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PHYSIOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF GANGA WATER AT KANPUR WITH SPECIAL REFERENCE TO BIOREMEDIATION

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Abstract: The polluted water from tannery effluent exposed area of Ganges River from Kanpur at various site were collected for physicochemical and microbiological analysis. Physicochemical analysis of Ganga water from Bithoor's sites, pH was recorded as highest (8.60). D.O (8.8 mg/L) was quite optimal however chloride was high (131.24 mg/L). The B.O.D was recorded as (10.0 mg/L) whereas conductivity (0.426 mg/L) was the lowest and it has same value of salinity (0.1 ppt) like Jajmau. Sarsaiya Ghat site revealed B.O.D (13.92 mg/L), chloride values (62.66 mg/L), highest value of alkalinity (103.7 mg/L) and possess same conductivity (0.45 ppt) as in Shuklaganj. The value of soluble phosphate of Shuklaganj site was recorded as 1.465 mg/L whereas the value of total alkalinity was the lowest among all the four sites (15.25 mg/L). The pH value of Jajmausite is the lowest (7.72) whereas B.O.D and T.D.S of the sample was the highest 15.2 mg/L and 221.2 mg/L respectively. T.S was 232.0 mg/L. The chloride was recorded as (185.44 mg/L), which was the highest among all the sites. It has same value of salinity like Bithoore site. A total of thirty bacteria isolated from Ganga River, out of these five were selected for present study on the basis of their distinct morphotypic characteristics. These bacteria also tested for their potential to solubilize phosphate and metal tolerance for Chromium. All four bacterial isolates were able to tolerate 75 ppm concentration of chromium except B. Bacterial strain B was able to tolerate up to 100 ppm. Three strains had phosphate solubilizing potential. Among all the strains S exhibit high phosphate solubilizing potential and reduced the level of BOD from initial 2.52 mg/L to 2.42 mg/L content in the sewage. The present study evaluates that the identified bacteria were used to bio-remediate contaminated waste water and sewage.
Keywords: Bioremediation; Heavy Metal tolerance; Phosphate solubilization; Sewage degradation.

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INTRODUCTION

Water is most abundantly available on Earth and required by all kinds of life. It is well established that water is important for life. Water is useful for several purposes including agricultural, industrial, household, recreational and environmental activities. The quality of water is of vital concern for mankind since it is directly linked with human welfare. India is the country which has rich and wide history of social and economic prosperity and of environmental richness. Ganga River (Goddesses in Indian Culture) has been facing the curse of pollution for a long time because of unplanned development, urbanization, industrialization, population density and agricultural activities (Waseem *et al.,* 2013). In Kanpur Ganga is always considered to be a holy river but on the other side it is also true that somehow it is now a day's getting very polluted by the people of its own city. The tannery industry has converted the Ganga

River into a dumping ground. It discharges different type of waste into the environment, primarily in the form of liquid effluents containing organic matters, chromium, sulphide, ammonium and other salts. As per an estimate, about 80-90% of the tanneries use chromium as a tanning agent. Of this, the hides take up only 50-70%, while the rest is discharged as effluent. The major components of the tannery effluents are the toxic trace metals. Several analysis reveal high concentrations of chromium even in supposedly treated effluents (Beg and Ali, 2008). In February 1985, the Ministry of Environment and Forest, Government of India launched the Ganga Action Plan, an environmental project to improve the river water quality. It was the largest single attempt to clean up a polluted river anywhere in the world and has not achieved any success in terms of preventing pollution load and improvement in water quality of the river. Failure of the Ganga Action Plan may be directly linked with the environmental planning without proper understanding of the human-environment interactions. According to the Central Pollution Control Board of the 12,690 kilometers length of the river Ganga including its tributaries: (Gangotri to Ganga Sagar is 2,500 killometers), 42% is moderately or severely polluted (BOD greater than 3 mg/L) and hence unfit for bathing and drinking based. This is almost 40 % of India's polluted riverine length. Bottom line is that if we address pollution in the Ganga, then we have solved 40 % of the problem. Ganga River is life line of Kanpur and its water is utilized for residential and horticulture purposes along these lines, successful support of water quality is needed through proper estimations. Physico-compound and smaller scale organic attributes may depict the nature of water, in this way, an investigation on physicochemical and microbiological-parameters of Ganga Water have been studied by various researchers. Pollution due to heavy metal ions and pesticides is dangerous as they tend to bioaccumulate and therefore toxic to the plant, human and animal health. Pesticides are the chemicals used in agriculture, in order to protect the crops from the attacks of pests,

diseases and rodents. They are toxic and cause environmental contamination as well as generate public health problems (Sabale *et al.,* 2012). The analysis of pesticide residue in water is difficult since these compounds occur at very low concentration (Brondi *et al.,* 2005; Agrawal *et al.,* 2010). Organochlorine and organophosphorus pesticides are the most important pollutants among the toxicants in India. Bioremediation provides an efficient and economical way to reduce environmental toxins, using indigenous or introduced microbes that naturally degrade contaminants water was made by numerous researchers (Mehrotra, 1990; Sinha *et al.,* 2000). Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process. Algae and protozoa reports are scanty regarding their involvement in biodegradation (Das and Chandran, 2011). Biodegradation processes vary greatly, but frequently the final product of the degradation is carbon dioxide (Pramila *et al.,* 2012). Recent advancements have also proven successful *via* the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants (Chowdhury *et al.,* 2012). Use of microorganism is considered to be economical, energy efficient and environmental friendly with minimal disposal problems. Effective microbes can completely degrade and oxidizes toxic organic compounds; are characterized by low cost and offer the possibility of *in-situ* treatment. The present study is therefore, focused on the water quality in samples collected tannery industry exposed area of Ganga River at Kanpur in order to find current status of pollutants discharged from the mushrooming tannery industries of the city and other sources. Conventional Water purification technology is often complicated and requires sophisticated equipment. It is also expensive to run and maintain. The Effective bioremediation technology could prove a simple answer to the problem. Bioremediation technology could be used to remove toxic chemicals, bacteria, viruses and other hazardous materials from water much more effectively and at lower cost than other conventional water purification

methods. Considering these points present study was planned to develop a biological treatment system to treat sewage water.

EXPERIMENTAL

Sample collection: Water sample were collected from tannery effluent exposed area of Ganges River in Kanpur at various site such as Bithoore, Sarsaiya Ghat, Shuklaganj, Jajmau. The sampling started after a few minutes of arrival at the sampling station, to minimize the disturbance in water for the analysis of physicochemical characteristics, samples are collected from the fixed stations of the site which were being investigated. Samples were immediately transported aseptically and stored at 4°C for further analysis. Water temperature, pH, and electrical conductance (EC) were recorded at the time of sample collection by using a thermometer, pocket digital pH meter and conductivity meter. The TDS, chlorides, DO BOD and phosphate were analyzed in the laboratory using standard methods.

Physicochemical analysis of polluted Ganga water: The physico-chemical characteristics of water were analyzed to determine the quality of Ganga water. The physical variables such as temperature, pH and EC were analyzed at the site and the other parameters such as dissolved oxygen (DO), total hardness (TH), calcium, chloride and phosphate were analyzed in the laboratory (APHA, 2005). Dissolved oxygen was determined by titration against sodium thiosulphate, total hardness (TH) was analyzed by titration using a standard EDTA solution; with eriochrome black T as an indicator. Calcium content was evaluated by titration with EDTA using murexide as an indicator. Chloride (Cl^-) content was measured using potassium chromate as an indicator relatively to silver nitrate. Phosphate ($^3-PO_4$) was determined using the turbidimetric method, comparing the results with the standard graph using stannous chloride as reagents.

Isolation of bacterial colonies from Ganga Water: For isolation of bacteria, water samples were first collected from various polluting sites of Ganga River at Kanpur. Isolation was conducted mainly by serial dilution agar plating

method (Agrawal *et al.,* 2013). A ten-fold serial dilution could be used and take appropriate dilution (10^{-5}) of this sample was spread plated in triplicate on nutrient agar plate. Cultures were incubated at 37°C±2 for 2 days. For experimental use, different colony was isolated and streak in nutrient agar plate.

Phenetic characterization of bacterial isolates: Bacterial isolates were thus randomly selected morphologically from Ganga water samples. Recovered bacterial isolates were phenotypically (morphotypic and physiological) characterized.Colony morphology of isolates was studied under a stereoscope microscope (Leica). This included shape, edge, elevation, surface and pigmentation. Cellular morphology was based upon cell shape and Gram staining (Leica fluorescent microscope). Cellular morphology and biochemical characteristics were determined based upon Bergey's manual of systematic bacteriology (Clauss and Berkeley, 1986, Agrawal *et al.,* 2013).

Determination of heavy metal tolerance: The tolerance of the selected bacterial isolates against heavy metals was also tested in nutrient agar media with the salts of heavy metals $K_2Cr_2O_7$ (for chromium) is considered for the study.The above mentioned metal supplemented with media was prepared with concentration of 100,75,50 and 25 ppm. Cultures were incubated at 37°C for 24 hours and cell growth observed. The result of growth was rewarded visually as positive or negative in terms of growth or no growth.

Qualitative estimation of Phosphate solubilization: Solubilization of tri-calcium phosphate was detected in Pikovskaya's Agar (Pikovskaya, 1948; Johri et al., 2003; Agrawal and Johri, 2014). All bacterial isolates were spotted on the surface of Pikovskaya agar medium (g/L) (glucose 10g, $Ca_3(PO_4)_2$ 5g, $(NH_4)2SO_4$ 0.5g, KCl 0.2g, $MgSO_4$ 0.1g, $MnSO_4$ traces, $FeSO_4$ traces, yeast extract 0.5g, agar agar 20g, pH 7.0) and phosphate solubilizing activity was estimated after 5 days of incubation at room temperature. A clear zone surrounding a growing colony indicated phosphate solubilization and was measured as phosphate solubilization index (SI). Phosphate solubilization index (SI) was determined by

formula (Edi-Premono *et al.,* 1996) given below.

$$SI = \frac{Colony\ diameter + Halo\ zone\ diameter}{Colony\ diameter}$$

Quantification of Phosphate solubilizing capacity of isolated bacterial strains: The bacterial isolates positive for Tri-calcium phosphate solubilisation on agar medium were further analyzed for their ability to solubilize phosphate in liquid medium was carried out as per standard methodology (Mehta *et al.,* 2001), by inoculating 1 ml of bacterial suspension (3×10^7 cells/mL) in 100 ml of National Botanical Research Institute Phosphate broth (NBRIP) in Erlenmeyer flasks (250ml) that contained the following ingredients (g/L): glucose 10.0g, $Ca_3(PO_4)_2$ 10.0g, $MgCl_2.6H_2O$ 5.0g, $MgSO_4.7H_2O$ 0.25g, KCl 0.2g, $(NH_4)2SO_4$ 0.1g and incubating the flasks for 5 days at 30°C. At the end of the incubation period, the cell suspension was centrifuged at 10,000 rpm for 10 minutes, and the phosphate content in the supernatant was spectrophotometrically determined by the ascorbic acid method (Murphy and Reily, 1962; Mehata and Nautiyal, 2001). Uninoculated broth was used as control. All the studies were repeated on three times to confirm the results. Sterile water inoculated media was treated as blank.

Sewage degradation by using bacterial inoculants: Among all the bacterial strains after various analysis strain S (Sarsaiya Ghat) was appeared as superlative strain so that it was utilized for one week experiment to check its sewage degrading potential. For this a set was planned to study the sewage degradation property of this particular strain. Firstly fresh sewage was analyzed for physicochemical parameters and then autoclave for inoculation of S bacterial strain. Inoculation was done in duplicate for 100% concentrated sewage. Non inoculated autoclaved sewage was studied as control. All the physicochemical parameters were investigated in all the sets after 7 days of inoculation. For this, the physicochemical analysis was done for fresh as well as and inoculated sewage sample. The sewage was inoculated for 7 days with S bacterial strain.

RESULTS AND DISCUSSION

Physiochemical analysis of Ganga Water

The pollution of the environment with toxic heavy metals is escalating throughout the world along with industrial revolution. Microorganisms and microbial products can be highly efficient bioaccumulators of soluble phosphate and particulate forms of metals. Microbe related technologies may provide alternative bioremedial techniques to the conventional method of metal removal or metal recovery. The present study deals with physicochemical analysis of Ganga water, isolation, identification and characterization of isolated bacteria from the Ganga water collected from various sites in Kanpur region and application of selected bacterial strain in sewage degradation. Some physiochemical parameters of collected polluted Ganga water like pH, temperature, conductivity, salinity, chloride, dissolved oxygen; biological oxygen demand and soluble phosphate were analyzed by various laboratory instruments under optimum condition just after the sampling. The physicochemical analysis of the water sample of various sites is given in Table 1. During physicochemical analysis of Ganga water from Bithoor's sites, pH was recorded 8.60 which was the highest pH value in comparison to rest of the sites. Dissolved oxygen (8.8 mg/L) was quite optimal however the value of chloride was high (131.24 mg/L).The B.O.D was recorded as 10.0 mg/L whereas the conductivity of this sample (0.426 mg/L) was the lowest among all the samples and it has the same value of salinity (0.1 ppt) like Jajmau (Table 1). Physicochemical analysis of this Sarsaiya Ghatsite revealed that it had the biological oxygen demand (13.92 mg/L) and chloride values (62.66 mg/L).This site had the highest value of alkalinity (103.7 mg/L) and this site possess the same conductivity (0.45 ppt) as in Shuklaganj (Table 1). The value of soluble phosphate of this Shuklaganj site was recorded as 1.465 mg/L. Shuklaganj had same value of conductivity like Sarsaiyaghat (0.45 ms/cm).Whereas the value of total alkalinity was the lowest among all the four sites (15.25 mg/L) (Table 1). The pH value of Jajmau site is the lowest one (7.72). On the other hand, the biological oxygen demand

(B.O.D) of the sample was the highest one (15.2 mg/L).It had the highest value of the total dissolved solid (221.2 mg/L) and total solid(232.0 mg/L).The chloride was recorded as 185.44 mg/L, which was the highest among all the sites. It has same value of salinity like Bithoore site (0.1 ppt) (Table 1).

Analysis of upstream and downstream water and sediment revealed a 10-fold increase in chromium level in the sediment at Ganaga river of Kanpur showing unchecked release of untreated tannery effluent (Khwaja *et.al.,* 2001). The tanning industry discharges different types of waste into the environment, primarily in the form of liquid effluents containing organic matters, chromium, sulphide ammonium and other salts. Initially physicochemical analysis was done for all Ganga water samples from all four sites of Kanpur exposed to tannery industry. Bithoor site possess the highest pH value which is an important factor because most of the chemical and biochemical reactions are influenced by the pH, so it is of great practical importance. Generally the sewage pH was slightly alkaline. Jajmau site possess the highest B.O.D value, which were very important parameter of water quality and represent an index of physical and biological process going in water. BOD has been used as a measure of the amount of organic materials in an aquatic solution which support the growth of microorganisms (Khanna *et al.,* 2012). BOD determines the strength or polluting power of sewage, effluents and other polluted waters and provides data on the pollution load in natural waters. A higher value of BOD indicates a higher consumption of oxygen and a higher pollution load. These sites also possess the highest value of soluble phosphate and chloride. Phosphorus is usually present in orthophosphate, polyphosphate and organic phosphate forms. Phosphorus is very important polluting constituents of sewage because of their role in algal growth and eutrophication of water bodies. Phosphate determination is highly useful in measuring the water quality since it is an important plant nutrient whereas chloride is one of the major inorganic anions in water and waste water. The alkalinity was found to be highest in

Sarsaiyaghat, as alkalinity measures the acid-neutralizing capacity of a water sample. It is an aggregate property of the water sample and can be interpreted in terms of specific substances only when a complete chemical composition of the sample is performed. The alkalinity of surface waters is primarily due to the carbonate and hydroxide content and is often interpreted in terms of the concentrations of these constituents. The higher the alkalinity, the greater the capacity of the water to neutralize acids; conversely, the lower the alkalinity, the less the neutralizing capacity. Alkalinity constitutes an important parameter in determining the quality of water.

Phenetic Characterization of bacterial isolates: A total 30 bacterial isolates have been recovered from different polluted sampling sites *viz.* Bithoor, Sarsaiyaghat, Shuklaganj and Jajmau of Ganga River. In which a total 5 different bacterial morphotypes were used for further study. The strains that are isolated mentioned as B Strain (Bithoor), S Strain (Sarsaiyaghat), U Strain (Shuklaganj), and J Strain (Jajmau) (Table 2).

Biochemical Characterization of Isolated Bacterial Strain

After physicochemical analysis of all four samples, a total of thirty bacterial strains were isolated in form from the Ganga water. In which only five isolates were used for further study on the basis of their distinct morphotypic characteristics. Various biochemical tests were performed to study the biochemical characterization of all the bacterial strains. Biochemical characterization of all the bacterial strains has been reflected in (Table 3). During the test it is showed that the S strain gives positive result only for Nitrate Reduction test, H2S Production test and for Sorbitol utilization. It gives negative result for the citrate utilization, lysine utilization, glucose utilization, phenylalanine deamination and for some other test (Table 4). All bacterial isolates were biochemically characterized. On biochemical characterization it was clear that the S strain (Sarsaiya ghat) gives the best result in comparison to the rest of the bacterial strain. S strain gives positive result to the methyl red test, vogusproskauer and indole acetic acid.

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When indole acetic acid test was performed positive result was shown because it was able to split tryptophan into indole and pyruvic acid using the hydrolase called tryptophanase. This strain also showed positive result to the catalse test as it produce high amount of catalase. Catalases catalyse conversion of H_2O_2, a powerful and potentially harmful oxidizing agent to water and molecular oxygen. Catalase also uses H_2O_2 to oxidise toxins including Phenols, Formic Acid, Formaldehyde and Alcohols. There are over 300 forms of catalase, found in most organisms (Chelikani *et al.,* 2004). S strain gives positive result to the amylase test because Amylase is an exoenzyme that hydrolyzes (cleaves) starch, a polysachharide into a disachharide and some monosachharide such as glucose. During my study it was found that S strain was gram negative and rod shaped bacteria therefore further the biochemical test kit was performed only for that strain. During the test it is showed that the S strain gives positive result only for Nitrate Reduction test, H_2S Production test and for Sorbitol utilization. Nitrate represents the end product of oxidation of nitrogenous matters and its concentration may depend on the nitrification and denitrification activities of micro-organisms. S strain gives negative result for the citrate utilization, lysine utilization, glucose utilization, phenylalanine deamination and for some other test. Simmons Citrate Agar detects the ability of certain organisms to utilize citrate as its sole source of carbon in a medium containing inorganic ammonium salts as its only source of nitrogen.

Table 1: Physiochemical characteristics of polluted Ganga water sample

Physico-chemical properties	Bithoor (B)	Sarsaiyaghat(S)	Shuklaganj (U)	Jajmau (J)
pH	8.60	8.23	8.28	7.72
Temperature ($^\circ$C)	23.6	22.9	23.4	24.0
D.O. (mg/L)	8.8	5.72	6.8	2.98
B.O.D (mg/L)	10.0	13.92	8.74	15.2
Soluble Phosphate (mg/L)	1.837	2.167	1.465	3.57
Chloride estimation (mg/L)	131.24	62.66	117.8	185.44
Conductivity (ms/cm)	0.426	0.45	0.45	0.48
Salinity (ppt)	0.1	0.23	0.17	0.1
Total Alkalinity (mg/L)	67.1	103.7	15.25	61.25
Total Solid (TS) (mg/L)	50.4	128.8	64.0	232.0
Total dissolved solid (T.D.S) (mg/L)	35.6	111.5	45.6	221.2

Table 2: Morphological characterization of isolated bacterial strain

Name of site	Bacterial strain	Gram's stain	Colour of Colony	Margin	Elevation	Surface	Form
Bithoor	B	+ve	Off White	Repand	Flat	Dry	Irregular
Sarsaiyaghat	S	-ve	Transparent	Entire	Convex	smooth	Round
Shuklaganj	U$_1$	-ve	Creamy White	Entire	Raised	Mucoid	Round
Shuklaganj	U$_2$	-ve	Creamy White	Entire	Umbonate	Mucoid	Round
Jajmau	J		Creamy Translucent	Undulate	Flat	Dry	Irregular

Table 3: Biochemical characterization of isolated bacterial strain

Strain	Methyl Red	Voges Proskauer	Indole Acetic acid	Catalase Production	Amylase test
B	+ ve	-ve	-ve	-ve	+ ve
S	+ ve	+ ve	+ + ve	+ +ve	+ + +ve
U$_1$	+ ve	-ve	+ve	-ve	+ + ve
U$_2$	+ ve	-ve	+ve	-ve	+ + ve
J	+ ve	-ve	-ve	-ve	- ve

Table 4: Biochemical Test (Hi Assorted Biochemical Test Kit for Gram Negative Rods) for S Strain

S.No.	Test	Reagents to be added	Principle	Original colour of the medium	Positive Result	Negative Result
1	Citrate utilization	_	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
2	Lysine utilization	_	Detects lysine decarboxylation	Olive green to light purple	Purple/ Dark purple	Yellow
3	Ornithine utilization	_	Detects ornithine decarboxylation	Olive green to light purple	Purple/ Dark purple	Yellow
4	Urease	_	Detects urease activity	Orangish yellow	Pink	Orangish yellow
5	Phenylalanine deamination	2-3 drops of TDA reagent	Detects Phenylalanine deamination activity	Colourless	Green	Colourless
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N,N-dimethyl-1-Napthylene	Detects Nitrate reduction	Colourless	Pinkish red	Colourless
7	H$_2$S production	_	Detects H$_2$S production	Orangish yellow	Black	Orangish yellow
8	Glucose	_	Glucose utilization	Pinkish red/red	Yellow	Red/Pink
9	Adonitol	_	Adonitol utilization	Pinkish red/red	Yellow	Red/Pink
10	Lactose	_	Lactose utilization	Pinkish red/red	Yellow	Red/Pink
11	Arabinose	_	Arabinose utilization	Pinkish red/red	Yellow	Red/Pink
12	Sorbitol	_	Sorbitol utilization	Pinkish red/red	Yellow	Red/Pink

Table 5: Minimum inhibitory concentration of Cr on different bacterial strains

Dose (µg/mL)	B Strain	S Strain	U Strain	U$_2$Strain	J Strain
25	+ +	+ + +	+ +	+ +	++
50	+ +	+ + +	+ +	+ +	+
75	+ + +	+ + +	+ +	+ +	+
100	+	-	-	-	-

Table 6: Qualitative estimation of phosphate solubilization efficiency of PSB isolates

Bacterial Isolates	Growth behavior for phosphate Solubilization	Colony diameter (mm)	Phosphate solubilization zone (mm)	Solubilization Index (SI)
S	+ + +	7	15	3.14
U$_1$	+ +	8	12	2.50
U$_2$	+	8	11	2.37
B	-	-	-	-
J	-	-	-	-

Table 7: Treatment with selected bacterial strain (S strain) for sewage degradation

Physicochemical properties	Before inoculation	After inoculation and one week treatment
pH	7.92	8.63
Temperature (^{0}C)	31.7	34.9
D.O. (mg/L)	4.8	3.96

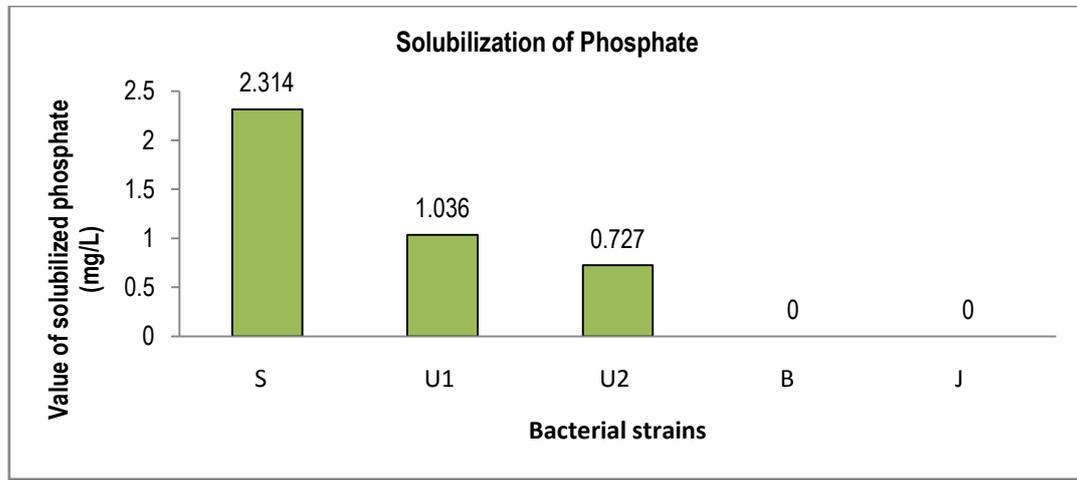|		
B.O.D (mg/L)	2.52	2.24
Soluble Phosphate (mg/L)	2.60	4.25
Chloride estimation (mg/L)	158.33	39.76
Conductivity (ms/cm)	1.472	1.734
Salinity(ppt)	0.4	0.6
Total Alkalinity (mg/L)	158.6	18.3
Total Solid (TS)(mg/L)	17.2	51.2
T.D.S (mg/L)	16.0	46.4

Figure 1: Quantification of phosphate solubilizing bacteria

Minimum Inhibitory Concentration (MIC) test for all selected bacterial strain

The tolerance capacity of the all morphtypically distinct bacterial strains against heavy metals like Chromium was scrutinize by analyzing minimum inhibitory concentration on Nutrient Agar Media. In case of Cr, 75 ppm was minimum inhibitory concentration for strain-S, U_1, U_2 and for J strain. B strain was able to tolerate up to 100ppm of Cr concentration (Table 5). The ability of microbial strains to grow in the presence of heavy metals would be helpful in the waste water treatment where microorganisms are directly involved in the decomposition of organic matter in biological process for waste water treatment, because often the inhibitory of heavy metal is a common phenomenon that occurs in the biological treatment of waste water and sewage (Filali *et al., 2000*).

Screening of phosphatesolubilizing bacteria

Out of five only three isolates showed significant Phosphate solubilizating by using plate assay. Present data showed that S, U_1 and U_2 bacterial strains were able to solubilize significant amount of phosphate. B strain and J strain did not solubilised phosphate while S strain solubilized maximum PO_4 (Table 6).

Isolates S showed maximum solubilisation index 3.14 followed byU_1 (2.50).and isolates U_2showd least solubilisation index 2.37.

Phosphate-solubilizing capacity of isolated bacterial strains

Phosphate solubilization ability of all three bacterial isolates was further evaluated in NBRIP liquid broth medium. Reasonably, all isolates showed consistent results in solubilizing phosphate from calcium phosphate in both liquid broth and agar assays. Similar consistent results of phosphate solubilization by PSB in both agar and broth assay were observed earlier (Nautiyal, 1999).The isolate S showed maximum solubilization index, which differed non-significantly from U1 and U2 (Table 6). These isolates were confirmed for their P solubilization ability by phospho-molybdate test. Phospho-molybdate test for quantitative determination of available phosphorous indicated that the isolates S solubilized significantly higher phosphate than all other bacterial strains. The isolates S showed maximum phosphate solubilisation 2.314 mg/l followed by U1 (1.036 mg/L) and U2 showed least P-solubilization which was0.727 mg/L. Microorganisms are effective in releasing phosphorus from inorganic and organic pools

of total phosphorus through solubilization and mineralization. The selection of Phosphate solubilizating bacteria was carried out and quantification was done by NBRIP medium. S bacterial strain solubilize significant amount of phosphate. Generally strains from bacterial genera *Pseudomonas, Bacillus, Rhizobium* and *enterobacter* are found to be most powerful phosphate solubilizers along with *Penicillium* and *Aspergillus* (Whitelaw, 2000). Phosphate solubilizing bacteria are found in sewage, including both aerobic and anaerobic strains such as *Bacillus megaterium, B.subtilis, B.polymyxa, B.sircalmous* (Subbarao, 1988; Kucey*et al*, 1989) and new strains are constantly being discovered. *Cupriavidus basilensis* was proven to have the ability to decompose organic phosphate (Qian*et al.,* 2010). *A nematofungus, Arthrobotrys oligospora* also has the ability to solubilize the phosphate rocks (Duponnois *et al.,* 2006). Bacteria are generally more effective in phosphorus solubilization than fungi (Alam*et al.,* 2002). In this study various bacteria are able to solubilize phosphate and were proven an important role in solubilization. All were isolated from polluted Ganga water. Generally some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphate solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.,* 1998). Phosphate solubilization process takes place through various microbial processes including organic production and proton extrusion (Dutton and Evans,1996; Nahas,1996). It is generally accepted that the mechanism of mineral phosphate solubilization by phosphate solubilization bacterial strains is associated with the release of low molecular weight organic acids (Goldstein,1995; Kim *et al,* 1997) which through their carboxylic groups chelate the cations (mainly Ca) bound to phosphate converting them into the soluble forms (Kpomblekou and Tabatabai, 1994).

Microorganisms possess phosphate mineralization potential under the action of phosphatases (Cosgrove,1967; Tarafdar *et al.,* 1988) .Phosphate solubilizing microorganisms produce a variety of organic acids (Rashid *et al.,*2004) from simple carbohydrates by virtue of which they solubilize insoluble inorganic phosphates (Banik and Dey 1982, Vazquez et al.,2000). Besides releasing organic acids some microorganisms produce auxins (Leinhos and Vacek, 1994; Gutierrez-Manero *et al.,* 1996; Yasmin *et al.,* 2004), gibberellins (Gutierrez-Manero *et al.,* 2001) and cytokinins (Timmusk *et al.,* 2005). Thus phosphate solubilizing bacteria not only improve the quality of sewage water but also possessing various plant growth promotion abilities, suitable for development of a better constructed wetland.

Treatment of phosphate solubilizing bacterial isolates for sewage degradation
Among all the bacterial strains after various biochemical test strain S (Sarsaiya ghat) was appeared as superlative strain so that it was utilized for one week experiment to check its sewage degrading potential. For this, the physicochemical analysis was done for fresh as well as and inoculated sewage sample. The sewage was inoculated for 7 days with S strain. On doing the physicochemical analysis it was found that the soluble phosphate, dissolved oxygen and conductivity get increased in one week old inoculated sewage sample. On the other hand pH, alkalinity, chloride and biological oxygen demand get decreased due to the degradation of sewage by the isolated bacterial strains. One week inoculation of selected bacterial strain was also done to study degradation of sewage. Among all the bacterial strains after various analyses, strain S (Sarsaiyaghat) was appeared as best strain (most potent) so that it was utilized for one week experiment to check its sewage degrading potential. All the physicochemical analysis was done in all sets after 7 days of inoculation. During the analysis it is revealed that the phosphate gets solubilized. Phosphate plays a role of limiting factor among all other plant nutrient so its determination is useful. The pH also get increased which is of great

practical importance as it influences the chemical and biochemical reactions (Singh, 2010). According to these results, the present study evaluates that the identified bacteria were used to remediate contaminated waste water and sewage. The bacteria could be used in bioremediation process as it seems to be a good alternative to conventional clean up technologies as it has a great potential for dealing with certain types of contaminants.

CONCLUSION

The Ganga water samples were analyzed for physicochemical and microbiological properties. Recovered bacterial isolates were tested for their potential to solubilize phosphate and their level of heavy metal tolerance for Chromium. Since these bacterial strains grow in the sewage already fortified with many heavy metals like Fe, Pb and Cr etc.Therefore the MIC of these bacterial strains showed tolerance towards the metals. Three strains had phosphate solubilizing potential. Among all the strains S exhibit high phosphate solubilizing potential and reduced the level of B.O.D content in the sewage.It could be concluded from the present study that different kind of bacteria are present in the sewage, some of which possess phosphate solubilizing potential. In some newer approaches, however, the sewage is inoculated with a specific microorganism, which has been specially selected for that particular sewage treatment process. Such organisms might be called 'starter cultures'. The use of starter cultures increases the efficiency of sewage degradation so that the process of phosphate solubilisation can be done easily and faster. In this way we can increase the potential of bioremediation. Use of S strain phosphate solubilizing bacteria as bio-inoculants will increase the available phosphorus, reduces heavy metal pollution and promotes sustainable agriculture.The present investigation suggested that bacterial isolates can be useful to remediate polluted Ganga water. The treated waste water can be used in agriculture and irrigation process. Effective microorganisms convert a degraded ecosystem to productive.

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