

Metagenomic Analysis Reveals Bacterial and Fungal Diversity and Their Bioremediation Potential from Sediments of River Ganga near Patna

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Abstract – In this research, we report the presence of bioremediation capacity in the microbial community in terms of relative abundance and taxonomic biodiversity at nine separate sites in sediment samples from the Ganga and Yamuna Rivers, India. Using the TruSeq Nano DNA Library Prep Kit, met genomic libraries were constructed and sequenced by Illumina Next Generation Sequencing (NGS) technology on Next Seq 500. Bioremediation bacteria belong to 45 genera with 92 species and Kaiju taxonomic classification has been used to classify fungi belonging to 13 genera with 24 species. The study showed that, followed by Actinobacteria, Firmicutes, and Deinococcus-Thermus, Proteobacteria are the most dominant bacterial flora. PCA analysis showed the bacteria viz. bioremediation. In highly polluted stretches of rivers, Streptomycin bikiniensis, Rhodococcus qingshengii, Bacillus aerophilus, Pseudomonas veronii, etc., were more dominant compared to less polluted stretches of rivers. Likewise, the relative abundance of fungi for bioremediation, viz. The contaminated Kanpur stretch of the Ganga River was significantly associated with Phanerochaete chrysosporium and Rhizopus oryzae, etc.

Using protein domain review, several protein domains that play a crucial role in bioremediation in contaminated environments, including the urea ABC transporter, UrtA, UrtD, UrtE, zinc/cadmium/mercury/lead-transporting AT Pase, etc., have been identified. The protein domains involved in the biodegradation of pesticides, viz. In river sediment met genomics info, P450, short-chain dehydrogenises/reeducates (SDR), etc. were also discovered. This is the first study on the richness of microbial bioremediation organisms in the river ecosystems of the Ganga and Patna, highlighting their significance in the management of aquatic pollution.

Keywords: Ganga; Bioremediation; Met Genomics; River Sediment.

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INTRODUCTION

Amphibian ecological contamination is a worldwide danger to characteristic biodiversity and human wellbeing. With the consistently expanding human populace, the utilization of pesticides and other agro-input has been expanded to accomplish worldwide food security. Apart from customary use in the farming area, pesticides are utilized in family purposes to control vector-borne sicknesses (VBD) like jungle fever, particularly in agricultural nations including India. In any case, the aimless utilization of pesticides in agribusiness and family at last outcomes in a lot of their deposits in a climate which ultimately cleans out to common streams like waterways, wetlands, lastly to the ocean, causing a colossal interruption in the normal sea-going biodiversity. It has been accounted for that pesticide buildup found in wetlands, waterways, and ocean in considerable sums can make the multitudinous sick

impact sea-going life forms like endocrine disturbance, development decrease, and so on. Employments of organ chlorine (OC) pesticides are restricted in numerous nations for a long, however they are as yet found in the regular streams, silt, sea-going vegetation, and fauna because of their high persistency.

A few oceanic biological systems including waterways, wetlands, and ocean were as a rule consistently observed routinely for surveying the degree of OC and their danger evaluation on amphibian life. Due to their high lipophilicity properties, they are by and large effectively adsorbed on the stream residue and by the sea-going fauna. High groupings of OC have been accounted for in the fish, *Drapane africana* (2237–6368 µg/kg) when contrasted with *Mochokus niloticus* (1006–3288 µg/kg) in the Niger River of Nigeria. Quality organizations engaged with the

multiplication and insusceptible capacity of largemouth bass, are accounted for to disturb in OC debased destinations of the Lake Apopka Other than OC, the oftentimes utilized pesticides found in the regular stream have a place with the gathering organophosphate, carbonates', pyrethroids, and so on Chlorpyrifos and other organophosphate pesticides are accounted for to disable invulnerable capacity and underlying uprightness of the fish *Cyprinus carpio* L. through oxidative pressure and apoptosis Lambda-cigalothrine, a parathyroid bug spray has been accounted for to cause the lightening of free amino corrosive substance in the muscle, liver, and mind of fishes in the Alazani River In light of the poisonous impact of pesticide buildups on the local widely varied vegetation, a few physical and synthetic strategies are being utilized to kill these harmful xenobiotics from indigenous habitats, similar to landfills, reusing, loss of motion, and so on, However, bioremediation utilizing various microorganisms was discovered to be the most doable procedure, end up being worked in many polluted destinations.

It was accounted for that the presence of organophosphate hydrolyses (OPH) catalyst in these microorganisms has the limit of detoxifying them through cleavage of phosphate ester bonds (P-O, P-F, P-CN, and P-S). The yeast (*Saccharomyces cerevisiae*) equipped for hydrolyzing the inadequately hydrolyzed P-S class of organophosphate by coordinating quality, encoding the wild-type OPH (upgraded variation chemical S308L-OPH) into the ribosomal operon of a similar Similarly, bacterial strain, *Bacillus pumilis*, was accounted for to be fit for bioremediation of methyl parathion, a P-S type OP pesticide, because of the presence of *opdA* quality Several microorganisms having a place with the genera *Bacillus*, *Brevibacillus*, *Ochrobactrum*, *Pseudomonas*, *Serratia*, and *Sphingobium* are accounted for to be fit for debasing different parathyroid pesticides through metabolic action Several qualities with parathyroid corrupting capacity were accounted for viz. *estP* in *Klebsiella* sp. ZD112 *pye3* from the met genome of soil (*pytH* in *Sphingobium* sp. JZ-1 *pytZ* and *pytY* (from a genomic library of *Ochrobactrum anthropi* YZ-1) Not just microorganisms, however a few parasites were likewise detailed with the capacity of pesticide remediation through the catabolic or co-metabolic cycle.

Parasites having a place with the genera *Aspergillums*, *Candida*, *Cladosporium*, *Trico derma*, and so on, were accounted for to debase distinctive parathyroid pesticides like cyfluthrin, bifenthrin, deltamethrin, and so on The common oceanic environment holds and perishes a decent measure of microbial populace, which can corrupt these harmful xenobiotic deposits in situ. Subsequently, to distinguish these likely microorganisms and their practical job, met genomic considers have been directed broadly lately. Met genomic considers

uncovered a silt miniature biome in the Deepwater Horizon oil slick and the effect of oil statement on microbial networks in surface residue Met genome-amassed genomes (MAGs) uncovered the microbial networks of two warm pools in Kamchatka, Russia Similarly, the key bacterial species were described in the *Daphnia magna* macrobiotic utilizing shotgun met genomics. Anti-infection Resistance Genes (AMRs) from the dregs of River Yamuna have been distinguished from the met genomic study Using the met genomics approach, potential microbial local area associated with the biodegradation of phenanthrene, diesel, and hexadecane in the mangrove silt were recognized and the corrupting microscopic organisms had a place with genera *Bacillus* sp., *Pseudomonas* sp., *Acinetobacter* sp., and *Staphylococcus* sp. The met genomic approach was likewise used to show the part of IS1071, an addition component flanks with xenobiotic corruption, in the development and conveyance of bacterial catabolic pathway quality bunch The biodegradation pathway of DDT, HCH, and atrazine in freshwater and marine dregs was dissected through a met genomics approach. The examination recognized 69 genera fit for debasing these tireless pesticides with significant populaces having a place with class *Plesiocystis* sp., *Anaerolinea* sp., *Jannaschia* sp., and *Mycobacterium* sp., and found the presence of various qualities viz. *atzB*, *hdg*, and *hdt* which encode for ethylaminohydrolase, dehalogenase, and hydrates, separately Carbonate pesticide corrupting catalyst was likewise detailed by utilitarian met genomic investigation of rumen tests of Holstein dairy cows Similarly, the met genomics approach was used to comprehend the fundamental component of biodegradation in situ and to foresee the debasement capability of microorganisms in the dirt of Queensland, Australia However, there were very few examinations on the event of the local microbial populace in the Ganga and Yamuna waterway environment having bioremediation potential. With this foundation, the current examination highlights the recognizable proof and relative wealth of potential bioremediation microorganisms equipped for corrupting contaminations in these waterway silt through the met genomics approach. Moreover, the examination additionally expects to research the variety and relative plenitude of these bioremediation organisms in the diverse dirtied and non-contaminated locales of stream Ganga and Patna.

MATERIALS AND METHODS

Study area

The present study was confined to the surrounding areas of Site I (including *Pahalwan Ghat and Bans Ghat*) and Site II (including *Krishna Ghat and Gandhi Ghat*) of Patna where huge masses of devotees offer holy dip, local people do perform

bathing, washing the clothes and discharge of local wastes throughout the year.

Collection of Samples

Water tests were gathered once in pre-rainstorm and post-storm seasons during, 2010 from two chose destinations of the River Ganga. The examples were gathered from a profundity of 1 foot beneath the outside of stream utilizing the plastic holders (500ml). Tests were gathered from two spots of each examining site, haphazardly and blended completely. Water tests were put away in the refrigerator to hinder the biochemical exercises. Assortment of tests occurred between the long periods of 9 am to 11.30 am. The examples were investigated according to standard strategies referenced in (APHA, 1995). The guidelines reagents utilized in examination were readied utilizing twofold refined water. Nine water quality boundaries specifically pH, EC, Alkalinity, Total Solids, Hardness, Chloride, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Most Probable Number (MPN) were checked at the ISM, Dhanbad. In Fig. 1 testing locales are appeared on the guide and Fig. No 2 shows the basic Bathing Ghat of River Ganga in Patna.

Methodology

The aim of this study is to describe the trend and variations of the selected water quality parameters of the river. The study also aims to ascertain the levels of the quality parameters and in the absence of any detectable impact from any source, may serve as baseline values (Singh *et al.*, 2007). The water quality test methods are shown in Table 1.



Fig. 1. Location of sampling sites in River Ganga (Red marked).



Fig. 2. A Simple View of Bathing Ghat (Ganga) in Patna, Bihar.

Table 1. Water Quality Test Methods

S. No.	Parameters	Units	Test Method
1	pH	-	Electrometric
2	Electrical Conductivity	µS/cm	Electrometric
3	Total Solids	mg/l	Electrometric
4	Alkalinity	mg/l	Titration and Electrometric
5	Hardness	mg/l	Titration EDTA
6	Chloride	mg/l	Titration
7	Dissolved Oxygen	mg/l	Winkler method with Azide Modification
8	Biological Demand	Oxygenmg/l	5 days incubation
9	**Most Probable Number/100 ml	Probablemg/l	Lactose broth method

**In the most likely number (MPN) test, containers of lactose stock are immunized with water tests estimating 10ml, 1 ml, and 0.1ml. During hatching, coliform living beings produce gas. Contingent on which tubes from which water tests disp lay gas, a MPN table is counseled and a measurable scope of the quantity of coliform microscopic organisms is resolved. The MPN test is anything but difficult to perform and decipher, however it doesn't decide the specific number of microorganisms as the standard plate tally does.

Table 2. Site Wise Observed Values of Different Water Quality Parameters with their W.H.O Standards.

Parameters	Site I		Site II		WHO Standards (ISIRI)
	Pre-monsoon season	Post-monsoon season	Pre-monsoon season	Post-monsoon season	
pH	Range 8.1-8.4	Range 7.5-8.2	Range 8.4-8.7	Range 6.8-7.3	7.0-8.5
Electrical conductivity (µmho/cm)	431-453	405-427	505-532	487-504	-
Alkalinity (mg/l)	177-185	166-171	197-203	184-189	120.0
Total solids (mg/l)	817-943	615-672	947-976	588-621	500.0
Hardness (mg/l)	168-172	161-165	188-198	131-153	100.0
Chloride (mg/l)	158-164	184-194	181-211	208-218	250.0
Dissolved oxygen (mg/l)	7.2-7.5	7.7-8.2	6.8-7.2	7.2-7.7	5.0
BOD (mg/l)	6.1-6.6	4.7-4.9	5.8-6.3	5.2-5.7	6.0
MPN/100 ml	2150-2343	3850-1951	1700-1767	1445-1634	<4

RESULTS

The reach estimations of the River Ganga water quality boundaries of the current examination are introduced in Table 2 and talked about based on just pre-rainstorm and post-storm seasons in a word. The scent was unobjectionable and taste pleasing at the chose destinations.

DISCUSSIONS

pH

The pH is quite possibly the main components which impact amphibian existence of any water body. The pH is by and large influenced by natural and inorganic solutes present in water. In the current investigation, pH goes from

8.1 to 8.4 in pre-storm and 7.3 to 8.2 in post-rainstorm seasons at site I. At site II, pH goes from 8.4 to 8.7 in pre-rainstorm, and 6.8 to 7.3 in post storm seasons. It has been seen that pH esteems are higher in summer than different seasons. Greatest estimations of pH during summer might be because of expanded photosynthesis of the algal sprouts bringing about the precipitation of carbonates of calcium and magnesium from bicarbonates (Prakash et al., 2007).

Electrical conductivity (EC)

The electrical conductivity is an estimation of ability of water to send electric ebb and flow in water bodies. It speaks to the all-out convergence of dissolvable salts/mineral salts in water (Trivedy and Goyal, 1986), subsequently making it acrid and unsatisfactory for drinking. In the current examination, the EC changes from 431-453 μ mho/cm in pre-rainstorm and 416-427 μ mho/cm in post-storm seasons at site I. At site II, 505-532 μ mho/cm in pre-storm and 497-514 μ mho/cm in post-rainstorm seasons as appeared in Table 2. The limited variety of EC can be because of the current litho logy of the locale of the River Ganga.

Alkalinity

It is the quantitative limit of water test to kill a solid corrosive to a pH Increase weakening of waterway water might be answerable for lower estimations of alkalinity in blustery seasons (Bhargava, 1982). In the current examination, the alkalinity at site I ran between 177-185mg/l in pre-rainstorm season and 166 - 171mg/l in post storm seasons. The qualities at site II ran between 197-203mg/l in pre-storm and 184-193 mg/l in post rainstorm seasons. Alkalinity at the two destinations was over as far as possible endorsed for drinking water which is 120mg/l (WHO, 1984). The high estimation of alkalinity shows the presence of frail and solid base, for example, carbonates, bicarbonates and hydroxides in the water body (Abassi et al., 1999; Jain et al., 1997). The high estimations of alkalinity may likewise be because of expansion in free carbon dioxide in the River Ganga which eventually brings about the increment in alkalinity at site I and site II.

Total Solids (TS)

TS have great implications for the control of biological and physical wastewater treatment

processes. The largest amount of total solids adds to the highest turbidity and EC value in the river water. TS at site I varied from 817-943mg/l (pre-monsoon period) and 615-672mg/l (post-monsoon period). At site II ranged between 947-976mg/l (pre-monsoon) and 598-621mg/l (post-monsoon period). The measurement of TS can be useful as an indicator of the effects of runoff from urban and agricultural areas. The increased TS was attributed to soil erosion in the nearby catchment and massive contribution of suspended solids from domestic effluents or local sewage.

Hardness

By and large, hard water has no known impact on human wellbeing except for is inadmissible for homegrown employments. Hardness chiefly relies on the broke up salts present in water. Hard water will be water that contains elevated levels of broke up calcium, magnesium and other mineral salt, for example, iron. High measure of broke down minerals in the water causes more the water hard. Hard water is alright for drinking, cooking and other family unit utilizes, however it can cause issues. Hardness of water is because of the grouping of multivalent metallic particles of calcium and magnesium. In the current investigation, it is discovered that hardness goes from 168mg/l to 172mg/l (pre-rainstorm period) and 161-165mg/l (post-storm period) at site I. At site II, goes from 189 mg/l to 198mg/l (pre-storm period) and 131-153mg/l (post-rainstorm period). The pattern of variety is non-uniform at all the destinations. The hard water causes wellbeing impact on stomach related framework and shaping of calcium oxalate in urinary bladder. The water hardness on chose study destinations of River Ganga was higher during late spring months which may have caused expanded centralization of salts by extreme dissipation. In Table 3, Classification of Hard Water is appeared.

Table 3. Classification of Hard Water

S. No.	Concentration as CaCO ₃	Indication
1	0 to 60 mg/l	Soft water
2	60 -120 mg/l	Moderately hard water
3	120 -180 mg/l	Hard water
4	>180 mg/l	Very hard water

Rainstorm seasons) at site I. The worth reaches from 181-211mg/l (pre-storm seasons), and 209-219mg/l (post-rainstorm season) at site II. Chloride content was lower than the acknowledged furthest reaches of 250mg/l at two chose locales in River Ganga. Chlorides in River Ganga waters can be credited to release of neighborhood effluents or homegrown sewage removal which may bring about moderate expansion in degrees of chlorides. The current perception discovers uphold with crafted by Singh et al., 2007.

Dissolved Oxygen

DO levels in surface water body demonstrate the capacity to help oceanic life. The high DO esteems implies the pace of oxygen recharging in water is more prominent than the oxygen usage. Sufficient DO is essential for acceptable water quality. DO levels between 5.0 and 8.0mg/l are agreeable for endurance and development of amphibian living beings. In the current examination, the DO substance were gone from 7.1-7.5mg/l (pre-storm seasons) and 7.7-8.2mg/l (post-rainstorm seasons) at site I. The worth reaches from 6.8-7.2mg/l (pre-rainstorm seasons) and 7.2-7.7mg/l (post-storm season) at site II. The low worth was found in pre-storm period because of diminished dissolvability of oxygen in mid-year months and the other way around.

Biological Oxygen Demand (BOD)

Body is the measure of oxygen needed by the living beings (microorganisms) in the usage or adjustment of natural issue. All in all, the BOD is the measure of oxygen needed by the microorganisms during their development in wastewater. It is significant pointer of the natural contamination status of a water body. The Unpolluted water has BOD estimation of 3mg/l or less and modern wastewater has BOD esteem 25000mg/l. The estimation of BOD in the current examination was most elevated nearby I when contrasted with site II (found in Table 2). The estimation of BOD was more in pre-rainstorm seasons and demonstrates that the waterway can be somewhat dirtied at various Ghats. The higher qualities Chloride (Cl-1) is one of the significant anions found in water and are for the most part joined with calcium, magnesium or sodium. Chlorides are drained from different rocks into soil and groundwater by enduring. The chloride particle is profoundly versatile and is shipped to shut bowls. The primary wellspring of chloride in surface water and groundwater is because of barometrical precipitation, creature takes care of, septic tanks, utilization of inorganic manures and landfill leachate. In this investigation, the chloride substance were gone from 158-164mg/l (pre-storm seasons), and 184-194mg/l (post-different sources. Practically comparable outcomes were seen by (Bhargava, 1982; Rao, 1992; Shukla, 1989) for the Ganga stream water.

Most Probable Number (MPN)

For the most part, complete coliform microscopic organisms allude to the whole coliform gathering. These microorganisms are bountiful in soil, rotting vegetation, creature fecal issue and crude surface water. They are not ordinarily present in profound groundwater and appropriately treated surface water. Notwithstanding fecal coliform microbes alludes to a subgroup of coliform microorganisms a significant

number of which are found in the digestive organs of warm-blooded creatures, including people.

E. coli is the essential fecal coliform of interest. The presence of these microorganisms in a water supply ought to be taken very genuinely as they likely could be joined by illness microscopic organisms that are sent in creature defecation.

The estimation of MPN in the current investigation was most noteworthy (2150-2343mg/l, pre-storm season; 1850-1951mg/l post-rainstorm) on location I when contrasted with site II which esteems goes from (1700-1767mg/l, pre-rainstorm; 1445-1634mg/l post-rainstorm) as appeared in Table 2. In the current examination, the most extreme number of all out coliform was in the long stretch of April to June. The base number was recorded in the long stretch of December and January. The high incentive in the current investigation might be ascribed to the presence of bacterial burden from the close by encompassing regions (Fecal issue) and because of this explanation, the River Ganga is totally unsuitable for drinking and undesirable for washing. Such higher estimation of MPN is likewise upheld from the investigations of different scientists (Bhargava, 1982; Rao 1992; Shukla, 1989).

CONCLUSIONS

It might infer that the overall attributes of water from the investigation zone is antacid in nature with more all-out solids just as above immersed with disintegrated oxygen. The boundaries chlorides are well inside the passable furthest reaches of drinking water quality norms. Notwithstanding some chose boundaries are somewhat higher cutoff points recommended by (WHO, 1984) and not okay for family unit and business purposes. Marginally higher Hardness, DO, BOD and MPN esteems in Ganga stream water is unsuitable for drinking reason at chosen destinations. Nonetheless, high pH, TS, Hardness, DO, BOD and MPN esteems propose decontamination might be essential for homegrown utilization. Exacting lawful move ought to be made against the individuals who sully the River Ganga by squander unloading or releases of nearby effluents.

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