Mapping Microbial Diversity of the Holy Ganga

atya kapley

Chief Scientist,
Head, Environmental Biotechnology and Genomics Division
CSIR- National Environmental Engineering Research Institute (NEERI), Nagpur

‘Namami Gange’ to ‘Arth Ganga”: Building Commonomics

Water Resource Program’ at **GeoSmart India 2022** conference, a collaborative effort and Co-hosted by Indian Society Remote Sensing and Indian Society of Geomatics and Geospatial World, to be held on 16th of November in HICC Hyderabad. **Focusing on the integration of Geospatial technologies with Space, Drones, Iot/ IIoT, AI/M:, SAR, LiDAR and 3D Mapping**

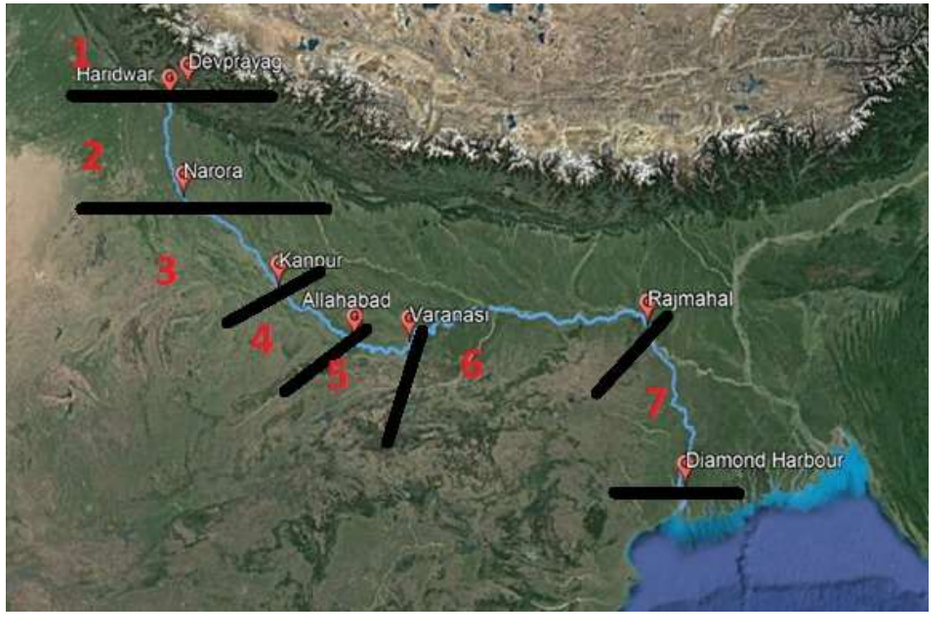
Microbes – The Unseen Warriors

1. They are indicators of contamination
2. They evolve with the surroundings and can become more pathogenic / multi-drug resistant (horizontal gene transfer)

1. They are the major force of bioremediation
2. They evolve with the surroundings and can biodegrade pollutants (horizontal gene transfer)
3. They are responsible for cycling of nutrients (bio-geo-chemical cycles)

GIS based mapping of microbial diversity across the Ganges for ecosystem services

National Mission for Clean Ganga



Project Team

CSIR-NEERI,
MNNIT,
Charotar University,
M/s PhiXgen,
M/s Xceleris

Target 6 major Aspects:

1. **Creating a baseline**
2. Ecosystem services of the river (nutrient recycling, fisheries etc)
3. Define health of the river/ rejuvenation efficiency
4. Threat to human health (antibiotic resistance surge)
5. Identification of the source of E. coli (Human v/s Animal)
6. Indicators of type of contamination (Sewage and Industrial)

River Ganga Sampling Details

Segments	Sampling Locations			
Segment I	Gangotri(Gan)	Devprayag (DP)	Rajaji National Park (RNP)	Haridwar (Har)
Segment II	Bijnor (Bij)		Hastinapur (Has)	Narora (Nar)
Segment III A	Badaun (Bad)		Kannauj (Kan)	Kanpur (Knp)
Segment III B	Fatehpur (Fat)		Prayagraj (P)	Prayagraj Sangam (PS)
Segment III C	Tela (Tel)		Mirzapur (Mir)	Varanasi (Var)
Segment IV A	Buxar(Bux)		Patna (Pat)	Rajmahal (R_M)
Segment IV B	Katwa (Kat)		Howrah(How)	Diamond Harbour (DH)

186 samples (biomat, sediment and water) in 3 replicates collected from 21 locations across 7 Segments of river Ganga were analysed using ShotGun Metagenomic Approach

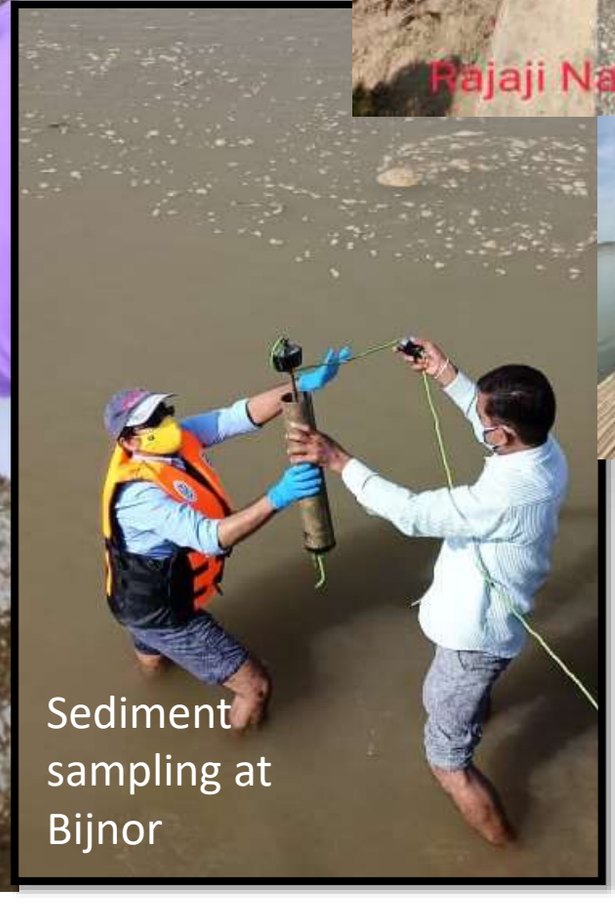
11 - States

2,500 km - Length from source to sea

500 million - People in Ganga Basin

520 persons/km^2 - Highest population density among all basins

26% - Of India's landmass



Katwa

Sampling

Gangotri

Devprayag

Physico-chemical parameters

Physical Parameters										
Sr. No	Segment	Sample Name	pH	Temp (°C)	DO (mg/L)	Time (Hr)	EC (uS/cm)	TDS (mg/L)	SS (mg/L)	Turbidity (NTU)
(1)	Segment (I)	Devprayag	7.3	19.7	8.54	12:40	120	48	3	1.88
(2)		Rajaji National Park	7.2	20.3	7.69	12:07	101	86	2	1.44
(3)		Haridwar	7.3	18.1	9.92	15:50	156	86	3	3.69

Inorganic Parameters												
Sr. No	Segment	Sample Name	Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Ca as Ca	Mg as Mg	Fluoride (F^-)	Na^+	K^+	Sulphate (SO_4^{2-})	Chloride (Cl^-)	Nitrate (NO_3) as N
(1)	Segment (I)	Devprayag	64	72	22.4	3.8	0.3	4	1.9	35.6	2	0.07
(2)		Rajaji National Park	60	100	27.2	7.7	0.25	9.6	2.5	30.3	4	0.01
(3)		Haridwar	62	96	27.2	6.7	0.24	5.6	2.4	14.2	12.9	0.01

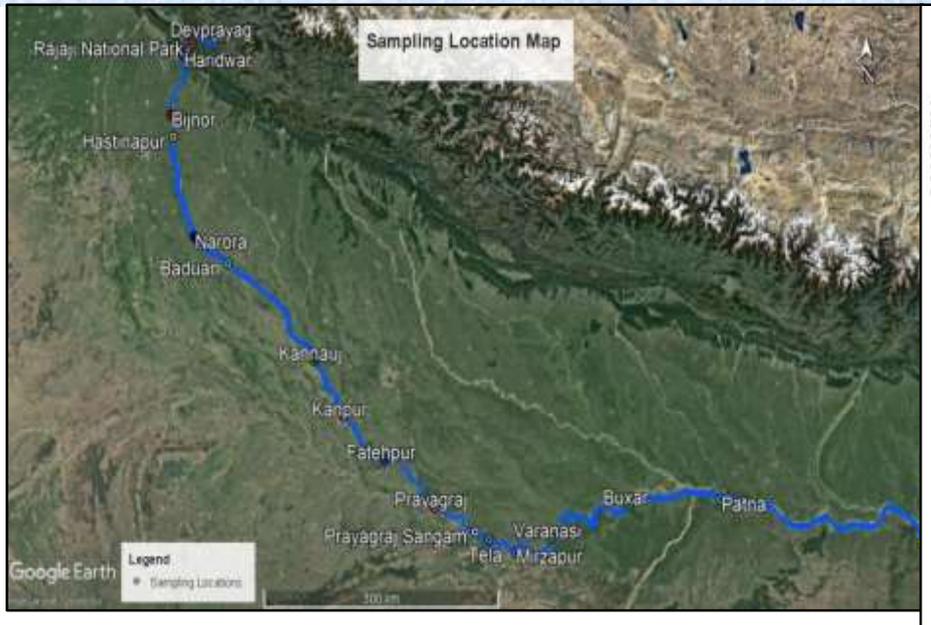
Organic & Nutrients Parameters								
Sr. No		Sample Name	COD	TOC	BOD	TKN as N	Total NH_3 as N	Phosphate as P
(1)	Segment (I)	Devprayag	20	12.4	2	3.6	2.2	1.0
(2)		Rajaji National Park	24	18	2	4.2	2.8	1.6
(3)		Haridwar	24	16.8	2	3.6	1.7	4.3

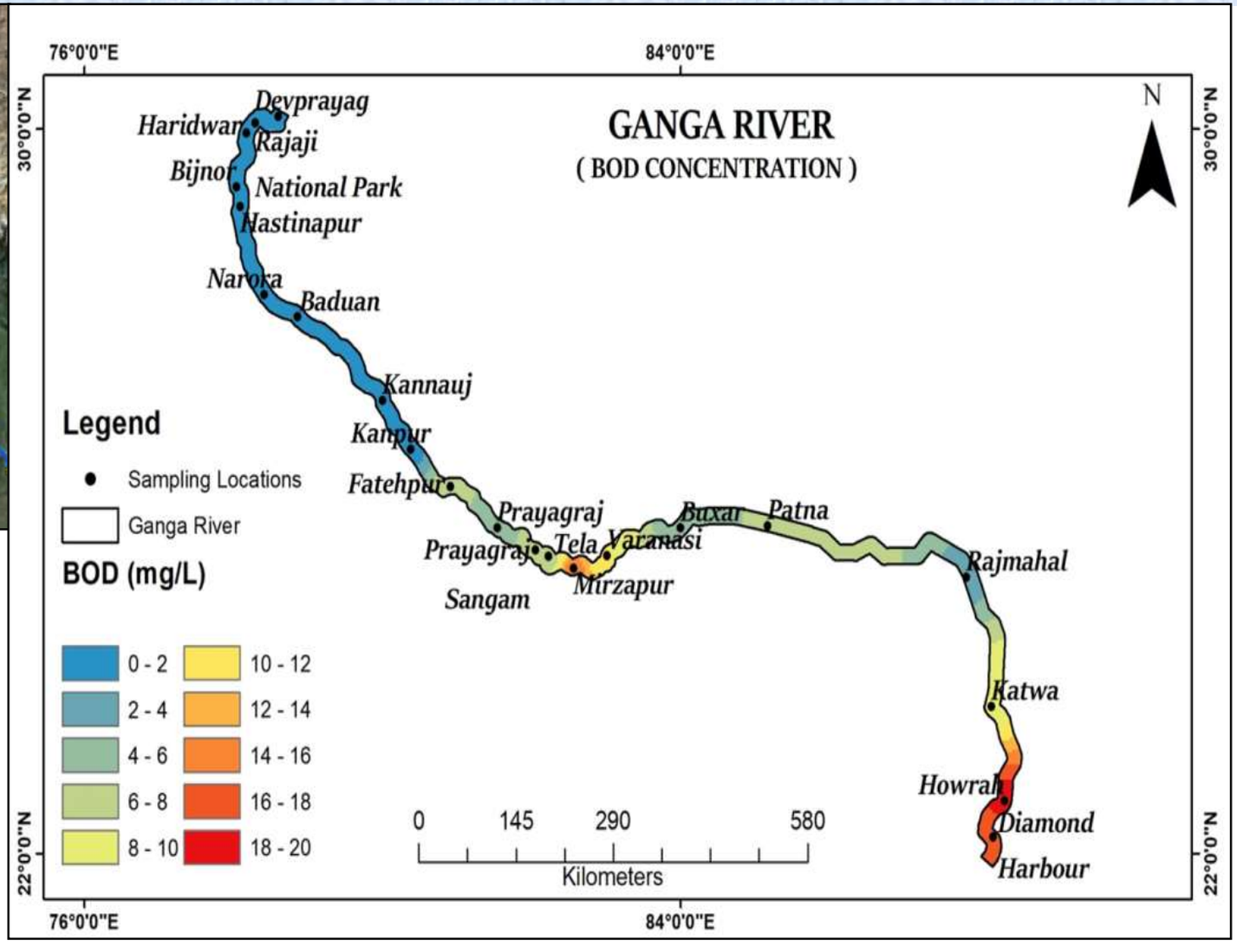
*All parameters in mg/L

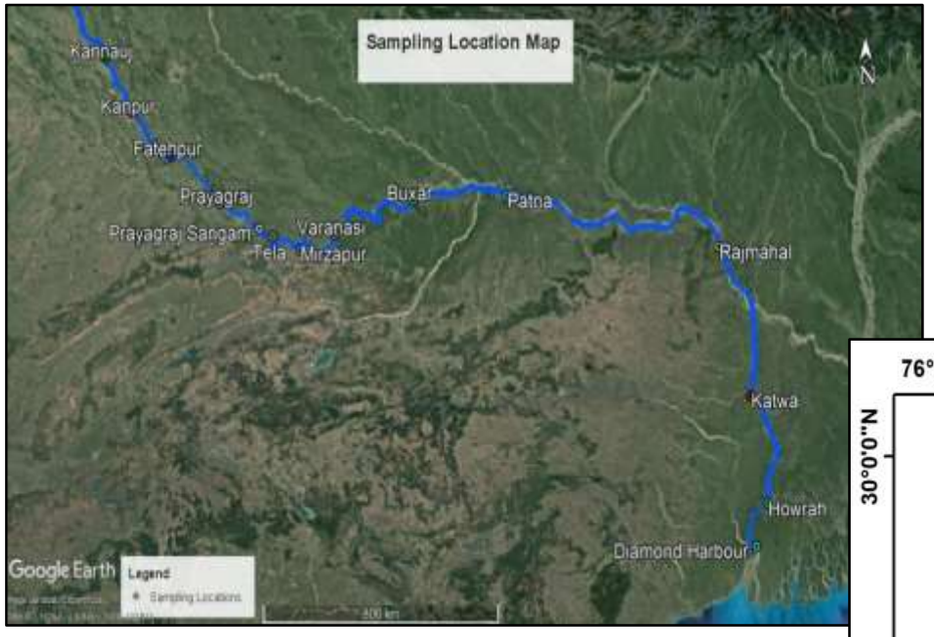
Heavy Metal Analysis													
SAMPLE		Al	B	Cd	Co	T. Cr	Cu	Fe	Mn	Ni	Pb	Zn	As
IS 10500:2012		0.03/0.2	0.5/1.0	0.003	-	0.05	0.05	0.3	0.1/0.3	0.02	0.01	5	0.01/0.05
Detection Limit		0.011	0.0015	0.0015	0.003	0.0015	0.0034	0.0003	0.0008	0.0008	0.0067	0.0133	-
Devprayag	Segment (I)	BDL	0.0080	BDL	0.004	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0046
Rajaji National Park		BDL	0.0066	BDL	0.004	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0023
Haridwar		BDL	0.0148	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.015	0.0040
Detection Limit		0.009	0.0015	0.0006	0.003	0.0045	0.0004	0.0003	0.018	0.005	0.009	0.001	0.007

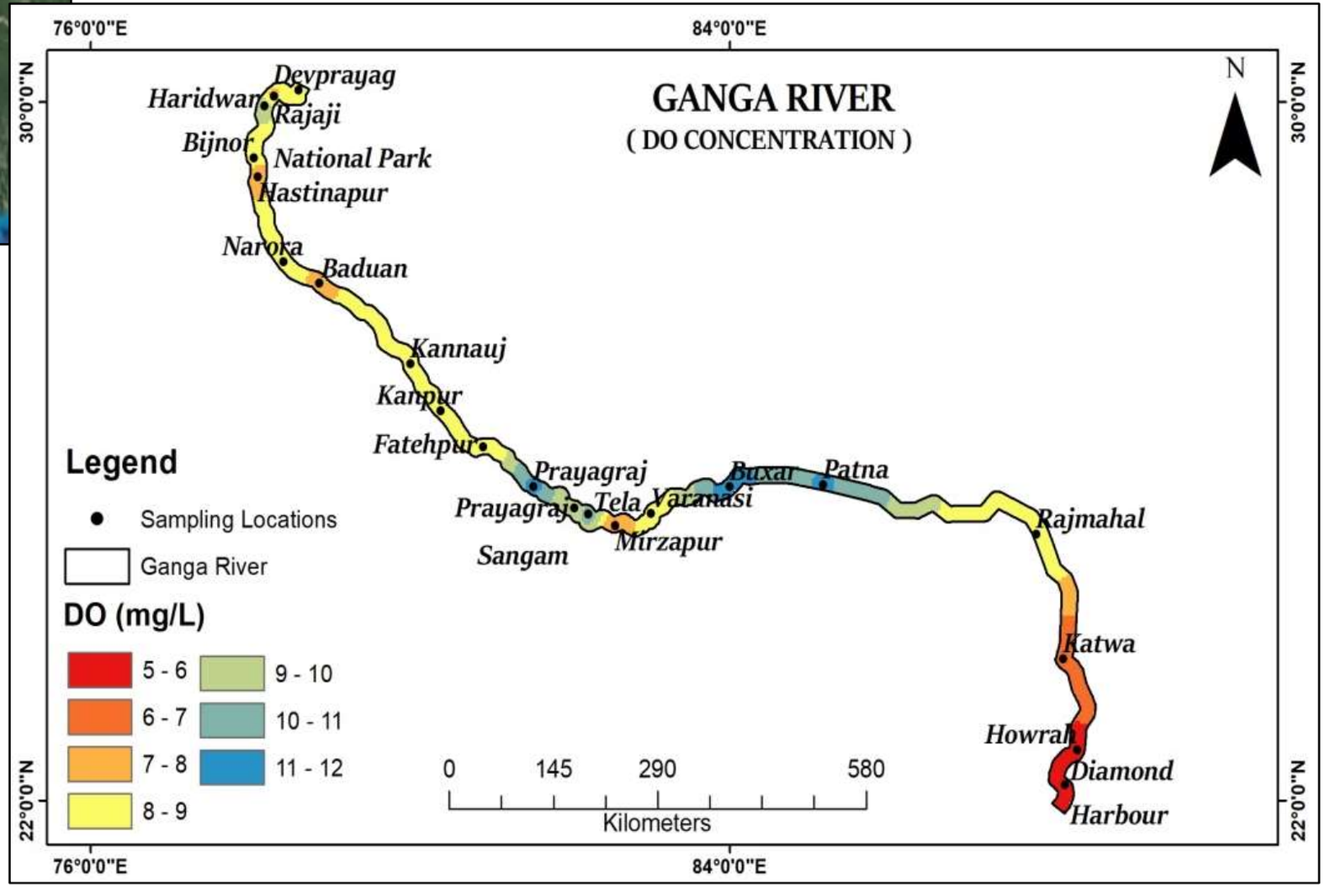
Designated-Best-Use	Class of water	Criteria
Drinking Water Source without conventional treatment but after disinfection	A	• Total Coliforms Organism MPN/100ml shall be 50 or less • pH between 6.5 and 8.5 • Dissolved Oxygen 6mg/l or more • Biochemical Oxygen Demand 5 days 20C 2mg/l or less
Outdoor bathing (Organised)	B	• Total Coliforms Organism MPN/100ml shall be 500 or less pH between 6.5 and 8.5 Dissolved Oxygen 5mg/l or more • Biochemical Oxygen Demand 5 days 20C 3mg/l or less
Drinking water source after conventional treatment and disinfection	C	• Total Coliforms Organism MPN/100ml shall be 5000 or less pH between 6 to 9 Dissolved Oxygen 4mg/l or more • Biochemical Oxygen Demand 5 days 20C 3mg/l or less
Propagation of Wild life and Fisheries	D	• pH between 6.5 to 8.5 Dissolved Oxygen 4mg/l or more • Free Ammonia (as N) 1.2 mg/l or less
Irrigation, Industrial Cooling, Controlled Waste disposal	E	• pH betwwn 6.0 to 8.5 • Electrical Conductivity at 25C micro mhos/cm Max.2250 • Sodium absorption Ratio Max. 26 • Boron Max. 2mg/l
•	Below-E	Not Meeting A, B, C, D & E Criteria

GIS Based Mapping



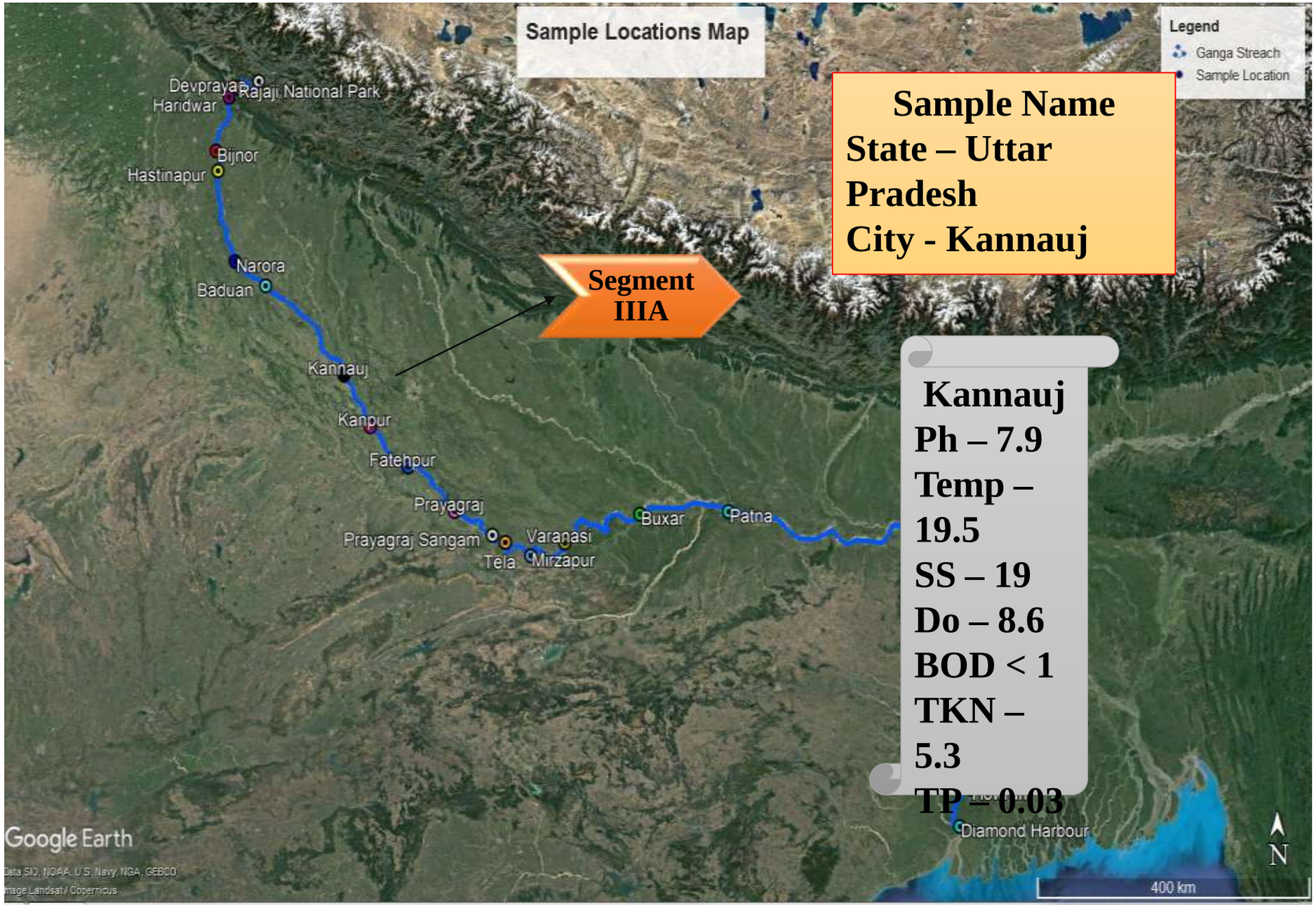



Sampling Location Map
Kannauj
Kanpur
Fatehpur
Prayagraj
Buxar
Patna
Prayagraj Sangam
Varanasi
Tela
Mirzapur
Rajmahal
Katwa
Howrah
Diamond Harbour
N
Google Earth
Legend
Sampling Locations


GANGA RIVER
(DO CONCENTRATION)
76°0'0"E
84°0'0"E
30°0'0"N
22°0'0"N
N
Devprayag
Haridwar
Rajaji
Bijnor
National Park
Hastinapur
Narora
Baduan
Kannauj
Kanpur
Fatehpur
Prayagraj
Prayagraj Sangam
Tela
Varanasi
Mirzapur
Buxar
Patna
Rajmahal
Katwa
Howrah
Diamond Harbour
Legend
Sampling Locations
Ganga River
DO (mg/L)
5 - 6
6 - 7
7 - 8
8 - 9
9 - 10
10 - 11
11 - 12
0
145
290
580
Kilometers

Active Link

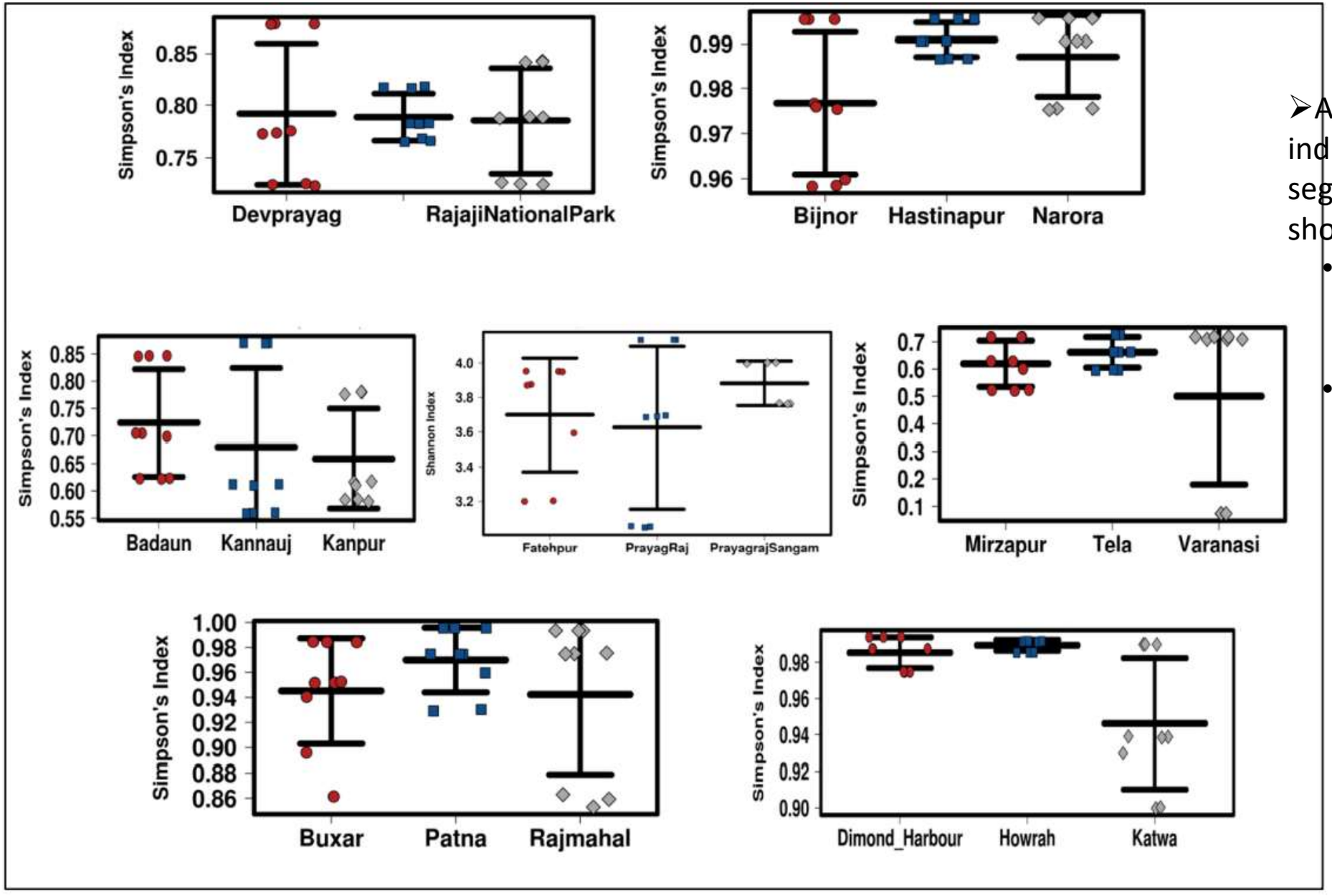
https://www.google.com/maps/d/u/0/edit?mid=1_gxTWHAuB_xSfORVsNL-wpu-H8gHlTRh&usp=sharing


Sample Locations Map
Legend
Ganga Streach
Sample Location
Sample Name
State – Uttar Pradesh
City - Kannauj
Segment IIIA
Devprayag
Haridwar
Rajaji National Park
Bijnor
Hastinapur
Narora
Baduan
Kannauj
Kanpur
Fatehpur
Prayagraj
Prayagraj Sangam
Tela
Varanasi
Mirzapur
Buxar
Patna
Diamond Harbour
Kannauj
Ph – 7.9
Temp – 19.5
SS – 19
Do – 8.6
BOD < 1
TKN – 5.3
TP – 0.03
Google Earth
N
400 km

Metagenomic tools to identify bacteria and other microbial inhabitants of the river

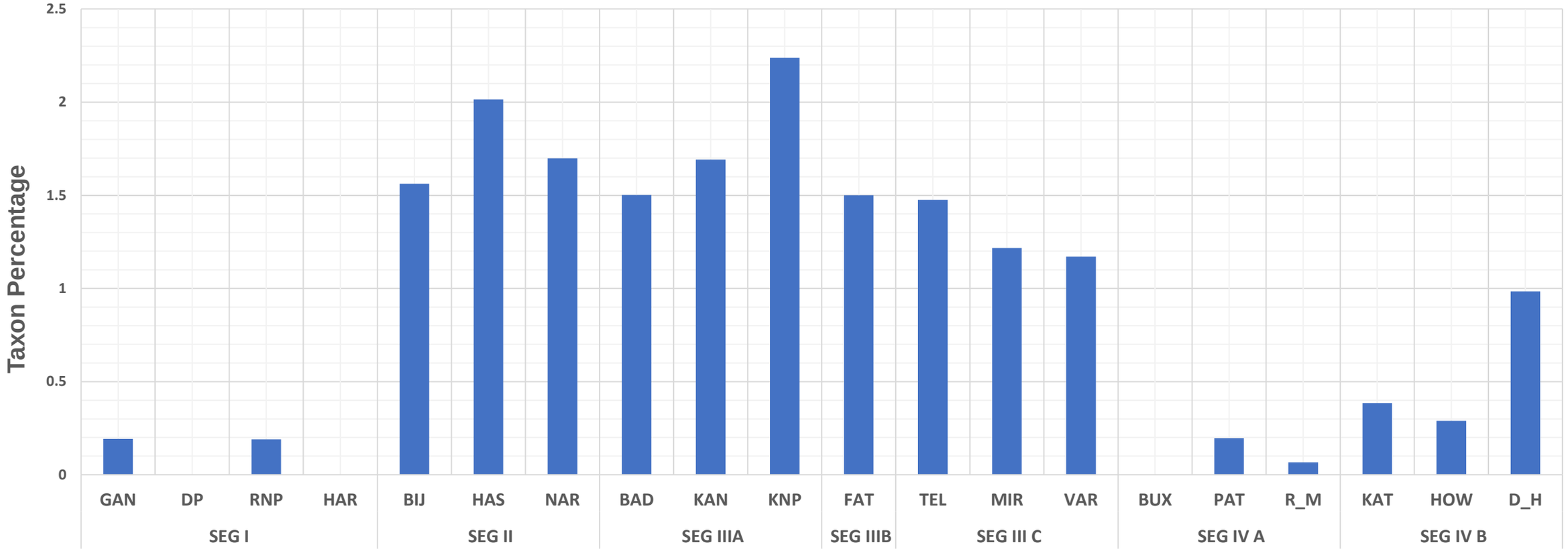
Segments	Location	Overall Abundant Diversity	Biomat	Sediment	Water
SEGMENT I	**Devprayag**	Proteobacteria	Bacteriodetes (17%), Verrucomicrobia (2%)	Fermicutes (1%), Actinobacteria (16%), Acidobacteria (2%), Chloroflexi(1%) , Planctomycetes (2%) and Nitrospinae (1%)	Planctomycetes (2%) and Nitrospinae (1%)
	Rajaji National Park	Proteobacteria (50-60%), Bacteriodetes (13%) Actinobacteria (1%Biomat 4%Sediment/Water),		Firmicutes (2%), Verrucomicrobia(1%), Planctomycetes(1%)	
	Haridwar	Proteobacteria (30-40%), Firmicutes (3%), Actinobacteria (26%Biomat, 7-9%Sediment andWater.), Planctomycetes (1%).	Verrucomicrobia(3%), Planctomycetes(1%)	Firmicutes (4%), Bacteriodetes (16%) and Nitrospinae (2%)	Bacteriodetes (16%) and Nitrospinae (2%)
SEGMENT II	**Bijnor**	Proteobacteria (40%), Fermicutes (2%), Verrucomicrobia(2%)	Bacteriodetes (17%)	Actinobacteria (6%), Acidobacteria (3%), Chloroflexi(2%), Planctomycetes (7%)	Planctomycetes (7%), Nitrospinae (1%)
	Hastinapur	Proteobacteria (30-40%), Bacteriodetes (6%) Actinobacteria (1%Biomat, Sediment, 4%-Water), Firmicutes (4%), Acidobacteria (1%)	Planctomycetes(1%)	Cyanobacteria (4%), Spirochaetes (1%)	Cyanobacteria (2%), Verrucomicrobia(1%), Euryarchaeota (3%)
	Narora	Proteobacteria (60%-Biomat; 30% Sediment/water), Verrucomicrobia(2%), Firmicutes (1%), Actinobacteria (26%-		Cynobacteria (1%), Gemmatimonadetes(1%), Bacteriodetes (5%), Planctomycetes(2%),	Bacteriodetes (5%), Planctomycetes(2%), Acidobacteria (2%) and Chloroflexi (2%)

Diversity Indices across River Ganga



- Alpha and beta diversity indices (Simpson) at both segment and location levels, showed:
  - Segment-IIIB had the highest inter-sample variations.
  - While location-wise samples variations analysis showed Fatehpur, Prayagraj, Kannauj, Katwa, Devprayag, rajmahal and Bijnor samples were highly heterogenous.

Identification of Escherichia coli Taxon in Biomat samples (Phase 2)

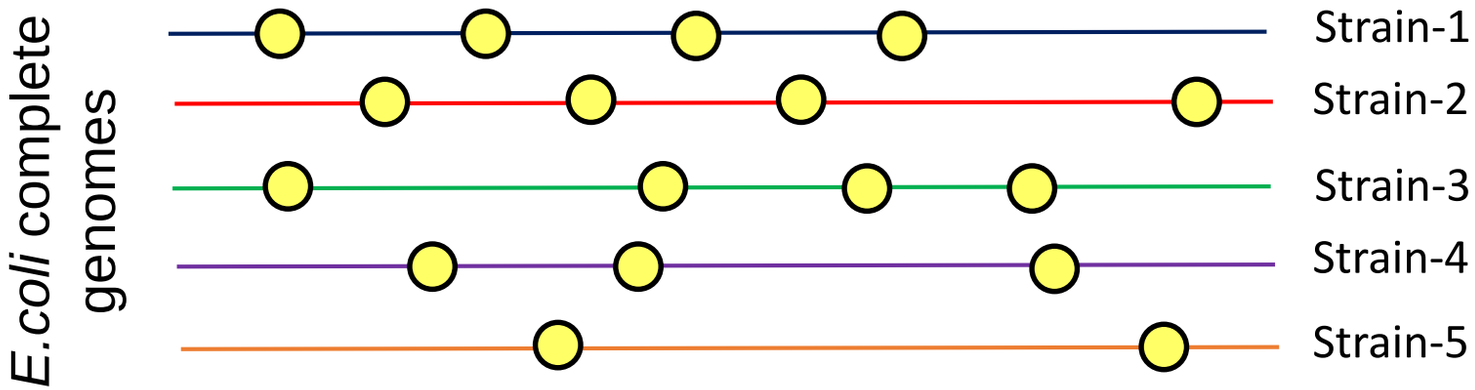


Based on taxonomic diversity, distribution (%) of Escherichia coli in Biomat samples across 21 location

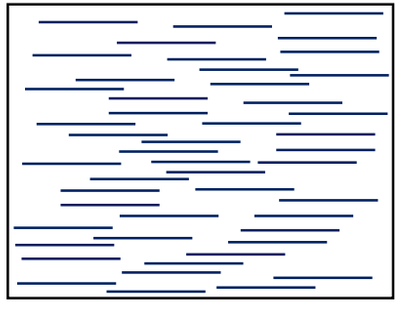
***Next: E.coli* Specific Analysis to be done**

Analyses overview

StrainFLAIR[1] was used for strain level analyses



Analyse genomic level variations across different strains



NMCG sample
E.coli specific reads

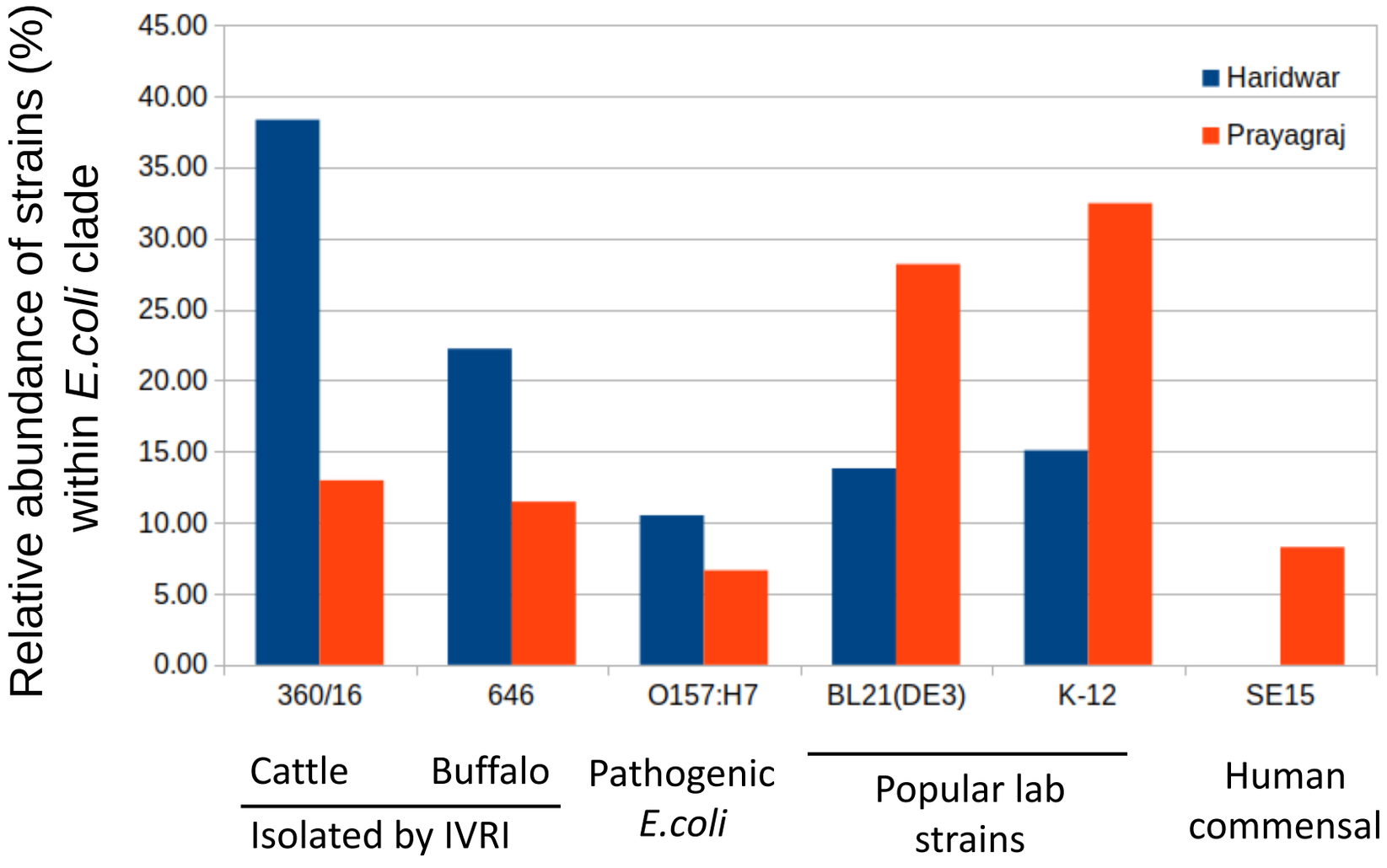
Variation graph construction

Strain-1: 27%
Strain-2: 12%
Strain-3: 5%
Strain-4: 39%
Strain-5: 17%

Abundance of
E.coli strains in sample

[1]Silva et al., 2021, PeerJ

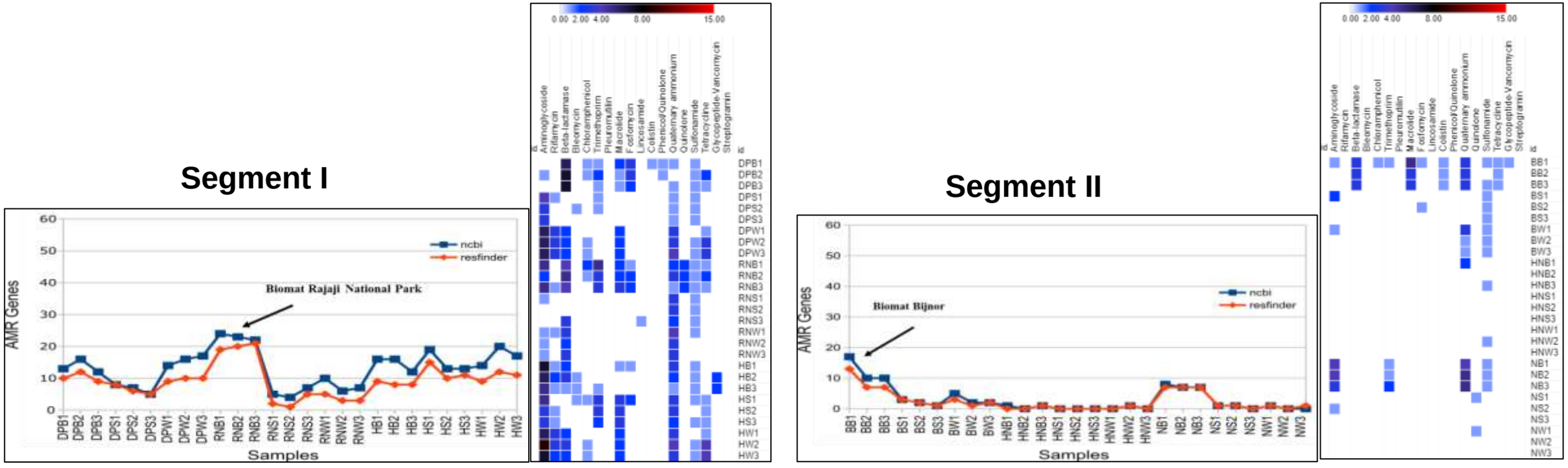
RESULTS



O157:H7 – Cattle is the major reservoir. Causes severe disease in humans

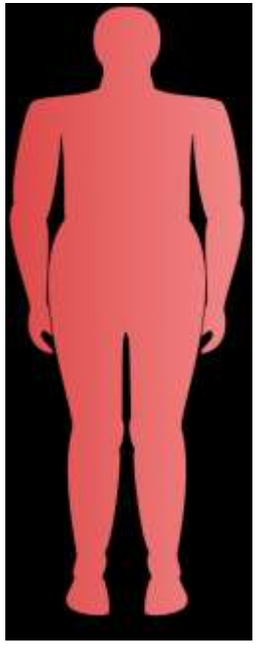
K-12 – Originally isolated from human

ARG Diversity across River Ganga



Number of AMR associated genes (y- axis) found in each sample (x-axis). (B) Heatmap showing the number of AMR associated genes determined in each sample for resistance to different classes of antibiotics.

- Overall, the genes for resistance against **18 classes of antibiotics** were determined in the samples.
- The most abundant genes were found for resistance against aminoglycoside (20.7%), beta-lactamase (15.9%), quaternary ammonium (13.0%), macrolide (10.9%), tetracycline (8.2%) and sulfonamide (7.2%) classes of antibiotics suggesting contamination of these antibiotics in the river Ganges.
- Not surprisingly, in majority of the locations, biomat samples harboured a relatively high number of AMR genes as compared to water or sediment samples.

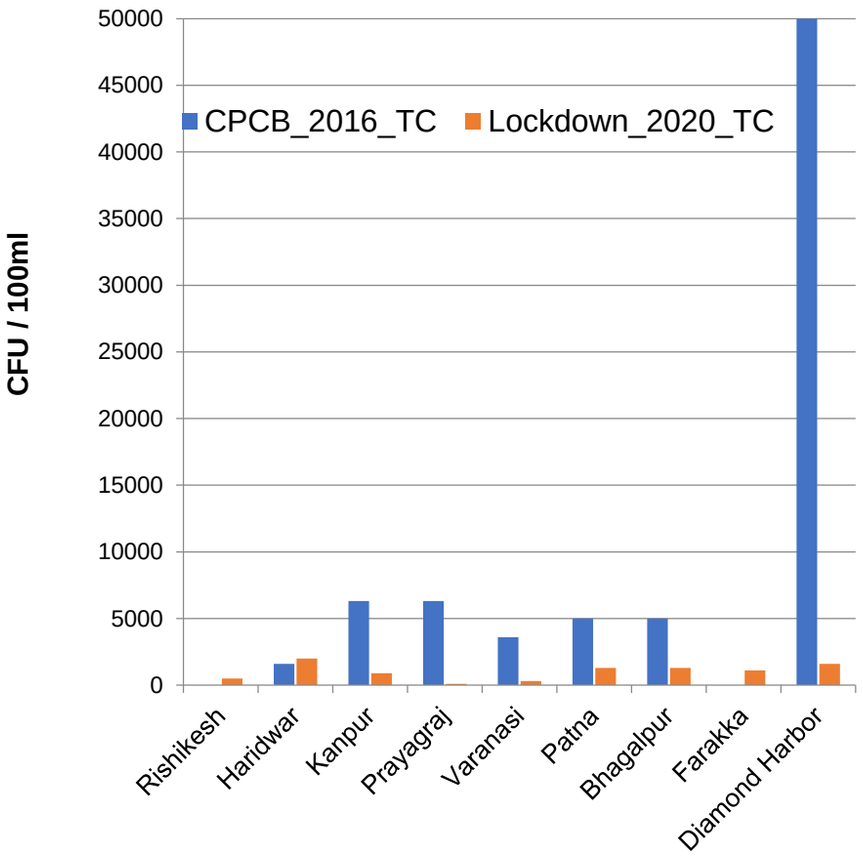


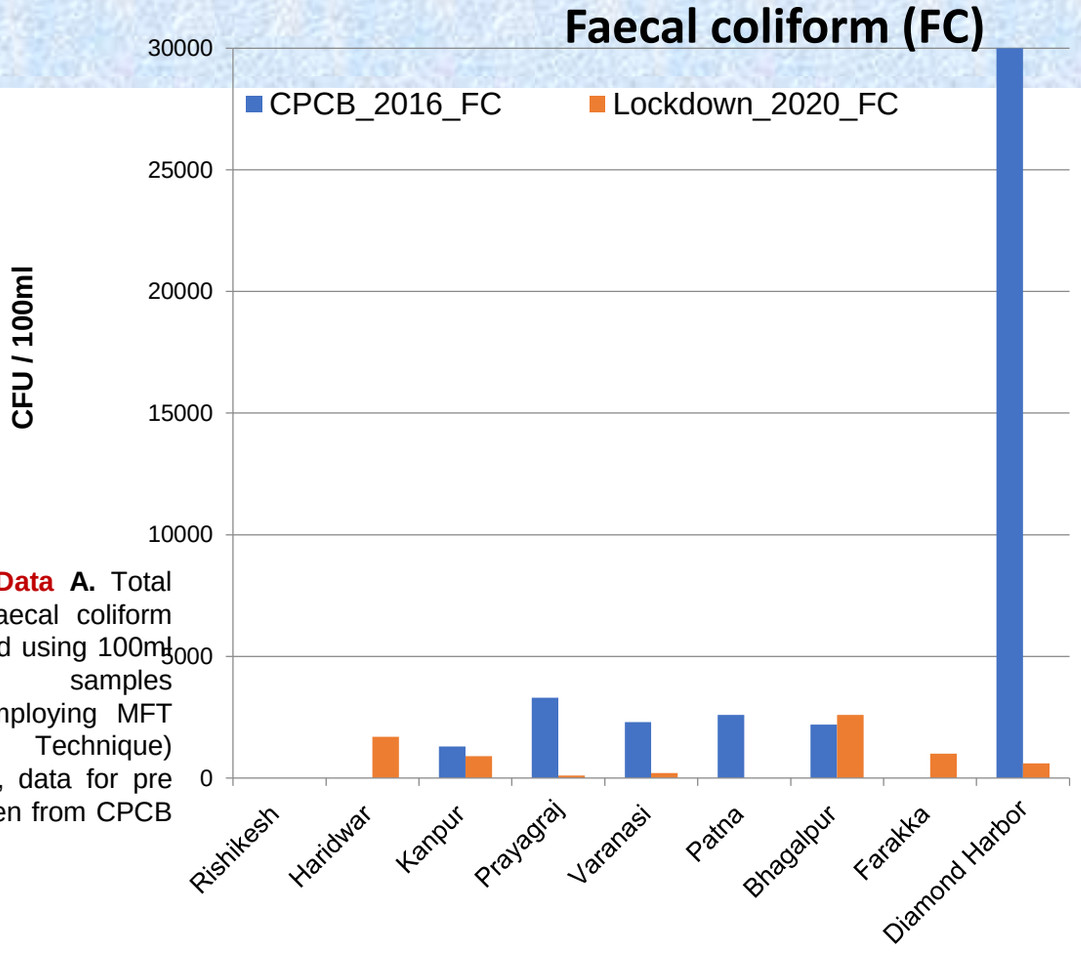
Anthropogenic Activities

Health Issues


HOTEL

Microbiological Parameters: Coliforms Analysis (Different Study)

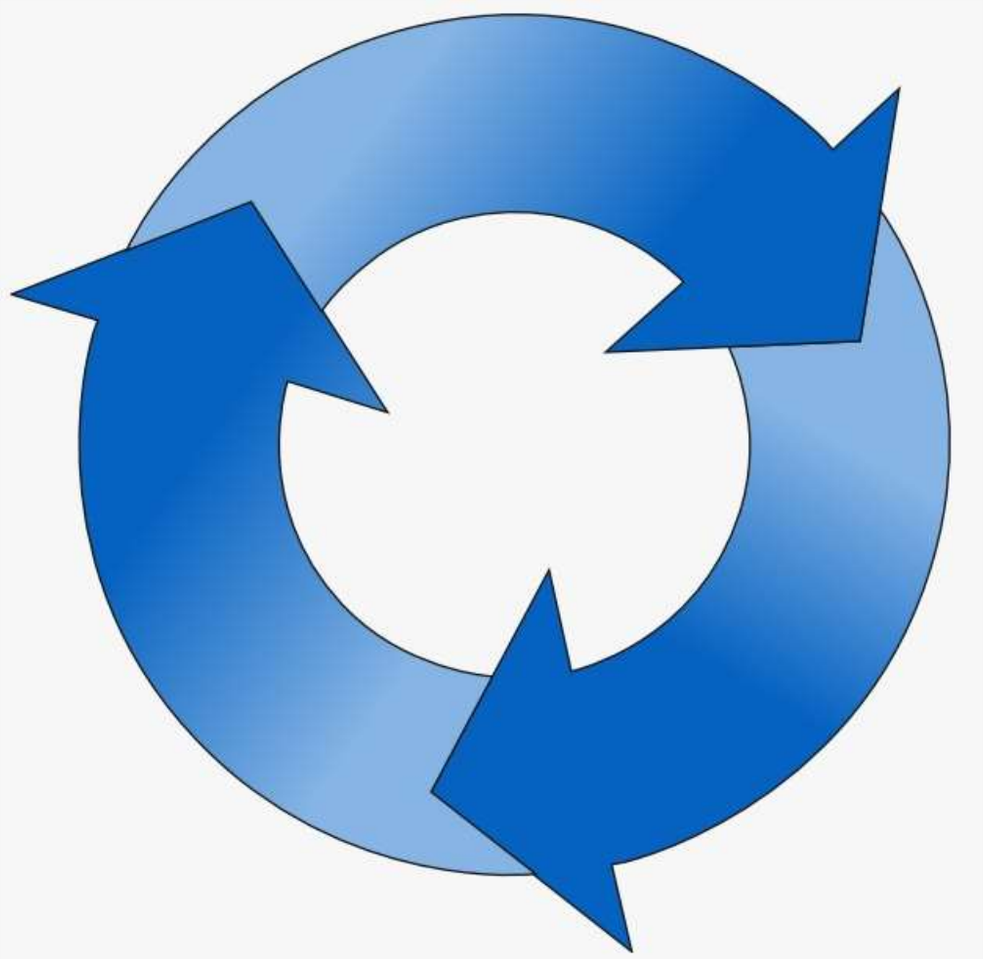




Comparative Coliform Data **A.** Total coliform (TC) and **B.** Faecal coliform (FC) count was performed using 100ml of Ganga water samples (2020_Lockdown) by employing MFT (Membrane Filtration Technique) method. For comparison, data for pre lockdown period was taken from CPCB 2016 water quality report.

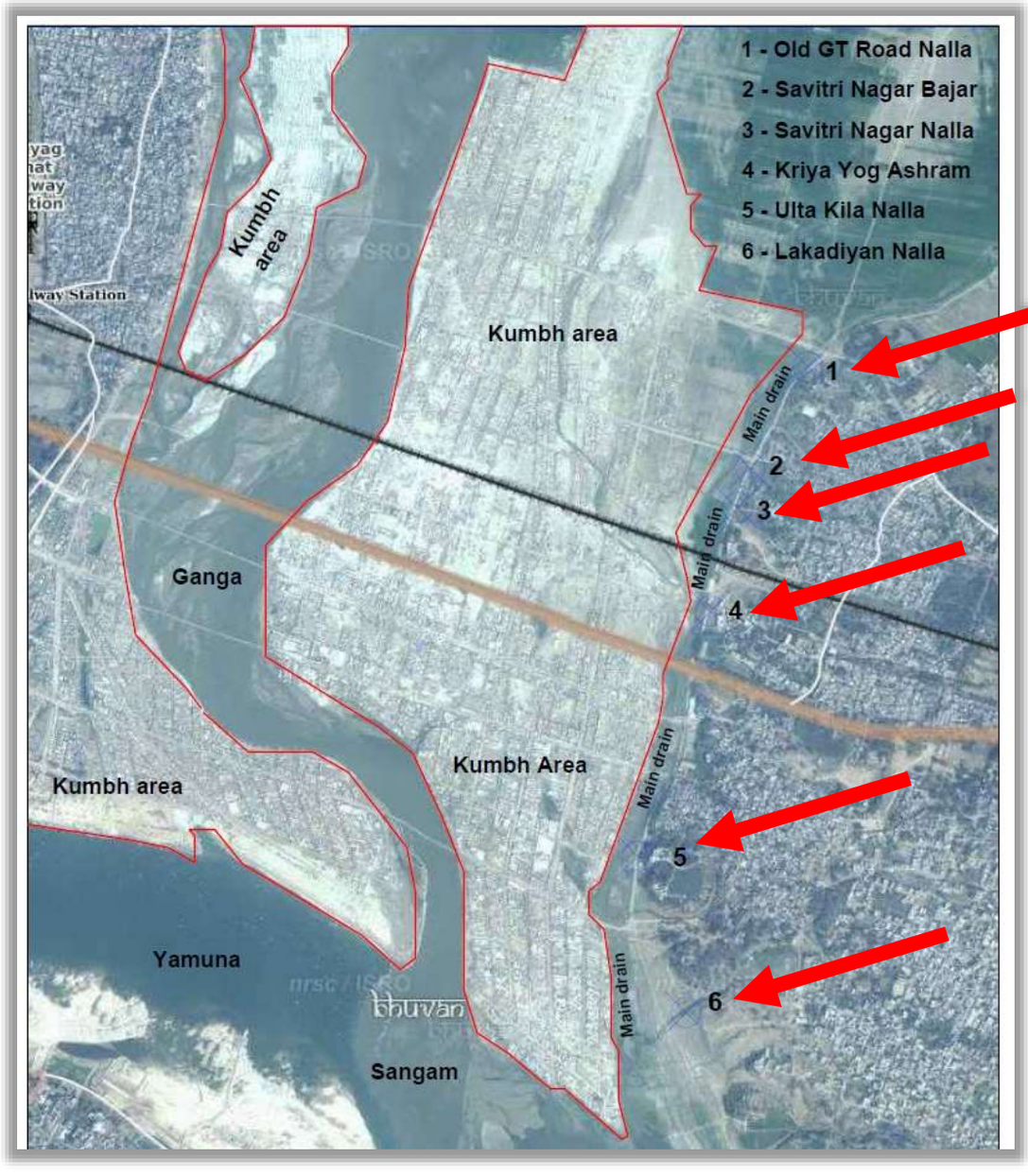
1. **For upper stretch cities not much impact was observed.**
2. **For mid and lower stretch cities decrease in total coliform and faecal coliform was observed, may be linked to decline in the anthropogenic activities at these region due to strict lockdown conditions.**

River restores itself



- Don’t exceed its carrying capacity
- Surveillance is paramount
- Community participation is a MUST

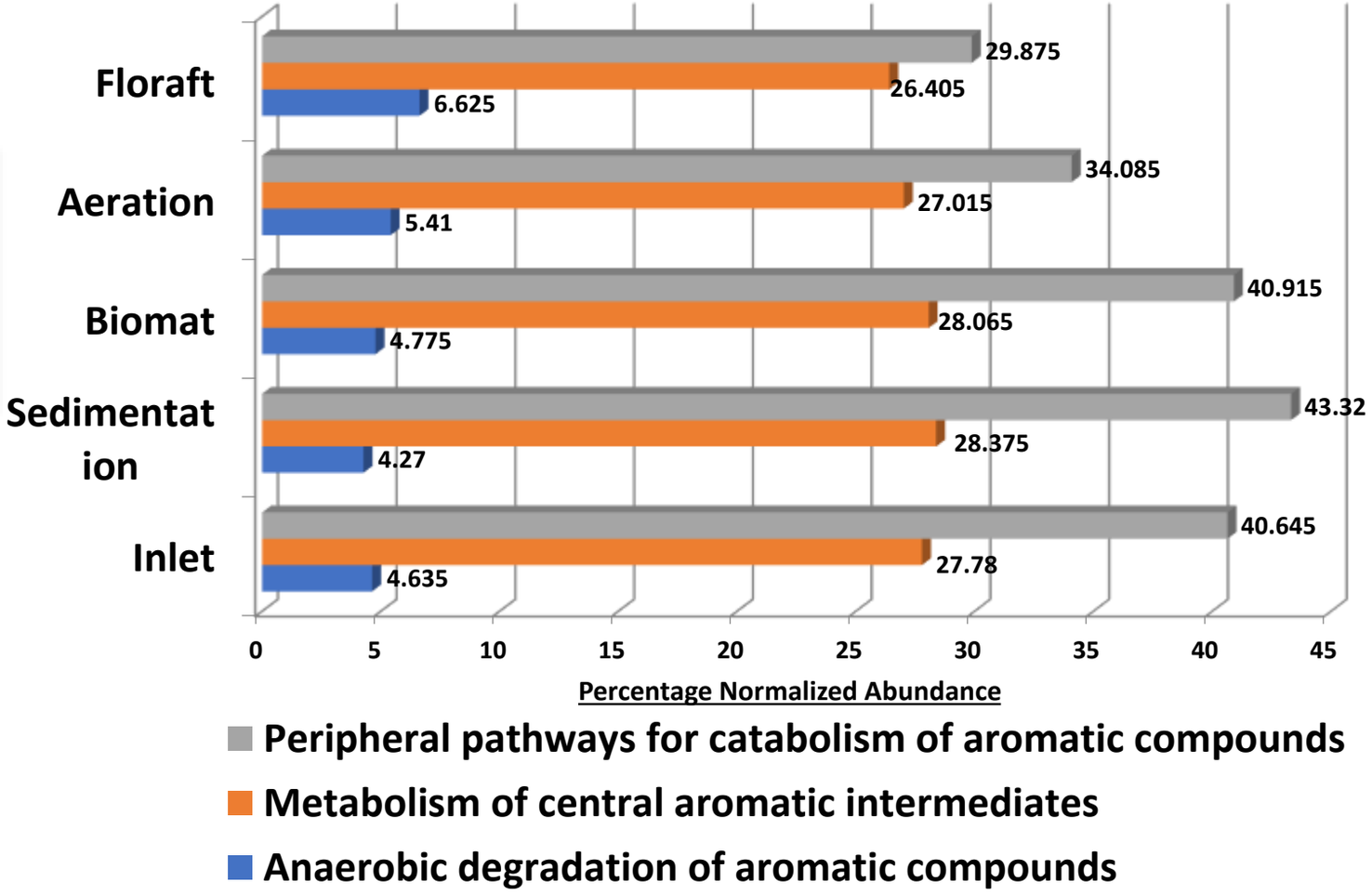
In-situ drain treatment By CSIR-NEERI



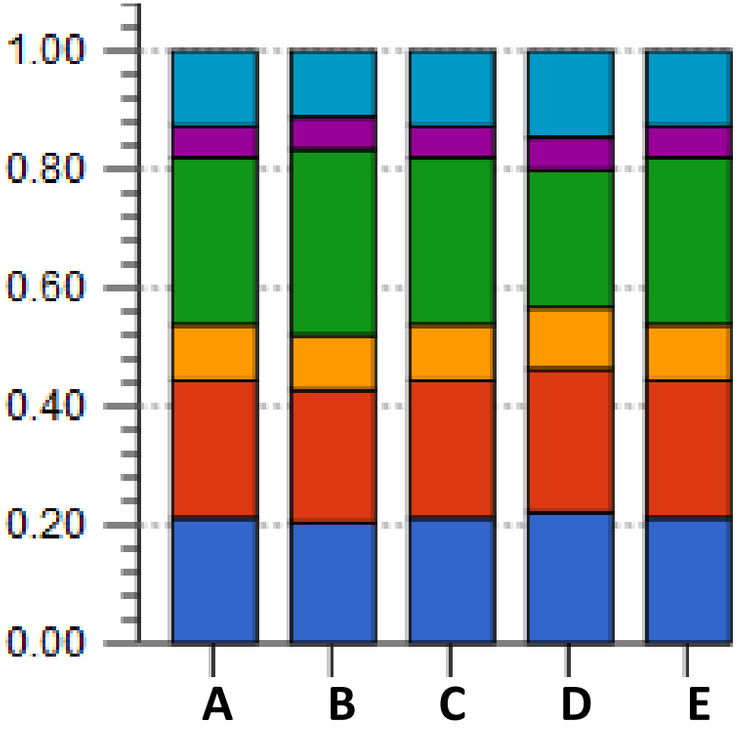
NMCG and SMCG, U.P. Project in readiness for the Kumbh Mela 2019

Metabolism of Aromatic Compounds

COD / BOD

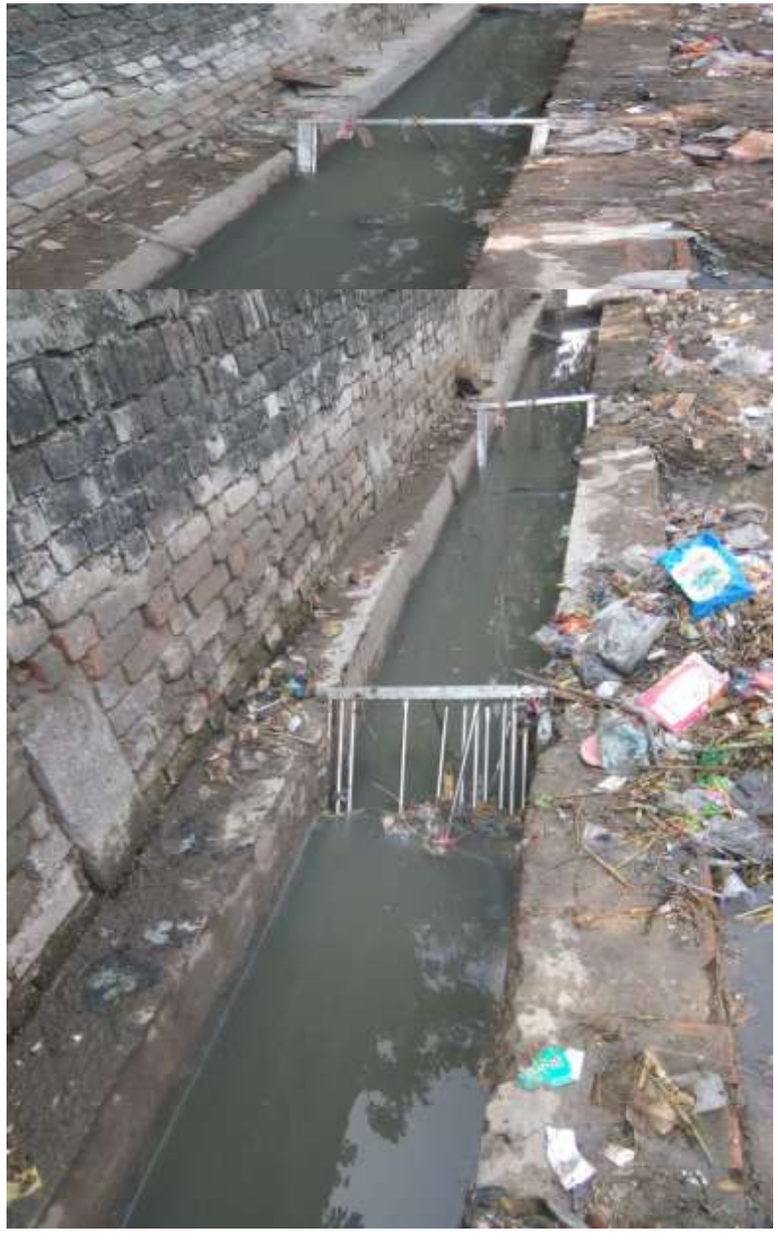


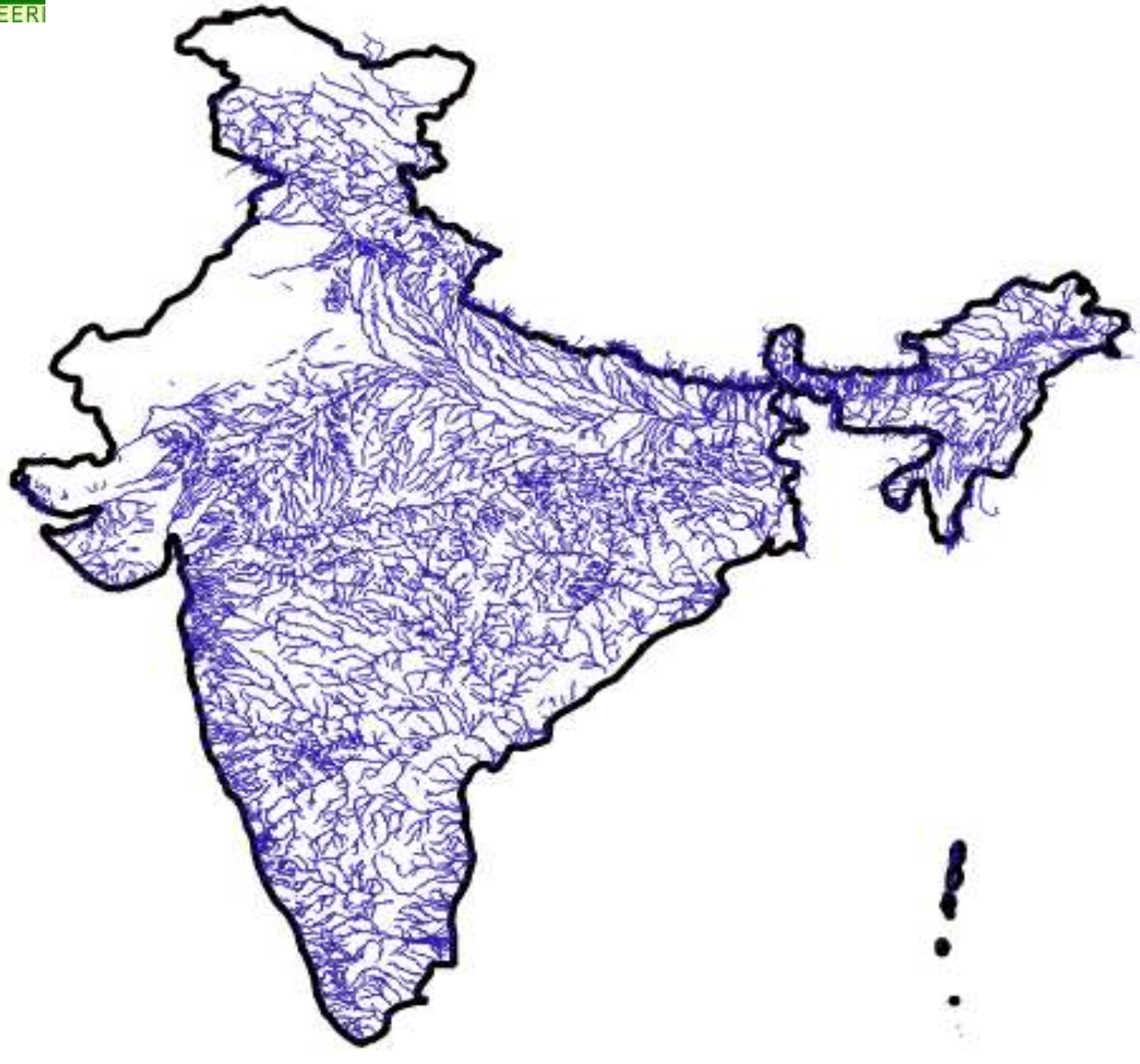
Multidrug Resistance



- Multidrug transporter MdtB
- Multidrug transporter MdtC
- Multidrug transporter MdtD
- Probable RND efflux membrane fusion protein
- Response regulator BaeR
- Sensory histidine kinase BaeS

A: Inlet
B:Sedimentation
C:Biomat
D:Aeration & Phyto-trap
E:Floraft





Surveillance of our rivers and tributaries

- Ground verification, esp of the sources of contamination
- Surveillance technology
- GIS based mapping

Thank You.