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## Research Article

# ISOLATION OF IRON REDUCING MICROBES FROM CONTAMINATED ZONES OF GOMTI RIVER

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**ABSTRACT**

Water pollution s the imajor universal threats for environment pollution. The industrial effluent iand untreated sewage water is discharge n to the water ibodies, which creates a serious problem to the living systems. Gomti River is polluted with different hazardous contaminants including heavy metals like iron, cadmium, zinc etc. As iron is essential component for good health of living organisms, but the ingestion of large quantities of iron results in haemochromatosis. This problem has lead to study the biosorption of iron. The present study is carried out to isolate microbes from the metal contaminated zone of Gomti river. The isolation of microbes was done to study the resistance patterns of microbe against iron metal and to evaluate iron biosorption potential. Samples were collected from five locations namely Hussainabad, Pakkapul, Hanuman setu, Lakshman mela ground, Baikunth dham . The parameters like total suspended solids (TSS), total dissolved solid (TDS), pH, hardness, dissolved oxygen (DO), chlorine, total coliforms and heavy metals like iron, cadmium, copper and arsenic were determined. High concentration of Iron (Fe) contamination was observed in these locations. The isolation of bacteria, fungi and yeast was performed by using standard plate techniques on specific media having concentration of iron. Among these three microbes, only three bacterial were present . Amongst these three the isolated bacterial strain was used identified as E coli, and used for the biosorption of iron. The results of present study revealed that the minimum % biodegradation of concentration of iron after 5 days was 6.10 ( GWS2)and maximum was 14.9 (GWS1) ,after 10 days minimum %35.37 (GWS2) and maximum was 47.62 (GWS1) and after 15 days minimum % was 68.29 (GWS2) and maximum was 76.19 (GS1) The maximum reduction of iron was observed over a period of 15 days . These results indicate that the isolated E coli are potential strain for iron biosorption.



## INTRODUCTION

Today environmental pollution is a very serious issue, as because of hazardous waste. The world is facing problems with a wide variety of pollutants both inorganic and organic in nature. Good and health soil, uncontaminated water and pure air are the soul of life. This pollution has led to scarcity of clean water and soil quality, which limits crop production [1].The industries play very important role in the economic growth of the country. Gomti receives waste from sugar and distillery industries, milk industry etc According to previous studies it is evident that drains are the major supply of water contamination particularly for rivers flowing within the city hold industrial effluent, household waste, sewage and medical waste results in poor water quality [2]. Industrial effluents and untreated sewage are discharged in water bodies, which may contain different types of pollutants like carcinogenic compounds, drugs, dyes, pigments, preservatives, various chemical compounds and heavy metals Study of water quality of the river Gomti of Jaunpur City was carried out by [3].The impurities of water consist of sewage, mixture of both organic and inorganic contaminants together with oils, phenols .plastics plasticizers, greases, , suspended solids, , acids, greases, salts, dyes, metallic wastes cyanides, DDT and some heavy metals like copper,iron,chromium cadmium lead are also discharged from various industries [4]. High concentration of all metals like Cr, Cu, Ni, Pd, Fe and Zn were noticed in River Gomati from 2006-2008 i[5].These unrestricted effluents causes problem in water bodies are change in colour, bad aromas, change in pH value, high organic materials, depletion of oxygen content, which may leads to dreadful conditions of basal ganglia of brain, hepatic and renal system damages and mental retardation etc [6,7]. iAccording to study carried out by previous researchers on some of the important rivers, it has been observed that in recent years, the water of most of rivers

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has become contaminated. Many sources of heavy metals including tannery, sugar, beverages, paints, chemicals, fertilizers, batteries, automobiles, factories, food processing units, cement thermal power plants, petroleum refineries and sewage disposal water. Heavy metals reveals a huge amount of problems having high density but physical properties are quit meaningless [8].Heavy metals causes ecological pollution and are phytotoxic in nature [9]. The pollution of the surroundings with toxic metals has be converted into a global problem, affecting crop yields, soil biomass and fertility, contributing for the bioaccumulation and biomagnifications in the chain [10]. Drinking water containing traces of heavy metals and is dangerous for health. Fresh water fishes also get affected due to bioaccumulation of heavy metals [11].Various conventional physicochemical methods are been used for the removal of pollutants. These methods are highly expensive and create lot of problems for the environment. Bioremediation is proved as an effective environment friendly affordable technology for the removal of various pollutants. Bioremediation uses biological agents, mainly microorganisms i.e., bacteria, fungi and yeast to clean up environment as well as contaminated water bodies [12,13].In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources [14,15].The bioremediation uses natural microorganisms present in soil, water, and other parts of the environment [16]. The heavy metal are the major pollutants causing various problem associated with health and environmental issues. Metal content at high level can inhibit various metabolic activities of microbes. Microbes can also develop resistance or tolerance towards the higher concentration of metals. The bioremediation process reduces the toxicity, mobility and bioavailability of metals, like other pollutants. It is difficult to remove heavy metals from contaminated environment. Metals like Copper, Chromium, Nickel,Iron and Zinc are essential micronutrients for animals, plants, and microorganisms [17].

Biosorption is an innovative and cost effective technique for the removal of heavy metals from aqueous solution. The microorganisms are used as an effective biosorbents for heavy metal removal [18].Microorganisms have evolved coping strategies to either transform the element to a less harmful form or bind the metal intra- or extracellular, thereby preventing harmful interactions in the host cell. On the other hand they are able to actively transport the metal out of the cell cytosol [19,20].

Iron is one of the essential elements in human nutrition but it should be consumed within the permissible limits. It leaches into the water resources from rock, soil formations and other anthropogenic activities. The iron above 0.3 mg/L limit causes changes in taste and odor of drinking water; it also affects water staining, plumbing fixtures, dishware and clothes. According to United States Environmental Protection Agency (USEPA) has suggested iron limits of 0.3 mg/L for secondary drinking water [21,22].Iron in ground river water makes it toxic for humans, plants as well as natural microbes therefore it is necessary to reduce the amount of Iron that is the biggest issue of environment health. The ingestion of large quantities of iron results in haemochromatosis, which interfere normal regulatory mechanisms which leads to tissue damage [23].

The remediation of heavy metals leads to isolate various strains having potential for the biosorption of heavy metals. These isolated microbes can be effective for biosorption of various metals as well as for other pollutants also. According to a previous research isolated E. coli from river water, has proved its efficiency in bioremediation of zinc [24].Pseudomonas aeruginosa isolated from soil effectively bioremediated chromium, copper, iron and zinc. Since researches have reported that pseudomonas species is potential strain for the bioremediation of heavy metals even at higher concentration [25,26].The environment polluted with heavy metals can be suitable region for the isolation of potential microbes. The microbes growing in heavy metal polluted region are having more resistance towards the particular metals. The current study is focused on identification and isolation of microbes from river water having metal contamination, which can be a potentially source for biosorption of various heavy metals. I

## MATERIALS AND METHODS

### *Selection of site for Sample Collection*

The Gomti river is branch of River Ganga. Five different locations of Gomti River were selected for the sampling on the basis of literature survey. These locations namely Hussainabad, Pakkapul, Hanuman setu, Lakshmanmela ground, Baikunth dham were almost within range of 10 km distances.The 10 L plastic container was used for the collection of samples.

### *Detection of Physicochemical parameters of water samples*

Different physicochemical parameters namely Temperature, pH, Total Hardness, D.O., Nitrate, Nitrite, TSS, TDS, Chloride, Heavy metals namely Copper, Iron, Arsenic and Cadmium were analysed. The quality of collected water sample was checked for their physiochemical properties using BIS and APHA methods [27,28].Heavy metals namely Copper, Iron, Arsenic and Cadmium were analyzed using spectrophotometric method.

### *Isolation of Microorganisms*

The microorganisms were isolated from the water sample by serial dilution plate method. LB agar was used for the isolation of bacteria and SDA was used for isolation of fungi. The 1 ml of 10-3 and10-4 water sample dilutions were transferred to petridishes with the help of sterile pipettes containing SDA and LB media for isolation of fungi and bacteria respectively. To isolate iron tolerance microorganism, 1 ml iron solution of 1mg/ml concentration was added to the SDA and LB media. The incubation temperature was 28oC day for fungi and 37oC for the bacteria. The incubation time period was 5 to 7 days for fungi and 24 to 48 hrs for bacteria. The growth of microorganism was observed in iron contaminated media with the help of colony counter.

### *Biochemical Identification of Isolated Strain*

The identification of isolated colonies was done by imvic test ( Indole production, Methyl Red, Voges – Proskauer,citrate utilization) [29].

### *Quantitative Estimation of Iron Metal*

The quantitative estimation of iron was done by spectrophotometric estimation at absorption 510nm. [30,31]

### *Bioremediation of Iron Metal by Isolated Strain*

Two sets of experiments were planned to study bioremediation of iron using isolated strain. In set I, 100ml of LB broth was prepared in the Gomti river water sample and then inoculated with isolated strain. In set experiment, LB broth was prepared with five different salt concentrations 0.5mg, 1.0mg, 1.5mg, 2.0mg and 2.5mg in 1ml of media. These media of five different concentrations were inoculated with the isolated strain. The incubation time of all these set of experiments was 5days. The absorption was checked at 510nm. The sampling for estimation of metal concentration was done at the interval of 5days to 15 days incubation. The absorption was checked at 510nm.

## RESULTS AND DISCUSSION

The River Gomti on different location loses its clarity and becomes hazy due 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. The source of the Total 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 7.2-7.8. 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 pakkapul and hussainabad area are more than the three areas. (Table 1). The DO level in the water has significantly decreased and BOD was higher which was affecting flora and fauna. The suspended solids in the water were high, which was absorbs heat from the sun resulting in increase in temperature of water body. Gomati river water was hazy because of the suspended solids.

**Table 1** Physiochemical parameters of water samples collected from different sites

| Sr. No | Sampling Sites | pH | TDS (mg/l) | TSS (mg/l) | DO (mg/l) | Nitrate (mg/l) | Nitrite (mg/l) | Hardness (mg/l) | Chloride (mg/l) | BOD (mg/l) | Color | Turbidity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GWS1 | 7.2 | 340 | 520 | 3.5 | 57.05 | 0.1405 | 185 | 10.85 | 115.5 | Hazy | Present |
| 2 | GWS2 | 7.8 | 410 | 480 | 2.7 | 52.25 | 0.1139 | 190.5 | 52.17 | 89.5 | Hazy | Present |
| 3 | GWS3 | 7.6 | 480 | 430 | 3.2 | 68.54 | 0.1606 | 178.5 | 22.65 | 78.5 | Hazy | Present |
| 4 | GWS4 | 7.5 | 425 | 510 | 1.9 | 79.80 | 0.6705 | 165 | 16.62 | 102.4 | Hazy | Present |
| 5 | GWS5 | 7.4 | 405 | 475 | 4.2 | 55.20 | 0.4202 | 175 | 15.25 | 68.3 | Hazy | Present |

*GWS: Gomati Water Sample, GWS1: Hussainabad, GWS2: Pakkapul, GWS3: Hanuman Setu, GWS4: Lakshaman mela ground, GWS5: Baikunth Dham

Further, dust input in river water increases the heavy metal concentration. High quantity of crude sewage increase load of different metals. The value of copper was maximum at site Hussainabad i.e. 0.51 mg/l and minimum at site baikunthdham i.e.0.022 mg/l. (Table 2) The concentration of copper at most of sites was within the permissible limit i.e.is 0.05 mg/l. The maximum desirable limit for arsenic is 0.05 mg/l. The maximum value of arsenic was at pakkapul i.e. 0.075mg/l and minimum at Hanuman setu i.e. 0.037mg/l.(Table 2).Arsenic contamination in water in river is major factor of human health risk. The problem can be solved or relieved by supply of clean water Iron in natural water is controlled by both physicochemical and microbiological factors. Iron level was maximum at pakkapul i.e. 0.82 mg/l and minimum 0.21mg/l at Hussainabad (Table 2).The permissible limit of Iron is 1.0 mg/l. The maximum value of cadmium was at Hussainabad i.e.0.027 mg/l and minimum at site Baikunth Dham i.e.0.015 mg/l .(Table 2) The maximum desirable limit for Cadmium is 0.02 mg/l.

**Table 2** Showing Heavy Metal estimation of River Gomti in Lucknow

| Sr. No | Sampling Sites | As (mg/l) | Cu (mg/l) | Fe (mg/l) | Cd (mg/l) |
|---|---|---|---|---|---|
| 1 | GWS1 | 0.072 | 0.051 | 0.21 | 0.025 |
| 2 | GWS2 | 0.075 | 0.033 | 0.82 | 0.027 |
| 3 | GWS3 | 0.037 | 0.027 | 0.70 | 0.022 |
| 4 | GWS4 | 0.050 | 0.028 | 0.78 | 0.020 |
| 5 | GWS5 | 0.040 | 0.022 | 0.54 | 0.015 |

All the water samples collected were positive for bacteria and negative for fungi (Table 3). As per further biochemical identification Three bacterial were identified with biochemical characterization namely Escherichia coli, Bacillus sp. and Pseudomonas sp.(Table 4).E coli strains were isolated from all the samples collected from different locations.( Table 5) It was found that the relative abundance of E. coli was influenced by two independent variables (water quality parameters), namely, DO and TDS . Among these three, only single colony of bacterial strain was observed. showing resistance towards iron metal, whereas fungi and yeast growth was not observed on the media plates due to the toxicity of heavy metals.

**Table 3** Isolation of microbes from different sampling sites

| Sr.No | Microbes | Hussainabad | pakkapul | Hanuman setu | Lakshmanjhoola | Bakunthdham |
|---|---|---|---|---|---|---|
| 1 | Fungi | _ | _ | _ | _ | _ |
| 2 | Bacteria | + | + | + | + | + |

**Table 4** Biochemical identification of bacteria isolated from different sampling sites of gomati river

| Sr.No. | Isolates | Indole production | Methyl Red | Voges – Proskauer | citrate utilization |
|---|---|---|---|---|---|
| 1 | Bacillus sp. | _ | _ | _ | + |
| 2 | E.Coli | + | + | _ | + |
| 3 | Pseudomonas sp. | _ | _ | _ | + |

**Table 5** Isolation of bacterial isolates in samples collected from different sites

| Sr.No. | Sampling sites | Pseudomonas sp. | Bacillus sp. | E. coli. |
|---|---|---|---|---|
| 1 | GWS1 | + | _ | + |
| 2 | GWS2 | _ | _ | + |

| | | | | |
|---|---|---|---|---|
| 3 | GWS3 | + | + | + |
| 4 | GWS4 | + | _ | + |
| 5 | GWS5 | _ | + | + |

The biodegradation study was carried out on iron metal with the isolated E.Coli strain The maximum biodegradation was observed in Gomti water sample of Hussainabad (GSW1), as the iron concentration was very low at this point of source. The gradual decrease in iron concentration was observed in all the samples starting from 5 days upto 15 days. The maximum degradation was observed after 15 days incubation almost in all the Gomti water samples (Table 6). The maximum % biodegradation was 76.19% in GWS 1 sample after 15 days of incubation. The maximum % biodegradation of iron sample was observed in water sample having low concentration of iron and as the concentration was increasing, the % biodegradation was decreasing. In all the Gomti water samples, the highest % biodegradation was observed after 15 days incubation. The rate of biodegradation was almost constant throughout the time period. The concentration of iron in the water sample of pakkapul was high but % biodegradation (68.29% in GWS2) was low as compared to other samples. (Fig 1) This indicates that as the concentration of iron was increasing the % biodegradation was decreasing.

**Table 6: Kinetic study of biosorption of Iron in different water samples**

| S.No | Water Samples | Initial Iron Concentration (mg/L) | Concentration of Iron after interval of Time | | |
|---|---|---|---|---|---|
| | | | 5 Days | 10 Days | 15 Days |
| 1 | GWS 1 | 0.21 | 0.18 | 0.11 | 0.05 |
| 2 | GWS 2 | 0.82 | 0.77 | 0.53 | 0.26 |
| 3 | GWS 3 | 0.7 | 0.62 | 0.43 | 0.21 |
| 4 | GWS4 | 0.78 | 0.72 | 0.5 | 0.24 |
| 5 | GWS5 | 0.54 | 0.47 | 0.31 | 0.13 |

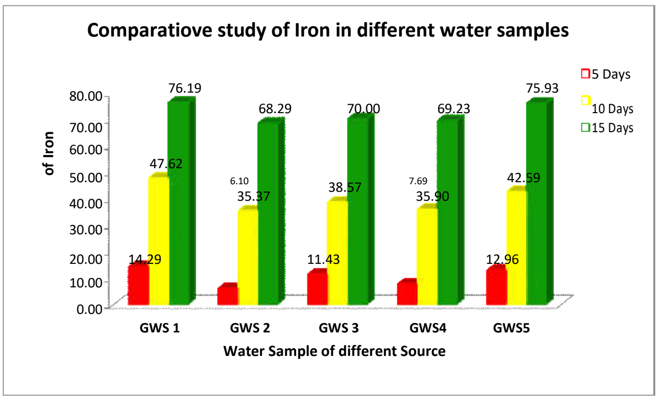


**Figure 1** Comparative analysis of % biodegradation in different concentration of iron metal

To study the effect of % biodegradation on higher concentration of iron, the different concentration of iron samples were prepared ranging from 1.0 mg/l to 5.0 mg/l. The biodegradation of iron was also observed in sample having higher concentration upto 5.0mg/l. The biodegradation was 65.72% in sample of iron having concentration 1.0mg/l and 55.52% in sample having iron concentration 5.0mg/l. (Table 7).

**Table 7** Kinetic study of bioremediation of Iron in different concentration

| S. No. | Incubation Time (Days) | % Biodegradation of iron sample of different concentration | | | | |
|---|---|---|---|---|---|---|
| | | FeS1 | FeS2 | FeS3 | FeS4 | FeS5 |
| 1 | 5 | 6.2 | 5.91 | 5.87 | 5.23 | 4.94 |
| 2 | 10 | 32.81 | 30.47 | 29.89 | 28.46 | 26.33 |
| 3 | 15 | 65.72 | 62.56 | 59.21 | 56.85 | 55.52 |

The *E. coli* was showing promising biodegradation capabilities in remediating iron metal even at high concentration though it was less as compared to sample having low concentration of iron. This indicates that bacteria is absorbing or metabolizing iron for its growth and survival. This isolated bacterial strain was used for the bioremediation of iron, which was showing gradual decrease in the concentration of metal. The percentage bioremediation was increasing as the metal concentration was increasing and reached very high till $15^{th}$ day incubation. The iron resistant bacteria were isolated from the water of the river itself. These bacteria were used to bio remediate the iron and well as were potential source for remediation of other metals too.

Water pollution due to heavy metals through different sources remains a serious ecological and common trouble in developing countries. There must be some important ecological rules and regulations should be passed on the release of wastes from industries without any treatment that make it essential to build up a variety of technologies for the elimination of pollutants. Hence various technologies were implementing for the treatment of effluent before released into the environment. In the present study the water collected from different location of gomati nagar lucknow The result indicated that different parameters were analysed . Heavy metals were also higher than the permissible limit. It is also evident from the study of [32]
.Heavy metals such as aresenic , Cadmium and iron can bio collect and all the way through the food chain to lethal levels in humans [33] This study reveals that the E.Coli is a best isolated strain for the degradation of heavy metal iron in water collected from different locations of gomti river.

## CONCLUSION

The water from the River Gomti is supplied to the whole of city of Lucknow. Therefore, the quality of the water of River Gomti is significant. Though the Physio-chemical techniques of metal remediation are in practice but these are expensive and not having good impact on environment. The results indicate that the isolated bacteria is a potential biological tool for bioremediation of iron. The isolated bacteria *E.coli* from Gomti river water can effective for the bioremediation of many other metals also. Bioremediation is most hopeful economically and eco-friendly process to treat heavy metal contamination. The area having high concentrations of iron can poses danger to the human life. The present study concluded that the best isolated strain from water collected from different location of river Gomati to degrade the heavy metal of is E.Coli when compared to other organism. Bioremediation is most positive cost-effectively and eco-friendly method to get rid of heavy metal contamination.

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