bacterial species *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC 1743, *Rhizobium leguminosarum* EMCC 1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* were evaluated their potential activity in bioremediation of zinc ion. Our results showed that five bacterial species have great variation potential for zinc bioremediation. The aim of our study was to evaluation the bioremediation capacity of zinc as heavy metals by five different bacterial species with glucose and ammonium oxalate as carbon and nitrogen energy sources to use them in further study in removal of Zn(II) from plating waste water. This results is important to be well understand the bioremediation mechanism of *Bacillus megaterium* EMCC, and is significant for its pilot test and future practical application. In addition *Bacillus megaterium* EMCC as the most potent Zn (II) resistant microorganisms will very useful in biotechnology for the remediation of metal contaminated environments with Zn (II)and can also be used in the construction of biomarkers for the detection of zinc ions.

Keywords:Bioremediation, *Bacillus megaterium*, heavy metal, Zinc

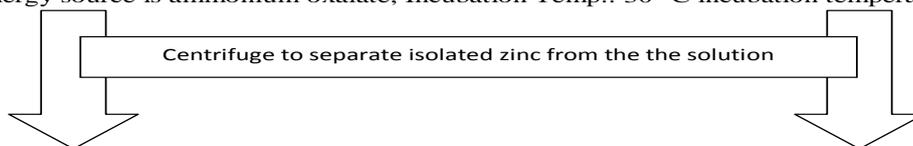
Graphical Abstract



Optimum condition

Time: 24 h, Inoculums size 0.1×10^{29} cfu, pH: 7, Carbon energy source is glucose

Nitrogen energy source is ammonium oxalate, Incubation Temp.: 30 °C incubation temperature



INTRODUCTION

Heavy metals are present in the environment in different concentrations in water, soil, and in all biological objects. So, Exposure to heavy metals has been linked with development retardation (Brooks *et al.*, 2010). Zinc consider one of the metals found in effluents discharged from industries involved in galvanization,

electroplating, manufacturing of batteries, and metallurgical industries. Zinc in its metallic form has limited bioavailability and poses no ecological risk. However, zinc can react with other chemicals like acids and oxygen to form compounds, which can be potentially toxic and can cause serious damage to biological systems (Fosmire, 1990)

Wide paying attention on management of environmental pollution and its control due to hazardous materials like heavy metals was been interested. Heavy metals bioremediation inwater behind industrial factories has been a challenge for a long time. A lot of physicochemical strategies, such as, membrane technology, ,electrochemical treatment, ion exchange, oxidation/reduction, filtration and reverse osmosis, have been developed for bioremediation of heavy metals from the polluted water (Tang et al., 2008).

A lot of recordshad indicated thatmicrobe–plantsymbioses and native microbesresist heavy metal concentrations in different ways and may play a significant role in the restoration of polluted water soil (Ge et al., 2009)

Bioremediation involves the utilize of microorganisms to remove and detoxifyenvironmental contaminants, has received increasing attention to remove up a contaminated environment (Malik, 2004)

The bioremediation of heavy metals from contaminated environments and reducing their toxicity by applying different microorganisms was developed, so, *Bacillus megaterium* has great role in the biogeochemical cycle of heavy metals and processes involved in bioremediation (Shazia et al., 2002). Microorganisms are using their secondary metabolites that participate in the bioremediation of heavy metals by production of organic and inorganic acids, oxidation or reduction reactions or excretion of chelating agents (Kumar and Achyuthan, 2007). The aim of this paper was to evaluation the bioremediation efficacy of heavy metal zinc as by different species of microorganisms to use them in further study in removal of zinc from waste water plating industries

MATERIAL AND METHODS

Microorganisms

Three bacterial species were purchased from Egyptian Microbial culture collection, Ain shams university (*Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC 1743, *Rhizobium leguminosarum* EMCC 1130). *Azotobacter vinelandii* was obtained by El -Badry et al 2016 and *Nocardioopsis Dassenvillei* was obtained by Elbarbary et al., 2015

Chemicals and instrumentation

Zinc stock solution:

Zn(II) (1 mg/ml) stock solution was prepared using by dissolving 4.398 g of $ZnSO_4 \cdot 7H_2O$ in distilled water containing a few drops of conc. H_2SO_4 and standardized by 8-hydroxyquinoline (Vogel, 1989). Using this stock solution with different zincppm concentration was prepared for culture media supplements.

Zn(II)bioremediation Experiments:

LB (Luria-Bertani)liquid medium (Oxoid) was used as basal media consists of different ppm concentration of Zn (II) solution. Different pH was prepared by adjustment 0.1(N) HCl and 0.1(N) NaOH solutions. After that media was autoclaved in 250 ml conical flasks containing 100 ml media. The media was inoculated with five different bacterial species. After incubation time samples were collected and centrifuged at 6000 rpm for 10 minutes. Supernatant was assayed for the zinc removal by Optical Emission Spectrometer Model: Optima 2000 DV Perkin Elmer (Inductive Couple Plasma). Bioremediation of zinc ion in basal media inoculated with five different bacterial species separately were evaluated by following equation Bioremediation of Zn (II) % = $\frac{S_{cont} - S_{sampl}}{S_{cont}} \times 100$. All the glassware was cleaned with 5% HNO_3 .

Relative effects of different Zn(II) concentration bioremediation on microbial growth

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different concentration (10, 15, 20, 25, 30, 35, and 40) ppm of Zn(II) for each bacterial species. After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

Relative effects of different inoculum size on Zn(II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different inoculum size (0.1×10^{29} , 0.5×10^{29} , 1×10^{29} , 3×10^{29} and 5×10^{29}) cfu of each bacterial species. After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

Relative effects of different Temperature on Zn(II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different incubation temperature (20° , 25° , 30° , 35° and 40°) C. After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

Relative effects of different PH on Zn(II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different PH (4, 5, 6, 7 and 8). After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

Relative effects of different Carbon sources on Zn(II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different carbon sources (glucose, starch, sucrose and dextrose). After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

Relative effects of different Nitrogen sources on Zn(II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different nitrogen sources (ammonium chloride, ammonium sulphate, ammonium oxalate, glycine and asparagine). After 24 h of incubation the remediation percentage of Zn(II) concentration on each bacterial growth was assessed

RESULTS AND DISCUSSION

Bioremediation is elimination processes that apply microbial way to minimize cytotoxicity of heavy metal pollutant to limit of an acceptable value. In this method, biotransformation by microorganisms for different heavy metal pollutants in the environment was carried out. Bioremediation follows of various biochemical reactions which help in activity, growth and reproduction of microorganisms. This microbial metabolism system allows microorganisms to obtain carbon, electrons and other necessary components for their existence. During bioremediation process, heavy metals are penetrated into the cells, get attached to intracellular proteins and then are associated with vacuoles or other intracellular sites (Malik, 2004; Srivastava and Majumder, 2008)

Different industries as metal cutting, milling, mining and surface finishing are the largest source of many toxic heavy metal ions such as Zn(II). It is very harmful for humans as they adversely affect the liver, brain and skin, kidney and respiratory tracts. Recently, the levels of zinc metals in drinking water have increased at an alarming rate. Thus the removal, recovery and recycling of these metals have greater significance.

Relative effects of different PH on Zn(II) bioremediation

Effect of different PH for zinc bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC 1743, *Rhizobium leguminosarum* EMCC 1130, *Azotobacter vinelandii* and *Nocardiaopsis Dassenvillei*. *Bacillus megaterium* EMCC 1013 was the most potent Zinc II bioremediation percentage with 65 % at PH 7 by using glucose as ammonium oxalate as carbon and nitrogen energy sources. But in case of using Piptone and Beef extract as carbon and nitrogen source the adsorption of zinc ion reach to 54% only as tabulated in table 1 and 2. It has been shown that low pH affect the network or chemistry of the cell wall as well as its physio-chemistry

and the hydrolysis of the heavy metals (Sag et al., 2000). From the results of this work, the maximum bioremediation percentage rates was observed in all five different bacterial species at Neutral pH 7 which agrees with the evidence that the optimal pH range for bioremediation by bacteria is 6.0-8.5 With increase in pH, there will be a resulting increase in negative charge on the surface of the cell which favoured electrochemical attraction and adsorption of metal (Van Nostrand et al., 2007). Li et al., 2017 reported that the optimum pH for Zinc removal by *Rhodobacter sphaeroides* was 7 which agree with our work by using *Bacillus megaterium* EMCC 1013. Many of authors have indicated that optimum removal efficacy for microbial biomass is indicated between 6 and 8, while little removal is recoded at pH less than 3 due to the cation competition effects with oxonium (hydronium) ion H_3O^+ (Al-Gheethi et al. 2014).

Table 1: Effect of pH on different bacterial Species on zinc ion adsorption from wast water.

Bacteria	<i>Nocardiopsis</i>	<i>Azotobacter vinelandii</i>	<i>Bacillus megaterium</i> EMCC 1013	<i>Rhizobium rhizogenes</i> EMCC 1743	<i>Rhizobium leguminosarum</i> EMCC 1130
pH					
4	50.86616	50.86616	52.75964	50.79333	51.07904
5	50.848	50.848	52.7408	50.7752	51.0608
6	51.5744	51.5744	53.49424	51.50056	51.79024
7	63.0152	63.0152	65.36092	62.92498	63.27892
8	39.044	39.044	40.4974	38.9881	39.2074

Table 2: Effect of pH on different bacterial Species without energy source on zinc ion adsorption from wast water.

Bacteria	<i>Nocardiopsis</i>	<i>Azotobacter vinelandii</i>	<i>Bacillus megaterium</i> EMCC 1013	<i>Rhizobium rhizogenes</i> EMCC 1743	<i>Rhizobium leguminosarum</i> EMCC 1130
pH					
4	39.16078	44.22779	43.86366	46.04844	41.78252
5	39.1468	44.212	43.848	46.032	41.7676
6	39.70604	44.8436	44.4744	46.6896	42.36428
7	48.51407	54.7913	54.3402	57.0468	51.76199
8	30.05915	33.9485	33.669	35.346	32.07155

Relative effects of different Zn(II) concentration bioremediation on microbial growth

Five different bacterial species *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC 1743, *Rhizobium leguminosarum* EMCC 1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* were evaluated for their potential percentage of Zn (II) bioremediation under different concentration of Zn (II) with 80, 76, 77, 77 and 77 % respectively for 10 ml of 650ppm of Zn (II) in using glucose and ammonium oxalate as carbon and nitrogen sources. In the case of using piptone and beef extract as carbon and nitrogen sources it is reach to 66.5% removal of zinc ion by bacillus megatirium. Its removal decreased in bioremediation for all tested microorganism by increase in Zn (II) concentration as listed in Tables 3 and 4. Rhizobia species have great efficacy to removal heavy metals Zn elements was studied with high potential to removal of heavy metal resistance as proved by Khalid and Abdel-lateif, 2017. On the other hand, the most potent isolates from contaminated soil that showed multiresistance to all heavy metals tested were identified as *A. chroococcum* as reported by Ali, et al., 2013. On the other hand The removal of heavy metals from polluted environments of their toxic potential can be realized by *Bacillus megaterium*, so it plays an important role in the biogeochemical cycle of heavy metals and processes involved in bioremediation was reported by Kumar and Achyuthan 2007. Ahemad and Malik (2012) characterized and identified five Zn (II) resistant *Bacillus* spp. from Indian agricultural soils irrigated with metal polluted wastewater.

Table 3: Effect of zinc ion concentration on different bacterial Species on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Zinc ion conc.					
10	77.07104	77.07104	79.93998	76.9607	77.39358
15	51.3928	51.3928	53.30588	51.31922	51.60788
20	38.19048	38.19048	39.61211	38.1358	38.35031
30	18.77744	18.77744	19.47642	18.75056	18.85602
40	9.55216	9.55216	9.907736	9.538484	9.592136

Table 4: Effect of zinc ion concentration on different bacterial Species without energy source on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Inoculum Size					
10	59.33536	67.01276	66.46104	69.77136	63.30775
15	39.56623	44.6857	44.3178	46.5252	42.21511
20	29.40204	33.20637	32.93298	34.57332	31.37045
30	14.45635	16.32686	16.19244	16.99896	15.42418
40	7.354006	8.30554	8.23716	8.64744	7.846342

Relative effects of different inoculum size on Zn(II) bioremediation

Different inoculum size of five bacterial bioremediation evaluated test organisms was studied on zinc bioremediation as tabulated in tables 5 and 6 with 10ml of 650 ppm concentration of Zn (II). The results indicated as increase in bacterial cell count decrease percentage of Zn (II) bioremediation. The highest bioremediation was by using inoculum size 0.1×10^{29} cfu of five different bacterial species as *Bacillus megaterium* EMCC 1013 was 81. From the above results *Bacillus megaterium* EMCC 1013 showed the most potent Zn (II) bioremediation organism as in table 5 while in case of using another energy sources of peptone and beef extract as carbon and nitrogen energy sources the adsorption of zinc iron reach to 67.34 by bacillus megaterium as in table 6.

Table 5: Effect of Inoculum Size on different bacterial Species on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Inoculum Size					
0.1	78.088	78.088	80.9948	77.9762	78.4148
0.5	29.2376	29.2376	30.32596	29.19574	29.35996
1	47.0344	47.0344	48.78524	46.96706	47.23124
3	24.1528	24.1528	25.05188	24.11822	24.25388
5	25.2424	25.2424	26.18204	25.20626	25.34804

Table 6: Effect of Inoculum Size on different bacterial Species without energy source on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Inoculum Size					
0.1	60.1183	67.897	67.338	70.692	64.1431
0.5	22.50941	25.4219	25.2126	26.4684	24.01637
1	36.21079	40.8961	40.5594	42.5796	38.63503
3	18.59473	21.0007	20.8278	21.8652	19.83961
5	19.43359	21.9481	21.7674	22.8516	20.73463

Relative effects of different Temperature on Zn(II) bioremediation

Effect of different incubation temperature for copper bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei*. *Bacillus megaterium* EMCC 1013 was the most potent Zn(II)bioremdation percentage with 88 % at 30° C followed by *Rhizobium leguminosarum* EMCC 1130 by 85 % Zn(II)bioremediation at 30° C tabulated in table 7 and 8. As mentioned by Rajeshkumar et al., 2011 Temperature can affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. The binding sites on the isolated bacterial species might be simultaneously affected by these factors and may cause reduction in metal removal. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature (Gulay and Yakup, 2003). Li et al., 2017 reported that the optimum temperature for Zinc removal by *Rhodobacter sphaeroides* was 40 °C which larger than form our work by using *Bacillus megaterium* EMCC 1013. These results was explained by the influence of incubation temperature on the microbial cells and in this case the bioremoval process appeared as endothermic process, where the bio-removal process increase with the increasing of temperature to limited values, or based on the influence of incubation temperature on the metal ions where the removal process decreased with the increasing of temperature due to the releasing of metal ions form the active site to the solution (Padmavathy et al. 2003; Selatnia et al. 2004).

Table 7: Effect of Temperature on different bacterial Species on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Temp.					
20	73.3664	73.3664	76.09744	73.26136	73.67344
25	84.444	84.444	87.5874	84.3231	84.7974
30	84.9888	84.9888	88.15248	84.86712	85.34448
35	67.192	67.192	69.6932	67.0958	67.4732
40	21.2472	21.2472	22.03812	21.21678	21.33612

Table 8: Effect of Temperature on different bacterial Species without energy source on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Temp.					
20	60.1183	67.897	67.338	70.692	64.1431
25	22.50941	25.4219	25.2126	26.4684	24.01637
30	36.21079	40.8961	40.5594	42.5796	38.63503
35	18.59473	21.0007	20.8278	21.8652	19.83961
40	19.43359	21.9481	21.7674	22.8516	20.73463

Relative effects of different Carbon sources on Zn(II) bioremediation

Effect of different carbon sources for Zn(II) bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC 1743, *Rhizobium leguminosarum* EMCC1130 *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* was evaluated. *Bacillus megaterium* EMCC 1013 was the most potent Zn(II) bioremediation percentage with 95 % followed by other tested bacterial species by 92 % Zn(II) bioremediation with glucose utilization as carbon source. While zinc ion adsorbtion by bacillus megaterium without glucose was 79.7. Utilization of starch and sucrose as carbon source showed sharply decrease in Zn(II) bioremediation with 2 % with all tested bacterial species as Tabulted in tables 9 and 10. Our results was agree with results As reported by El badry et al., 2016 *Azotobacter vinelandii* isolate grows well on modified PVK liquid medium containing different carbon sources. Whereas, high amounts of soluble phosphate is detected only in the culture filtrate of bacterium with glucose which reaches to 52.8% then dextrose with low pH value, while starch and sucrose exhibited low amount of soluble phosphate with high pH value. The bacterial growth exhibited remarkable variation according to the utilized carbon source, the best bacterial growth to produce enzyme and organic acids reached when glucose is utilized as a carbon source.

Table 9: Effect of Carbon source on different bacterial Species on zinc ion adsorption from wast water.

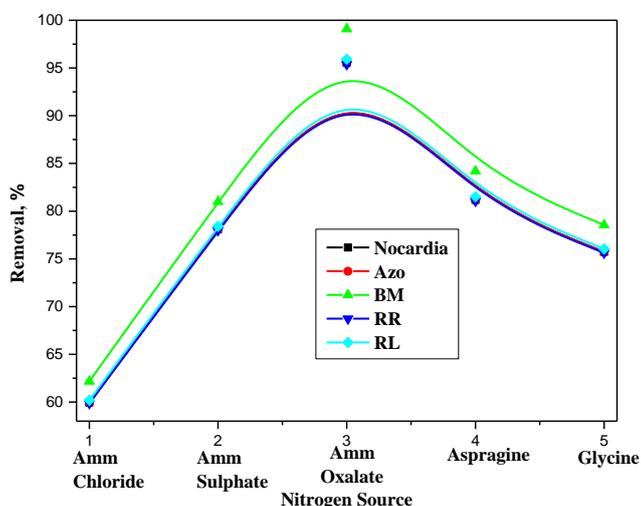
Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Temp.					
Glucose	92.4344	95.87524	92.30206	92.82124	71.16329
Starch	4.92136	5.104556	4.914314	4.941956	3.788851
Sucrose	2.27	2.3545	2.26675	2.2795	1.747625
Dextrose	89.892	93.2382	89.7633	90.2682	69.20595

Relative effects of different Nitrogen sources on Zn(II) bioremediation

Effect of different nitrogen sources for zinc bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei*. *Bacillus megaterium* EMCC 1013 was the most potent Zn(II) bioremediation percentage with 99 % followed by other tested bacterial species by 95 % Zn(II) bioremediation with ammonium oxalate utilization as nitrogen source. Utilization of ammonium chloride as nitrogen source showed decrease in Zn(II) bioremediation with 59 % with all tested bacterial species as presented in figure No 1 and in table 10. As a nitrogen source, ammonium oxalate was found to give maximum soluble Phosphate. Oxalate ions have the ability to form stable complexes with calcium, iron and aluminum to liberate phosphates (Khan et al., 2009). As reported by Elbarbary et al., 2016 *Nocardiopsis dassenvillei* solubilized high amount of phosphorus from rock phosphate ore ammonium oxalate was found to be the best nitrogen source utilized by *Nocardiopsis dassenvillei* isolate for maximum phosphate solubilization that reached to 53.5% followed by ammonium sulphate and lowest dissolution of phosphate content of the ore at using glycine as nitrogen source.

Table 10: Effect of nitrogen sources on different bacterial Species on zinc ion adsorption from wast water.

Bacteria	Nocardiopsis	Azotobacter vinelandii	Bacillus megaterium EMCC 1013	Rhizobium rhizogenes EMCC 1743	Rhizobium leguminosarum EMCC 1130
Temp.					
Ammonium Chloride	59.94616	59.94616	62.17764	59.86033	60.19704
Ammonium Sulphate	78.088	78.088	80.9948	77.9762	78.4148
Ammonium oxalate	95.5216	95.5216	99.07736	95.38484	95.92136
Aspragine	81.1752	81.1752	84.19692	81.05898	81.51492
Glycine	75.7272	75.7272	78.54612	75.61878	76.04412

**Figure No 1:** Relative effects of different nitrogen sources on Zn(II) bioremediation by different bacterial species

CONCLUSION

It was found that the optimum condition for adsorption of zinc ion at the pH 7 and the inoculum size is 0.1×10^{29} , the temperature 30°C , and the amount of zinc is 10ml from 650 ppm and using Glucose as carbon source and Ammonium oxalate as nitrogen source, it reaches to 99% removal of zinc from the waste water of electroplating baths.

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