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RESEARCH ARTICLE

BIOREMEDIATION OF ZINC BY ISOLATED BACTERIAL STRAINS

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ABSTRACT

Heavy metals and other trace elements are important for proper functioning of biological system, but also their deficiency or excess could lead to a number of disorders. Zinc is essential metal for human health and plants growth. But in case of excess amount it can cause toxicity, as they change their form and become hazardous in the nature. In this study, the microbes like bacteria, yeast and fungi were isolated from soil for remediation of metals present in Gomti River. Among these three microbes only single colony of bacteria was observed where as no fungi and yeast growth was reported due to the toxicity of heavy metals. Two set of experiments were planned to check the efficiency of isolated bacterial. In the first set of experiment, zinc present in water collected from three different sources was degraded upto 93.7% by isolated bacteria. In the second set different concentration of zinc solutions (5, 10, 20, 30, 50, 70 mg/L) were prepared. The isolated bacteria was degrading zinc 80.8% of 5mg/l solution and 96.5% of 30mg/l solution after 72 hr incubation, which was showing gradual increase as the zinc concentration was increasing from 5mg/l upto 30 mg/l. The Isolated bacteria were efficiently degrading the zinc metal.

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INTRODUCTION

Water is the lifeline of our environment. Rivers are one of the pure sources of water on earth. Gomti River is a significant tributary of river Ganga, which flows within the urban area of Lucknow. Lucknow is exponentially growing both side of the Gomti River which has led to severe pollution. The Gomti River is seriously affected by pollutants which enter through industrial effluents, domestic discharge and sewage system that pump untreated effluents into the river during its journey through the city. There is a high concentration of heavy metals because of industrial and domestic waste water, besides other pollutants. High concentration of all metals like Cr6+, Cu2+, Ni+, Cd+ and Zn2+ were noticed in River Gomti. Heavy metals are natural constituents of the earth's crust, but by the revolution of industry production of heavy metal has been increased. Industrial activities and their technological practices along with in field development posing major threats to the environment and living health system because of heavy metal contamination and their toxicity, bioaccumulation and non-degradability. A few metals like copper (Cu), Iron (Fe), Zinc (Zn), Cadmium (Cd), Lead (Pb) and Arsenic (As) are used in environment in trace amount but when they present in bio surface at excessive level then they have potential to become toxic to the plants as well as to the animal/human health. The presence of heavy metals in access in any of the environment has been a subject of great concern due to their toxicity, non-biodegradable nature and the long biological half-lives for their elimination from biological tissues (Kumar *et al*., 2013 & 2014). Pollutants deteriorate the quality of soil and crops produced. Excessive metal concentrations and pesticides in contaminated soils can result in decreased soil microbial activity and soil fertility, and yield losses (Kumar *et al*., 2013 & 2014). Zinc is an essential requirement for health of human but excess zinc is harmful, especially when it is in the form of free ion, it shows the reverse effect on human, plants and other aquatic life. As a result of weathering of these materials, soluble compounds of zinc are formed and may be released to water (National Academy of Sciences (NAS), 1980). 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). All through the previous two decades, wide consideration has been paid on management of environmental pollution and its control due to hazardous materials like heavy metals. Numerous methods have been proposed to remove heavy metals from sewage sludge, including chlorination, use of chelating agents and acid treatments at high temperatures (Olatunji *et al*., 2009; Faryal *et al*., 2007). A lot of physicochemical strategies, such as filtration, electrochemical treatment, oxidation/reduction, ion exchange, membrane technology, and reverse osmosis, have

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been developed for removing heavy metals from the polluted water (Tang *et al*., 2008). But nearly all of them showed to be expensive, less efficient, labour intensive operation, or lack of selectivity in the treating process (Xiao *et al*., 201). Some reports have shown that indigenous microbes and plant–microbe symbionts tolerate high heavy metal concentrations in different ways and may play a significant role in the restoration of contaminated soil (Ge *et al*., 2009). It is important to study the indigenous microorganisms in heavy metal-polluted sites. It may provide new insight into bacterial diversity under unfavourable conditions, new isolates, and probably new genetic information on heavy metal resistance, which could be exploited in decontamination process in the future (Fabienne *et al*., 2003). An alternative way to replace chemical methods in removing heavy metals is bioremediation through microbial isolates. The bioremediation techniques are effective and efficient for remediation of pollutants so as the bioremediation technology from laboratory to field to clean up the environment can be taken up (Hamby, 1996). Bioremediation, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Malik, 2004)

To make bioremediation effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products (Pandey and Fulekar, 2012). As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. These factors include the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (Mueller *et al*., 1996). Therefore, this study was under taken to isolate and characterize microbes from waters source, which are from metal (Zinc) contaminated river area (Zinc and lead) to study the zinc resistance patterns and the zinc bioremediation potential of the selected microorganism.

MATERIALS AND METHODS

Collection of Samples

The water sample was collected from Gomti River, Lucknow. After the literature survey three locations were decided for the sampling. All the three areas are at about 5 km to 10 km distances from each other. The three locations were: Daliganj, Hazratganj (behind Clark Avadh Hotel), Gomti Barrage (near Ambedkar Park, Gomtinagar). The samples were collected using a 10 L plastic container.

Tests for Physicochemical Properties

The physiochemical and chemical properties of the water were estimated by using BIS methods (IS: 3025(Part 15)-1984; APHA, 1985).

Preparation of Media and Plates for isolation of Microorganisms

The microorganisms were isolated from the water sample. LB agar was used for the isolation of bacteria were as SDA was isolated from fungi. To isolate zinc tolerance microorganism, a fixed concentration of zinc is added in the media. 1ml of zinc solution of 1mg/ml concentration was added to 100ml of media (LB and SDA).

Biochemical Test

Biochemical testing was done for the identification of isolated colonies (Dubey and Maheshwari, 2004).

Quantitative test for Zinc concentration

This estimation was done by spectrophotometric method at absorbance 460 nm (Kiran, 2012).

Bioremediation of zinc using isolated strain

Three sets of experiments were planned to study bioremediation of zinc using isolated strain. In set I, 100ml of LB broth was prepared in the water sample and then inoculated with isolated strain. In set II, blank was prepared as LB broth was prepared in distilled water and inoculated with isolated strain. In set III experiment, LB broth was prepared with three different salt concentrations 0.5g, 0.1g and 0.15g in 100ml of media. These media of three different concentrations were inoculated with the isolated strain. The incubation time of all these set of three experiments was 72 hours. The absorption was checked at 400nm. The absorption was measured for estimation of metal concentration at 24 hr, 48hr and 72 hours incubation intervals.

RESULTS AND DISCUSSION

Estimation of Physical and Chemical Parameters of water samples

The samples collected from different location of Gomti River were first subjected to estimate its physical and chemical parameters to check the quality of water samples. The River Gomti on different location loses its clarity and becomes hazydue to the sewage and industrial effluent discharge from the city. The TDS in the all the three samples are below the permissible limit i.e.500mg/L, although TSS is far above the permissible limit of 20 mg/L. Haziness and turbidity observed was due to the presence of the suspended solids in the water samples. As the water with TSS level 80-90 mg/L appears to be cloudy while the water with the TSS level 150 mg/L appears to be dirty. The source of the suspended solids is due to the discharge of sewage and household waste in the water of the river. The pH of the samples lies within the permissible range 6.5-8.5. The dissolved oxygen (DO) is in the range from 3-7 mg/L which is lower than that required by the fishes to survive, this decline in DO is due to the increase in the suspended solids. The BOD of the water samples are above the level of the normal limits. For moderately polluted rivers the BOD should be in the range of 2 to 8 mg/L. but value of BOD is significantly higher than the normal limits. The total hardness of the water is in the permissible limit of 200 mg/L, but it can be seen that the hardness at the Daliganj area is more than the other two areas. This is because Daliganj area is a "dhobighat", which means that the water is used for washing clothes. Therefore, the salts of calcium and magnesium are higher in concentration as compared to the other two places. It was found that the relative abundance of Streptococcus and E. coli was influenced by two independent variables (water quality parameters), namely, DO and TDS (Basu *et al*., 2013).

Table 1. Physical and Chemical Parameters of Gomti River in Lucknow

S.No.	Parameters	S1	S2	S3	Permitted limit (22)
1	Colour	Transparent	Hazy	Hazy	Transparent
2	Odour	Odourless	Odourless	Odourless	Agreeable
3	Turbidity	Present	Present	Present	Agreeable
4	Total Dissolved Solids, mg/l	240	140	280	500
5	Total Suspended Solids, mg/l	160	480	100	20
6	pH	7.2	7.1	7.1	6.5-8.5
7	DO, mg/l	6.12	1.68	3.6	3-7
8	BOD, mg/l	115.2	14.4	16.6	2 to 8
9	Chlorides, mg/l	4.963	4.963	4.963	250
10	Hardness, mg/l	185	146.66	168.33	200

Estimation of Zinc concentration in water samples

The zinc concentration was determined in the water samples at absorbance 460 nm. The concentration of zinc is highest at Daliganj area (S1) about 0.78mg/L, which is less than the permissible limit of 5mg/L. The concentration of zinc was lower in the samples of other two sites also (0.65 mg/L in Hazratganj (S2) and 0.36 mg/L in Gomti Barrage location (S3)). The zinc is coming in high concentration in Gomti stream from different source like domestic discharge and industrial effluent. It seems from the results of zinc concentration of different sites, that microbes present in Gomti River are remediating zinc due to which the zinc concentration is lower even though the zinc is coming in the river stream through various sources.

Table 2. Concentration of water sample from different location

S.No.	Water Sample	Zinc Concentration (mg/l)
1	S1	0.78
2	S2	0.65
3	S3	0.36

Isolation and identification of microbes from water samples

Bacteria and yeast both the microorganism were isolated from the Gomti river water samples. No growth was observed in the SDA plates for the fungi growth.

Table 3. Isolated microbes from water samples

S.No.	Microbes	Sample 1	Sample 2	Sample 3
1	Bacteria	+ve	+ve	+ve
2	Yeast	-ve	-ve	-ve

+ve = growth observed
-ve = no growth observed

The growing colonies were seen in the LB agar plates after 24-48 hours, where as in the SDA plates, no growth was seen after incubation of 6 to 7 days. This result indicates that the microbes present in the Gomti River are affected by zinc toxicity and due to this no fungal growth was found during isolation from water sample. The isolated bacterial strain was showing resistance against zinc toxicity and was showing potential for bioremediation of zinc. The biochemical testing was done for identification of bacteria isolated from the water samples. The four tests were performed to detect isolated bacteria. The tests were to determine the family Eneterobacteriaece family and differentiate it with *E.coli* both of which are likely to be present in the sample water. The first test that was performed indole production by tryptophan producing bacteria. If the pink colour appears in the tube this means that the bacteria is indole producing and is most likely to be *E.coli*, since it is indole producing. If there is no colour this means the bacteria is indole negative which is most likely to be *K. pneumonae* and *E. aerogens*. Further confirmation was done by Methyl Red (MR) and VogesProskaeur (VP) test. Both the tests were performed on the same broth, hence it is called MR-VP test. If the bacterium produces substantial quantity of organic acids as the end products, on addition of methyl red the medium will acidic pH of the medium. This is the positive test, if the medium remains yellow in colour, and then it is negative test. In the VP test, the pink colour shows the positive test and the appearance of faint brown or no colour then it is a negative test. E.coli gives positive MR test and negative VP test while K. pneumonae and E. aerogens shows a negative MR test and positive VP test.

The control gives the positive MR test and the negative test which means E.coli is present in the control. The final test was done for the citric acid producing bacteria. If the bacteria produce citric acid the media turns blue. This is the positive test for the citric acid. If the colour of the media remains green in colour then it a negative test for the citric acid bacteria. From the table, it can be seen that the control media, in which no salt was added gives positive test for the bacteria E.coli. Since the water from the river contains sewage water it is the bacteria which are most likely to be present.

Table 4. Biochemical testing of isolated microbes from water samples

S.No	Water sample	Biochemical Test			
		Indole test	MR	VP	Citric acid
1	Sample 1	+	+	-	-
2	Sample 2	-	-	-	-
3	Sample 3	-	-	-	-

Biodegradation of zinc

Bioremediation of zinc in water sample of Gomti River

The zinc degradation in sample was observed for the incubation period of 24, 48 and upto 72 hours, it was seen that the concentration of zinc (S1) after 24 hours was reduced to 0.29 mg/L from 0.78 mg/L and finally after 72 hours it was reduced to 0.05 mg/L. Therefore, the bacteria which were isolated from the water reduce zinc about 93.7%. Similarly the zinc bioremediation was observed 73.8% in water sample S2 and 91.7% in water sample S3. This result indicates that bacteria is reducing or metabolizing zinc for its growth and survival. These results also indicated that for high zinc concentration bioremediation rate is high.

Table 5. Bioremediation of zinc in water sample of Gomti River

S.No.	Water sample	Zinc concentration, mg/L			
		Initial	24 hr incubation	48 hr incubation	72 hr incubation
1	S1	0.78	0.29	0.09	0.005
2	S2	0.65	0.31	0.18	0.12
3	S3	0.36	0.15	0.06	0.03

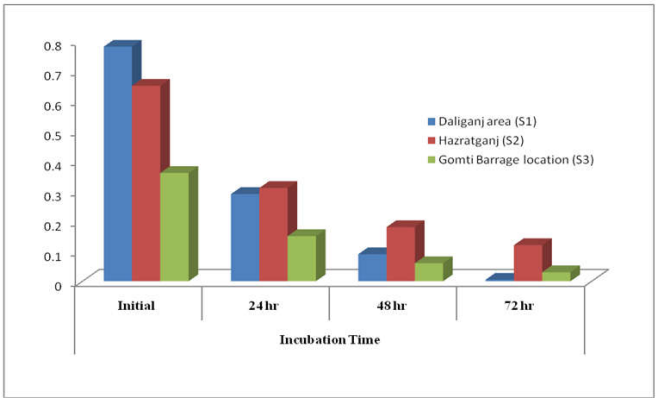

Fig.1. Comparative analysis of percentage Bioremediation of zinc in water sample of Gomti River

Bioremediation of zinc in designed sample of different concentration

To check the effect of isolated microbe (bacteria) on zinc bioremediation, three different concentrations of zinc solutions in media was designed. The concentration of zinc was reducing as the incubation time was increasing from 24 hr up to 72 hr in the case of all DS (designed sample) of known zinc concentration. The bioremediation of zinc was highest (96.5%) in solution having 30 mg/L concentration of zinc, which was showing the gradual increase till 30 mg/L from 5 mg/L after 72 hr incubation, zinc bioremediation was showing which was gradual increasing as the incubation times was increased from 24 h to 72 hr. This indicated that the zinc get remediate by microbes slowly as it is incubated for a longer time.

Table 6. Bioremediation of zinc in water sample of Gomti River

S.No	Sample code	Initial zinc concentration in designed sample mg/L	Zinc bioremediation (%)		
			after 24 hr incubation	after 48 hr incubation	after 72 hr incubation
1	DS1	5	68.4	72.5	80.8
2	DS2	10	70.6	75.2	84.3
3	DS3	20	80.8	85.2	93.7
4	DS4	30	88.3	90.8	96.5
5	DS5	50	65.5	69.5	70.9
6	DS6	70	56.5	60.5	63.9

DS= Designed sample of known zinc concentration

Since, when the media was contains 50mg/L concentration of a zinc salt, it restricts the growth of bacteria in the plate and gradually reduction in zinc bioremediation was observed at 70mg/L concentration. This indicates that the higher concentration of zinc causes toxicity towards the bacterial growth and reduces the bioremediation capability of bacteria. Since zinc is a toxic metal hence, only those bacteria which can metabolize zinc can survive on the plate to a certain concentration of zinc and then they slowdown their activity. Researchers Basha, S. A. and Rajaganesh K, in 2014, reported that *Escherichia coli, Salmonella typhi, Bacillus licheniformis* and *Pseudomonas fluorescence* have a potential to reduce maximum of 98.34% of cadmium, 94.83% of lead and 96.14% of zinc from the effluent samples (Basha and Rajaganesh, 2014). Karnwal and Bhardwaj, in 2014, worked on degradation of zinc and chromium, in his study zinc was showing more degradation as compare to chromium. The maximum amount of Zinc removed was 95% while minimum was 67.5% and the chromium degradation was recorded 55% for 10 ppm and 20 ppm and 47.5% for 40 ppm concentration. This shows that the percentage degradation was reducing as the metal toxicity was increasing (Karnwal and Bhardwaj, 2014). Awasthi G *et al*. in 2015 reported that *Pseudomonas aeruginosa* also effectively bioremediate metal like Cu, Cr, Zn and Fe and helps in reducing their toxicity. This indicates that microbial remediation is advantageous over conventional remediation methods (Awasthi *et al*., 2015; Anjali Chester *et al*., 2014). These various studies show that microbes can tolerate heavy metals (like zinc chromium etc) upto certain concentration, they also have resistance and catabolic potentials, which help in biodegradation of toxic metals. These techniques can be used for the removal of toxic compounds and to clean the environment, which will be beneficial for mankind.

Conclusion

The discharge of industrial effluents and household sewage in the river has increased the level of toxic substances in the river. The BOD is significantly higher than the normal limits in all the three samples. The total suspended solids are high were as chlorides are very low in concentration in almost all the three samples, which was indication of high concentration of microbes in water samples. But only bacteria was isolated which was showing the tolerance against the metal toxicity. The effluents have also increased the level of heavy metals in the river water. Since the Gomti River is contaminated with sewage water it is likely that the *E.coli* must be present in the water. *E. coli* was having the capacity to bioremediate metal zinc upto the concentration of 30mg/L and then while increasing the concentration above this bioremediation rate was constant and they slowly decreased. Isolated *E. coli* was showing zinc bioremediation in all the water samples collected from Gomti River.

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