University of Delhi

INNOVATION PROJECTS 2015-16

FINAL REPORT

SHC-303

Assessing Microbial Diversity of Yamuna Water: A Step Towards Environmental Restoration

(Bioinformatics Applied in Bioremediation)

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FINAL REPORT

1. **PROJECT CODE** : **SHC 303**

2. **PROJECT TITLE** : **Assessing Microbial Diversity of Yamuna Water: A Step Towards Environmental Restoration (Bioinformatics Applied in Bioremediation)**

3. **NAME OF COLLEGE/INSTITUTION** : **Shivaji College (Accredited with NAAC "A" Grade), University of Delhi**

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Certificate of Originality

This is to certify that the research work carried out and the final report submitted by the Project Investigators and the students of Innovation Project having **Project code SHC 303** and title **Assessing Microbial Diversity of Yamuna Water: A StepTowards Environmental Restoration (Bioinformatics Applied in Bioremediation)** of Shivaji College is original. Any plagiarism/academic dishonesty reported at any stage will be our responsibility.

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Final Report

1. **Project Title : Assessing Microbial Diversity of Yamuna Water: A step towards Environmental Restoration (Bioinformatics Applied in Bioremediation)**

2. **Project Code : SHC 303**

3. **Abstract**

The Yamuna river is one of the important rivers of our country but with the increase in population it has undergone severe deterioration in the quality of water. The daily discharge of municipal waste and industrial effluents into the river has contributed immensely to the present scenario. The increased level of toxic metals viz., Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Nickel (Ni) and Arsenic (As) were observed at ISBT and ITO. The consumption of this water leads to the bioaccumulation and bio-magnification, which disturbs the balance of our ecosystem. In human beings causes damage to the central nervous system, lungs, kidneys, liver, endocrine glands and bones. The water quality was then analysed by testing the biological and physico-chemical parameters. Upon screening the water samples for the presence of microorganisms, along with other microbes the presence of *E. coli* and *Bacillus Subtilis* were indicated using Hi media Biochemical Test Kits.

The presence of plethora of microorganisms in the river has been exploited for the bioremediation studies and construction of Microbial Fuel Cell (MFC). The significant uptake of Chromium (Cr) and Zinc (Zn) was observed by *E. coli* and *Bacillus Subtilis*. The uptake of Zn and Cr (IV) showed significant results and corroborated well with TEM images. The sample collected from ISBT was also used for the generation of electricity using a Microbial Fuel Cell (MFC). The parameters considered like single and dual electrodes, stirring, proton exchange systems and mediators contributed in the increased efficiency of MFC operation.

A comparative study was done for assessing the microbial diversity in Yamuna river water and sludge samples using Next Generation Sequencing Technology (NGS). The NGS holds immense potential for employing this microbial diversity information in the water treatment processes. In addition, paucity of literature on the microbial diversity in polluted Yamuna river encouraged us to carry out this study.

4. Introduction

The rate at which effluents are discharged into the environment especially into the rivers is increasing as a result of urbanization. Most of these effluents contain toxic substances especially heavy metals (Gambhir *et al*., 2012; Lone *et al*., 2003). Heavy metals make a significant contribution to environment as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and melting operations. Some heavy metals e.g., Mn, Fe, Cu, Zn, Mo and Ni are essential as micronutrient for microorganisms, plants and animals, while others have no known biological role. It has been known that heavy metal ions such as Pb, Cu, Zn, Cd, Cr and Cd when present at higher levels may enter our food chain through the vegetables grown on the banks of the river and accumulate in different parts of the body. This eventually causes reduced growth and impaired metabolism (Ray et al, 2014; Schwartz, 1994). All heavy metals at high concentrations have strong toxic effects and regarded as environmental pollutants (Nedelkoska& Doran, 2000). Industrial activities cause fast and considerable degradation of soil and vegetation cover, which necessitate pursuing the methods of managing derelict industrial lands.

The Yamuna river, which is the lifeline of Delhi, is one of the most-polluted river in the country. There is a severe deterioration in the quality of water due to the discharge of municipal and industrial effluents into the rivers. They both represent about 85 percent of the pollution of the river. The attempts made till now are generally conventional using more manpower and less effective. Lately, Bioremediation has proven to be a safe, effective and environmentally friendly alternative. It is a biological process which uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances, thus treating polluted water or soil. Bioremediation processes are very attractive in comparison with physicochemical methods for heavy metal removal because they can have lower cost and higher efficiency at low metal concentrations (Gaur *et al*, 2014).

The Microbial Fuel Cells (MFCs) provides an attractive option for the production of clean energy and bioremediation by the use of river water or other sources of water with high organic content (Du *et al*, 2007). MFC is a bio-electrochemical system, which converts the chemical energy stored in the biodegradable substrate to electrical energy via microbial catalyzed redox reaction (Lovely, 2006; Davis and Higson, 2007).

One of the objective of this innovation research project was to design and develop more cost effective MFC, identify native accumulator bacteria and examine their ability in remediation of heavy metals in the highly polluted water bodies such as Yamuna river. Application of Bioinformatics tools like BLAST, CLUSTAL and PHYLIP software for identification and to analyze the evolutionary relationship along with construction of metabolic pathways (Khan et al, 2013).

5. Research problem/hypothesis/objectives:

i. Analysis of the various physico-chemical parameters.

ii. Quantitative estimation of toxic heavy metals in the river water samples.

iii. Isolation of pure colonies and biochemical identification of two microbes.

iv. Remediation of heavy metals by the microorganisms present in polluted water.

v. Use of Dual Electrodes in Microbial Fuel Cell (MFC) for enhanced ENERGY generation using Yamuna water. Efforts are made to make the MFC more cost-effective (Preliminary work done in Innovation Project SHC 203).

vi. Accessing the microbial diversity of the water and the sludge samples collected from ISBT, Delhi.

vii. Application of bioinformatics tools like blast, clustal-w software for identification and to analyze the evolutionary relationship by extracting the data using GEN BANK.

viii. Heavy metal uptake by the microbes, estimation by atomic absorption spectophotometry (AAS).

ix. Transmission electron microscopy (TEM) based studies.

6. Methodology Techniques/Sampling /Tools/Materials:

1. <u>Sampling of Yamuna water</u>

   The samples were collected from the ISBT location of the river Yamuna. Water samples were collected in sterilized bottles and stored at 4°C till further processing.

2. Analysis of biological and physico-chemical parameters of Yamuna water

The water was processed immediately post collection, to ensure that the water profile is not affected by storage. The various parameters like pH, turbidity, DO, BOD and COD were estimated using pH meters and titrimetric methods (APHA, 2005).

3. Quantitative estimation of toxic heavy metals in water

The estimation of metals (Iron, Copper, Chromium, Zinc and Cadmium) in collected water samples were carried out by standard spectrophotometric methods (APHA, 1998; APHA, 2005)

4. Construction of Dual Electrode Microbial Fuel Cell

The Dual electrode Microbial Fuel Cell was constructed as follows:

Separate cathode and anode chambers were constructed and sealed after the addition of the required sample. The anaerobic conditions in the anode chamber were maintained by sparging with nitrogen. Nafion®117 membrane (Sigma) was used as a proton exchanger for the transfer of H+ ions. The electrodes were prepared using carbon cloth. Glucose (3g/L) was used as substrate in the anode chamber containing 500 ml of Yamuna water. Methylene blue (300μM) was used as a mediator as per the requirement of set-up. The electricity generated was measured for 96 hours, using a multimeter (Sanwa CD770) at a regular interval of 2 hrs.

The electricity generated was measured for the Yamuna water, Pure *E.coli, Bacillus subtilis* culture and Consortium (in the presence and absence of mediator).

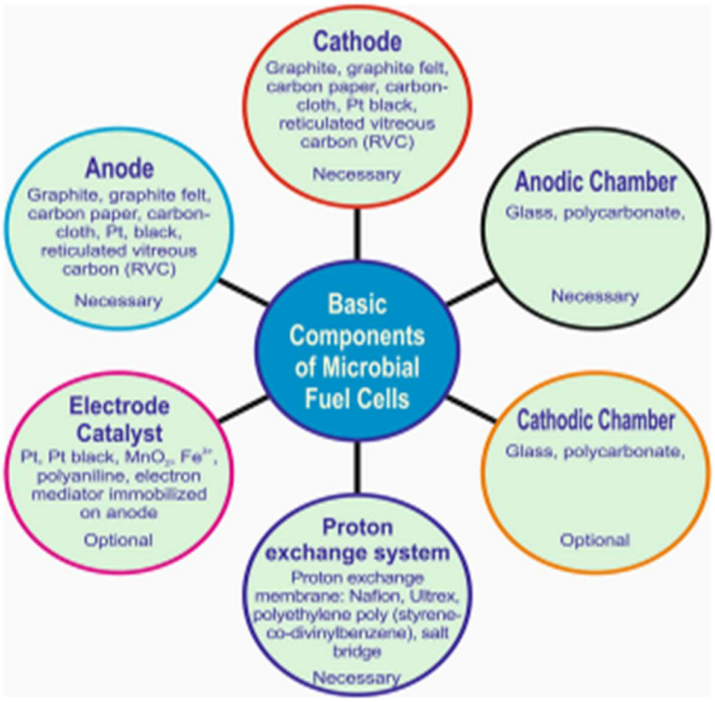


Figure 1: Basic components of Microbial Fuel Cells (MFC)

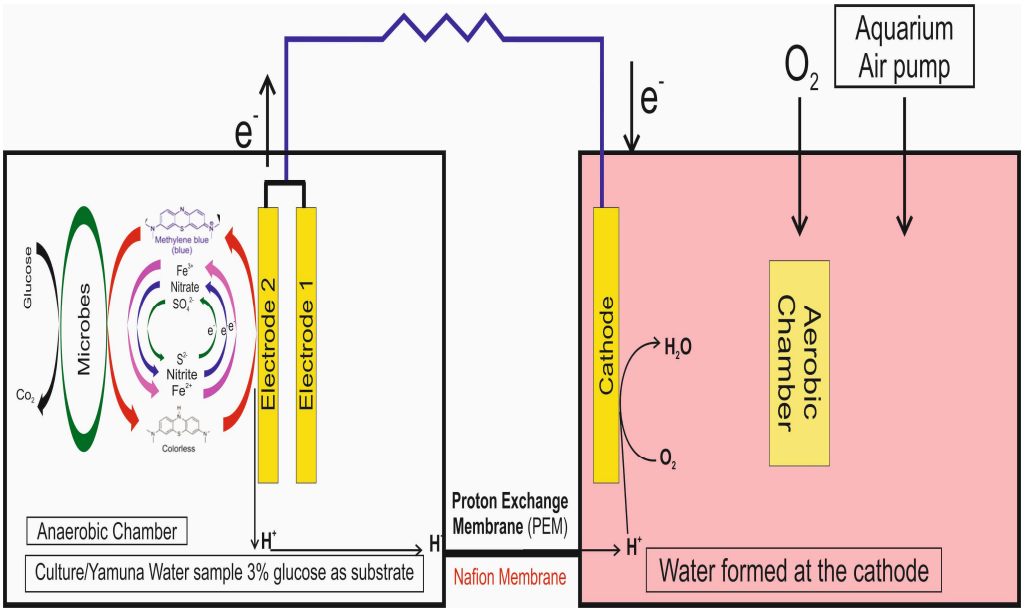


Figure 2: Schematic representation of Dual electrodes MFC assembly consisting of anodic and cathodic chambers. Nafion membrane & Methylene blue are used as proton exchanger and redox mediator for enhanced bioenergy generation.

4. Isolation and selection of microbes for bioremediation studies

The collected water samples were stored at 4°C, serially diluted and plated on LB Agar plates (37°C, overnight) for isolation of bacteria. Biochemical tests were carried out to identify bacteria.

5. Efficacy of bioremediation of heavy metals in selected microbes

To estimate the efficacy of bioremediation of metals by the chosen bacteria, it was grown in the presence of metals (Zinc & Chromium). The culture was collected at different time points (0, 24, 48,72 and 96 hrs). The bacteria was pelleted by centrifugation and supernatant used for metal uptake estimation. The amount of metal present in supernatant sample was estimated in college laboratory by spectrophotometric analysis.

The results were then further corroborated by Atomic Absorption Spectrophotometric (AAS) analysis and Transmission Electron Microscopy (TEM, TECNAI 200 Kv).

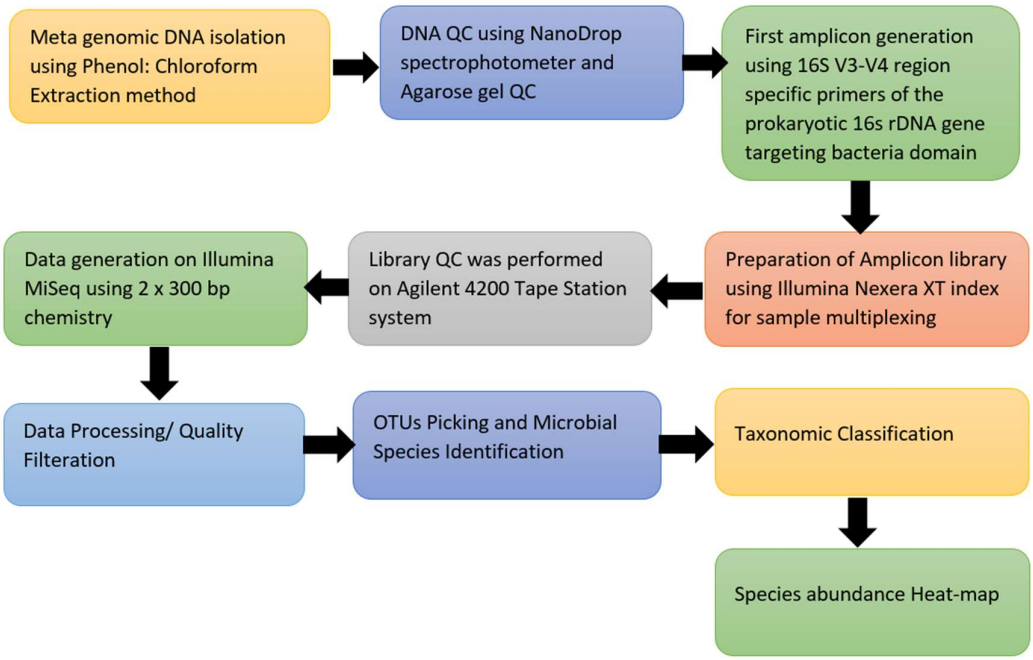


Figure 3: Flowchart for 16S based Metagenomics Diversity and analysis of bacteria using MiSeq Platform.

6. **Isolation , Qualtitative and Quantitative analysis of gDNA**

Yamuna water and sludge samples for bacterial diversity enumeration were collected from ISBT location. Samples were kept at 4°C.

The genomic DNA was isolated from the water and sludge samples using the Phenol: Chloroform extraction procedure and processed further for the amplicon generation using 16S V3-V4 region specific Primers.

Protocol for gDNA isolation:

- Spin down the water and sludge samples at 3900 RPM for 15 min and discard the supernatant.
- Dissolve the pellet with 750µl of Lysis Buffer (50mM Tris, pH 8.0; 10mM EDTA; 1.2% SDS) and 250µl of 2% c-TAB and transfer to a 1.5- or 2.0- Eppendorf tube
- Add 20µl of Proteinase K (20mg/ml) solution to the lysis mixture and incubated at $56^{O}C$ for 2 hours.
- Add 10µl of RNase A (10mg/ml) solution to above mixture and mix by inverting the tubes followed by incubation at $37^{O}C$ for 30 min.
- Add equal volume of Phenol:Chloroform:Isoamylalcohol (25:24:1) and mix slowly by inverting the tubes.
- Centrifuge at Room Temperature for 10 min at 12000 RPM.
- Transfer the clear supernatant into a fresh Eppendorf tube by using cut tips.
- Add equal volume of Choloroform:Isoamylalcohol (24:1) to the supernatant and mix properly by inverting the tubes.
- Centrifuge at 12000 RPM for 10 min at Room Temperature.
- Transfer the clear supernatant into a fresh Eppendorf tube by using cut tips and add equal volume of Chloroform to the tube and mix by inverting the tube.
- Centrifuge at 12000 RPM for 10 min at Room Temperature.
- Transfer the clear supernatant into a fresh Eppendorf tube by using cut tips and add $1/10^{th}$ volume of 3M potassium acetate followed by 2 volume of ice cold ethanol (100%) and incubate at $-20^{O}C$ for at least 15 min.
- Centrifuge at 12000 RPM for 15 min at $4^{O}C$.
- Discard the supernatant and wash the pellet with ice cold 70% Ethanol (1 ml) by centrifuge at 12000 RPM for 5 min.
- Discard the supernatant and air dry the pellet and dissolved with 20-30µl of Nuclease free water.

V3-V4 Specific Amplicon Generation:

- gDNA sample: 25 – 50 ng
- Primers used:

16S rRNA F	GCCTACGGGNGGCWGCAG
16S rRNA R	ACTACHVGGGTATCTAATCC

- Amplicon Size ~ 460bp
- PCR Condition:

$94^{O}C$ for 5 min

$94^{O}C$ for 30 sec } 25 cycles

58^{O}C for 30 sec 28 cycles
72^{O}C for 1 min
72^{O}C for 5 min

PCR generated first amplicons were resolved on 1.2% Agarose gel at 120V for approximately 60 min or till the samples reached 3/4th of the gel. 1 µl of each extracted gDNA were also checked in NanoDrop 2000 for determining A260/280 ratio.

Preparation of 2 x 300 PE MiSeq Library:

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) Nextera XT DNA Library Prep Kit (Part # 15044223 Rev. B). These primers were synthesized at Eurofins Genomics lab facility. The amplicons with the Illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by 1X AMpure XP beads and quantified using Qubit fluorometer.

Quantity and quality check (QC) of library on Agilent 4200 Tape Station:

The amplified libraries were analyzed in 4200 Tape Station system (Agilent Technologies) using D1000 Screen tape as per manufacturer instructions.

Cluster Generation and Sequencing

Mean Peak size from Tape Station profile, libraries were loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing, fragments to be sequenced in both the forward and reverse directions on Miseq.

Picked up Operational Taxonomic Units (OTUs) based on sequence similarity within the reads, and pick a representative sequence from each OTU. Assigning the OTU to a taxonomic identity using reference databases.

Calculated diversity metrics for each sample and compared the types of communities, using the taxonomic assignments.

The results were further analyzed by picking up nucleotide sequences from the Gen-Bank in the FASTA format and constructing the Phylogenic trees (CLUSTAL-OMEGA).

7. Result and Discussion

1. Analysis of physico-chemical parameters of Yamuna river sample

The samples collected in triplicate were analysed for the temperature, pH, Dissolved oxygen (DO), Conductivity, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Fecal coliform and total coliform. The DO was found to be zero with significant increase in BOD and COD (Table 1). Bacteriological water quality status in terms of total coliform count was studied. The coliform analysis of the samples indicated that the most probable number (MPN) of coliforms are highest in all the three samples. The level of these parameters correlates well with the Delhi as a major contributor of pollution in the Yamuna river followed by Agra and Mathurs (Misra, 2010). The sewage treatment plants (STP) due to power failure or technical fault also release the untreated sewage into the river thus further contributing to the present status. (CPCB, 2006).

Table 1: The analysis of Physico-chemical parameters of Yamuna river at ISBT location.

Parameters	Unit	Location (ISBT)	Normal Range
Temperature	^{0}C	27	27-30
DO	mg/l	Nil	3
pH	-	7.38	7
Conductivity	mmhos/cm	688	75-150
COD	mg/l	**106**	1-50
BOD	mg/l	**30**	1-3
Fecal coliform	MPN/100ml	2351950	-
Total coliform	MPN/100ml	31786556	-

2. Quantitative estimation of toxic metals

The quantitative estimation of metals were done using spectrophotometric methods and the results were also corroborated with AAS. The heavy metals viz., iron, copper, nickel, chromium, zinc and cadmium were found varying from borderline to very high (Figure 4). The increased levels causes deleterious health diseases.

Electricity generation from *E. coli, B. Subtilis* and Consortium using MFC

The pure cultures of *E. coli & B. Subtilis* which are found to be present in the Yamuna water, were considered for the electricity generation in the anaerobic chamber individually. The results were compared with the consortium culture. The significant increase was obtained for *B. Subtilis* by addition of methylene blue as a mediator led to increased power generation (maximum of 9.66 volts was generated in the presence of mediator as compared to 7.42 volts in the absence of mediator).

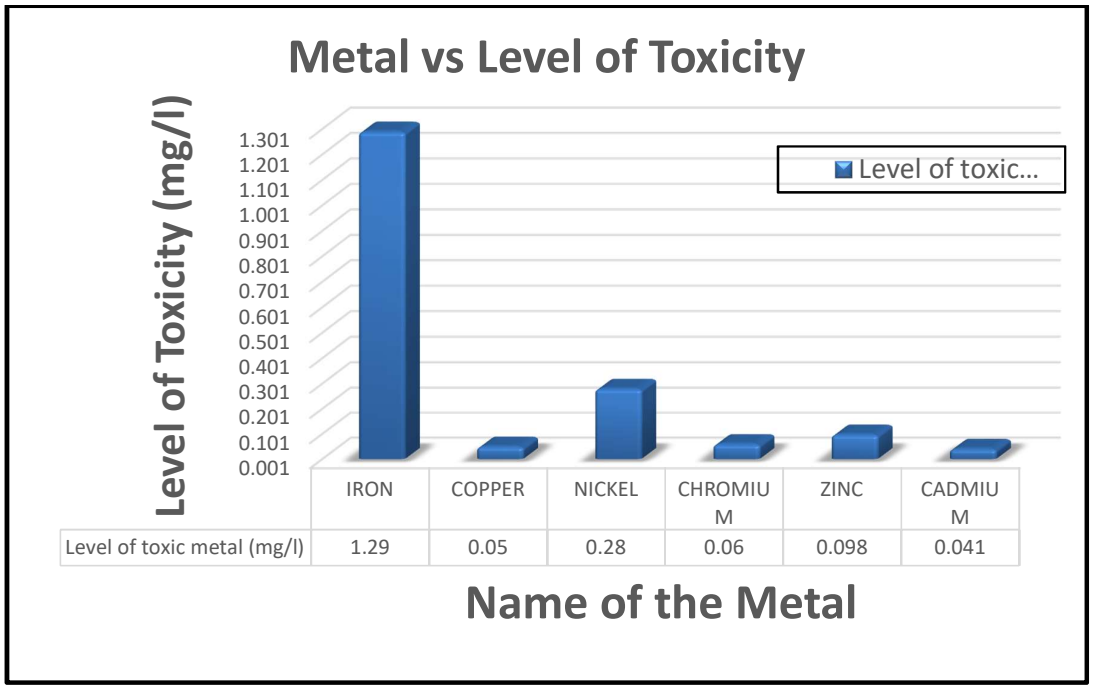


Figure 4: Quantitative estimation of toxic heavy metals in water samples

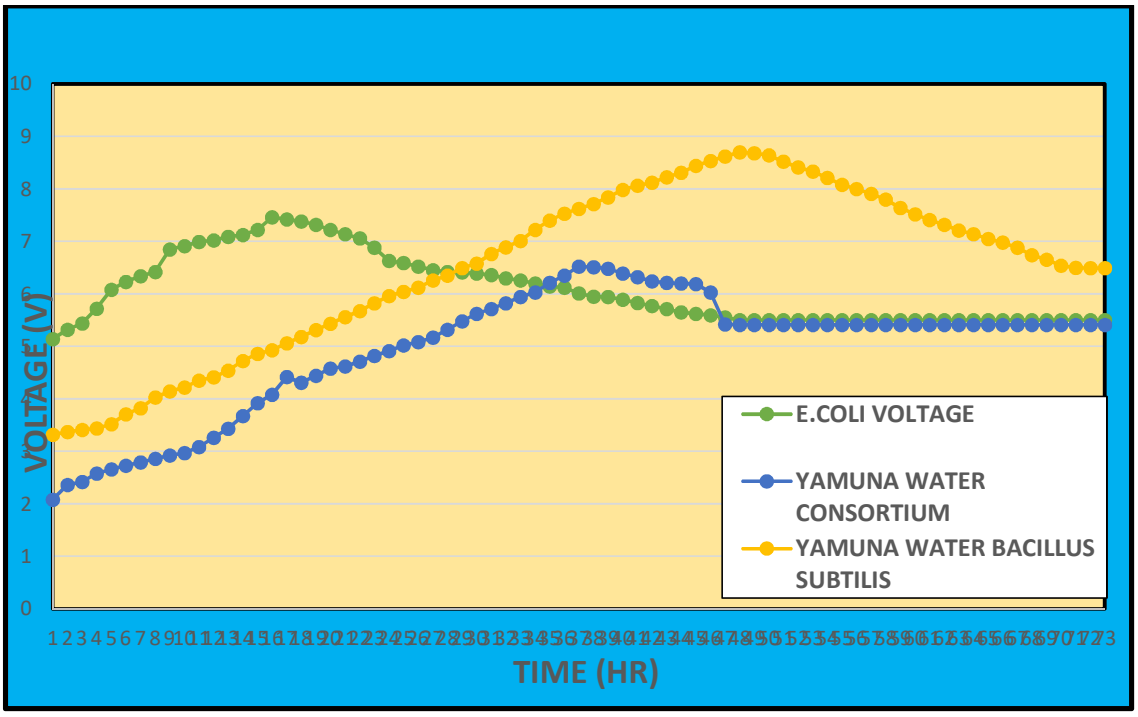


Figure 5: The electricity generation was seen for 72 hours using the *Bacillus subtilis, E.coli* and the consortium culture

Efficacy of Bioremediation of heavy metal uptake (Chromium and Zinc)

The uptake of heavy metals by *E,coli and B.subtilis* and consortium culture was studied for 0 -96 hrs. The Chromium uptake of 65, 72 & 25 % were shown by the microbes respectively (Table 2). A decrease uptake of 27, 12 & 23 % was observed in the case of Zinc (Table 3). Thus for the mentioned cultures bioremediation can be applied for the heavy metal uptake (Figure 6 & 7). The surface morphology variations with respect to metal uptake were observed using TEM (Figure 8 & 9).

Table 2: The initial and final concentration of Chromium estimated by AAS for the three microbes.

Chromium (Cr) uptake			
Bacteria	**Conc. at 0h (mg/l)**	**Conc. At 96h (mg/l)**	**% uptake**
B. subtilis	37.81	13.14	65.25
E. coli	31.17	8.81	71.73
Consortium	32.07	24.19	24.57

Table 3: The initial and final concentration of Zinc estimated by AAS for the three microbes.

Zinc (Zn) uptake			
Bacteria	**Conc. at 0h (mg/l)**	**Conc. At 96h (mg/l)**	**% uptake**
B. subtilis	0.33	0.24	27.27
E. coli	1.01	0.89	11.88
Consortium	0.9	0.71	22.82

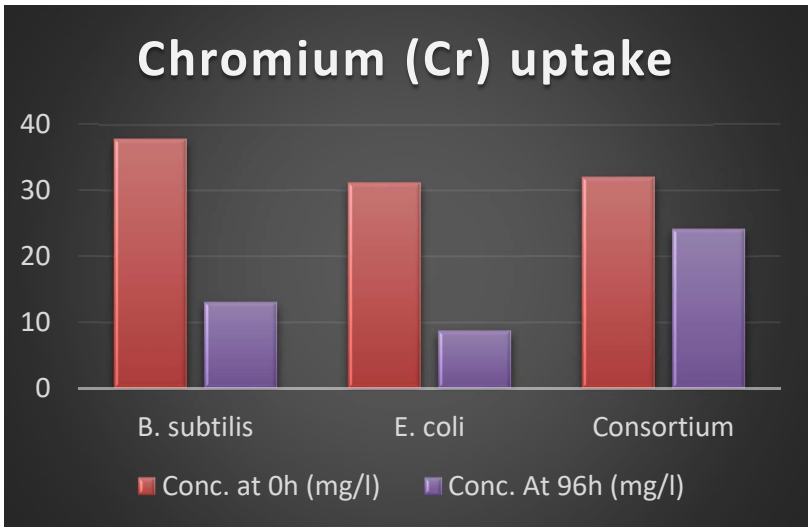


Figure 6: Chromium metal uptake by the three cultures of microbes were estimated by the amount of residual metal present in the collected supernatant.

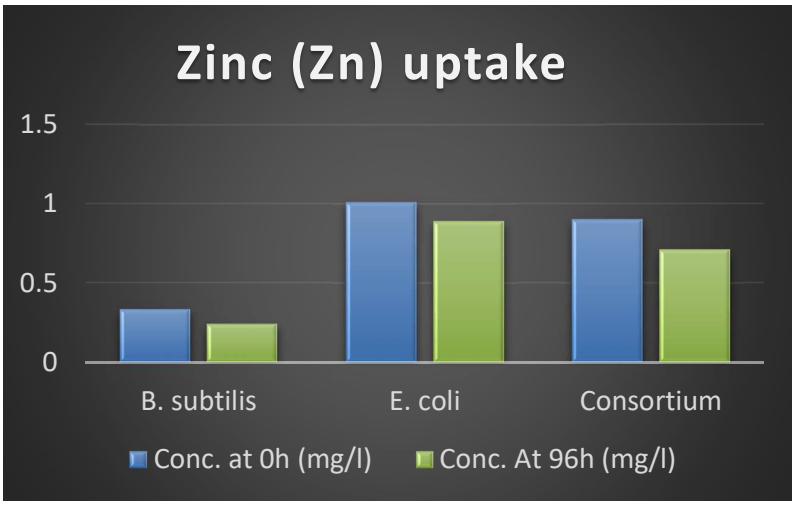


Figure 7: Zinc metal uptake by the three cultures of microbes were estimated by the amount of residual metal present in the collected supernatant.

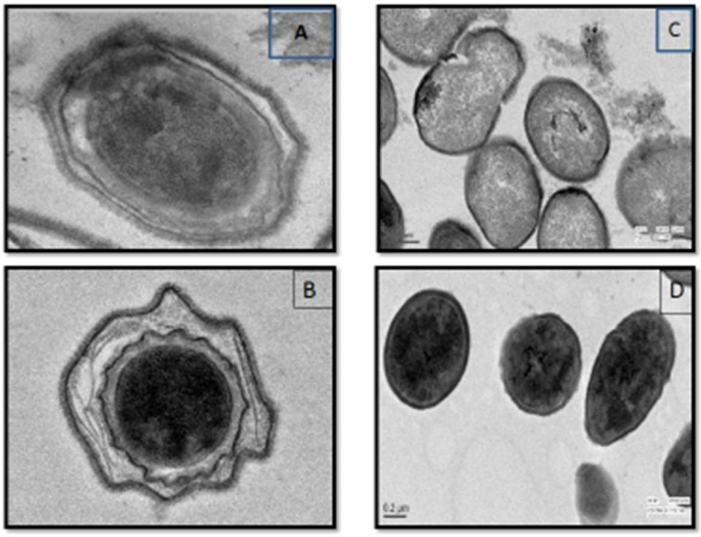


Figure 8: TEM images of A) *B. subtilis* **control; B)** ***B. subtilis*** **with Cr; C)** ***E. coli*** **control; D)** ***E. coli*** **with Cr. Dis-organised cell envelope in the presence of chromium and cell grown in the presence of chromium showed the electron dense particles in the cytoplasm which was not shown in the control cell.**

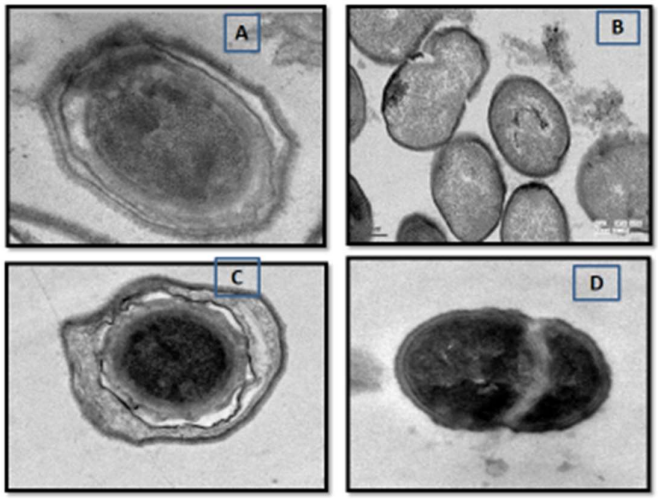


Figure 9: TEM images of A) ***B. subtilis*** **control; B)** ***B. subtilis*** **with Zn; C)** ***E. coli control*****; D)** ***E. coli*** **with Zn.**

Quality and quantity check of DNA isolated

The quality check of first amplicon of the v3-v4 region for both the sludge and water samples was done using the 1.2%agarose gel with the using suitable markers. The bands obtained on the gel confirmed the presence of the specific and known amount of the genomic DNA, to carry forward the work of the library preparation using the metagenomic approach.

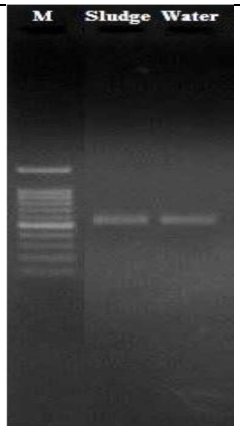


Figure 10 : Quality of first amplicon checked on 1.2 % Agarose gel

The quality check of the isolated genomic DNA was also done using the NanoDrop (an instrument that works on the same principle as the spectrophotometer, but can also measure in the minute quantity. The OD ratios at A260/280 and A260/230 were measured to check for the presence of impurity. Both the considered sample passed the quality check to carry the work further.

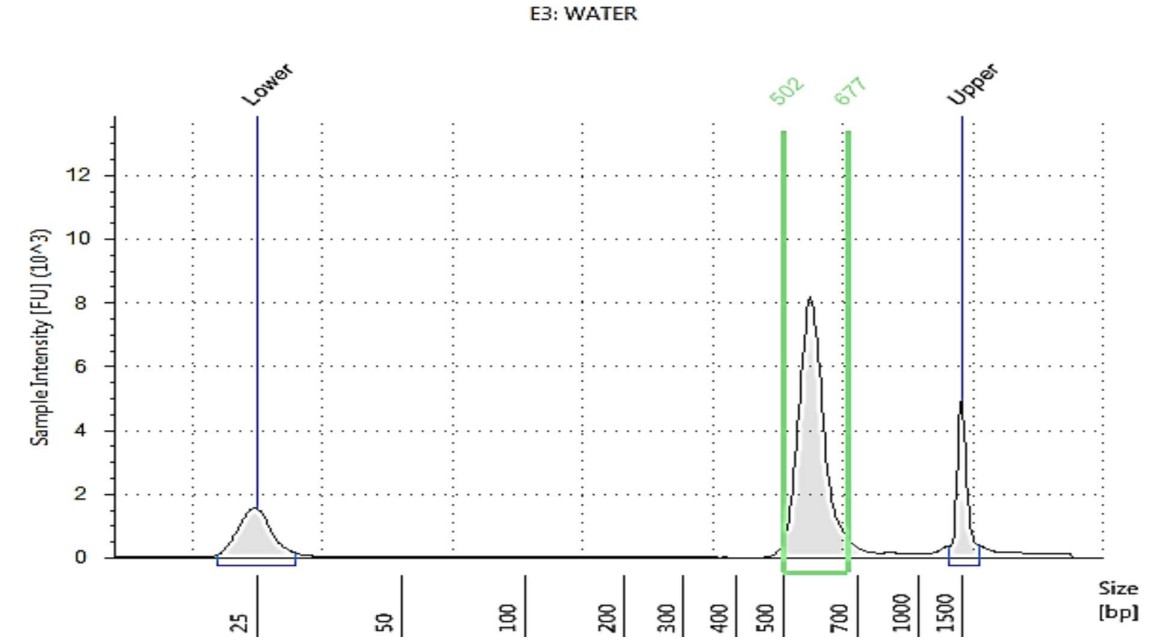
Table 4: NanoDrop reading of received Plasmid DNA

Sr. No.	Sample ID	Concentration (ng/µl)	NanoDrop OD A260/280	NanoDrop OD A260/230	Remark
1	Sludge	127.7	1.84	2.01	QC Pass
2	Water	199.4	1.66	1.20	QC Pass

Tape-Station profiles of library

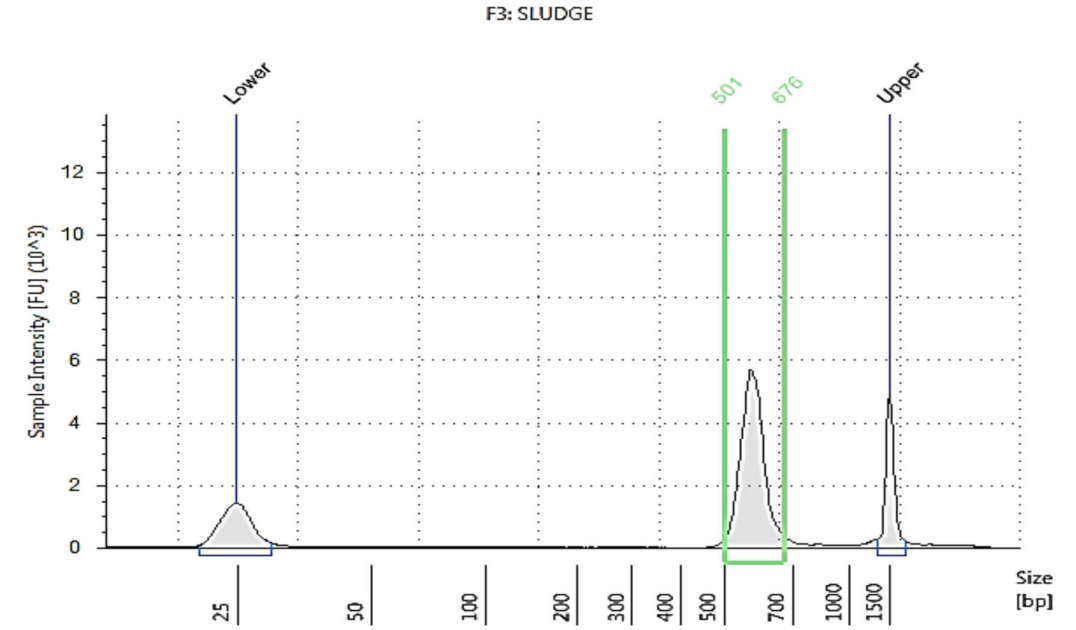
The libraries were prepared from the water and sludge samples after amplifying the V3-V4 region of the prokaryotic target bacteria domain. The mean peak from the tape station, which is actually a sequencing tool was taken ranging from 502 to 677 bp for the water and the sludge sample libraries prepared with approximately ~0.3 gb of the data generated for each of the sample. The tapestation mean peaks were further run on Miseq, which is a sequencer producing

the 2X300 paired end reads from the single run.This further enables the target variant with unmatched accuracy.



From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nm	% of Total
502	677	582	26.6	70.5	86.18

Figure 11: Library Profile of water sample on Agilent Tape Station using D1000 Screen Tape



From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nm	% of Total
501	676	586	19.5	51.4	87.96

Figure 12: Library Profile of sludge sample on Agilent Tape Station using D1000 Screen Tape

Table 5: Summary of the samples indicating the No. of raw reads, total bases and data in (mb).

Sr. No	Sample name	Number of Raw Reads	Total bases	Data In mb
1	Sludge	449,431	217,722,745	~ 217
2	Water	245,168	118,136,279	~ 118

The Bioinformatics Approach

The paired end stitching was performed to stitch together the reads that we got for the forward and reverse stitching, thus generating a single read of 2X300 bp.

The Operational Taxonomic Units (OUT's) were further picked up from the reads based on the sequence similarities within the reads. The reads were again sought for similarity with the picking up of representative OUT from each sequence.

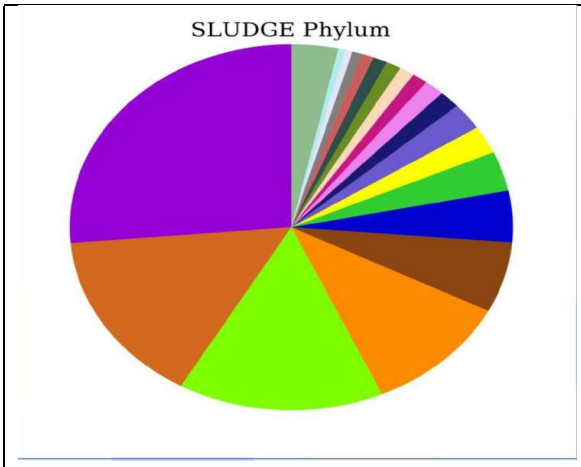


Figure 13: Pie chart showing the Absolute abundance of each phylum within each microbial community. From the figure it can be inferred that most abundant phylum is *Proteobacteria* followed by *Chloroflexi*.

Legends	Taxonomy	Abundance
	k__Bacteria;p__Proteobacteria	26.37%
	k__Bacteria;p__Chloroflexi	15.33%
	k__Bacteria;p__Firmicutes	15.01%
	k__Bacteria;p__Bacteroidetes	10.73%
	k__Bacteria;p__Thermotogae	6.24%
	k__Bacteria;p__Synergistetes	4.55%
	k__Bacteria;p__Spirochaetes	3.49%
	k__Bacteria;p__Actinobacteria	2.46%
	k__Archaea;p__Euryarchaeota	2.37%
	k__Bacteria;p__Acidobacteria	1.56%
	k__Bacteria;p__OP3	1.46%
	k__Bacteria;p__WS6	1.14%
	k__Bacteria;p__Nitrospirae	1.1%
	k__Bacteria;p__Verrucomicrobia	1.09%
	k__Bacteria;p__Cyanobacteria	1.06%
	k__Bacteria;p__Planctomycetes	0.84%
	k__Bacteria;p__OP8	0.75%
	k__Bacteria;p__TPD-58	0.52%
	k__Bacteria;p__Unclassified	0.51%
	Others	3.42%

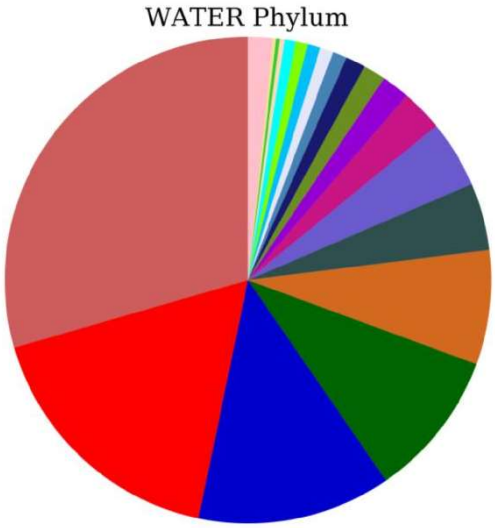


Figure 14: Pie chart showing the Absolute abundance of each phylum within each microbial community. From the figure it can be inferred that most abundant phylum is *Proteobacteria* followed by *Chloroflexi*.

WATER Phylum legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Proteobacteria	29.48%
	k__Bacteria;p__Chloroflexi	17.26%
	k__Bacteria;p__Bacteroidetes	12.86%
	k__Bacteria;p__Planctomycetes	9.79%
	k__Bacteria;p__Firmicutes	7.59%
	k__Bacteria;p__Verrucomicrobia	4.48%
	k__Bacteria;p__Cyanobacteria	4.46%
	k__Bacteria;p__Acidobacteria	2.78%
	k__Bacteria;p__OP3	1.85%
	k__Bacteria;p__Chlorobi	1.5%
	k__Bacteria;p__NKB19	1.31%
	k__Bacteria;p__TM7	0.95%
	k__Bacteria;p__Actinobacteria	0.88%
	k__Bacteria;p__Spirochaetes	0.86%
	k__Bacteria;p__WS6	0.79%
	k__Bacteria;p__WPS-2	0.75%
	k__Bacteria;p__BRC1	0.32%
	k__Archaea;p__Euryarchaeota	0.24%
	k__Bacteria;p__OD1	0.2%
	Others	1.65%

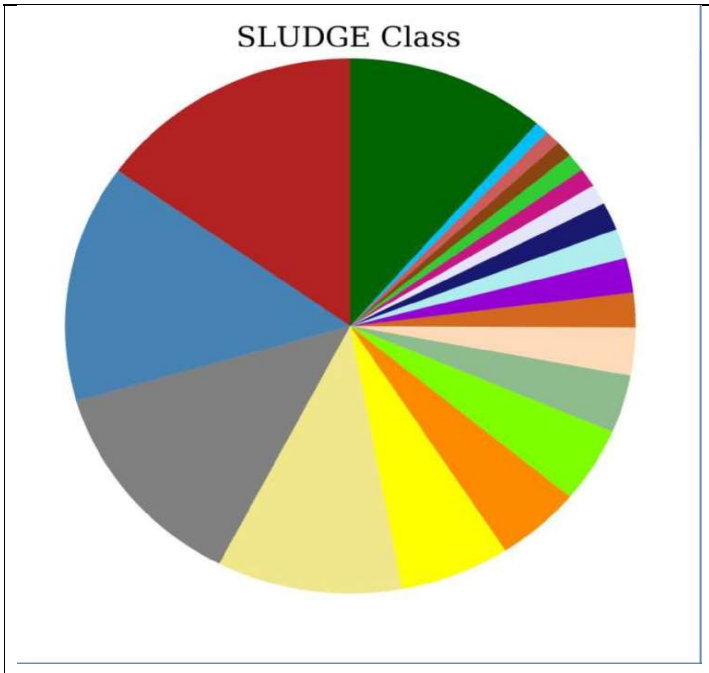


Figure 15: Pie chart showing the Absolute abundance of each class within each microbial community. From the figure it can be inferred that most abundant class is *Anaerolineae* followed by *Deltaproteobacteria*.

SLUDGE Class legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Chloroflexi;c__Anaerolineae	15.14%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	14.38%
	k__Bacteria;p__Firmicutes;c__Clostridia	12.89%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	10.51%
	k__Bacteria;p__Thermotogae;c__Thermotogae	6.24%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	4.83%
	k__Bacteria;p__Synergistetes;c__Synergistia	4.55%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	3.5%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	2.87%
	k__Archaea;p__Euryarchaeota;c__Methanomicrobia	2.1%
	k__Bacteria;p__Spirochaetes;c__[Brachyspirae]	2.09%
	k__Bacteria;p__Firmicutes;c__Bacilli	1.79%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria	1.7%
	k__Bacteria;p__Spirochaetes;c__Spirochaetes	1.21%
	k__Bacteria;p__WS6;c__SC72	1.14%
	k__Bacteria;p__Nitrospirae;c__Nitrospira	1.1%
	k__Bacteria;p__OP3;c__koll11	0.97%
	k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae]	0.88%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria	0.78%
	Others	11.34%

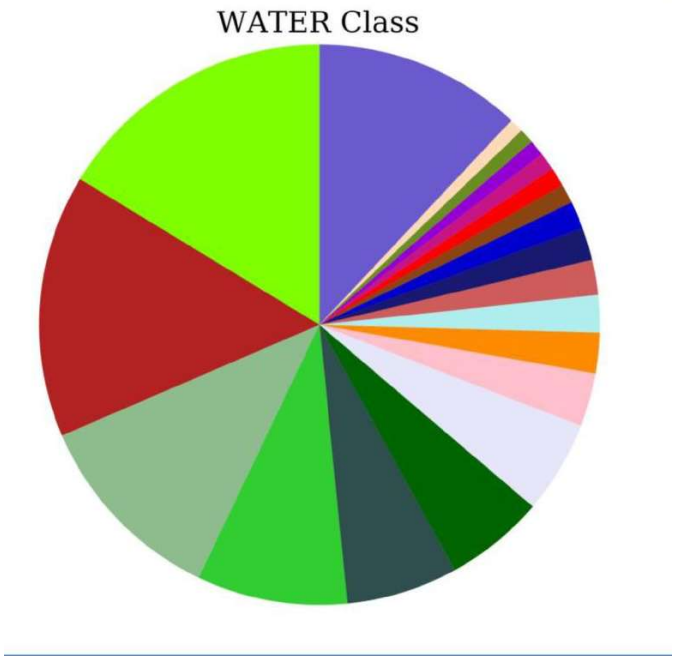


Figure 16: Pie chart showing the Absolute abundance of each class within each microbial community. From the figure it can be inferred that most abundant class is *Anaerolineae* followed by *Betaproteobacteria.*

WATER Class legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Chloroflexi;c__Anaerolineae	16.3%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	15.17%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	11.44%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia	8.68%
	k__Bacteria;p__Firmicutes;c__Clostridia	6.44%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	5.74%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	5.36%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	3.08%
	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae	2.35%
	k__Bacteria;p__Cyanobacteria;c__Chloroplast	2.15%
	k__Bacteria;p__Cyanobacteria;c__4C0d-2	2.02%
	k__Bacteria;p__Acidobacteria;c__Solibacteres	1.89%
	k__Bacteria;p__OP3;c__PBS-25	1.58%
	k__Bacteria;p__Firmicutes;c__Bacilli	1.11%
	k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae]	1.09%
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae]	1.03%
	k__Bacteria;p__TM7;c__TM7-1	0.9%
	k__Bacteria;p__Verrucomicrobia;c__Opitutae	0.86%
	k__Bacteria;p__NKB19;c__TSBW08	0.81%
	Others	12.0%

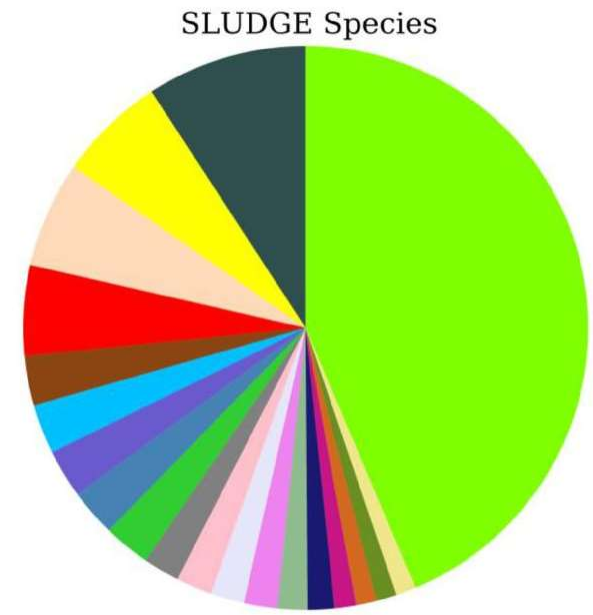


Figure 17: Pie chart showing the Absolute abundance of each species within each microbial community. From the figure it can be inferred that Unclassified species from *Bacteroidales* order is most abundantly present.

SLUDGE Species legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Unclassified;g_Unclassified;s_Unclassified	9.19%
	k_Bacteria;p_Thermotogae;c_Thermotogae;o_Thermotogales;f_Thermotogaceae;g_Kosmotoga;s_mrcj	6.14%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_T78;s_Unclassified	6.09%
	k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophaceae;g_Syntrophus;s_Unclassified	5.17%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_Longilinea;s_Unclassified	2.85%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_SMB53;s_Unclassified	2.84%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Unclassified;s_Unclassified	2.77%
	k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophaceae;g_Unclassified;s_Unclassified	2.7%
	k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophorhabdaceae;g_Unclassified;s_Unclassified	2.67%
	k_Bacteria;p_Spirochaetes;c_[Brachyspirae];o_[Brachyspirales];f_Brachyspiraceae;g_Unclassified;s_Unclassified	2.09%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_TTA_B6;g_E6;s_Unclassified	2.06%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_SHD-231;s_Unclassified	1.93%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_WCHB1-05;s_Unclassified	1.91%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_Methanosarcinales;f_Methanosaetaceae;g_Methanosaeta;s_Unclassified	1.72%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Chromatiales;f_Chromatiaceae;g_Allochromatium;s_Unclassified	1.51%
	k_Bacteria;p_Synergistetes;c_Synergistia;o_Synergistales;f_Dethiosulfovibrionaceae;g_PD-UASB-13;s_Unclassified	1.23%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Chromatiales;f_Ectothiorhodospiraceae;g_Unclassified;s_Unclassified	1.19%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Unclassified;f_Unclassified;g_Unclassified;s_Unclassified	1.16%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptostreptococcaceae;g_[Clostridium];s_difficile	1.15%
	Others	43.65%

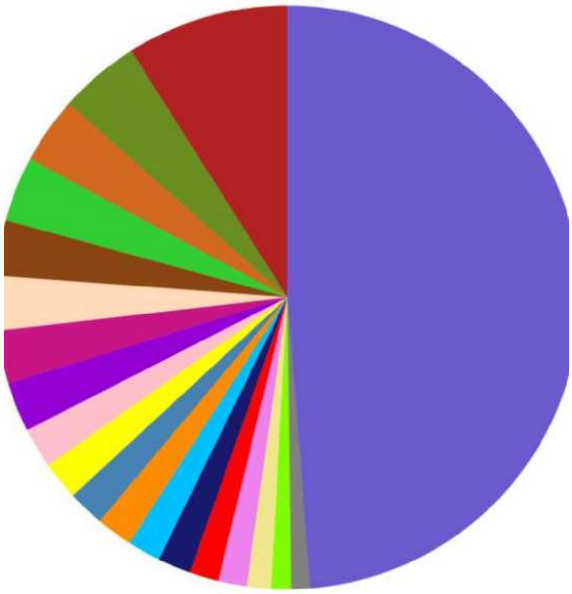


Figure 18: Pie chart showing the Absolute abundance of each species within each microbial community. From the figure it can be inferred that Unclassified species from *Bacteroidales* order is most abundantly present.

WATER Species legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Unclassified;g_Unclassified;s_Unclassified	8.98%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Planctomycetales;f_Planctomycetaceae;g_Planctomyces;s_Unclassified	4.47%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Caldilineales;f_Caldilineaceae;g_Unclassified;s_Unclassified	3.69%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Unclassified;f_Unclassified;g_Unclassified;s_Unclassified	3.6%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Rhodocyclales;f_Rhodocyclaceae;g_Dok59;s_Unclassified	3.06%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Nitrosomonadales;f_Nitrosomonadaceae;g_Unclassified;s_Unclassified	3.03%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Rhodocyclales;f_Rhodocyclaceae;g_Unclassified;s_Unclassified	2.95%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_envOPS12;f_Unclassified;g_Unclassified;s_Unclassified	2.73%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Gemmatales;f_Isosphaeraceae;g_Unclassified;s_Unclassified	2.25%
	k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_Prosthecobacter;s_debontii	2.12%
	k_Bacteria;p_Cyanobacteria;c_Chloroplast;o_Chlorophyta;f_Unclassified;g_Unclassified;s_Unclassified	2.02%
	k_Bacteria;p_Cyanobacteria;c_4C0d-2;o_MLE1-12;f_Unclassified;g_Unclassified;s_Unclassified	2.01%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_SMB53;s_Unclassified	1.84%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Pirellulales;f_Pirellulaceae;g_Unclassified;s_Unclassified	1.83%
	k_Bacteria;p_Acidobacteria;c_Solibacteres;o_Solibacterales;f_Unclassified;g_Unclassified;s_Unclassified	1.61%
	k_Bacteria;p_OP3;c_PBS-25;o_Unclassified;f_Unclassified;g_Unclassified;s_Unclassified	1.58%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_SB-1;g_Unclassified;s_Unclassified	1.33%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Caldilineales;f_Caldilineaceae;g_Caldilinea;s_Unclassified	1.1%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae;g_Unclassified;s_Unclassified	1.04%
	Others	48.75%

Table 6: COMPARATIVE STUDIES OF THE WATER AND SLUDGE SAMPLES

Sample	Sludge	Water
Phylum	Proteobacteria (26.37%)	Proteobacteria(29.48%)
Class	Anaerolineae(15.14%)	Anaerolineae(16.3%)
Order	Anaerolineales(13.5%)	Bacteroidales(11.44%)
Family	Anaerolinaceae(13.5%)	Unclassified family of
		Bacteroidales order
		(8.98%)
Genus	Unclassified genus of	Unclassified genus of
	Bacteroidales order (9.19%)	Bacteroidales order
		(8.98%)
Species	Unclassified species of	Unclassified species of
	Bacteroidales order (9.19%)	Bacteroidales order
		(8.98%)

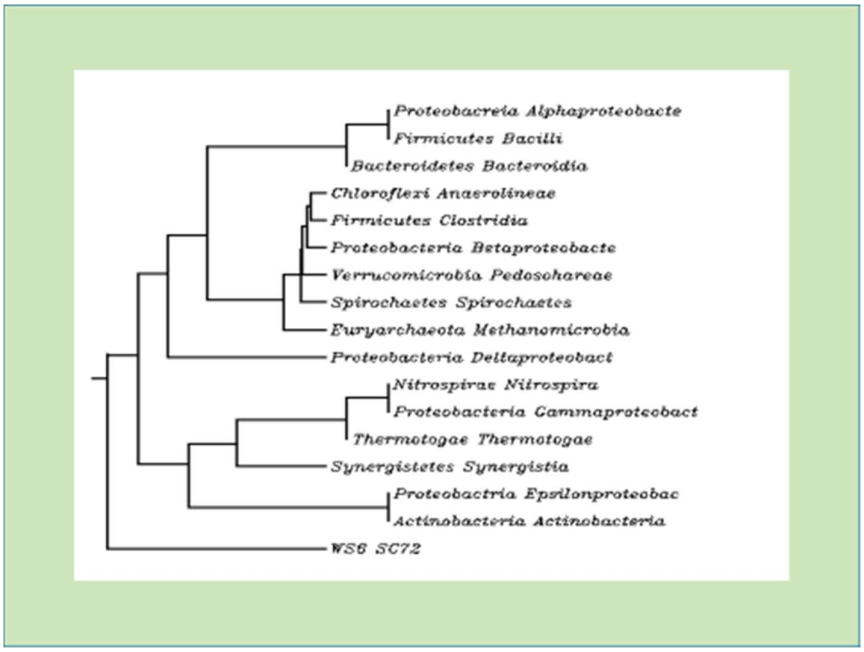


Figure 19: Phylogenetic tree for the sludge sample (Rooted phylogenetic tree with branched length (UPGMA).

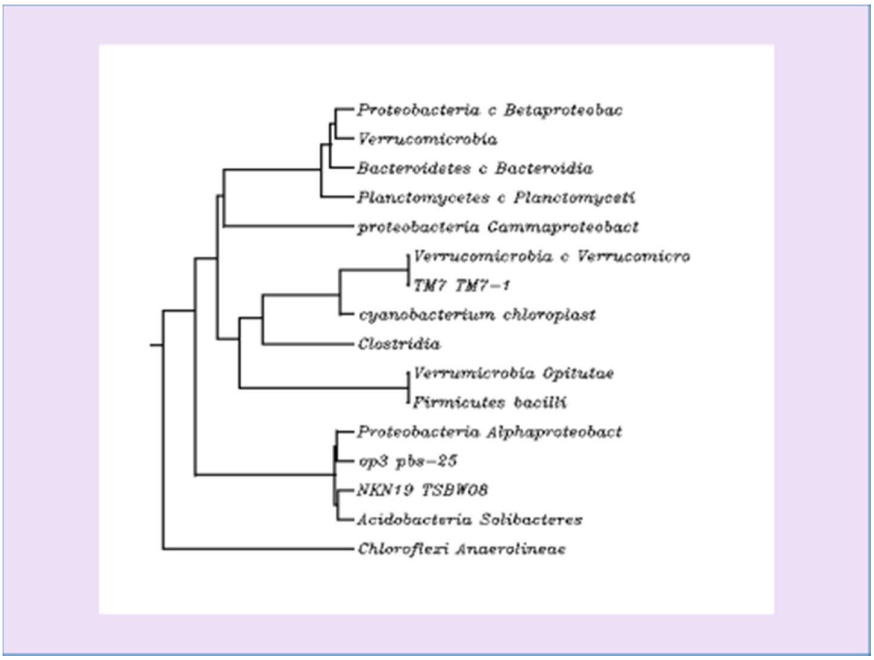


Figure 20: Phylogenetic tree for the species present in water sample (Rooted phylogenetic tree with branched length (UPGMA).

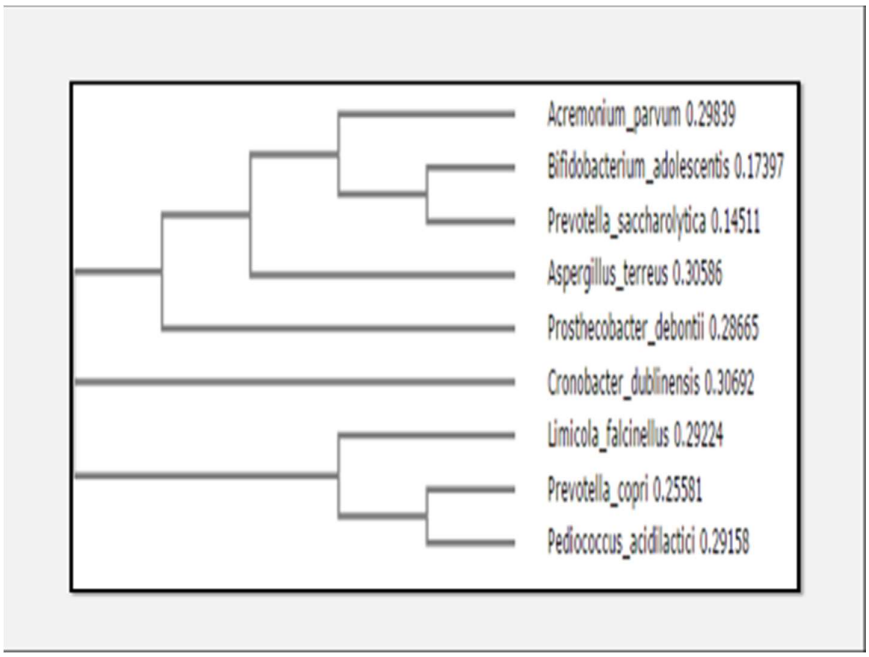


Figure 21: Phylogenetic tree for the species present both in water and sludge samples (Rooted phylogenetic tree with branched length (UPGMA).

The phylogenetic trees (Figure 19-21) were prepared to infer the evolutionary relationships the obtained microbial community. The construction of these trees gave an access to understand the similarities and differences in the physical or genetic characteristics of them. The conclusion about their common ancestor could be concluded. In a rooted phylogenetic tree, each node with descendants represents the inferred most recent common ancestor of the descendants, and the edge lengths in some trees may be interpreted as time estimates

8. Innovations shown by the project

The modified MFC holds the key to future energy problems and will also provide the future generations a clean and green planet.

Accessing the microbial diversity using 16S rRNA metagenomics is an innovative move for the welfare of human beings as this can further be extended to identify pathogenic and non-pathogenic microbes.

9. Conclusion and Future direction

The water at ISBT location was found to be of poor quality, with high biological and heavy metal content. The coliform levels of the water is so high that it is not fit for even bathing, let alone drinking. The study has shown that *E.coli* & *B. subtilis*, which are found to be present in the water of Yamuna have been found to be the potential candidates for bioremediation. It was shown that the use of nafion membranes for proton exchange, methylene blue as mediator and Nano-composite electrodes would lead to significant increase in the generation of electricity. The novel modifications applied in this project can be used for increasing the electricity generated through MFCs.

Further works that can be pursued include under MFC:

- New potent electrogenic microorganisms can be screened to improve the power output of the MFC will be a great challenge in future.
- Microbes in pure culture or a mixed culture forming a synergistic microbial consortium would lead to better performance of the MFC.
- An effort can be made to make the MFC more cost-effective.

The microbial diversity was studied and the abundant microbes were submitted for the sequence to GENE BANK. The Phylogeny trees were constructed to decipher the diversity. The points mentioned below may further be taken up.

- The deciphering of the microbial population in Yamuna river can further be explored with respect to seasonal variations and the pathogenic and non-pathogenic strains can be identified.
- Whole metagenome study with focus on Gene taxonomic - functional and microbial abundance in water and sludge samples from different locations of Yamuna stretch will be of great significance for the human welfare.

The preliminary work on **WATER QUALITY APP** has been initiated, which will help in assessing the water quality with complete information awareness of general public at lower price. The smart phone app is designed to work seamlessly with the waterproofed unit with parameter information (viz., pH, temperature, Dissolved Oxygen, total dissolved solids, HARDNESS, RESIDUAL ALKALINITY, Chloride levels) data storage and GPS tracking. The app will be paired with photometer with blue tooth, which will give the results and renders the data sharing via email and the built-in GPS feature allows for easy retrieval of water testing locations. The app will be **comp**atible with iPhone, iPad, and iPod touch with the minimum iOS / OS X system.

10. References

1. Gambhir RG, Kapoor V, Nirola, A, Sohi, R, & Bansal, V (2012) Water Pollution: Impact of Pollutants and New Promising Techniques in Purification Process, *J Hum Ecol*, 37(2): 103-109.
2. Lone MI, Saleem S, Mahmood T & Saifullah K (2003) Heavy metal contents of vegetables irrigated by sewage/tubewell water, *Int. J. Agric. Biol.,* 5: 533–5.
3. Ray PD, Yosim A. and Fry R. C. (2014). Incorporating epigenetic data into the risk assessment process for the toxic metals arsenic, cadmium, chromium, lead, and mercury: strategies and challenges. Front Genet 5:201 (Published online Jul 16, 2014).
4. Schwartz J. (1994). Air pollution and daily mortality: A review and meta - analysis. Environ Res 64: 26-35.
5. Nedelkoska TV and Doran PM (2000) Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Mineral Eng., 13: 549–61.

6. Lovely DR., 2006. Microbial fuel cells: Novel microbial physiologies and engineering approaches. *Current Opinion in Biotechnology*. 17:327–32.
7. Gaur N, Flora G, Yadav M, Tiwari A (2014). A review with recent advancements on bioremediation-based abolition of heavy metals.*Environ. Sci. Process Impacts.* 16(2):180-93.
8. Du Z., Li H., Gu T., 2007. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnology Advances. 25: 464–482.
9. Davis, F. and Higson, S.P.J., 2007. Biofuel cells—recent advances and applications. Biosensors and Bioelectronics. 22 (7), 1224–1235.
10. Malik D, Singh S, Thakur J, Kishore R, Kapur A, Nijhawan S. Microbial Fuel Cell: Harnessing Bioenergy from Yamuna Water; International Journal of Science and Research; Vol. 3 (6): 1076-1081 (2014).
11. Darshan Malik, Sunita Singh, Jayita Thakur, Raj Kishore Singh, Anita Kapur, AmarjeetKaur, ShashiNijhawan and Anil Kumar (2014).Nanocomposite Electrode Microbial Fuel Cell: A Promising Technology for Enhanced Power Generation from Yamuna Water;International Journal of Science and Research Vol. 3(7): 641-646.
12. Buszewski B, Jastrzębska A, Kowalkowski T, Górna-Binkul A (2000) Monitoring of Selected Heavy Metals Uptake by Plants and Soils in the Area of Toruń, Poland Polish Journal of Environmental Studies 9: 511-515.
13. APHA, Standard methods for examination of water and waste water. American Public
14. Health Association, New York (1989).
15. Fulekar, M.H. and Sharma J. 2008. “Bioinformatics Applied in Bioremediation”.Innovative Romanian Food Biotechnology. Vol. 2 No. 2. pp 28-36.
16. Khan F, SajidM and Cameotra SS. (2013) In Silico Approach for the Bioremediation of Toxic Pollutants, J Pet Environ Biotechnol 4: 161.doi:10. 4172/2157-7463.1000161.
17. Basak P.,Pramanik A. , Sengupta S. , Nag S., Bhattacharyya A., Roy D., Pattanayak R., Ghosh A., Chattopadhyay D. and Bhattacharyya M. Bacterial Diversity Assessment of Pristine Mangrove Microbial Community from Dhulibhashani, Sundarbans using 16S r RNA Gene Tag Sequencing , Genomics data 7 (2016) 76-78.
18. Bhattacharyya A., Majumdar M., Basak P., Roy D., Nag S., Haldar A., Mukherjee S., Chattopadhyay D., Mitra S., Bhattacharyya M. and Ghosh A. Diversity and Distribution of Archaea in the Mangrove Sediment of Sundarbans. Archaea (2015)

19. Chakraborty A., Bera A., Mukherjee A. and Basak P. Change in Bacterial Profile of Sundarbans, The World Heritage Mangrove : Impact of A nthropogenic Interventions. 31(2015) 593-610.
20. Ma X., Feng C., Zhou W. and Yu H. Municipal Sludge-Derived Carbon Anode with Nitrogen and Oxygen containing Functional Groups for High Performance Microbial Fuel Cells. Journal of Power Sources 307 (2016) 105-111.
21. Mishra A. K., (2010), A river about to Die: Yamuna, Journal of water resource and protection, 2: 489-500.
22. CPCB, 2006, Water Quality Status Of Yamuna River (1999 – 2005), Central Pollution Control Board, Ministry Of Environment & Forests, Assessment and Development of River Basin Series: ADSORBS/41/2006-07
23. Lenntech Water Treatment and Air Purification (2004) Water treatment. Lenntech, Rotterdamseweg, Netherlands (http://www. excelwater. com/thp/filters/Water-Purification.htm).
24. Nedelkoska, T.V. and P.M. Doran, 2000. Characteristics of heavy metaluptake by plant species with potential for phytoremediation andphytomining. Mineral Eng., 13: 549–61
25. Boxall A.B.A., Comber S.D., Conrad A.U., Howcroft J., Zaman N., (2000) Inputs, monitoring and fate modelling of antifouling biocides in UK estuaries. Marine Pollution Bulletin 40: 898-905.
26. Gaur N, Flora G, Yadav M, Tiwari A (2014). A review with recent advancements on bioremediation-based abolition of heavy metals. Environ. Sci. Process Impacts. 16(2):180-93
27. Davis, F. and Higson, S.P.J., 2007. Biofuel cells—recent advances and applications. Biosensors and Bioelectronics. 22 (7), 1224–1235.
28. American Public Health Assoociation. 2005. Standard Methods for the Examination of Water & Wastewater. Port City Press; Baltimore, MD, USA: American Water works Association; Water Environment Federation.
29. Standard Method, for the examination to water and waste water. APHA 20th edition, 1998.
30. Shrivastava J.N., Raghav N. and Singh Am 2012. Laboratory Scale Bioremediation of the Yamuna Water with Effective Microbes (EM) Technology and Nanotechnology, J Bioremed. Biodeg. 3(8) 1000160.

11. Publication/s from the work: In the process of submitting three research papers.

12. Conference Presentation/s:

Oral Presentation on "Assessing the Bioenergy using the Microbial Fuel Cell" in **2nd International Conference on Public Health: Issues, challenges, opportunities, prevention, awareness" (Public Health-2016) organized by** Jawaharlal Nehru University, New Delhi on 21st May, 2016.

Sunita Singh, Sristhi Sharma & Rashmi

Poster Presentations in International Conference/ National Seminar

Yamuna River A Toxicity Reservoir: Environmental Restoration Through Bioremediation Approach

International Conference on "Public Health: Issues, Challenges, Opportunities, Prevention, Awareness (Public Health: 2016)" organized by Daulat Ram College, University of Delhi, New Delhi, India and Krishi Sanskriti, New Delhi, India from January 15-16, 2016.

Srishti Sharma, Rashmi Singh and Kiran

First Prize

Bioremediation: A step towards environmental restoration

National Symposium on Water and Air Quality in Urban Ecosystem organized by ECO CLUB SHIVAJI COLLEGE, University of Delhi, New Delhi, India on March 22, 2016

Srishti Sharma, Rashmi Singh and Kiran

Department of Biochemistry & Department of Computer Sciences

Shivaji College (NAAC Accredited "A" Grade), University of Delhi, New Delhi 110027

First Prize

HARNESSING ELECTRICITY USING MICROBIAL FUEL CELL

National Symposium on Water and Air Quality in Urban Ecosystem organized by ECO CLUB SHIVAJI COLLEGE, University of Delhi, New Delhi, India on March 22, 2016

Harsimran[1], Ankit[2], Satvinder Singh[1] and Smriti Babbar[2]

Department of Biochemistry[1] & Department of Computer Sciences[2]

Shivaji College (NAAC Accredited "A" Grade), University of Delhi, New Delhi 110027

FIRST PRIZE

METAL TOXICITY: AN ISSUE OF ENVIRONMENTAL CONCERN

National Symposium on Water and Air Quality in Urban Ecosystem organized by ECO CLUB SHIVAJI COLLEGE, University of Delhi, New Delhi, India on March 22, 2016

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13. Patent/s and Technology Transfer: NIL

14. Media Coverage (attach copies): NIL

15. Acknowledgement

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We are also grateful to **Prof. Punit Kaur** (Head, Department of Biophysics, AIIMS) for providing the guidance and help throughout the project. We thank Mr. Puru Sachan for the experimental assistance in MFC.

Pictures related to the project:

Yamuna River A Toxicity Reservoir

Environmental Restoration Through Bioremediation Approach

Sunita Singh, Rashmi Singh, Srishti Sharma and Kiran

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ABSTRACT

The Yamuna river, which is the lifeline of Delhi, is one of the most-polluted river of India. There is a severe deterioration in the quality of water due to the discharge of municipal and industrial effluents into the rivers. The river is causing multidimensional harm to the human health, flora and fauna. Heavy metals make a significant contribution to environment as a result of human activities such as mining, smelting, electroplating, intensive agriculture, sludge dumping and melting operations. The heavy metals viz., Mn, Fe, Cu, Zn, Mo and Ni are essential as micronutrient for microorganisms, plants and animals, while others have no known biological function. It has been known that heavy metal ions such as Pb, Cu, Zn, Cd, Cr and Cd when present at an elevated level may enter our food chain through the vegetables grown on the banks of the river and accumulate in different parts of the body. This eventually causes reduced growth and impaired metabolism. All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants. Deciphering the microbial diversity using bioinformatics tools, adopting suitable bioremediation approach and public awareness are very important steps to be undertaken for making the Yamuna river healthy.

INTRODUCTION

The rate at which effluents are discharged into the environment especially into the rivers is increasing as a result of urbanization. This eventually causes serious health implications including carcinogenesis, reduced growth and impaired metabolism.

Industrial activities cause fast and considerable degradation of soil and vegetation cover, which necessitate pursuing the methods of managing derelict industrial lands.

The Yamuna water is reported to contain a consortium of bacteria like *E. coli, Bacillus subtilis* etc. The microbes and the reported metals may constructively be used in a biological process called Bioremediation, which uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances, thus treating polluted water or soil. Bioremediation processes are very attractive in comparison with physicochemical methods for heavy metal removal because they can have lower cost and higher efficiency at low metal concentrations.

METHODOLOGY

1. Sampling of Yamuna water

The Yamuna water samples were collected from Wazirabad Barrage, ISBT, ITO and Okhla Barrage. Water samples were collected in sterilized bottles and stored at 4^{0}C till further processed.

2. Analysis of biological and physico-chemical parameters

The water was processed immediately after the collection, to ensure that the water profile is not affected by storage. The various parameters like pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Dissolved Oxygen (DO) were estimated using pH meters, titrimetric methods (APHA, 2005).

3. Estimation of toxic heavy metals in water

The estimation of metals like Zinc and Chromium were carried out by AAS.

4. Isolation and selection of microbes for bioremediation studies

The collected water samples were stored at 4^{0}C, serially diluted and plated on LB Agar plates (37^{0}C, overnight) for isolation of bacteria. Biochemical tests were carried out for the identification, bacteria were subjected to the IMVic tests (consisting of Indole, Methyle Red, Voges-Proskauer and Citrate tests). The chosen microbe tested positive for Indole and MethyleRed, and negative for Voges-Proskauer and Citrate tests. This indicated the bacteria to be *E. coli*, and not *Shigella, Klebsiella, Salmonella* etc. The single *E. Coli* colonies were picked and cultured further for the bioremediation work.

5. Uptake of Heavy metals by Bioremediation

To estimate the efficacy of bioremediation of metals by the *E. coli,* it was grown in the presence of Zinc and Chromium. The bacteria ware pelleted by centrifugation at different time durations of 0, 24, 36, 48, 60, 72 and 90 hrs. The amount of metal present in supernatant sample was estimated by Atomic Absorption Spectrophotometric (AAS) analysis.

RESULTS AND DISCUSSION

1. Analysis of physico-chemical parameters

Water samples collected from the mentioned locations were analyzed for various physico- chemical parameters. Figure 1 (A-D) indicates the variation in the pH, COD (mg/L), BOD (mg/L) and DO (mg/L) respectively.

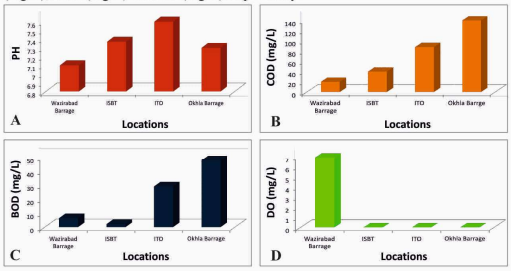

Fig.1 (A-D) Comparative analysis of the physico-chemical parameters of water samples

2. Quantitative estimation of Zinc and Chromium

A significant increase in the metal uptake by *E.coli* was obtained in the presence of Zinc and Chromium metal with time respectively. Figure 2 (A-B) indicated the significant efficacy of bioremediation.

- Decrease in Zinc levels in 0 to 90 hrs duration from 35.3 to 18.8 mg/L.
- Decrease in Chromium levels in 0 to 90 hrs duration from 38.2 to 21.0 mg/L.

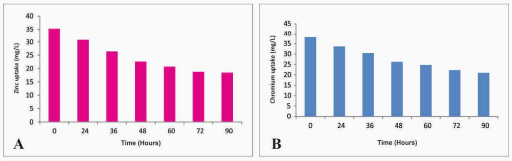

Fig. 2 (A-B) Metal uptake by bacteria estimated by the amount of residual metal in supernatant.

CONCLUSION

This study has shown that *E.coli*, which is present in the water samples of Yamuna has been a potential candidate for bioremediation.

The Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) studies will be conducted to study the effect and uptake of these metals.

REFERENCES

Gaur N, Flora G, Yadav M, Tiwari A (2014). A review with recent advancements on bioremediation-based abolition of heavy metals.Environ. Sci. Process Impacts. 16(2):180-93

Malik D, Singh S, Thakur J, Kishore R, Kapur A, Nijhawan S. Microbial Fuel Cell: Harnessing Bioenergy from Yamuna Water; International Journal of Science and Research; Vol. 3 (6): 1076-1081 (2014).

APHA, Standard methods for examination of water and waste water. American Public Health Association, New York (1989).

ACKNOWLEDGEMENT

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Poster Presented in International Conference on Public Health: Issues, Challenges, Opportunities, Prevention, Awareness (Public Health: 2016) organized by Daulat Ram College, University of Delhi, New Delhi, India And Krishi Sanskriti, New Delhi, India from January 15-16, 2016.

Received First Prize in the Poster Presentation

Received First Prize in the Poster Presentation

Shivaji College
(University of Delhi)
ACCREDITED BY NAAC WITH "A" GRADE

HARNESSING ELECTRICITY USING MICROBIAL FUEL CELL

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[1]Department of Biochemistry and [2]Department of Computer Sciences

ABSTRACT

Microbial fuel cell (MFC), is a bio-electrochemical system which is reported to convert chemical energy stored in the biodegradable substrate to electrical energy via microbial catalysed redox reactions. The performance of MFCs are dependent on various factors like type of organic molecule, proton exchange system, the type of electrodes, the use of mediators and the nitrogen gas sparging. Anode performance and proton exchange membranes are important factors in deciding the efficiency of MFCs for large scale applications. Highly efficient Ni-coated carbon cloth electrodes that are electrochemically and biologically stable have been reported to enhance the efficiency in turn synthesized at much lower cost by chemical vapour deposition. The MFCs composed of dual electrodes have also been reported to contributed significant increase in generation of electricity. Thus, it provides an attractive option for the production of clean energy, waste water treatment, as biosensors and bioremediation by the use of river water or other sources of water with high organic content. It thus leads us towards a system which generates green energy and carries out bioremediation of heavy metals at the same time. The MFC, establishes as an alternative future sustainable source of energy generation.

INTRODUCTION

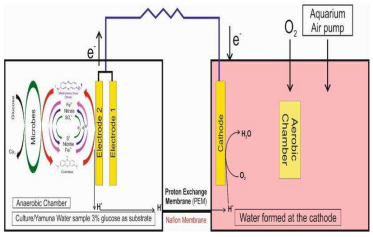
Yamuna which is called as lifeline of Delhi is one of the most polluted river of India (can be seen in Google map presented). Due to the continuous discharge of municipal and industrial effluents, the water is reported to contain a consortium of micro-organisms along with excessive amounts of heavy metals and poor physico-chemical parameters. The plethora of micro-organisms which also include *E. coli* and *B. subtilis* can be used as a fuel to MFC which generates electricity.

METHODOLOGY ADOPTED

Construction of Dual Electrode Microbial Fuel Cell

The Dual Electrode Microbial Fuel Cell was constructed as follows:

Separate cathode and anode chambers were constructed and sealed after the addition of the required sample. The anaerobic conditions in the anode chamber were maintained by sparging with nitrogen. Nafion®117 membrane (Sigma) was used as a proton exchanger for the transfer of H^+ ions. The electrodes were prepared using carbon cloth. Glucose (3g/L) was used as a substrate in the anode chamber containing 500 ml of Yamuna water. Methylene blue (300µM) was used as a mediator as per the requirement of set-up. The electricity generated was measured for 50 hours, using a multimeter (Sanwa CD770) at a regular interval of 2 hrs.

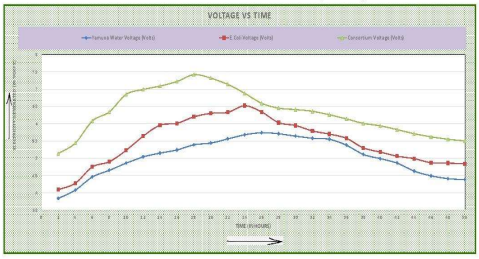


The electricity generated was measured for the Yamuna water, pure *E.coli* culture and Consortium (in the presence and absence of mediator).

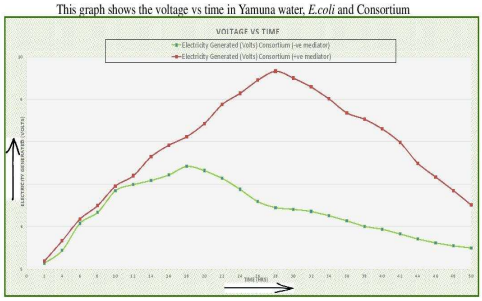
RESULTS AND DISCUSSION

Generation of electric current using overnight grown pure culture of E.coli

The *E. coli* is reported to be present in the in the Yamuna water, so the pure culture was taken for comparison with the collected samples in MFC. The maximum voltage generated by the Yamuna water, *E. col*, consortium (in the absence and presence of mediator) was found to be 5.74 Volts, 6.52 Volts, 7.42 Volts and 9.66 Volts respectively.



This graph shows the voltage vs time in Yamuna water, *E.coli* and Consortium



This graph shows the voltage vs time in Consortium (+ve mediator) and Consortium (-ve mediator)

DISCUSSION

MFC can also be used for waste water treatment, bio-hydrogen economy, biosensors etc. MFC can be further improved by various means like increasing the surface area of electrodes, using Ultra Centrifugation membrane as PEM and using ultra capacitor to store electric current.

REFFERENCES

Microbial fuel cell as new technology for bioelectricity generation: A review
Mostafa Rahimnejad [a,b,*], Arash Adhami [a,b], Soheil Darvari [a,b], Alireza Zirepour [a,b], Sang-Eun Oh[c]

Electricity generation using membrane and salt bridge microbial fuel cells
Booki Min[a], Shaoan Cheng[a], Bruce E. Logan[a]

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ABSTRACT

The water bodies have undergone severe deterioration in the water quality due to the discharge of municipal and industrial effluents into them. The presence of heavy metals viz., Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Nickel (Ni) and Arsenic (As) have been associated with adverse effects on human metabolism and health. Heavy metals have been reported to damage liver, kidneys, central nervous system, lungs, bones and endocrine glands. The Yamuna river is one of the most polluted river, a diverse range of pollutants are constantly added into the river and their toxicity has been a major challenge for ecology and environment. The water samples at various locations in Delhi were collected and analyzed for biological and physico-chemical parameters like temperature, pH, turbidity, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were estimated using pH meters and titrimetric methods The water at ITO was found to be of poor quality, with high biological and heavy metal contents. The ailing condition of Yamuna is of serious concern for lives of million people. Thus, it is important to check the influx of heavy metals into the river from various sources.

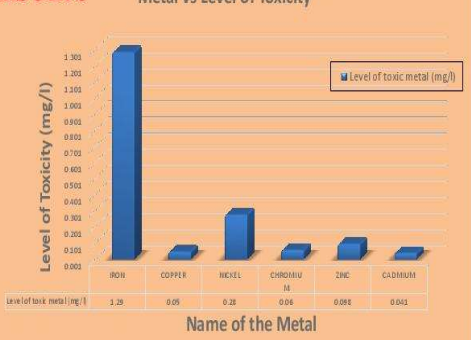
INTRODUCTION

Yamuna River water pollution in Delhi has reached to alarming proportions with low Dissolved Oxygen (DO), high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) and toxic levels of heavy metals like Cu, Cr, Fe, Zn and Ni due to increasing urbanization and industrialization.

Quantitative estimation of toxic heavy metals in water

The water sample were collected from the ITO, Delhi and the estimation of heavy metals (Iron, Copper, Chromium, Zinc and Cadmium) were carried out by standard spectrophotometric method (APHA, 1998; APHA, 2005)

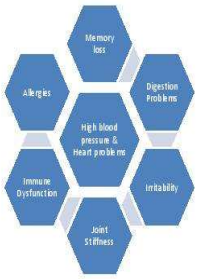
RESULTS



Quantitative estimation of toxic heavy metals in water samples

Effect of Heavy Metals on Human Health

Pollutants	Major sources	Effect on human health	Permissible level (mg/l)
Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis, poisoning	0.02
Cadmium	Welding, electroplating, pesticide fertilizer, Cd and Ni batteries, nuclear fission plant	Renal dysfunction, Lung disease, Lung cancer, Bone defects (Osteomalacia, Osteoporosis), increased blood pressure, kidney damage, bronchitis, gastrointestinal disorder, bone marrow, cancer	0.06
Lead	Paint, pesticide, smoking, automobile emission, mining, burning of coal	Mental retardation in children, developmental delay, fatal infant encephalopathy, congenital paralysis, sensor neural deafness and, acute or chronic damage to the nervous system, epilepticus, liver, kidney, gastrointestinal damage	0.1
Manganese	Welding, fuel addition, ferromanganese production	Inhalation or contact causes damage to central nervous system	0.26
Mercury	Pesticides, batteries, paper industry	Tremors, gingivitis, minor psychological changes, acrodynia characterized by pink hands and feet, spontaneous abortion, damage to nervous system, protoplasm Poisoning	0.01
Zinc	Refineries, brass manufacture, metal Plating, plumbing	Zinc fumes have corrosive effect on skin, cause damage to nervous membrane	15
Chromium	Mines, mineral sources	Damage to the nervous system, fatigue, irritability	0.05
Copper	Mining, pesticide production, chemical industry, metal piping	Anemia, liver and kidney damage, stomach and intestinal irritation	0.1



Symptoms of heavy metal toxicity

DISCUSSION

The adverse effects of excessive heavy metal consumption may lead to cancer as well as other deleterious health diseases. Thus, it is important to check the influx of heavy metals into the river from various sources. Bioremediation by bacteria may play an important role in correction of heavy metals toxicity of rivers .

References

- Singh S, Malik D, Thakur J, Kaur A, Singh,RK, Nijhawan S (2014) A Comparative Analysis of the Physico-chemical Properties and Heavy Metal Pollution In Three Major Rivers Across India. International Journal of Science and Research 3(8); 1936-41.

ACKNOWLEDGEMENT

The financial support provided under innovation project (SHC-303) by University of Delhi is sincerely acknowledged.

Received First Prize in the Poster Presentation

Utilization Certificate

Innovation Project 2015-16
SHC – 303

Project Title: Assessing Microbial Diversity of Yamuna Water : A Step Towards Environmental Restoration

Audited Financial Statement under Innovation Project scheme

College: Shivaji College

Project Investigators: Dr. Sunita Singh, Dr. Satvinder Singh, Dr. Smriti Babbar

Grant Sanctioned Rs	Rs. 6,00,000/- (Rupees Six Lacs Only)		
	Grant Received	**Grant Utilized**	**Unspent Grant**
Equipments/Consumables	3,25,000/-	2,72,475/-	52,525/-
Travel	55,000/-	24,519/-	30,481/-
Stipend	1,20,000/-	1,20,000/-	NIL
Honorarium	25,000/-	25,000/-	NIL
Stationery	20,000/-	66,891/-	(46,891)
Contingency	55,000/-	83,656/-	(28,656)
Total	**6,00,000/-**	**5,92,541/-**	**7,459/-**
Total amount utilized	Rs. 5,92,541/- (Rupees Five Lacs Ninety Two Thousand Five Hundred Forty One Only)		
Amount remaining Rs. (In figures and words)	Rs. 7,459/- (Rupees Seven Thousand Four Hundred Fifty Nine Only)		

Certified that out of **Rs. 6,00,000/- (Rupees Six Lacs Only)** sanctioned to Innovation Project Code **SHC-303, Rs. 5,92,541/- (Rupees Five Lacs Ninety Two Thousand Five Hundred Forty One Only)** has been utilized during the period of the project. The remaining amount **Rs. 7,459/- (Rupees Seven Thousand Four Hundred Fifty Nine Only)** and is being returned back to the University.

Note : Over expenditure under the head "Stationery and Contingency" has been met from unspent balance in Equipment/ Consumables and Travel with approval from the Innovation Desk.

1st Project Investigator **2nd Project Investigator** **3rd Project Investigator**

Principal

VIPUL DHAWAN & ASSOCIATES * Chartered Accountants * M. No. 518146 NEW DELHI

University of Delhi

RC/2015/9435 31 August, 2015

The Principal,
Shivaji College
Ring Road, Raja Garden,
New Delhi-27

Subject: - **Innovation Projects 2015-16**

Dear Principal,

The University of Delhi is pleased to announce the third round of the undergraduate research initiative in colleges, Innovation Projects 2015-16. You will be glad to know that the following project submitted by your college has been selected for award

Project Code: SHC 303
Project Title: Accessing Microbial Diversity Of Yamuna Water: A Step Towards Environmental Restoration

The distribution of grant under different budget heads as below:

Sr. No.	Budget Head	Amount
1.	Equipment/Consumables	Rs 3,25,000/-
2.	Stipends	Rs. 1,20,000/- (1000x10x12)
3.	Travel	Rs 55,000/-
4.	Honorarium	Rs 25,000/-
5.	Stationery/Printing	Rs 20,000/
6.	Contingency	Rs 55,000/-
	Total	Rs 600,000/-
Rs 6 lakhs (Rupees six lakhs only)		
Amount to be released in first phase by Finance Branch- Rs 450,000/		

Budget head No. 1 and half of the remaining grant will be released as the first instalment. The second and final instalment will be released after submission of half-yearly report (by 15 February 2016), satisfactory review and recommendation of release of the second instalment.
Please refer to the detailed guidelines for implementation of the project. Any queries may be addressed to- innovationprojects1516@gmail.com.

With best wishes,

Yours sincerely,

Prof. Malashri Lal