THESIS

on

ASSESSMENT OF EFFICACY OF NATURAL TREATMENT SYSTEM OF EAST KOLKATA WETLAND FOR TREATMENT OF MUNICIPAL SEWAGE OF KOLKATA CITY

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DECLARATION

This thesis titled "**Assessment of Efficacy of Natural Treatment System of East Kolkata Wetland for Treatment of Municipal Sewage of Kolkata City**" is prepared and submitted for the partial fulfillment of the continuous assessment of Master of Engineering in Civil Engineering at Jadavpur University for the session 2023 – 2024.

Date: 29.08.2024
Place: Civil Engineering Department
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CERTIFICATE OF RECOMMENDATION

I hereby approve this thesis report "**Assessment of Efficacy of Natural Treatment System of East Kolkata Wetland for Treatment of Municipal Sewage of Kolkata City**" is prepared and submitted for the partial fulfillment of the continuous assessment of Master of Engineering in Civil Engineering Course at Jadavpur University under my supervision and guidance by **Bodhayan Chakraborty** (Exam Roll No.- **M4CIV24015** and Registration No. – **163467 of 2022-2023**), it is also declared that no part of this thesis work has been presented or published elsewhere.

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After completion of the work and successful presentation, it may be accepted towards the partial fulfilment of the requirement for the award of the Master's Degree of Civil Engineering in Jadavpur University.

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TABLE OF CONTENTS

1 Introduction

1.1 General

East Kolkata Wetlands (EKW) known as the "kidney" of Kolkata naturally purifies the sewage water of the City and its surroundings through a resource recovery mechanism. EKW was included in the Ramsar list as a rare or unique wetland type under Criteria-01 of the Ramsar Convention on 19th August, 2002. Located between 22°25'N to 22°35'N and 88°20'E to 88°35'E, with the city of Kolkata on the west and River Kulti on the east, the EKW is a consortium of predominantly water dominated areas (used as fish farms) to land centric usages for agriculture, horticulture and settlements.

It is a world-renowned model of multiple use wetland complexes through resource recovery mechanism developed by local people with the passage of time. This wetland complex saves the city Kolkata from the huge costs of constructing and maintaining waste water treatment plants. It is a natural process for treating the city's waste water and utilizing the treated water for pisciculture and agriculture through the recovery of nutrients. As nutrient-rich effluent moves through the system, it is progressively cleaned, and nutrients are redirected to the growth of algae or agricultural products grown along the pond edges and agricultural lands. This wetlands complex naturally recycles nearly 910 Million Litres per day (MLD) of sewage water generated by Kolkata and its outskirts. Given that the core city area does not have a sewage treatment plant, EKW operates with minimal technology, purifies more than 80% of sewage, and has saved Kolkata Rs. 4680 million a year. (Dey D., Banerjee S., 2018).

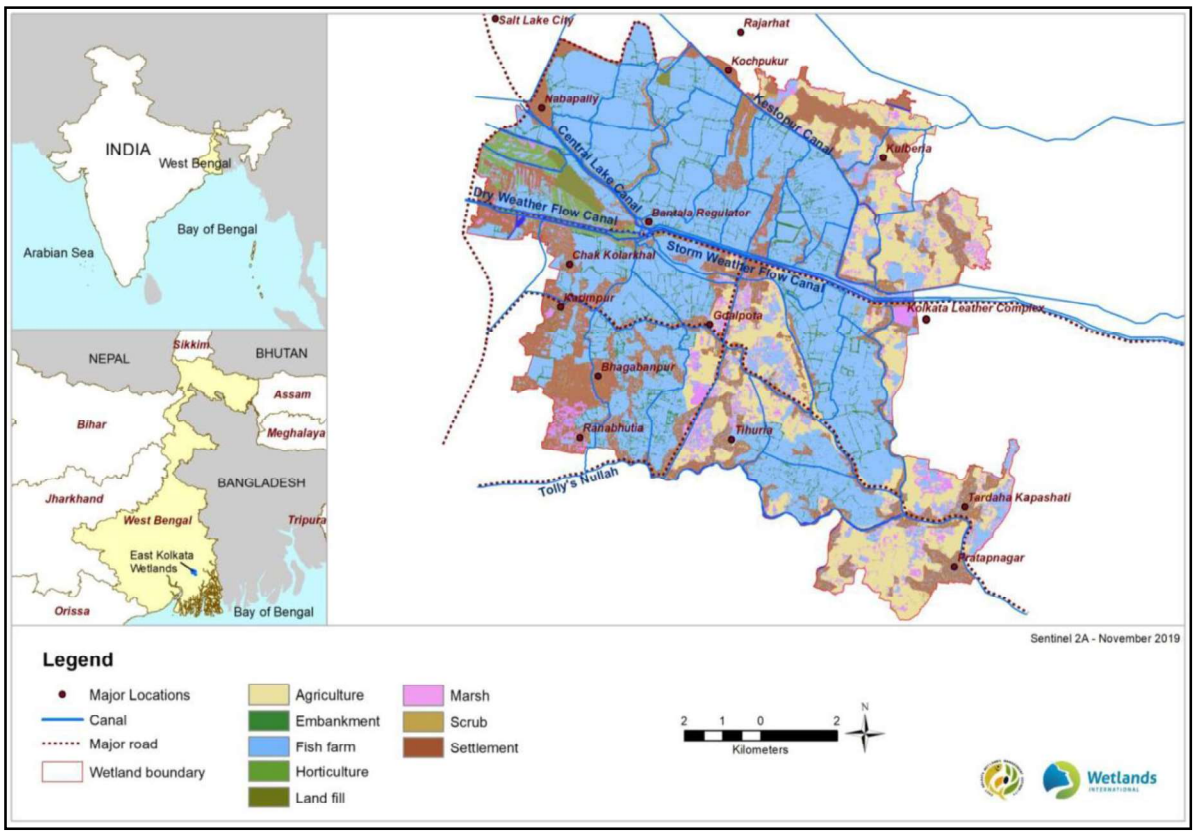

Fig.1.1: East Kolkata Wetland Map

Located to the eastern fringes of Kolkata City and spanning 12,500 Ha, the EKW lies between the river Hooghly on the West and the Kulti Ganga in the East. East Kolkata Wetlands (EKW) is a mosaic of landforms including predominantly water dominated areas (used as fish farms) to land centric usages for agriculture, horticulture and settlements. The existing wetland regime is a remnant of series of brackish wetlands connected to the freshwater as well as marine environments of the Gangetic Delta and the Bay

of Bengal, in an ecological continuum with the Sundarbans. Approximate 234 shallow fish ponds in the EKW receive over 900 MLD pre-settled sewage from the Kolkata Metropolitan region through a network of locally excavated secondary and tertiary canals, which is used to produce annually 20,000 MT of fish, 50,000 MT of vegetables and irrigate 2850 ha of paddy lands. As the nutrient-rich effluent moves through the system, it is progressively cleaned, and nutrients are redirected to the growth of algae or agricultural products grown along the pond edges and agricultural lands. Algae and other aquatic plants are used to feed up to 17 species of fish cultured in these ponds, which in turn create nitrogen and phosphorus-rich water to irrigate the adjacent rice fields. The traditionally evolved natural water purification waste recovery practice saves the Kolkata City nearly Rs. 4,680 million annually in terms of the treatment cost of up to 65% of the City's sewage. These wetlands also lock in over 60% of carbon from wastewater (Sudin Pal, 2017), thus reducing harmful Green House Gas emissions from the region.

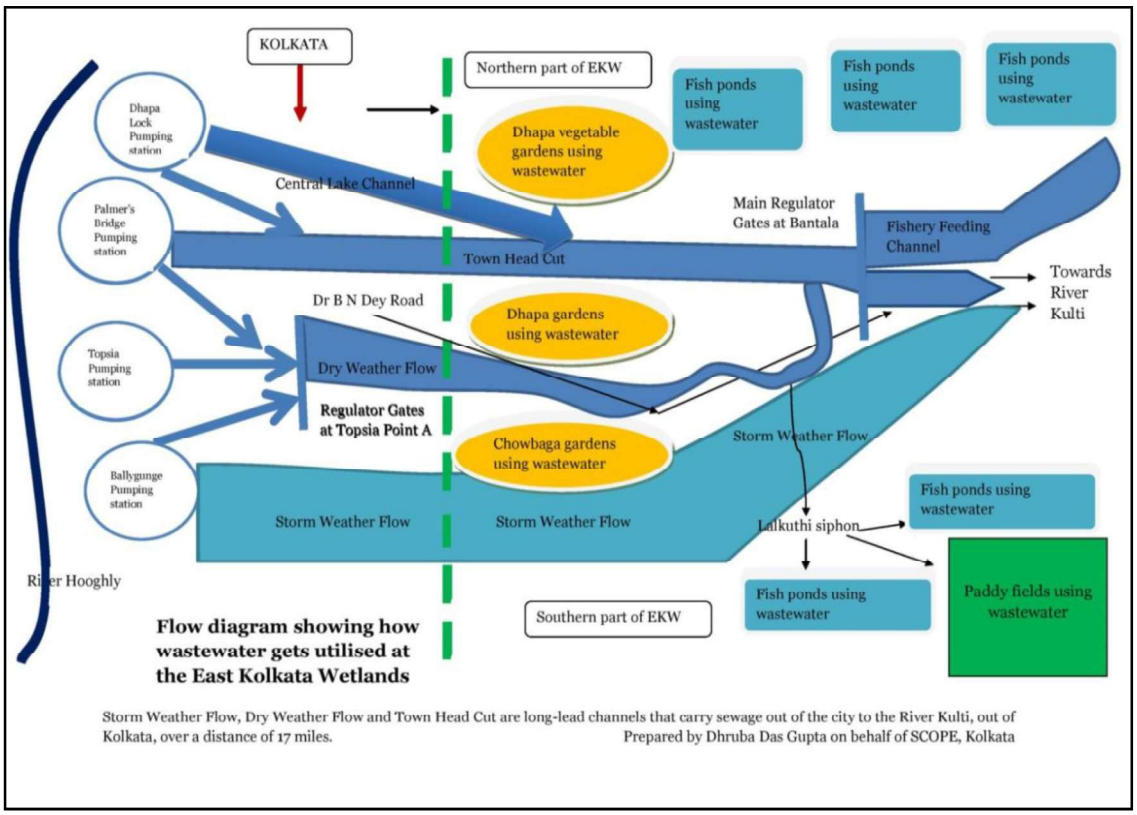

Fig.1.2 Treatment flow-diagram of East Kolkata Wetland resource recovery system

Article 3.1 of the Ramsar Convention commits the Contracting Parties to put in place management arrangements to ensure wise use of all wetlands within their jurisdiction. The text of the Ramsar Convention defines wise use as **"the maintenance of their ecological character, achieved through the implementation of ecosystem approaches, within the context of sustainable development".**

Wastewater of the city is discharged into the fish farms locally known as bheri. This wetland system exhibits immense potential in remediating the water quality by reducing the high amounts of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) it receives from the inflow on a daily basis. Each hectare of the shallow water body has the ability to remove 237 kg of BOD per day (Ghosh S., 2018). The bheri being shallow allows full vertical circulation of water to the surface where algal blooms occur. A solar radiation that is about 250 Langley's a day, allows sufficient photosynthesis which augments reoxygenation to allow for efficient BOD and pathogen/faecal Coliform reduction. Factors that play role in water purification are the shallow ponds acting as stabilization ponds, abundant water hyacinth that absorbs heavy metals, sun light penetrating to the bed of the water body and other microbial components that help in bioremediation (Raychaudhuri, 2008).

Chapter-2

2 Literature Review

2.1. Background

Through several DWF and SWF canals the sewage water flows into the Treatment Ponds (TP) of wetland for treatment purpose before disposal into Kulti river. The domestic sewage treatment process in single pond systems at EKW was developed by the local indigenous people through empirical observations by applying a **"Black-Box"** approach, over the preceding century and it involves a number of well-established phases,

i) The fishermen have constructed earthen barriers (with mud and clay) across the wetland area to separate the individual ponds. Inlets and outlets have also been constructed for each wetland for the inflow of untreated sewage (inlet) and outflow of treated water into outlet channels. Inlets and outlets have barriers made up of sustainable materials like bamboo sticks that regulate the flow of water into and out the individual wetlands.

ii) The first phase of sewage treatment is known as the Pond Preparation process. During this process, water from a particular single pond system is completely removed. Subsequently, the pond is air dried and then ploughed which leads to aeration of the soil. After that, the soil is treated with lime and left undisturbed for a span of few weeks.

iii) In the next step of treatment, untreated sewage from the city sewage canals is introduced into the single pond system. The pond is completely filled with the untreated sewage up to a depth of about 60-90 cm.

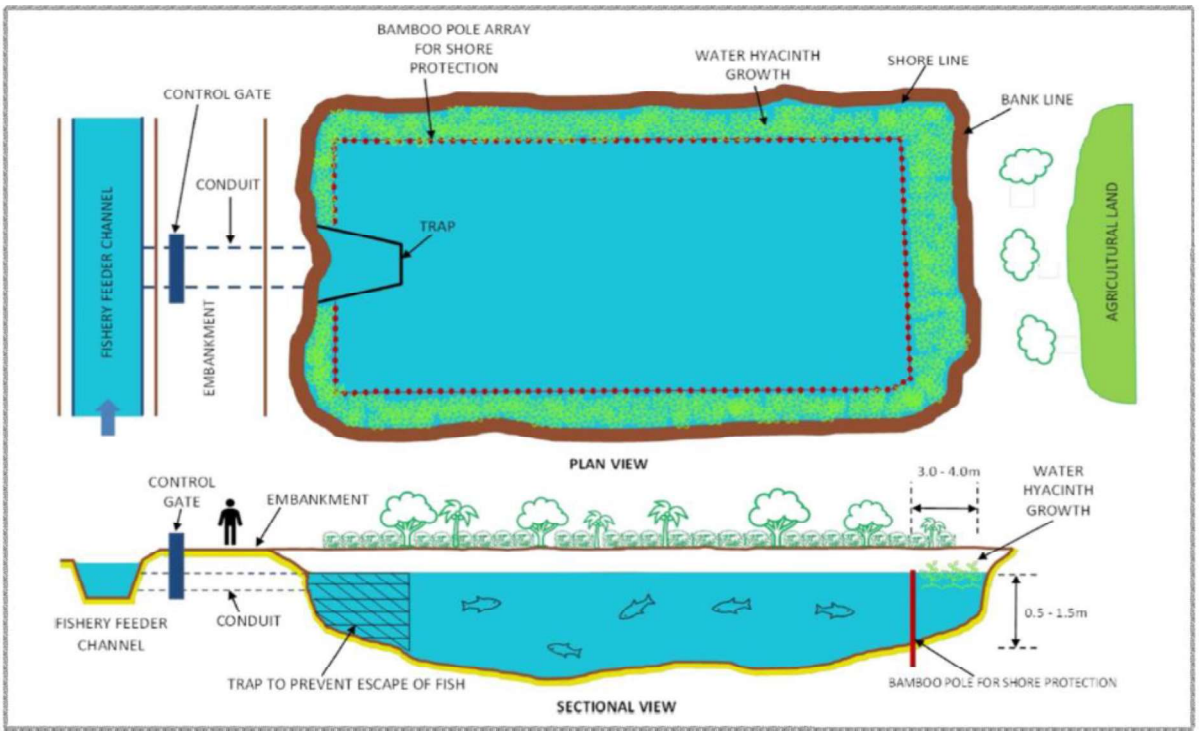

Fig. 2.1: A typical EKW fishpond showing the surrounding built and natural environment

Day 0 - The untreated sewage remains black in colour.

Day 3 to Day 4 - The colour turns green due to growth of photoautotroph like phytoplankton. These photoautotroph continue to grow.

Day 9 – The photoautotroph are removed by netting and increasingly the water turn clear within the following days. After that the water is utilized for large scale fishery and irrigation purposes

Fig. 2.2: Gradual changes of w/w colour at different time points during the purification process at EKW pond.

The primary surface water resource for Kolkata is the Hooghly River that skirts the western margin of Kolkata. In addition, the project area has a large number of water bodies and canals that are heavily used for everything: from bathing, washing, aquaculture and waste disposal. A large quantity of water is drawn from the Hooghly River for various uses and returns as wastewater to the river without little treatment. Industrial and domestic pollution along with runoff from adjoining areas has led to deterioration in river water quality. Summary chemical analysis Hooghly river water at Garden reach are given below in table.

Table 2.1: Water quality of Hooghly River at Garden Reach *(WBPCB, 2024)*

Sl. No.	Parameters	Unit	Test Results		
			21.02.2024	**11.03.2024**	**21.03.2024**
1	Temperature	°C	29	28	29
2	Conductivity	µs/cm	409.93	397.60	439.90
3	pH	-	7.35	7.08	7.21
4	DO	mg/L	7.81	6.00	5.65
5	BOD	mg/L	2.52	2.78	2.75
6	COD	mg/L	11.57	10.32	11.76
7	Nitrite-N	mg/L	0.05	0.05	0.16
8	Nitrate-N	mg/L	0.47	1.17	0.56
9	Phosphate-P	mg/L	0.12	0.11	0.13
10	Fecal Coliform	MPN/100ml	79,000	58,000	79,000
11	Total Coliform	MPN/100ml	170,000	110,000	13,0000
12	Heavy Metals	mg/L	-	-	-
13	TSS	mg/L	42.00	508.00	104.00
14	TDS	mg/L	306.00	234.00	260.00

The waste and storm water of the KMC area is carried by a system of natural and man-made canal system as follows:

i) Bagjola Canal system – flowing in easterly direction
ii) Kestopur Canal system – flowing in southerly direction
iii) Beliaghata (Circular) Canal system
iv) Storm Water Flow (SWF) – Dry Weather Flow (DWF) canal system flowing in easterly direction towards East Kolkata Wetlands carrying the pumped storm and sewage water of Kolkata
v) Tolly's nala system
vi) Monikhali system
vii) Churial system

The drainage canals in the southern part of the city are Kalagachia, Suti, Churial, Monikhali, Begore, Keorapukur, Western channel joining Keorapukur, Rania, TP Main canal, Intercepting channel, Suti khal

(eastern part), different Lead canals to TP Main, Mundapara khal etc. Chemical analysis of water of Suti, TP Main, Churial, Kalagachia and Keorapukur canals shows the following characteristics mentioned in the table below, water of these canals does not meet the primary water quality criteria for even bathing water.

Table 2.2: Quality of canal water collected from five selected boroughs of KMC *(KEIIP, 2012)*

SI. No.	Parameters	Suti khal	TP Main canal	Churial canal	Kalagachia canal	Keorapukur canal
1	pH	7.27	7.10	7.23	7.35	7.12
2	TSS (mg/L)	34.00	38.50	30.00	34.00	32.50
3	TDS (mg/L)	1735.00	1580.00	741.00	725.00	650.00
4	DO (mg/L)	4.60	4.60	4.60	5.20	5.20
5	COD (mg/L)	56.64	50.00	109.92	82.44	67.96
6	$(BOD)_{3days,\ 27°C}$ (mg/L)	20.00	23.00	35.00	20.00	18.00
7	Chloride (mg/L)	487.93	450.00	131.87	123.08	138.00
8	Sulphate (mg/L)	14.35	20.00	12.00	23.52	26.50
9	Nitrate (mg/L)	23.50	20.00	25.00	18.50	19.00
10	Sodium (mg/L)	250.00	230.00	80.50	62.50	70.00
11	Potassium (mg/L)	20.00	18.50	20.00	15.00	18.50
12	Calcium (mg/L)	137.47	130.00	66.77	58.92	51.06
13	Magnesium (mg/L)	58.80	50.00	28.22	35.28	23.52
14	Phosphorus (mg/L)	2.66	2.50	8.54	7.53	4.50
15	Lead (mg/L)	<0.3	<0.3	<0.3	<0.3	<0.3
16	Cadmium (mg/L)	<0.04	<0.04	<0.04	<0.04	<0.04
17	Mercury (mg/L)	<0.9	<0.9	<0.9	<0.9	<0.9
18	Arsenic (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01
19	Trivalent Cr (mg/L)	<0.2	<0.2	<0.2	<0.2	<0.2
20	Hexavalent Cr (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1
21	Zinc (mg/L)	0.13	0.20	0.04	0.50	0.80
22	Phenolic Comp. (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1
23	Cyanide (mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05
24	NH_4 - Nitrogen (mg/L)	4.28	5.30	6.80	3.50	3.00
25	Kjeldahl Nitrogen (mg/L)	20.77	15.00	23.50	9.34	8.50
26	Total Nitrogen (mg/L)	29.50	23.80	35.00	15.80	15.00
27	Total Ammonia (mg/L)	5.17	6.41	8.22	4.23	3.63
28	Total Coliform (CFU/100)	4.2×10^3	3.6×10^3	4.5×10^3	3.5×10^3	3.2×10^3

Different models or designs of wastewater utility and their features,

i) Obstructed flow system - Wastewater directly received by fishes; land is not a constraint.
ii) Waste stabilization system - Wastewater stored in oxidation ponds in order to suspend organic load and growth of plankton followed by its release to aquaculture ponds.
iii) Flow-through system - Wastewater treated through series of ponds and then used for fishes.
iv) Dilution of wastewater - If the BOD level of wastewater is more than 50 mg/l, then it is necessary to dilute by adding freshwater.

v) Duckweed culture - In absence of sufficient quantity of water and land to reduce BOD level below 50 mg/l, 'duckweed culture model' is a good option. Duckweed helps in reducing the pollutant level in wastewater and also serves as food for fishes.

vi) Effective microorganism (EM)-based system - In extremely land scarce situation, waste water can be treated by microorganism-based system [consisting of lactic acid bacteria (aerobic and anaerobic), yeast and actinomycetes etc.

Farmers of EKW usually adopt a specific aquaculture practice using traditional knowledge by the way of their experiences which is as follows,

i) Obstructed flow-through system with multiple stocking and multiple harvesting.
ii) Water column – 0.6–0.8 m generally, except ponds de-silted when water column is more than 2 m.
iii) Wastewater intake – 50–200 Cum./ha./day (100–200 days/year) due to lack of wastewater.
iv) Desired inflow rate of wastewater is 150–200 m^3/ha/day (not 400 Cum/ha/day that may create eutrophication).
v) Added wastewater should not consume more than 1–2 mg/l of dissolved oxygen/ hr.
vi) Avoid hot and cloudy day for intake of wastewater.
vii) Transparency limit – 25 to 35 cm should be maintained.
viii) Algal count – at least 100 million cells/L.

Not only that aquaculture practice in EKW produces fish by utilizing organic-laden water, but also it renders unbelievable environmental benefits to the entire vicinity and its people; otherwise, it creates both aquatic and land pollution that could hamper normal health of residents. The summary of benefits is mentioned below:

i) Each hectare of shallow water body can remove about 237 kg of biochemical oxygen demand/day.
ii) Organic loading rate in fishery is 20–70 kg/ha/day. It is a source of nutrients for plankton production, and it reduces the siltation rate in river mouth.
iii) Support biodiversity.
iv) Ecological environmental value of EKW is around Rs.3030 million/year (for flood control, siltation, extensive food chain, livelihood support, carbon sequestration and sanitation).

Table 2.3: Physicochemical parameters of aquaculture ponds of East Kolkata Wetlands (EKW) (Ghosh S., 2018)

Parameter	Raw sewage to fishery	Sewage-fed culture water	Outlet water for agriculture	Effluent standard for inland surface water[a]
Temperature (°C)	32.0	29.27	29.0	40
pH	7.2	7.70	7.5	5.5-9.0
Transparency	Nil	15	10	10
Total dissolved solid (ppm)	675	455	130.0	2100.0
TSS (ppm)	211	123	65.0	100.00
BOD (ppm)	128.4	25.0	17.9	30.0
DO (ppm)	Nil	3.9	4.5	4.0
Alkalinity (ppm)	273.7	130.0	130.0	83.0
Phosphate (ppm)	2.94	0.8	0.8	0.2
Nitrate (ppm)	3.7	2.41	2.41	0.8
Free ammonia	40.0	1.04	0.5	1.2
Lead (as Pb) (ppm)	0.57	Trace 0.09	BDL	0.1
Cadmium (as Cd) (ppm)	0.32	Trace 0.12	BDL	2.0
Chromium (as Cr) (ppm)	5.80	Trace 0.08	BDL	2.0
Zinc (as Zn) (ppm)	0.56	Trace 0.44	BDL	5.0
Coliform (cfu/100 ml)	$10^{4.5}$	$10^{1.5}$	$10^{1.5}$	<5000 cfu/100 ml
Faecal coliform (cfu/100ml)	$10^{3.5}$	10^{1}	10^{1}	<5000 cfu/100 ml
Salmonella sp. (cfu/100 ml)	10^{5}	10^{1}	10^{1}	<5000 cfu/100 ml

[a. CPCB, Govt. of West Bengal,]

From the above table the raw sewage is heavily polluted and exceeds almost every physicochemical parameter. But compare to raw sewage outlet water is much purified, it is only high alkaline in nature and is polluted with nitrate.

In no government report there is any mention about exceeding presence of Heavy Metals in waste water, sediments and also in fish muscles, tree stems and grains. But many autonomous reports indicate exceeding presence of Heavy Metals. Micro-plastics (MPs) pollution is one of the leading environmental problems due to its potential hazardous effect on both terrestrial and aquatic biota. MPs have been extensively studied as a vector of toxic heavy metals (Xuan Guo, 2021).

Important physicochemical parameters of standard water quality index have been chosen to detect the eutrophic status of the selective water bodies namely; temperature, electrical conductivity (EC), turbidity, total alkalinity, pH, total hardness, total dissolved solids (TDS), dissolved oxygen (DO), inorganic nitrogen, total phosphorus, potassium, ammonium-nitrogen (NH_4^+-N), biological oxygen demand (BOD), Secchidisk depth, chlorophyll - a.

Table 2.4: Calculation of mean and coefficient of variation (CV) of important physicochemical parameters (selective wetlands of EKW) for pre and post-monsoon season, year (2017–2019), source (Biswajit Bera, 2021)

Physicochemical parameters	Pre-monsoon (2017)		Post monsoon (2017)		Pre-monsoon (2018)		Post monsoon (2018)		Pre-monsoon (2019)		Post monsoon (2019)	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Temperature	33.40	1.30	22.40	3.20	33.70	1.10	22.50	3.00	34.00	1.20	22.80	3.10
Turbidity	87.80	0.80	74.40	1.20	88.40	0.70	75.00	1.10	88.80	0.70	75.30	1.10
Total Dissolved Solids	774.70	4.20	316.70	5.30	783.50	4.10	325.70	4.70	790.90	4.40	334.40	4.50
Electrical Conductivity	1211.80	4.00	1113.30	5.30	1220.60	3.80	1120.60	5.20	1231.50	3.90	1129.80	5.30
Total Alkalinity	119.80	10.30	133.40	14.60	128.50	9.10	141.40	14.10	136.90	7.80	149.70	13.10
Total Hardness	296.40	13.90	224.30	6.30	304.40	13.40	233.10	5.60	313.60	13.20	240.80	5.60
Inorganic Nitrogen	0.40	78.60	0.40	59.30	0.50	77.30	0.40	54.80	0.60	82.00	0.50	76.30
Total Phosphorus	0.03	176.90	0.01	133.50	0.04	165.10	0.02	141.00	0.05	157.30	0.03	130.90
Potassium	22.90	4.00	18.70	4.90	23.40	3.40	19.50	4.00	23.90	3.20	20.10	4.20
Ammonium-Nitrogen	21.00	0.90	11.90	1.40	21.10	0.90	12.10	1.30	21.20	0.80	12.20	1.10
Dissolved Oxygen	5.50	22.70	10.00	9.10	5.00	23.10	9.40	10.70	4.40	28.40	8.80	13.00
BOD	6.80	48.90	5.60	56.70	7.20	44.70	6.20	53.30	7.90	42.30	6.70	49.70
Secchi Disk Transparency	4.30	41.40	3.70	45.40	4.00	42.50	3.50	47.10	3.80	45.80	3.30	49.30
Chlorophyll-a	16.00	125.70	16.40	122.50	16.60	118.40	17.60	115.50	17.20	118.10	18.30	111.50

The water samples of consecutive three years 2017, 2018 and 2019 (pre and post monsoon) have been obtained from selective 20 wetlands in EKW. The important physicochemical parameters have been considered to analyze the water quality of these wetlands. The results portray that the magnification of inorganic nitrogen, total phosphorus, potassium and ammonium-nitrogen accelerates the mechanism of eutrophication in the selective wetlands. The DO level has been diminished during 2019 while the BOD level and chlorophyll concentration have been accelerated amidst 2019.The results reveal that about 40% of wetlands come under eutrophic and hyper-eutrophic stages respectively due to higher accumulation of nitrogen and phosphorus contents in the wetlands from various non-point sources and these principal components stimulate the processes of eutrophication of the water bodies (Biswajit Bera, 2021). Some relevant mitigation measures have been recommended to protect the “kidney of Kolkata Metropolitan City.”

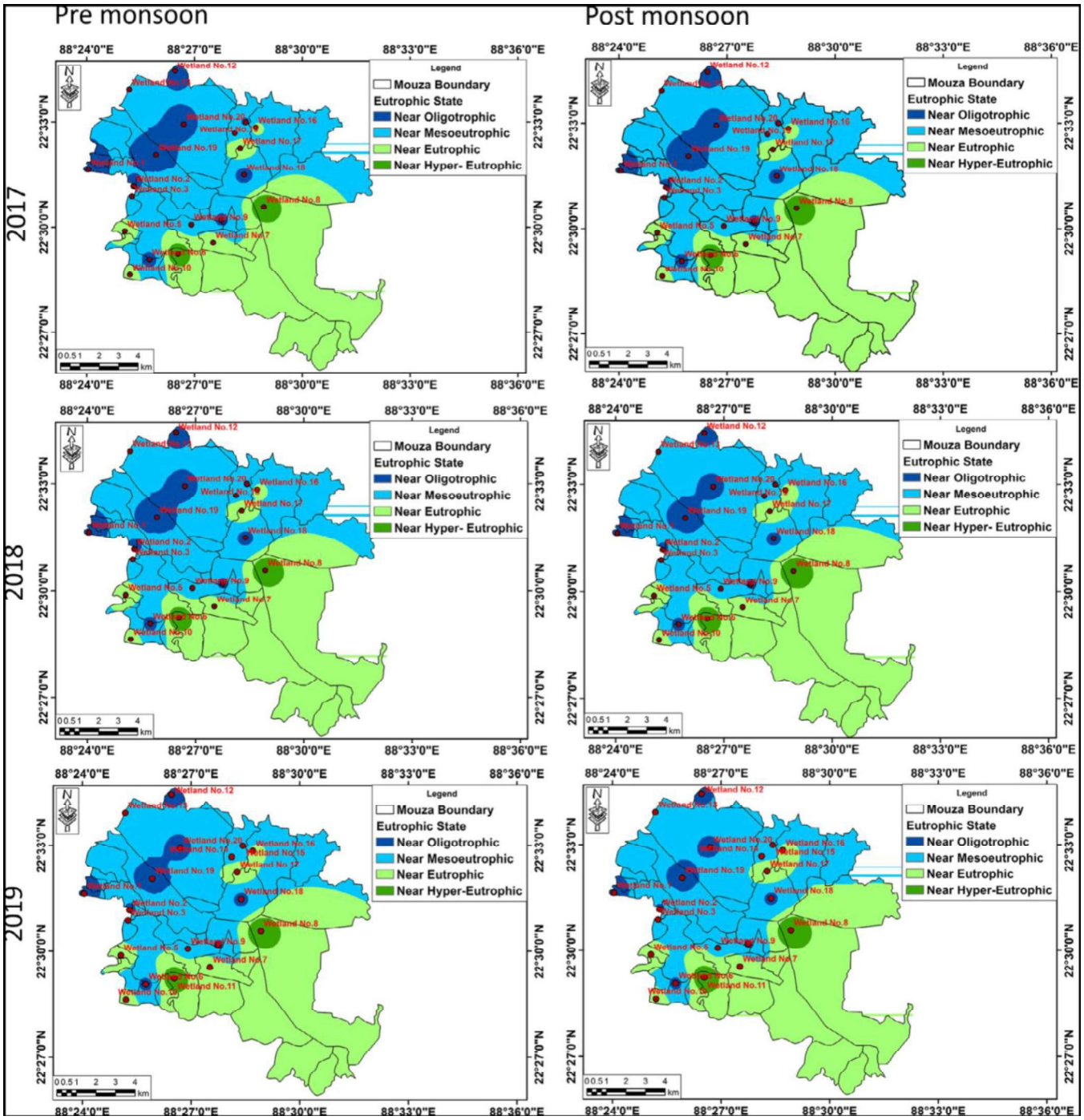

Fig: 2.3: Eutrophication index according to Carlson's TSI based on the analysis of Secchidisk transparency, total phosphorus and chlorophyll–a (Biswajit Bera, 2021)

Another serious pollutant toxic heavy metals concentration in fishes ring alarm bell with respect to ecological health of these wetlands. The persistence and the degree of heavy metal toxicity in ambient media (water and sediments) depends on a bouquet of factors such as water flow, water speed, frequency of tides, monsoonal run-off and same is true for EKW as well. The bulk of monsoonal run-off received by EKW from point and non-point sources is a main source of heavy metal load in the ambient media. High monsoonal run-off in consonance with lowering of pH levels lead to acidification. This increases dilution factor of toxic heavy metal in ambient media. The heavy metal locked up in sediment compartments undergo heavy dissolution in water and ultimately to the bodies of fishes and leads to bio-magnification.

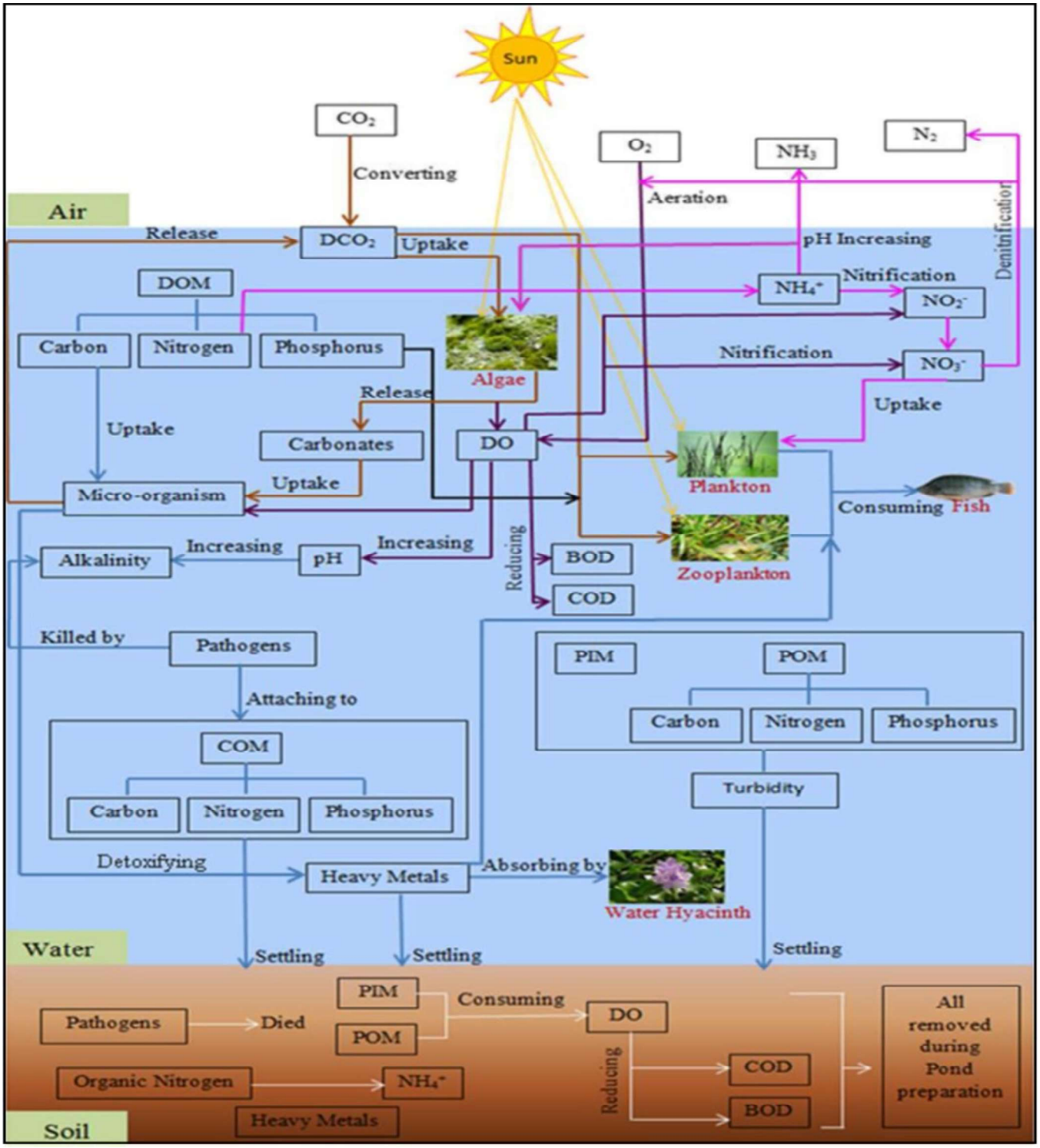

Fig: 2.4: Comprehensive interactions among water quality parameters in a typical fish pond of EKW (Anusha Nadella, 2021)

2.2. Related Works and their Findings

- **Literature review on** - Efficacy of Natural Treatment System of East Kolkata Wet Land for Municipal Sewage.
- **Period –** 2018-2022
- **Key Word –** East Kolkata Wetland, Biological Indicator, Natural Waste Water Treatment System, Fresh Water Wet Land, Physicochemical Parameters, Sewage Fed Fisheries, Green House Gas, Self Purification Capacity, Nutrient Removal.

Sl. No.	Title	Author	Methodology	Major Findings
1	Nutrient Removal Vis-à-vis Change in Partial Pressure of CO_2 During Post Monsoon Season in a Tropical Lentic and Lotic Aquatic Body: A Comparative Study	(Sourav Bhattacharyya A. C., 2018)	Surface water samples were collected from lotic estuarine system (Diamond harbour) and lentic aquaculture pond (EKW) on the same day of January (Post-monsoon) at 9:00am by two separate team. Two 300ltr. reservoirs made up of opaque polyvinyl chloride had 1 m depth were placed on the roof top which were filled up with waters collected from lentic and lotic system. Eight microcosm chambers, each of 6- litreand 1-control chamber (poisoning the chamber with 105µg $HgCl_2$ per ml of sample) transparent sealed polycarbonate bottles without any headspace were filled with the same surface waters of lotic and lentic system. These chambers were incubated from the day of sampling and kept floating 0.25m below the surface in the respective reservoirs under natural day-night cycle. A battery operated mechanized rotor was used only for lotic microcosm chambers, excluding the lentic microcosm twice a day (at 10a.m. and 8p.m. for 5 min) to avoid any biomass settlement at the bottom of the microcosm chambers. Initial data of all the parameters were measured within 6 h of collection of the water samples from both the locations. The microcosms of both lentic and lotic systems were sampled for eight consecutive days from the eight floating microcosm chambers. Experimental parameters: Salinity, conductivity, pH, temperature, DO, turbidity, underwater photosynthetically active radiation (PAR), water surface solar radiation, total alkalinity, Chlorophyll-a, NH_4–N, NO_2-N, NO_3-N, PO_4^{3-}, silicate and iron, gross primary productivity(GPP), community respiration (CR). Modelled parameters: The pCO_2 (water) and dissolved inorganic carbon (DIC) were computed using the data of measured salinity, water temperature, total alkalinity, pH, phosphate and silicate using CO_2SYS.EXE programme. Sulphate correction was applied for lotic system	According to the initial concentration of CO_2, lotic water was a substantial source of CO_2 and lentic water was a sink for CO_2. Efficiency of nutrient removal and pCO_2 reduction were significantly higher in lotic system (ΔpCO_2 - 90%) compared to lentic system (ΔpCO_2 - 78%). Dissolved NO_3–N followed by NH_4–N was the most utilized nutrients in both lentic and lotic water. Except silicate, other nutrients reduced to78–91% in lentic water and 84–99% in lotic water. Chlorophyll-a concentration steadily depleted in lentic samples during the experiment due to intense zooplankton grazing. On the contrary in lotic samples it increased rapidly with decreasing pCO_2 (water).

Sl. No.	Title	Author	Methodology	Major Findings
			Indexing: Net primary production, Trophic state index (TSI) for lentic system and trophic index (TRIX) for lotic system.	
2	Urban Wetlands CO_2 Sink or Source? A Case Study on The Aquaculture Ponds of East Kolkata Wetlands	(Protusha Biswas., 2018)	Field parameters: pH, conductivity, temperature, DO, underwater photosynthetically active radiation (PAR), CO_2 concentration in the ambient air was measured with a non-dispersive infrared (NDIR) sensor in association with air temperature, pressure and wind speed with a weather station. Laboratory parameters: Water samples were collected for three consecutive seasons in a year carefully with the help of 300ml BOD bottles and they were poisoned with saturated $HgCL_2$ solution and brought back to laboratory for analysis of total alkalinity. Modelled parameters: The partial pressure of carbon dioxide in water [pCO_2 (water)] and dissolved inorganic carbon (DIC) were computed from total alkalinity and pH using the software CO_2SYS.EXE. The measured mol fraction of CO_2 is converted to partial pressure of air [pCO_2 (air)] by using air temperature and pressure, and the Virial equation of state. Concentration of carbon dioxide in water and air were computed from pCO_2 water and pCO_2 air followed by CO_2 flux estimation of Air-water.	The EKW is acting as a CO_2 source in all the three seasons. The average CO_2 flux was4184 µmol m-2 h-1, 2897 µmol m-2 h-1 and 438µmol m-2 h-1 during pre-monsoon, monsoon and post-monsoon season respectively. Effective treatment of sewage water and avoiding wide spread eutrophication could lead to lowering of CO_2 effluxes or may turn it to a sink as well.
3	Characterizing nutrient dynamics with relation to changes in partial pressure of CO_2 in a tropical sewage-fed aquaculture pond situated in a Ramsar wetland	(Sourav Bhattacharyya S. H., 2019)	Surface water samples were collected from one of the aquaculture ponds of EKW around 9:00 a.m. in January (post-monsoon), in May (pre-monsoon) and in July (monsoon). Two reservoirs of 300 l with 1 m depth made up of opaque polyvinyl chloride were placed in the roof top and filled with EKW waters. Eight microcosm chambers (each being a 6-L transparent sealed polycarbonate bottle without any headspace) also filled with surface waters were incubated the same day and kept floating 0.25m below the surface in the respective reservoirs under natural day-night cycle. The bottles were tightly closed with plastic paraffin	A significant relationship between nutrient removal from and reduction in pCO_2 (water) was observed. These water bodies acted as significant sources of CO_2 in pre-monsoon and monsoon despite having substantial quantity of chlorophyll-a.

Sl. No.	Title	Author	Methodology	Major Findings
			film strips. One bottle was kept as a control by poisoning the microcosm chamber with 105µg mercuric chloride ($HgCl_2$) per ml. Each bottle was mixed gently every day with a magnetic stirrer for 5min at two particular times (at 9:00 a.m. and 9:00p.m.) in order to avoid the bottom settlement. The microcosms were sampled every day for eight consecutive days from the next day. Initially the parameters were measured within 6 hours of collecting the water samples from EKW. Experimental parameters: pH, water temperature, DO, underwater PAR, turbidity, incoming solar radiation, Chlorophyll-a, NO_3-N, NO_2-N, NH_4-N, phosphate, silicate, iron, total alkalinity, gross primary productivity and community respiration. Modelled and/or secondary parameters: pCO_2 (water) and dissolved inorganic carbon were computed by means of CO_2SYS.EXE programme using the water temperature, total alkalinity, pH, phosphate, silicate concentration. Indexing: Net primary production, trophic state index (TSI).	The study revealed that if conditions favourable for optimum photosynthesis can be maintained in these ponds, the CO_2 source character of these ponds can be reversed. In the post-monsoon season, when the pH of the water column was high, the system acted as sink for CO_2 which suggests the use of lime to prevent these systems from becoming hyper-eutrophic and carbon source at the same time.
4	Assessment of Self Rectification Capacity of the Main Sewage Canal While Passing Through the East Kolkata Wetlands, a Ramsar Site in West Bengal, India	(Susmita Mukherjee, 2019)	Sampling: Sample was collected from DWF canal at 6 different locations along a stretch of 16km during winter. Water samples were collected in separate plastic containers and immediately transferred into ice box. Surface soil/sediments samples were also collected from each location from within 0 to 5 cm depth and kept in plastic bags. Sampling was done Parameters: pH, DO, COD, BOD, TSS for water samples and organic carbon and percentage of organic matter in surface soil/sediment samples.	A trend of increasing pH, DO and decreasing TSS, BOD, COD was observed in its way towards the sea and/or downstream. The efficiency of rectification capacity has been calculated as more than 80% for all these parameters. Organic carbon and percentage of organic matter in the sediments have been found to increase gradually. This substantiates the fact that flocculation of

Sl. No.	Title	Author	Methodology	Major Findings
				organic matter from the overlying waste water and its subsequent sedimentation is possibly the main cause of self-rectification. Anaerobic condition at source triggers rectification by removing excess organic matter through sedimentation and finally carbon sequestration. The removal of organic matter and total suspended solids permit sunlight penetration into water column and activates algal community for O2 production through photosynthesis which accelerates the rectification process.
5	An Assessment of Direct and Indirect Emission Reduction Potential of Natural Wetland Systems of Kolkata, India	(Alokananda Banerjee Mukherjee, 2019)	Wetland based wastewater management system in EKW was analysed with following aspects like – 1. Geographic and historic context 2. Hydraulic regime 3. The biological process in sewage fed fisheries 4. Reduction of direct and indirect greenhouse gas (GHG) emission – a. Calculation of CO_2 eq. emission from the STPs of Kolkata city. b. Estimation of annual carbon sequestration potential (CO_2 eq.) of the sewage fed fishponds of EKW c. A Comparison of the sewage fed fishponds of EKW with the STPs was done Calculation: tCO_2e/y) = Power.	Tonnes of CO_2eq. emission from 3 STPs of Kolkata city namely, Keorapukur, Gardenreach and Bangur for total 137.5MLD capacity is 1594.64 tCO_2 e/y Indirect component of emission reduction: Tonnes of CO_2 eq. emission from EKW is 3479 tCO_2e/y (Considering 11.6 tCO_2 e/y per MLD, derived from aforesaid 3 STPs of Kolkata city and

Sl. No.	Title	Author	Methodology	Major Findings
			consumption (Mwh/y) × emission factor (0.82 t CO_2/Mwh)	EKW had a treatment capacity of 300MLD) Direct component of emission reduction: Annual carbon sequestration potential (tCO_2 e/y) for EKW is 6000 tCO_2 e/y (Considering aquaculture ponds sequester 1.5 tCO_2/ha/y and EKW has an aquaculture ponds area of 4000 ha)
6	Assessing soil and sediment organic carbon sequestration potential of selected wetlands at different physiographic regions of West Bengal, India.	(Shuvadip Adhikari, 2020)	19 wetlands were selected for soil and water sampling within West Bengal and were categorized as follows – 1. Wastewater-fed fishponds 2. Floodplain wetlands 3. Wetlands from sub-Himalayan forested hill region 4. Wetlands from Darjeeling Himalayan region 5. Wetlands from Chotanagpur Plateau and adjacent lateritic area 6. Coastal wetland **Soil sampling:** Bank surface soil samples were randomly collected from five sites of each wetland, above the water table, 0–10 cm depth using a handheld core sampler in January, May and September. Bottom sediment samples were collected by Ekman dredge from a depth of 1.5±0.45 m. **Parameters for water samples:** pH, electrical conductivity (EC), total dissolved solids (TDS)and salinity were measured on the spot. **Parameters for soil samples:** Bulk density, and percentage of sand and silt-clay, soil organic carbon, percentage of organic carbon. Data analysis: ANOVA, Levene's test, Post–hoc analysis, Hierarchical cluster analysis Past 4.01 software.	Soil and sediment organic carbon content of both bank soil and bottom sediments of the Wetlands varied widely. Highest amount of bank SOC were recorded from Himalayan region wetlands (50.54 ± 6.65 t/ha) whereas bottom SOC for floodplain wetlands (36.81±17.80 t/ha). Sewage water, runoff from catchment area, wetland macrophytes, sediment texture, physicochemical properties of water influence C sequestration potential of wetlands. All of the mentioned wetland types collectively constitute 247674 ha. area which

Sl. No.	Title	Author	Methodology	Major Findings
				represents 22.35% of total wetland area of the state can sequester up to 6.63 Mt carbon in soils and sediments.
7	Summer methane emissions from sewage water fed tropical shallow aquaculture ponds characterized by different water depths	(Sania Shaher, 2020)	**Sampling:** Air and water sampling were done in summer months of April and May from two adjacent aquaculture ponds of EKW having almost equal area and a depth of 1.1 m and 0.6 m respectively. Hence the ponds are adjacent. It was assumed that sewage of same organic load entered in both of the ponds during the sampling period. The hydraulic retention time and flow rate of the ponds were 33.8 ± 2.9 days and 0.06 – 0.33 m^3/s respectively. In addition, same fish species was cultivated in the ponds and no supplementary foods for fishes were added during the study period. Samples were taken on 5 days covering the entire extent of summer. Sampling in all the 5 days done at 2h interval for 24 h duration. The atmospheric parameters were monitored with the help of a weather station and most of the physicochemical parameters were measured in-situ by standard probes. For other parameters like air and water CH_4 concentration and chlorophyll-a, samples were collected and transferred to laboratory with appropriate measures of preservation. **Experimental parameters** **Air parameters:** Air temperature, wind speed, CH_4 **Water parameters:** Conductivity and pond surface temperature, DO, pH, underwater PAR, turbidity, Chlorophyll-a, BOD, GPP, CR, CH_4 **Sampling of CH_4 in surface water:** Surface water samples were collected in 40ml glass vials fitted with rubber septum leaving no headspace. Collected water samples were preserved with 8% $HgCl_2$ solution. Before analysis, 20ml of water from the glass vial was removed with a disposable syringe and the remaining 20 ml of water sample in the	pCH_4(water) in both of the ponds had significant positive correlation with water temperature (R2 = 0.68 and 0.71, $p < 0.05$), daily mean chlorophyll-a, turbidity, BOD and GPP. This indicated that higher primary production and presence of turbid materials acted as substrates for methanogenesis, which favoured water to air CH_4 effluxes. Mean water to air CH_4 fluxes in the ponds having depth of 1.1 m and 0.6 m were 24.79 ± 12.02 mg m−2 h−1 and 6.05 ± 3.14 mg m−2 h−1 respectively. Hence higher pond depth facilitated higher CH_4 production due to lower pH level, lower DO level, lower CH_4 oxidation rate and reduced photosynthesis.

Sl. No.	Title	Author	Methodology	Major Findings
			glass vial was purged with 99.99% pure nitrogen gas. After 2 h, 5 ml of headspace gas of the vial was collected by a Hamilton syringe and analysed using a gas chromatograph. **Sampling of CH_4 in ambient air:** Air samples above the respective pond surface were collected with a battery-operated pump in glass sampling bulbs, which were evacuated before collection and carefully air tightened and covering the stopcock by parafilm. Air samples were brought to the laboratory and analysed in GC as described earlier. **Modelling:** Air-Water CH4 flux estimation **Data analysis:** Descriptive statistics, one-way ANOVA, repeated measures ANOVA, independent samples Student's t test, Pearson correlation coefficients using SPSS v16.0	
8	Impact of physicochemical characteristics of wastewater on nitrogen dynamics: A case study in Kolkata, India	(Sen, 2021)	**Sampling:** Water sample were collected seasonally from the SWF channel at a depth of 50 - 60cm below the water surface in May (summer),August (monsoon)and in December (winter) for one-year at 8 different locations between the pumping stations to the tidal creek, the river Kulti (a tidal creek),covering a stretch of 30 km (The SWF channel is trapezoidal in a section with an average bottom width, water depth and flow velocity were of 40m, 2.5m and 0.4m/s respectively). All eight samples were collected and preserved accordingly one after another along the channel's length and the samples were tested in laboratories for the concentration of different nitrogen forms. Temperature, pH, and dissolved oxygen (DO) were measured in situ using a multi-parameter probe. **Parameters:** Nitrogen forms, such as organic nitrogen (Org-N), NH_3-N, NO_2-N, NO_3-N, temperature, pH and DO. **Data analysis:** Correlation analysis, one-way ANOVA.	Dynamics of all forms of nitrogen, such as Org-N, NH_3-N, NO_2-N, and NO_3-N are strongly related to internal changes of physicochemical characteristics of wastewater in the SWF channel. It is also observed that NH_3-N dynamics are strongly dependent on temperature whereas, dynamics of NO_2-Nand NO_3-N are strongly dependent on pH and dissolved oxygen levels in the wastewater. It was also observed that wastewater in the SWF channel is not

Sl. No.	Title	Author	Methodology	Major Findings
				undergoing fully bio-treatment and not meeting the permissible limits of the receiving waters.
9	Water quality assessment of East Kolkata Wetland with a special focus on bioremediation by nitrifying bacteria.	(Mousumi Saha, 2021)	**Sampling:** A total of 196 water samples were collected from inlet to outlet namely, Topsia sewage pumping station (inlet), Ambedkar bridge, Bantala, Bamunghata, Ghosher khal Ghusighata (outlet) and two ponds connected to the inlet and outlet namely Nalban fish ponds (from 3 sites) and Jhagrasisa bhery (from 3 sites) in order to assesses wage purification capacity of EKW. **Water parameters:** Temperature, pH, TDS, DO, BOD, COD, NH_3-N, NO_3-N **Bacteriological parameters:** Total heterotrophic bacteria (from column water sample), Nitrosomonas sp., and Nitrobacter sp. (from bottom water sample) **Data analysis:** Descriptive statistics, ANOVA	Significant variation in all the physicochemical parameters across the selected water bodies of EKW was observed. Nitrosomonas sp. and Nitrobacter sp. were isolated and enumerated. Their ability to degrade trichloroethylene (TCE) was tested. In two microbial isolates Nitrosomonas sp. and Nitrobacter sp. displayed superior TCE degradation ability at pH 5. Application of these strains as probiotics were found to improve the quality of water and survival rate of fishes in the treated experimental tanks.
10	Metabolic Dynamics of Soil Microorganisms of the Aquatic Ecosystem is a Key Component for Efficient Sewage Purification in Single Pond Natural Treatment Wetlands at	(Anirban Das Gupta, 2022)	**Sampling:** Water (5 cm below the surface of water) and sediment (top sediment horizon) samples were collected and preserved accordingly from three different single pond systems on Day0, Day3, Day 6, Day9, Day12 and Day15 at inlet, middle and outlet. Top soil samples were also collected three days before the pond was filled with untreated sewage. Ponds did not receive any sewage after the onset of treatment and until the end of the process. **Water parameters:** BOD, DO, TSS, specific oxygen uptake rate of total solids (SOUR) and specific gravity of water.	Moisture content of the wetland soil increased markedly upon introduction of sewage. Rewetting of soil led to hydrolytic activity of anaerobic soil microorganisms. In addition, anaerobic electron acceptors were abundantly available and anaerobic metabolic activity of soil

Sl. No.	Title	Author	Methodology	Major Findings
	East Kolkata Wetland		**Soil/sediment parameters:** Moisture content, CO_3^{2-}, HCO_3^-, NO_3^- **Parameters (12 assays) for metabolic dynamics of sediment soil microorganisms:** Catalase enzyme activity (1), basal soil respiration (2), substrate-induced soil respiration (3), arginine ammonification activity(4), nitrate reductase activity(5), anaerobic ammonification activity (6), histidine ammonia lyase activity (7), anaerobic dehydrogenase activity (8), protease activity (9), β- Glucosidase activity (10), asparaginase activity (11), fluorescein di-acetate activity (12). **Data analysis:** one-way ANOVA, two-tailed T-test (in SPSS v11)	micro-organisms increased several folds during the early phase of treatment. Aerobic microbial activity increased during the later phase of treatment which facilitate aerobic degradation. High oxygen demand of sewage was reduced competently through the metabolic activities of the wetland microorganisms within the soil-water interface without any addition of exogenous oxygen.

2.3. Critical Review

Following specific research studies are proposed to be commissioned to address the knowledge gaps in assessing status and trends in the ecological character of EKW.

i) **Carbon and GHG Flux assessment –** to assess the role of EKW in sequestering carbon and GHG, and this integrate role of these wetlands in climate change mitigation strategies.
ii) **Nutrient budget** - to assess the quantity of nutrients entering EKW, its uptake in resource recovery practices and discharges downstream.
iii) **Bioaccumulation studies –** to assess the chain of heavy metal contamination in EKW, quantity of toxic metals accumulating in fish, vegetables and crops cultivated in EKW and possible remedial measures.
iv) **Multiple values assessment –** to assess multiple values communities living in and around EKW associate with the wetland, the underlying reasons and the ways in which this value can be orientated towards behaviour change for wetland wise use.
v) Public awareness and stakeholder engagement
vi) Restoration and conservation techniques

Chapter-3

3. Objective and Scopes of the Research

3.1. Objective

Based on the research gap the objective of the present research can be summarized as ***to assess the efficacy of Natural Treatment System of East Kolkata Wetland for Treatment of Municipal Sewage of Kolkata City.***

3.2. Scope

Scopes of the proposed topic are as follows:

i) Identification of actual number of Treatment Ponds (TP) in EKW receiving domestic sewage of Kolkata city.
ii) Investigation into the possible linkage between ponds within the wetland.
iii) Identification of actual sewage flow pathway through the entire EKW area up to confluence.
iv) Qualitative and quantitative assessment of the wastewater entering into the fisheries of EKW.
v) Assessment of the treatment capacity of the sewage-fed fish ponds.
vi) Delineation of anthropogenic strategies currently adopted by the fishermen to utilize wastewater in fish cultivation.

4. Research Methodology

4.1. General

East Kolkata Wetland located between 22°25'N to 22°35'N and 88°20'E to 88°35'E, spanning over approximately 12,500 sq. km. where at present a total of 234 numbers of sewage fed Treatment ponds/Fishing Ponds are under conventional practice. Earlier during 19th century this number were near 900 and with passage of time due to heavy urbanization and rapid development of Greater Kolkata city this number reduced almost by 3 times. Among these 234 ponds the ponds located on the left side of Basanti Highway and SWF coming from Ballygunge Pumping Station are under intense practices of sewage fed pisciculture.

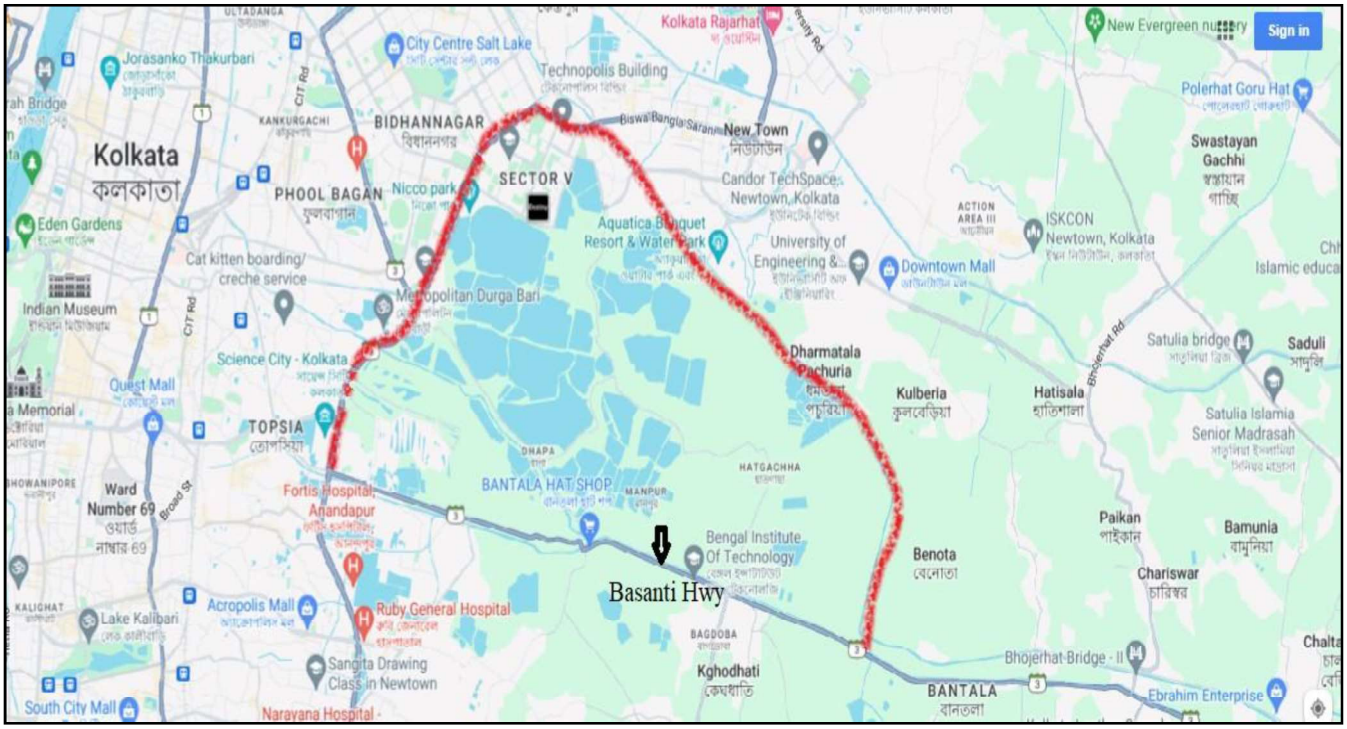

Fig. 4.1: Location of typical sewage fed fish pond of EKW where it has been practised intensely

There are two main DWF canals carrying the sewage of greater Kolkata into the EKW, one from Ballygaunge Pumping Station (i.e. flowing behind Science City, Kolkata and then running parallel to Basanti Hwy) and another from Pulmar`s Pumping Station (i.e. coming through Dhapa Dumping Yard, Kolkata) which are then getting mixed immediately after Bantala Lock Gate and flowing towards Bidyadhari river and getting discharged ultimately into Bidyadhari river at Ghushighata.

There are several numbers of pumping stations which are pumping the sewage coming through several drains carrying raw sewage of Greater Kolkata City into those two SWF canal and Kestopur canal (which is flowing making a boundary at north side of EKW).

There are many feeding canals and secondary canals which are drawn from those feeding canals are made to take raw sewage from the main DWF (i.e. flowing parallel to Basanti Hwy) into the treatment ponds at different location as per the convenience of local farmers. After proper utilization of the food value of the

raw sewage water the conventionally treated sewage water by means of pisciculture is discharged into same feeding or secondary canal which is being used to take raw water into Treatment pond.

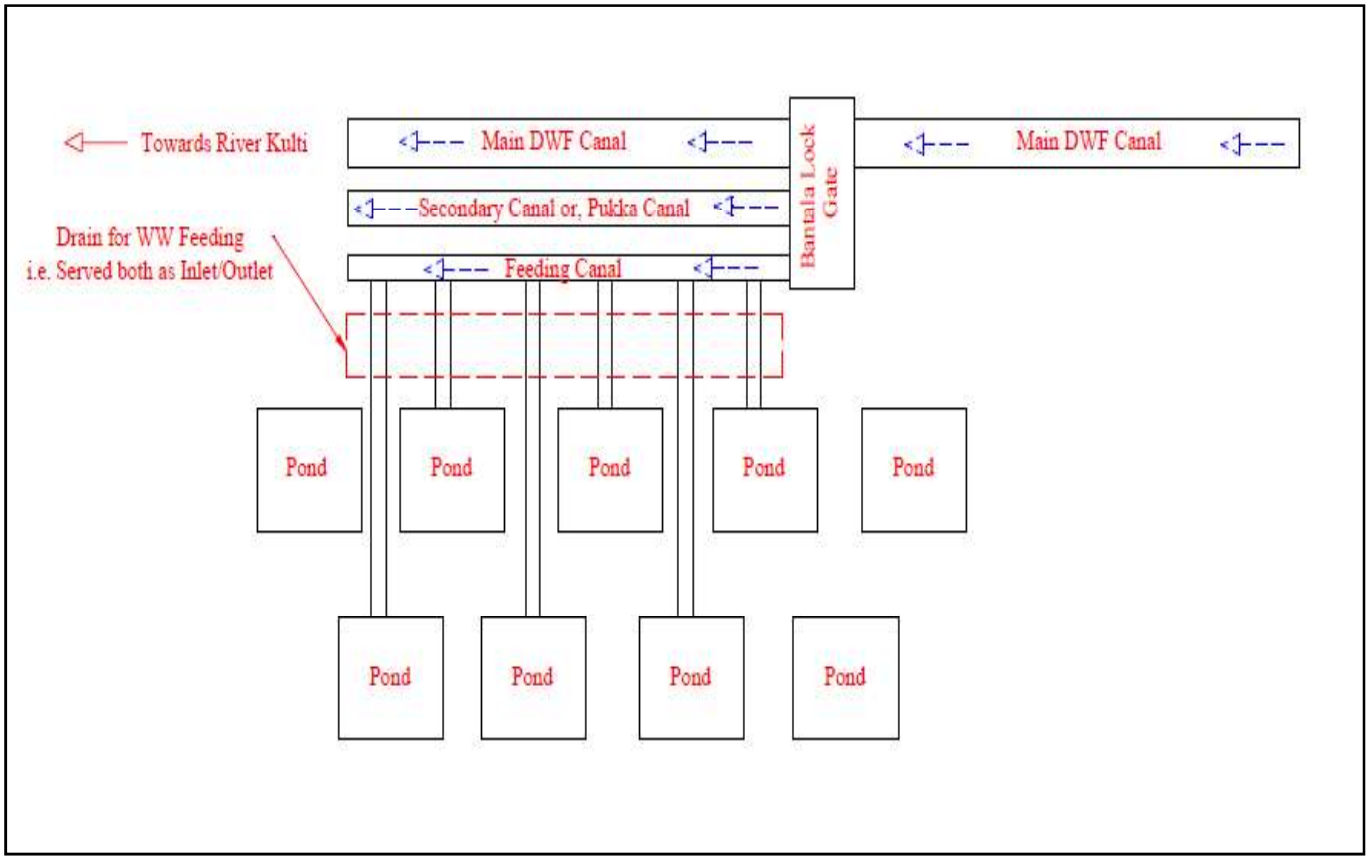

Fig. 4.2: Typical raw and treated sewage flow pathway in EKW

It has been tried to measure the natural treatment efficacy of the raw sewage coming from Greater Kolkata City and flowing through East Kolkata Wetland. For that we have collected raw sewage sample at different location of this sewage flow pathway. Details of sampling location, parameter tested, methods adopted are mentioned as follows:

a. Laboratory Used: Kolkata Laboratory, Central Pollution Control Board (CPCB)

b. Physicochemical Parameter Tested

a. pH
b. Total Dissolved Solid and Total Suspended Solid (TDS and TSS)
c. Biochemical Oxygen Demand (BOD)
d. Chemical Oxygen Demand (COD)
e. Total Nitrogen and Total Phosphate (TN and TP)
f. Total Coliform and Fecal Coliform (TC and FC)
g. Nitrite (NO_2^-) and Nitrate (NO_3^-)
h. Heavy Metals, if any

c. Sample Sources

a. SWF behind Forum Atmosphere (22.535787, 88.394198)
b. Pulmar's Bridge Pumping Station Outlet (22.551910, 88.404368)
c. Before Bantala Lock Gate (22.528405, 88.441479)
d. At Bantala Lock Gate (22.5276, 88.44222)

e. At a treatment pond outlet situated at the downstream of Bantala Lock Gate (22.517575, 88.478887)

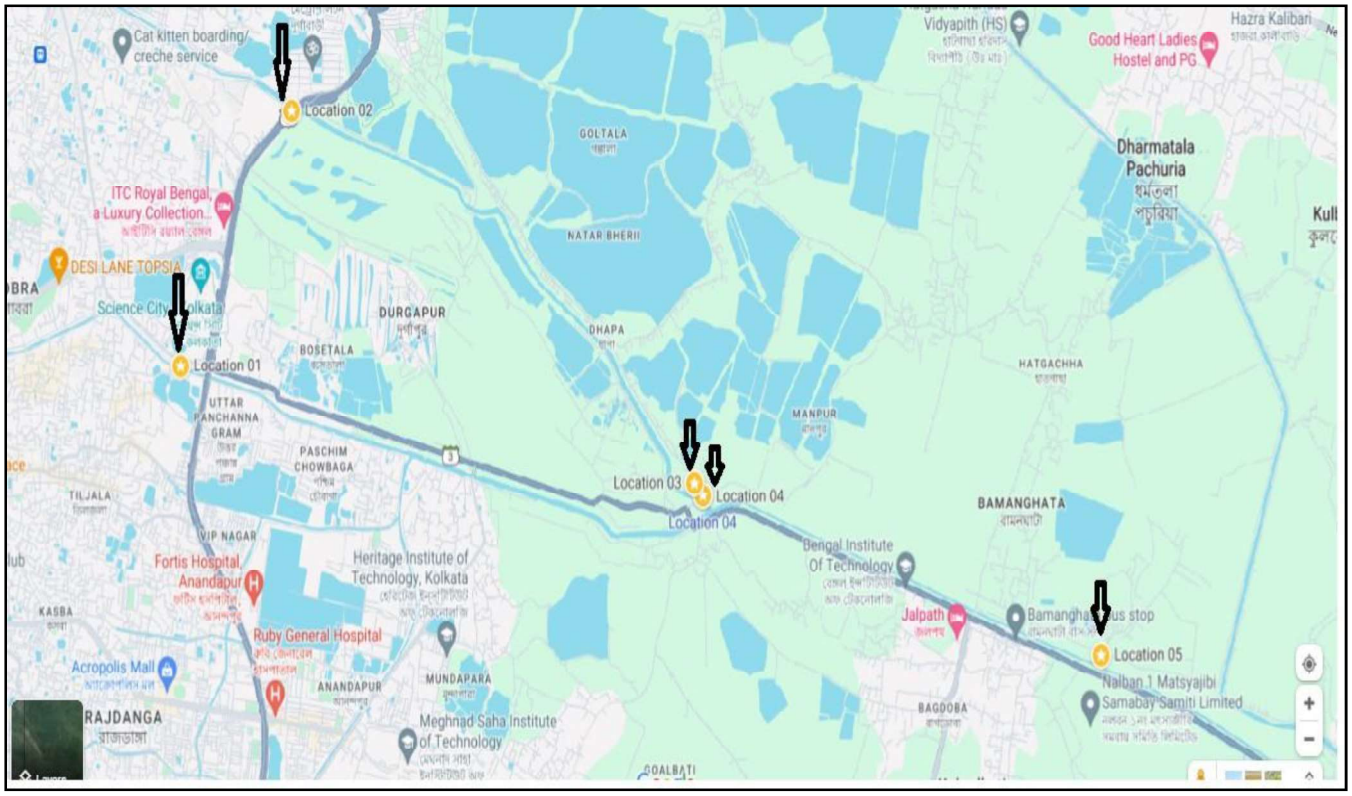

Fig. 4.3: Locations of raw sewage sample collection points

4.2. Details of the method adopted for the physicochemical tests

i) pH by Electrometric Method (Standard Methods 23rd Edition: 4500-H^+ B.)

A. Principle

The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 KPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (EMF) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation. Because single ion activities, such as aH^+, cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so:

$$pH_B = -\log_{10}(a_{H^+})$$

Where,

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_B \pm \frac{F \times (Ex - Es)}{2.303\ RT}$$

Where,

pH_x = potentiometrically measured sample pH
F = Faraday: 9.649 _ 104 coulomb/mole
E_x = sample emf, V
E_s = buffer emf, V
R = gas constant; 8.314 joule/(mole K)
T = absolute temperature, K

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between Ex and Es should be minimal. Samples must be dilute aqueous solutions of simple solutes (<0.2M). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic strength solutions.

B. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0.001 pH unit, but most instruments are not that precise. For routine work, use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment. Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagrammatically in Figures 4500-H_:1 and 2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7.0 buffer that has no change in potential with temperature. The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7.0). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7.0 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference electrode. Upon immersion of a new electrode in a solution, the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass–solution interface a potential that is a function of hydrogen ion activity in solution. Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a "low sodium error" electrode that can operate at high temperatures for measuring pH>10 because standard glass electrodes yield erroneously low values. For measuring pH<1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE* beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

C. Reagents

Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H^-:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2.0 µmhos/cm. To 50 mL, add 1.0 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts. Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, ensure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H^+:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500-H^+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 6 months.

D. Procedure

a. **Instrument calibration:** In each case, follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have conductivity greater than 4000 µmhos/cm. A pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (4500-H^+.B.2a). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature; a fixed temperature, such as 25°C; or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately 3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (4500-H^+.B.5a and b).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made, standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

b. **Sample analysis:** Establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

ii) Total Dissolved Solids (Standard Methods 23rd Edition: 2540 C.)

A. Principle

Filter a well-mixed sample through a standard glass-fiber filter. Then, transfer the filtrate to a pre-weighed dish, evaporate it to dryness, and dry it to constant weight in an oven at 180±2°C. The increase compared to the empty pre-weighed dish weight represents TDS. These results may differ from the theoretical value for solids calculated from chemical analysis of sample. Approximation methods for correlating chemical and physical analyses are available. The filtrate collected from the TSS determination (2540D) may be used to determine TDS.

To meet the LFB requirement (2540A.5), analysts can create a TDS standard as follows: Dry NaCl at 103–105°C for ≥1 h, weigh 50 mg, and dilute to 1 L with reagent water. This results in a 50-mg/L TDS standard.

B. Apparatus

a. Sample dishes: Dishes of approximately 90-mm dia. and 100-mL capacity made of one of the following materials:

1) Porcelain,

2) Platinum,
3) High-silica glass (may react with highly alkaline samples),* or
4) Other material shown to be resistant to the sample matrix and weight stable at the required evaporation and drying temperatures. Aluminum is NOT appropriate for this purpose.†

b. Wide-bore pipets,* Class B in glass, mechanical or electronic.
c. Graduated cylinders, Class A.
d. Steam bath (optional) for sample evaporation.
e. Hot plate or block (optional) for sample evaporation. Must be capable of maintaining a temperature <100°C without boiling samples.
f. Pre-drying oven (optional) for sample evaporation that operates at temperatures approximately 2°C below boiling to prevent splattering.
g. Drying oven that operates at 103–105°C.
h. Muffle furnace that operates at 550±50°C.
i. Desiccator, which includes either a desiccant whose color changes in response to moisture concentration or an instrument for measuring moisture (e.g., a hygrometer).
j. Analytical balance, capable of weighing to 0.1 mg.
k. Magnetic stirrer with TFE stirring bar (optional).
l. Blender or homogenizer (optional).
m. Glass-fiber filter disks, 22 to 125 mm dia, ≤2µm nominal pore size without organic binder.*
n. Filtration apparatus: One of the following, suitable for the filter selected:
1) Membrane filter funnel various capacities, to fit selected filter.
2) Gooch crucible 25 to 40 ml capacity, with Gooch crucible adapter.
3) Filtration apparatus with reservoir and coarse (40 to 60 µm) fritted disk as filter support.†
o. Suction flask with sufficient capacity for sample size selected.
p. Oven that operates at 180±2°C.

C. Procedure

a. Preparation of glass-fiber filter disk: Insert disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive volumes of ≥20 mL reagent-grade water. Continue suction to remove all traces of water. If using commercially prepared glass-fiber filter disks, the washing step may be skipped if the manufacturer certifies that the filters meet this method's requirements.

b. Preparation of evaporating dish: If measuring volatile solids, ignite cleaned evaporating dish at 550±50°C for ≥15 min in a muffle furnace. If only measuring TDS, then heat cleaned dish to 180±2°C for ≥1h in an oven. Cool dishes to ambient temperature and weigh. Store in desiccator or, oven until needed.

c. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If filtration will take >10 min to complete, then increase filter size or decrease sample volume. Identify any sample that yields residue <2.5 mg or >200 mg, and report the value as described in Sections 1020 and 2020.

d. Sample analysis: Stir or mix sample and use a pipet or graduated cylinder to transfer a measured volume onto a glass-fiber filter with applied vacuum. Wash the entire exposed surface of filter with three successive volumes of ≥10 mL reagent-grade water. Allow complete drainage between washings, and continue suction until all traces of water are removed. Transfer total filtrate (with washings) to a pre-weighed evaporating dish and evaporate to dryness on a steam bath, hot plate, or block, or in a drying oven. If necessary, add successive portions to the same dish after evaporation. Dry evaporated sample for

≥1 h in an oven at 180±2°C, cool in a desiccator to ambient temperature, and weigh. Repeat cycle (drying, cooling, desiccating, and weighing) until weight change is <0.5 mg.
If determining volatile solids, follow procedure in 2540E.

D. Calculation

Total Dissolved Solid (mg/lt) = (A-B) x 1000 / Sample Volume in ml.

Where,

A = final weight of dried residue + dish (mg)

B = weight of dish (mg)

iii)Total Suspended Solids (Standard Methods 23rd Edition: 2540 D.)

A. Principle
Filter a well-mixed sample through a pre-weighed standard glass-fiber filter, and then dry the filter and the residue retained on it to a constant weight in a 103–105°C oven. The increase in filter weight represents TSS. To estimate an unknown sample matrix TSS concentration, calculate the difference between TDS and total solids.
To meet the LFB requirement (2540A.5), a TSS standard can be created as follows: weigh 100 mg of Sigmacell® Cellulose Type 20 or Celite 545, dilute to 1 L with reagent water, and stir for ≥15 min to mix well. This results in a 100 mg/L TSS standard.

B. Apparatus

a. Sample dishes: Dishes of approximately 90-mm dia and 100-mL capacity made of one of the following materials:
   1) Porcelain,
   2) Platinum,
   3) High-silica glass (may react with highly alkaline samples),*or
   4) Other material shown to be resistant to the sample matrix and weight stable at the required evaporation and drying temperatures. Aluminum is NOT appropriate for this purpose.†

b. Wide-bore pipets,* Class B in glass, mechanical or electronic.
c. Graduated cylinders, Class A.
d. Pre-drying oven (optional) for sample evaporation that operates at temperatures approximately 2°C below boiling to prevent splattering.
e. Drying oven that operates at 103–105°C.
f. Muffle furnace that operates at 550±50°C.
g. Desiccator, which includes either a desiccant whose color changes in response to moisture concentration or an instrument for measuring moisture (e.g., a hygrometer).
h. Analytical balance, capable of weighing to 0.1 mg.
i. Magnetic stirrer with TFE stirring bar (optional).
j. Blender or homogenizer (optional).
k. Low-form beaker, Class B or better.
l. Glass-fiber filter disks, 22 to 125 mm dia, ≤2µm nominal pore size without organic binder.*
m. Filtration apparatus: One of the following, suitable for the filter selected:
   1) Membrane filter funnel—various capacities, to fit selected filter.

2) Gooch crucible—25- to 40-mL capacity, with Gooch crucible adapter.
3) Filtration apparatus with reservoir and coarse (40- to 60-_m) fritted disk as filter support.†

n. Suction flask with sufficient capacity for sample size selected.

C. Procedure

a. Preparation of glass-fiber filter disk: Insert filter with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive portions of ≥20 ml reagent-grade water. Continue suction to remove all traces of water. Remove filter from filtration apparatus and transfer to an inert weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in a 103–105°C oven for ≥1 h. Cool in desiccator to ambient temperature and weigh. Store filters (on inert dishes or pans) in desiccator or 103–105°C oven until needed. Adequate filter preparation is demonstrated by negligible weight loss or gain for method blanks.

If measuring volatile solids, ignite at 550±50°C for ≥15 min in a muffle furnace. Cool to room temperature before proceeding. (Alternatively, the ignition step may be performed after washing and drying at 103–105°C for ≥1 h, but before weighing.)
If using commercially prepared glass-fiber filters, the ignition, washing, and weighing steps may be eliminated if the manufacturer certifies that the prepared filters meet this method's requirements. Verify filters using method blanks. Filters are verified if the measured weight differs from the manufacturer's weight by less than ±0.5 mg.

b. Selection of filter and sample sizes: Choose sample volumes to yield between 2.5 and 200 mg dried residue. If filtration takes >10 min to complete, increase filter size or decrease sample volume. Identify any sample that yields residue <2.5 mg or >200 mg, and report the value as described in Sections 1020 and 2020.

c. **Sample analysis:** Stir or mix sample and use a pipet or graduated cylinder to transfer a measured volume onto a glass-fiber glass-fiber filter with applied vacuum. Wash filter with at least three successive volumes of ≥10 ml reagent-grade water. Allow complete drainage between washings, and continue suction until all traces of water are removed. When filtering samples with high dissolved solids concentrations, additional washings may be required to ensure that dissolved material is removed from all exposed filter surfaces.

Using forceps, carefully remove filter from filtration apparatus and transfer to an inert weighing dish or pan as a support. If using a Gooch crucible, remove crucible and filter combination from the crucible adapter. Dry for ≥1 h in a 103–105°C oven, cool in a desiccator to ambient temperature, and weigh. Repeat the cycle (drying, cooling, desiccating, and weighing) until the weight change is <0.5 mg.

D. Calculation

Total Suspended Solids (mg/lt) = (A-B) x 1000 / Sample Volume in ml.

Where,

A = final weight of Filter + dried residue, in mg.

B = weight of Filter in mg.

iv) 5-Day BOD (Standard Methods 23rd Edition: 5210 B.)

A. General Discussion

The BOD test is an indirect measurement of organic matter; it measures the change in DO concentration caused by microorganisms as they degrade organic matter in a sample held in a stoppered bottle incubated for 5 d in the dark at 20°C. Analysts measure DO before and after incubation, and compute BOD using the difference between DO measurements. Because initial DO is determined shortly after dilution, all oxygen uptake occurring after this measurement is included in the BOD measurement.

B. Apparatus

a. Incubation bottles: Use 60-mL glass bottles or larger (300-mL bottles with a flared mouth and ground-glass stopper are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use. Alternatively, use disposable plastic BOD bottles that are capable of meeting all method quality-control (QC) checks.

b. Air incubator or water bath, thermostatically controlled at 20±1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

c. Oxygen-sensitive membrane electrode, polarographic or galvanic, or oxygen-sensitive optical probe with appropriate meter.

C. Reagent

Discard reagents if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable, and different stock concentrations may be used if doses are adjusted proportionally. Use reagent grade or better for all chemicals and use distilled or equivalent reagent-grade water (see Section 1080) to make all solutions.

a. Phosphate buffer solution: Dissolve 8.5 g monopotassium phosphate (KH_2PO_4), 21.75 g dipotassium phosphate (K_2HPO_4), 33.4 g disodium phosphate (Na_2HPO_4).$7H_2O$, and 1.7 g ammonium chloride (NH_4Cl) in about 500 mL reagent-grade water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 and 1.7 g NH_4Cl in about 700 mL reagent-grade water. Adjust pH to 7.2 with 30% sodium hydroxide (NaOH) and dilute to 1 L.

b. Magnesium sulfate ($MgSO_4$) solution: Dissolve 22.5 g $MgSO_4$.$7H_2O$ in reagent-grade water and dilute to 1 L.

c. Calcium chloride ($CaCl_2$) solution: Dissolve 27.5 g $CaCl_2$ in reagent-grade water and dilute to 1 L.

d. Ferric chloride ($FeCl_3$) solution: Dissolve 0.25 g $FeCl_3$.$6H_2O$ in reagent-grade water and dilute to 1 L.

e. Acid and alkali solutions: 1N to neutralize caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc. sulfuric acid (H_2SO_4) to reagent-grade water. Dilute to 1 L.

2) Alkali—Dissolve 40 g NaOH in distilled water. Dilute to 1 L.

f. Sodium sulfite (Na_2SO_3) solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL reagent-grade water. This solution is unstable; prepare daily.

g. Nitrification inhibitor

1) 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Use pure TCMP or commercial preparations.*

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea ($C_4H_8N_2S$) in about 500 mL reagent-grade water and dilute to 1 L. Store at 4°C. The solution is stable for 2 weeks when stored at ≤6°C without freezing.

h. Glucose–glutamic acid (GGA) solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to reagent-grade water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile container. Store all GGA mixtures at ≤6°C without freezing unless manufacturer recommendations state otherwise. Commercial preparations may be used but concentrations may vary. Discard solutions if evidence of contamination is indicated (e.g., growth occurs in the stock bottle or GGA test results are consistently low).

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL reagent-grade water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

j. Source water for preparing BOD dilution water: Use de-mineralized, distilled, or equivalent reagent-grade water, tap, or natural water to make sample dilutions (see 5210B.4c).

D. Testing Procedure

a. Preparation of dilution water: Transfer desired working volume of source water (5210B.4c) to a suitably sized bottle (glass is preferred). Check to ensure that the DO concentration is at least 7.5 mg/L before using water for BOD tests. If not, add DO by shaking bottle or aerating it with organic-free filtered air. Alternatively, store the water in cotton-plugged bottles long enough for the DO concentration to approach saturation. Add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solution/L to prepared source water (5210B.4c). Mix thoroughly and bring temperature to 20 ±3°C. Prepare dilution water immediately before use, unless dilution-water blanks (5210B.6c) show that the water is acceptable after longer storage times. If dilution-water blanks show a DO depletion >0.2 mg/L, then improve purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light to try to bring the dilution blank into range.

b. Sample temperature adjustment: Bring sample temperature to 20 ±3°C before making dilutions.

c. Preparation of dilutions: Using dilution water prepared as in ¶ a above, make at least three dilutions of prepared sample estimated to produce, at the end of the test, at least one dilution that would result in a residual DO of ≥1.0 mg/L and a DO uptake of ≥2.0 mg/L after a 5-d incubation. Two dilutions are allowed if experience with a particular sample source produces at least one bottle with acceptable minimum DO depletions and residual limits (5210B.6a). Individual laboratories should evaluate the need for more than three dilutions when historical sample data are unavailable. A more rapid analysis, such as COD (Section 5220), may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following percentages of wastewater when preparing dilutions: 0.01 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters. The number of bottles to be prepared for each dilution depends on DO technique and number of replicates desired. Prepare dilutions in volumetric containers (Class A glass or equivalent) and then transfer to BOD bottles, or else prepare directly in BOD bottles. Either dilution method can be used to transfer sample to respective BOD bottles.

1) **Dilutions prepared in volumetric containers—**Using a widetipped pipet or graduated cylinder, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix sample well immediately before pipetting to avoid solids loss via settling. For dilutions greater than 1:300, make a primary dilution before making final dilution in volumetric cylinders or flasks. Fill cylinders or flasks at least two-thirds full with dilution water and sample without entraining air. Add appropriate amounts

of seed suspension (¶ d below) and nitrification inhibitor (¶ e below). Dilute to final level with dilution water (¶ a above). Mix well but avoid entraining air. Siphon mixed dilution into a suitable number of BOD bottles, taking care not to let solids settle in cylinder or flask during transfer. When a cylinder or flask contains >67% of sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (5210B.3*a–d*) directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle), or use commercially prepared solutions designed to dose the appropriate container size.

2) **Dilutions prepared directly in BOD bottles—**Using a wide-tip volumetric pipet or graduated cylinder, add desired sample volume to individual BOD bottles. Mix sample well immediately before pipetting to avoid solids loss via settling. For dilutions greater than 1:300, make a primary dilution before making final dilution in the bottle. Fill each BOD bottle approximately two thirds full with dilution water and/or sample without entraining air. Add appropriate amounts of seed suspension (¶ d below) and nitrification inhibitor (¶ e below) to individual BOD bottles. Fill remainder of BOD bottle with dilution water. When a bottle contains >67% of sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (5210B.3a–d) directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle), or use commercially prepared solutions esigned to dose the appropriate bottle size.

d. Sealing bottles: Completely fill each bottle by adding enough dilution water so insertion of stopper leaves no bubbles in the bottle. Mix sample by turning bottle manually several times unless immediately using a DO probe with a stirrer to measure initial DO concentration. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of water seal during incubation.

e. Determination of initial DO: Use the azide modification of the iodometric method (Section 4500-O.C), membrane-electrode method (Section 4500-O.G), or optical-probe method (Section 4500-O.H) to determine initial DO on all sample dilutions, dilution-water blanks, and, where appropriate, seed controls. Replace any displaced contents with enough diluted sample or dilution water to fill the bottle, stopper all bottles tightly, and water seal before beginning incubation. After preparing dilution, measure initial DO within 30 min. If using the membrane-electrode method or optical probe method, calibrate DO probe daily by following the manufacturer's calibration procedure. Make frequent calibration checks daily to ensure accurate DO readings and, ideally, perform a Winkler titration as needed to verify calibration. If using the azide modification of the titrimetric iodometric method, prepare an extra bottle for initial DO determination for each sample dilution.

f. Sample incubation: Incubate at 20 ±1°C the stoppered and sealed BOD bottles containing desired dilutions (¶ c above), seed controls (5210B.6d), dilution-water blanks (5210B.6c), and GGA checks (5210B.6b). Exclude light to avoid algae growth in bottles during incubation.

g. Determination of final DO: After 5 d ±6 h of incubation, determine DO in all sample dilutions, blanks, and checks as in 5210B.6g, using the azide modification of the titrimetric method (Section 4500-O.C), membrane-electrode method (Section 4500-O.G), or optical-probe method (Section 4500-O.H).

E. Quality Control Checks

The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

a. Minimum residual DO and minimum DO depletion: Only bottles (including seed controls) whose DO depletion is ≥2.0 mg/L and residual DO is ≥1.0 mg/L after 5 d of incubation are considered to produce valid data, because ≥2.0 mg oxygen uptake/L is required to give a meaningful measure of oxygen uptake and ≥1.0 mg/L must remain to ensure that waste constituents' oxidation rates were not limited by insufficient DO. However, there are exceptions—for reporting purposes only— when testing undiluted samples and all bottles' DO depletion is <2.0 mg/L and residual DO is <1.0 mg/L (see 5210B.7).

b. Glucose–glutamic acid check: The GGA check is the primary basis for establishing the BOD test's accuracy and precision, as well as the principal measure of seed quality and set-up procedure. Together with each batch of BOD or CBOD samples, check seed effectiveness and analytical technique by using procedures in 5210B.5 to make BOD measurements on an equal weight mixture of glucose and glutamic acid as follows: Add sufficient amounts of standard glucose and glutamic acid solutions (5210B.3*h*) to give 3.0 mg glucose/L and 3.0 mg glutamic acid/L in each of three test bottles (20 mL GGA solution/L seeded dilution water, or 6.0 mL/300-mL bottle). Commercial solutions may contain other GGA concentrations; adjust doses accordingly. Add nitrification inhibitor if seed is obtained from a source that is nitrifying, and also to all CBOD GGA checks. Evaluate data as described in 5210B.8. The resulting average BOD/CBOD for the three bottles, after correcting for dilution and seeding, must fall into the control-limit range established in 5210B.8*a*. If the average value falls outside this range, evaluate the cause and make appropriate corrections. Consistently high values can indicate too much seed suspension, contaminated dilution water, or nitrification; consistently low values can indicate poor seed quality or quantity or else the presence of a toxic material. If low values persist, prepare a new GGA mixture and check the dilution-water and seed sources.

c. Dilution-water-quality check: With each batch of dilution water, incubate two or more bottles of dilution water containing nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. Dilution water checks must be analyzed with each batch of samples; the dilution-water blank serves as a check on the quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO for each bottle (5210B.5e and i), and average results. The average DO uptake in 5 d must not be >0.2 mg/L and preferably ≤0.1 mg/L before making seed corrections. If average dilution-water blank is >0.2 mg/L, record the data and clearly identify such samples in data records.

F. Data Analysis and Reporting

a. Calculations

1) For each test bottle with at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO before seed correction, calculate BOD as follows:

$$BOD_5\,(mg/lt) = \{(D_1 - D_2) - (S)Vs\}/\,P$$

Where,

D_1 = DO of diluted sample immediately after preparation (mg/L)
D_2 = DO of diluted sample after 5 d incubation at 20°C (mg/L)
S= oxygen uptake of seed [ΔDO/mL seed suspension added per bottle (5210B.6*d*) (*S* = 0 if samples are unseeded)],
Vs = volume of seed in respective test bottle (mL)
P = decimal volumetric fraction of sample used; 1/*P* = dilution factor.

v) COD-Open Reflux Method (Standard Methods 23rd Edition: 5220 B.)

A. General Discussion

a. Principle: Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

b. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

B. Apparatus

a. Reflux apparatus, consisting of 500- or 250-mL Erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm^2 of heating surface, or equivalent.

b. Blender.

c. Pipettes, Class A and wide-bore.

C. Reagents

a. Standard potassium dichromate solution, 0.04167M: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000 mL. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is 6 x 0.04167M or 0.2500N.

b. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix.

c. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 .7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.*

d. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25M: Dissolve 98 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 mL conc. H_2SO_4, cool, and dilute to 1000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:

Dilute 25.00 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL conc. H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

$$\text{Molarity of FAS Solution} = \frac{\text{Volume of 0.04167M } K_2Cr_2O_7 \text{ Solution Titrated, mL}}{\text{Volume of FAS used in Titration, mL}} \text{x} 0.2500$$

e. Mercuric sulfate (HgSO4), crystals or powder.

f. Sulfamic acid: Required only if the interference of nitrites is to be eliminated (see 5220A.2).

g. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$: Lightly crush and then dry KHP to constant weight at 110°C. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD1 of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 µg O_2/ mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

D. Testing Procedure

a. Treatment of samples with COD of >50 mg O_2/L: Blend sample if necessary and pipet 50.00 mL into a 500-mL refluxing flask. For samples with a COD of >900 mg O2/L, use a smaller portion diluted to 50.00 mL. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$. Cool while mixing to avoid possible loss of volatile materials. Add 25.00 mL 0.04167M $K_2Cr_2O_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent.

[CAUTION: Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents.**]**

Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h. Cool and wash down condenser with distilled water. Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same volume for all titrations. Take as the endpoint of the titration the first sharp color change from blue-green to reddish brown that persists for 1 min or longer. Duplicate determinations should agree within 5% of their average. Samples with suspended solids or components that are slow to oxidize may require additional determinations. The blue-green may reappear. In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

b. Alternate procedure for low-COD samples: Follow procedure of ¶ a above, with two exceptions: (i) use standard 0.004167M $K_2Cr_2O_7$, and (ii) titrate with standardized 0.025M FAS. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause gross errors. If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux as follows: Add all reagents to a sample larger than 50 mL and reduce total volume to 150 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached. Compute amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4$:Cl^-, using the amount of Cl^- present in the original volume of sample. Carry a blank reagent through the same procedure. This technique has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost, but an improvement is gained over ordinary evaporative concentration methods. Duplicate determinations are not expected to be as precise as in ¶ a above.

c. Determination of standard solution: Evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

E. Calculation

$$\text{COD as mg } O_2/L = \frac{(B\text{-}A) \times M \times 8000}{\text{mL Sample}}$$

Where,
B = mL FAS used for Sample.
A = mL FAS used for Blank.
M = molarity of FAS.
8000 = milli-equivalent weight of Oxygen x 1000 mL/L

F. Precision and Bias

A set of synthetic samples containing potassium hydrogen pthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2/L in the absence of chloride, the standard deviation was ±13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O2/L and 100 mg Cl^-/L, the standard deviation was ±14 mg/L (coefficient of variation, 10.8%).

vi) Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus (Standard Methods 23rd Edition: 4500-P J.)

A. General Discussion

a. Principle: The oxidation of nitrogenous compounds for determining total nitrogen must occur in an alkaline medium. Conversely, the oxidation of phosphorus compounds for determining total phosphorus must occur under acidic conditions. Methods determining total nitrogen have used a persulfate-sodium hydroxide system to oxidize nitrogenous compounds to nitrate. Accordingly, methods determining total phosphorus have used persulfate in an acidic medium.
During the initial stage of the digestion, sample pH is alkaline (pH>12). In the final stage of the digestion, the sodium hydroxide is consumed, causing sample pH to become acidic (pH<2). By means of this broad pH range, the method allows for the oxidation of both nitrogen and phosphorus compounds. The digested sample is analyzed for nitrate and orthophosphate, yielding total nitrogen and total phosphorus results.

b. Selection of nitrate/orthophosphate measurement methods: Using a dual-channel auto-analyzer that performs nitrate-nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method, total nitrogen and total phosphorus can be measured simultaneously. Alternatively, other methods for orthophosphate and nitrate can be used.

B. Apparatus

Clean all glassware with HCl before use.

a. Autoclave, capable of achieving a temperature of 120°C for a minimum of 120 min.
b. Glass culture tubes, 13-mm-OD _100-mm-long with autoclavable caps.
c. Autopipettor, capable of pipetting a 6.0-mL portion.
d. Repeating pipettor, capable of pipetting 1.25-mL portion.
e. Erlenmeyer flask, 3000-mL.
f. Aluminum foil.

g. Automated continuous-flow instrument system for nitrate and phosphate determination: The suggested analytical instruments are described in Sections 4500-$NO3^-$.F.2 and 4500-P.F.2a.

C. Reagents

a. Deionized water, high-quality, free of phosphorus and nitrogen compounds. Prepare by ion-exchange or distillation methods as directed in Sections 4500-NH_3.B.3a and 4500-NO_3^-.B.3a.
b. Sodium hydroxide, 3N: Dissolve 120 g low-nitrogen NaOH in 800 mL deionized water in a 1000-mL volumetric flask. Cool and dilute to volume.
c. Oxidizing reagent: Dissolve 64 g low-nitrogen (<0.001% N) potassium persulfate, $K_2S_2O_8$, in 500 mL deionized water. Use low heat if necessary. Add 80 mL 3N NaOH, prepared from low-nitrogen sodium hydroxide, and dilute to 1000 mL. Store in a brown bottle at room temperature.
d. All of the reagents listed for determining nitrate + nitrite as indicated in Section 4500-NO_3^-.F.3.
e. All of the reagents listed for determining phosphate as indicated in 4500-P.F.3.
f. Nicotinic acid p-toluenesulfonate stock and working standards: Dry nicotinic acid p-toluenesulfonate in an oven at 105°C for 24 h. Dissolve 2.1084 g in deionized water and dilute to 100 mL; 1 mL = 1 mg N. To prepare a working standard, dilute 2.0 mL stock solution to 1000 mL; 1 mL = 2 µg N.
g. Adenosine triphosphate stock and working standards: Dissolve 0.6514 g adenosine triphosphate in deionized water and dilute to 1000 mL; 1 mL = 0.1 mg P. To prepare a working standard, dilute 20.0 mL stock solution to 1000 mL; 1 mL = 2 µg P. To prepare a low-range working standard, dilute 1.0 mL stock solution to 1000 mL; 1 mL = 0.1 µg P.

D. Procedure

a. Calibration curve: Prepare a minimum of five standards over the desired calibration ranges using a stock calibration standard containing both nitrate and orthophosphate. Treat standards in the same manner as samples. Include blanks in calibration curves.
b. Sample preparation: If necessary, dilute sample with deionized water so expected nitrogen and phosphorus concentrations fall within the range of the calibration standards. Samples preserved with acid cannot be analyzed by this digestion method.
c. Digestion check standards: Analyze quality-control standards containing organic nitrogen and phosphorus on each analytical run (see 4500-P.J.3f and g for suggested standards and preparation procedures). These standards provide reference checks on the calibration and test the efficiency of the digestion.
d. Digestion: Pipet 6.0 mL of sample or standard into the culture tubes. Add 1.25 mL oxidizing reagent to each tube using a repeating pipettor. Cover the tubes with loose-fitting plastic caps. Prepare autoanalyzer wash water in an Erlenmeyer flask by adding oxidation reagent to deionized water in the same proportion as was added to the samples. Cover flask with foil. Autoclave samples and wash water for 55 min at 120°C. Cool to room temperature. Add 0.05 mL of 3N NaOH to each tube before proceeding to nitrate + nitrite and phosphate analyses. Shake to mix. Add same proportion of 3N NaOH to digested wash water.
e. Final nitrate + nitrite measurement: Use the automated cadmium reduction method for the determination of nitrate-nitrite after digestion. See Section 4500-NO_3^- .F. Other nitrate analysis methods may be applicable; however, precision and bias data do not exist for these methods on this matrix at this time.

f. Final phosphate measurement: Use the automated ascorbic acid reduction method for the determination of phosphate after digestion. See 4500-P.F. Other phosphate analysis methods may be applicable; however, precision and bias data do not exist for these methods at this time.

E. Calculation

Prepare nitrogen and phosphorus standard curves by plotting the instrument response of standards against standard concentrations. Compute the nitrogen and phosphorus concentrations by comparing the sample response with the standard curve. Where necessary, multiply sample concentration by the appropriate dilution factor to determine final concentration.

vii) Nitrate by Cadmium Reduction Method (Standard Methods 23rd Edition: 4500-NO_3^- E.)

A. General Discussion

a. Principle: Nitrate (NO_3^-) is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate ($CuSO_4$) and packed in a glass column.
The NO_2^- is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to form a highly colored azo dye that is measured colorimetrically. To correct for any NO_2^- present in the sample before NO_3^- reduction, samples must be analyzed without the reduction step. The applicable range of this method is 0.05 to 1.0 mg NO_3^--N/L. The method is recommended especially for NO_3^--N levels <0.1 mg N/L for which other methods lack adequate sensitivity.

b. Interferences: Suspended matter in the column may restrict sample flow, so filter turbid samples (see 4500-NO_3^-.A.l). Iron, copper, or other metals concentrations above several milligrams per liter lower reduction efficiency; add ethylenediaminetetraacetic acid (EDTA) to the buffer reagent to eliminate this interference. If necessary, pre-extract oil and grease with an organic solvent (Section 5520) to prevent it from coating the Cd surface. Hydrogen sulfide in water or wastewater samples from anoxic water bodies may de-activate the column; treat acidified odorous samples by bubbling with air for 15 min. Residual chlorine can oxidize the Cd column, reducing its efficiency, so check samples for residual chlorine (see DPD methods in Section 4500-Cl) and, if needed, remove by adding sodium thiosulfate (Na_2SO_3) solution (Section 4500-NH_3.B.3d). Sample color that absorbs at about 540 nm interferes with results; dilute samples or measure absorbance of treated samples to which color reagent has not been added and subtract from the absorbance after addition of the color reagent.

B. Apparatus

a. Reduction column: Purchase or construct the column* (Figure 4500-NO_3^- :1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-inner diameter (ID) tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve1 to control flow rate.

b. Pipets: If adjustable pipets are used, verify the calibration according to manufacturer's directions.

c. Colorimetric equipment: One of the following is required:
1) Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.
2) Filter photometer, with light path of 1 cm or longer and a filter whose maximum transmittance is near 540 nm.

C. Reagents

a. Reagent water: See 4500-NO_3^-.B.3a. Use for all solutions and dilutions.

b. Copper-cadmium granules: Wash 25 g new or used 20- to 100-mesh Cd granules† with 6M HCl and rinse with water. Swirl Cd with 100 mL 2% $CuSO_4$ solution for 5 min or until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with ammonium chloride-EDTA solution (¶ d below) to remove all precipitated Cu. Store activated Cd covered with dilute ammonium chloride–EDTA solution (¶ e below).

c. Color reagent: To 800 mL reagent water, add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g NED. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in an amber bottle in a refrigerator. Discard if the solution is highly colored or dark or if a precipitate forms.

d. Ammonium chloride-EDTA solution: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL reagent water. Adjust to pH 8.5 with conc. ammonium hydroxide (NH_4OH) and dilute to 1 L with reagent water. This solution is stable for 1 year.

e. Dilute ammonium chloride-EDTA solution: Dilute 300 mL NH_4Cl-EDTA solution to 500 mL with water. Use this reagent as a cadmium-reduction-column storage solution.

f. Hydrochloric acid, 6M: Carefully add 500 mL concentrated HCl to~ 400 mL reagent water. Dilute to 1 L with reagent water. This solution is stable for 1 year.

g. Copper sulfate solution, 2%: Dissolve 20 g $CuSO_4$.5H2O in 500 mL reagent water and dilute to 1 L with reagent water. This solution is stable for 1 year.

h. Stock nitrate solution A: Prepare as directed in 4500-NO_3^- .B.3b or obtain from a commercial source.

i. Stock nitrate solution B: Purchase from a commercial source different from stock nitrate solution A or prepare as directed in 4500-NO_3^-.B.3b using KNO_3 from a commercial source different from that used to prepare stock nitrate solution A.

j. Intermediate nitrate solution A: Prepare as directed in 4500-NO_3^-.B.3c using stock nitrate solution A.

k. Intermediate nitrate solution B: Prepare as directed in 4500-NO_3^-.B.3c using stock nitrate solution B.

l. Stock nitrite solution: See Section 4500-NO_2^-.B.3e.

m. Intermediate nitrite solution: See Section 4500-NO_2^-.B.3f.

n. Working nitrite solution: Dilute 50.0 mL intermediate NO_2^--N solution to 500 mL with reagent water; 1.00 mL = 5 µg NO_2^--N. Prepare fresh daily.

D. Procedure

a. Preparation of reduction column: Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to avoid entrapping air. Wash column with 200 mL dilute NH_4Cl-EDTA solution. Activate column by passing through it, at 7-10 mL/min, several 100-mL portions of a solution composed of 1 part 1.0 mg NO_3^--N/L standard and 3 parts NH_4Cl-EDTA solution.
[CAUTION: Cadmium is toxic and carcinogenic. Collect and store all waste Cd. When handling Cd, wear gloves and follow the precautions described on Cd's safety data sheet.]

b. Treatment of sample:
1) Turbidity removal—Filter turbid samples (see 4500-NO_3^-.A.1).
2) pH adjustment—Adjust pH to between 7 and 9, as necessary, with dilute HCl or NaOH. This ensures a pH of 8.5 after adding NH_4Cl-EDTA solution.
3) Sample reduction—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first

25 mL. Collect the rest in a clean sample flask. There is no need to wash columns between samples, but if columns will not be reused for several hours or longer, pour 50 mL dilute NH_4Cl-EDTA solution onto the top nd let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

4) Color development and measurement - As soon as possible (≤15 min after reduction), add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 h afterward, measure absorbance at 543 nm against a distilled water-reagent blank.

[NOTE: If NO_3^--N concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.]

c. Calibration: Using intermediate nitrate solution A, prepare standards of 0.05, 0.10, 0.20, 0.50, and 1.0 mg NO_3^--N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. (This calibration range is just a suggestion; other concentrations may be used as long as they meet reporting requirements). Prepare a CVS using intermediate nitrate solution B. To 25.0 mL of each standard, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed standard into column and collect at a rate of 7 to 10 mL/min. Discard first 50 mL. Collect the rest in a clean sample flask. There is no need to wash columns between samples, but if columns will not be reused for several hours or longer, pour 50 mL dilute NH_4Cl-EDTA solution onto the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry. To verify reduction-column efficiency, compare at least one mid-range NO_2^- standard to a reduced NO_3^-standard at the same concentration. Reactivate Cu-Cd granules as described in 4500-NO_3^-.E.3b when reduction efficiency fails the quality control criterion (4500-NO_3^-.E.6).

E. Calculation

Use an electronic spreadsheet, calculator, or instrument software to find the slope, intercept, and correlation coefficient (r) or coefficient of determination (r^2) of the calibration curve by least squares linear regression. Calculate the NO_3^--N concentration from the following equation:

$$C = \frac{A - I}{S}$$

Where,

C = Concentration.
A = Absorbance.
I = Intercept of the regression line.
S = Slope of the regression line.

Report as milligrams oxidized N per liter (the sum of NO_3^-N plus NO_2^--N) unless the concentration of NO_2^--N is separately determined and subtracted.

viii) Nitrite by Colorimetric Method (Standard Methods 23rd Edition: 4500-NO_2^- B.)

A. General Discussion

a. Principle: Nitrite (NO_2^- is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10-1000 µg NO_2^--N/L. Photometric measurements can be made in the range 5 to 50 µg N/L if a 5-cm

light path and a green color filter are used. The color system obeys Beer's law up to 180 µg N/L with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

b. Interferences: Chemical incompatibility makes it unlikely that NO_2^-, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^+, chloroplatinate ($PtCl_6^{2-}$), and metavanadate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

c. Storage of sample: Never use acid preservation for samples to be analyzed for NO_2^-. Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. For short-term preservation for 1 to 2 d, freeze at -20°C or store at 4°C.

d. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

B. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

C. Procedure

a. Removal of suspended solids: If sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter.

b. Color development: If sample pH is not between 5 and 9, adjust to that range with 1N HCl or NH_4OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

c. Photometric measurement: Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO_2^--N concentrations:

Light Path Length (cm)	NO_2^--N (µg/L)
1	2-25
5	2-6
10	<2

D. Calculation

Prepare a standard curve by plotting absorbance of standards against NO_2^--N concentration. Compute sample concentration directly from curve.

Chapter-5

5. Results and Discussion

5.1. General

As per previous data in Greater Kolkata approximately 910ML sewage generates on daily basis which is then flows through different drains or, canals intercepted by pumps at various locations which regulates the discharge and also changes the flow direction. Some of the raw sewage got diverted and receives a primary or, secondary grade of treatment. Then the treated sewage got discharged in the same canal at a downstream interval from where the inlet source was intercepted and thus the sewage load got diluted in the canal flow. Eventually the confluence of treated and untreated sewage flows through a DWF towards the East Kolkata Wetland where the sewage load get reduced in manifolds by the means of pisciculture, horticulture, agriculture and foremost the sewage got settled by the long flow path through these canals and interconnected treatment ponds of EKW and finally the reduced sewage load submit itself in Bidyadhari River.

Here we tried to assess the background scenario of the sewage flow pathway by means of i) how much raw sewage is getting generated on daily basis, ii) how much among those quantity is getting partial treatment, iii) how much is diverted towards EKW for natural conventional treatment without any prior treatment.

5.2. Status of Sewage generation in Kolkata and its flow path

As per the West Bengal pollution Control Board provided data,

a. Quantity of Sewage generated in Kolkata = **910 MLD**

b. Quantity of Sewage Diverted into STPs = **155.33 MLD**

Table 5.1. Location and quantity of sewage receiving treatment for different STPs of Kolkata (*West Bengal Pollution Control Board*)

Sl. No.	Location	Type	Capacity (MLD)
1	Gardenreach	ASP	57.00
2	Baghajatin	Aerated Lagoon	15.00
3	Hatisur	WSP	10.00
4	Kamarhati-1	Trickling Filter	60.00
5	Maheshtala-1	STP	4.00
6	Budge Budge	WSP	9.33
			155.33

c. Quantity of sewage remains untreated (i.e. before reaching EKW) = (910-155) = **755 MLD**

Followings are the channel which carries the sewage load to different STPs and Pumping Stations and finally gets submitted into main DWF heading towards EKW, *(Source West Bengal Pollution Control Board)*

a. Tolly Nullah Drain (15.5km)
b. Kulti Ghat Drain (28.5km)
c. Circular Channel (8.5 km)

d. Cossipore Drain
e. Nimtala Burning Ghat.
f. Dhankheti Drain.
g. Munikhali Khal Drain.

5.3. Physicochemical characteristics of Sewage at different location along its flow

Sample collected from the selected 5 points are examined and the test results are mentioned in the table given below,

Table 5.2. Physicochemical parameters of 5 Nos. fixed location on DWF channel

Sl. No.	Parameter	16.05.2024				
		Before Lock Gate	Bantala Lock Gate	Pond Outlet	Pulmar's Bridge Pumping Station Outlet	SWF behind Forum Atmosphere
1	pH	6.89	6.87	6.95	6.90	6.89
2	COD (mg/lt)	76.00	72.00	72.00	68.00	133.00
3	BOD (mg/lt)	38.00	33.00	32.00	18.00	60.00
4	TSS (mg/lt)	24.00	16.00	28.00	29.00	163.00
5	TDS (mg/lt)	567.00	441.00	526.00	397.00	558.00
6	Nitrate-N (mg/lt)	0.01	BDL	BDL	BDL	0.01
7	Total-N (mg/lt)	21.00	26.00	18.00	14.00	21.00
8	Total-P (mg/lt)	1.60	1.50	1.50	1.50	2.20

As East Kolkata wetland covers a vast area and situated on the outskirts of the main city, due to constraints like transportation and limited course period of my thesis work we have also collected and analysed a secondary data from WBPCB spanning from January'22 to April'24, mentioned in the following table.

Table 5.3. Physicochemical characteristics of Sewage collected from 5 nos. fixed location on DWF channel, *West Bengal Pollution Control Board*

Sl. No.	Parameters	13.01.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.22	7.07	7.10	7.24	7.21
2	Conductivity(µS/cm)	743.30	724.20	976.40	1130.00	1779.00
3	COD (mg/lt)	100.00	75.00	50.00	31.00	30.00
4	BOD (mg/lt)	30.42	16.56	11.61	7.75	7.63
5	TSS (mg/lt)	28.00	16.00	20.00	48.00	54.00
6	TDS (mg/lt)	382.00	392.00	534.00	652.00	696.00
7	Nitrate-N (mg/lt)	1.73	1.74	0.83	0.73	0.50
8	Chloride (mg/lt)	110.07	124.42	167.50	201.00	220.14
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	09.02.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.04	7.01	6.96	7.10	7.01
2	Conductivity(µS/cm)	754.80	721.40	1255.00	1344.00	1281.00
3	COD (mg/lt)	56.00	51.00	50.00	40.00	35.00
4	BOD (mg/lt)	21.67	16.25	15.36	12.81	11.25
5	TSS (mg/lt)	26.00	22.00	70.00	56.00	32.00
6	TDS (mg/lt)	438.00	528.00	796.00	814.00	792.00
7	Nitrate-N (mg/lt)	0.40	0.86	0.69	0.95	1.29
8	Chloride (mg/lt)	81.36	86.14	210.57	248.85	229.72
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	16.03.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	6.99	7.00	7.21	7.41	7.54
2	Conductivity(µS/cm)	1049.00	1021.00	1423.00	2330.00	4001.67
3	COD (mg/lt)	60.00	60.00	64.00	41.00	35.00
4	BOD (mg/lt)	14.50	15.25	16.75	11.25	9.75
5	TSS (mg/lt)	74.00	42.00	100.00	64.00	156.00
6	TDS (mg/lt)	444.00	450.00	668.00	1120.00	2120.00
7	Nitrate-N (mg/lt)	1.39	1.34	1.38	1.36	1.22
8	Chloride (mg/lt)	105.29	110.07	201.00	421.15	909.29
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	08.04.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.17	7.32	7.25	7.55	7.64
2	Conductivity(µS/cm)	920.00	898.20	1574.00	3648.00	4652.00
3	COD (mg/lt)	84.00	65.00	90.00	44.00	28.00
4	BOD (mg/lt)	22.19	16.50	23.12	10.89	8.02
5	TSS (mg/lt)	28.00	42.00	52.00	108.00	156.00
6	TDS (mg/lt)	482.00	440.00	776.00	2040.00	2700.00
7	Nitrate-N (mg/lt)	0.59	1.98	0.67	1.37	0.59
8	Chloride (mg/lt)	109.96	99.97	259.92	899.72	1249.61
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	13.05.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.15	7.22	7.25	7.42	7.56
2	Conductivity(µS/cm)	877.30	828.10	929.00	5672.00	6689.33
3	COD (mg/lt)	160.00	60.00	50.00	100.00	70.00
4	BOD (mg/lt)	41.25	15.50	12.68	25.83	16.25
5	TSS (mg/lt)	72.00	46.00	92.00	128.00	140.00
6	TDS (mg/lt)	396.00	414.00	632.00	3412.00	4192.00
7	Nitrate-N (mg/lt)	3.34	3.95	4.66	8.38	7.96
8	Chloride (mg/lt)	119.64	114.86	134.00	1531.44	2010.02
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	07.06.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.50	7.46	7.40	7.55	7.69
2	Conductivity(µS/cm)	825.10	806.80	1361.00	1730.00	2076.67
3	COD (mg/lt)	68.00	50.00	42.00	52.00	42.00
4	BOD (mg/lt)	15.50	12.50	10.63	12.08	9.84
5	TSS (mg/lt)	36.00	24.00	36.00	56.00	36.00
6	TDS (mg/lt)	466.00	434.00	706.00	896.00	1112.00
7	Nitrate-N (mg/lt)	1.92	1.83	1.74	2.50	1.75
8	Chloride (mg/lt)	89.97	89.97	229.93	329.90	419.87
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	15.07.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.13	7.18	7.17	7.57	7.52
2	Conductivity(µS/cm)	936.40	731.80	1008.00	7428.00	2736.33
3	COD (mg/lt)	86.00	86.00	55.00	58.00	55.00
4	BOD (mg/lt)	21.25	19.69	12.71	13.31	12.71
5	TSS (mg/lt)	66.00	88.00	48.00	310.00	58.00
6	TDS (mg/lt)	360.00	388.00	382.00	4340.00	1410.00
7	Nitrate-N (mg/lt)	2.26	2.41	1.32	2.25	1.96
8	Chloride (mg/lt)	100.50	105.29	143.57	2201.44	717.86
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	10.08.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.11	7.12	7.15	7.26	7.15
2	Conductivity(µS/cm)	741.80	737.00	937.20	3701.00	4270.33
3	COD (mg/lt)	64.00	76.00	53.00	35.00	46.00
4	BOD (mg/lt)	18.50	21.25	15.63	9.25	11.07
5	TSS (mg/lt)	56.00	44.00	36.00	66.00	62.00
6	TDS (mg/lt)	442.00	504.00	682.00	2722.00	3118.00
7	Nitrate-N (mg/lt)	4.76	0.59	1.42	7.21	4.95
8	Chloride (mg/lt)	95.72	90.93	157.93	1100.72	1244.30
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	07.09.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.21	7.14	7.26	7.35	7.43
2	Conductivity(µS/cm)	911.50	887.80	963.40	1941.00	1866.67
3	COD (mg/lt)	70.00	75.00	55.00	36.00	41.00
4	BOD (mg/lt)	17.75	19.38	13.13	9.72	10.31
5	TSS (mg/lt)	28.00	12.00	36.00	38.00	22.00
6	TDS (mg/lt)	586.00	576.00	606.00	1388.00	1208.00
7	Nitrate-N (mg/lt)	1.64	1.58	1.79	1.40	2.23
8	Chloride (mg/lt)	117.25	124.43	129.22	406.79	526.43
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	13.10.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.33	7.31	7.36	7.52	7.47
2	Conductivity(µS/cm)	685.50	598.50	751.40	1122.00	1206.00
3	COD (mg/lt)	75.00	75.00	50.00	40.00	38.00
4	BOD (mg/lt)	15.31	16.56	12.65	11.09	8.19
5	TSS (mg/lt)	64.00	22.00	26.00	86.00	192.00
6	TDS (mg/lt)	466.00	434.00	706.00	896.00	1112.00
7	Nitrate-N (mg/lt)	1.48	2.12	1.20	2.07	3.33
8	Chloride (mg/lt)	79.97	64.98	77.48	249.92	474.85
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	30.11.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.89	7.65	7.49	7.35	7.32
2	Conductivity(µS/cm)	858.30	881.10	1357.00	1404.00	1557.33
3	COD (mg/lt)	70.00	80.00	60.00	55.00	40.00
4	BOD (mg/lt)	16.50	21.25	15.50	12.50	10.16
5	TSS (mg/lt)	56.00	74.00	32.00	30.00	66.00
6	TDS (mg/lt)	488.00	458.00	770.00	766.00	938.00
7	Nitrate-N (mg/lt)	2.58	1.59	2.06	4.78	2.77
8	Chloride (mg/lt)	104.97	99.97	219.93	249.92	449.86
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	05.12.2022				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.08	7.15	7.12	7.60	7.54
2	Conductivity(µS/cm)	813.40	858.90	1110.00	2544.00	2317.00
3	COD (mg/lt)	95.00	78.00	77.00	62.00	69.00
4	BOD (mg/lt)	27.92	24.38	24.69	21.50	19.25
5	TSS (mg/lt)	40.00	36.00	40.00	92.00	68.00
6	TDS (mg/lt)	460.00	440.00	700.00	880.00	1106.00
7	Nitrate-N (mg/lt)	1.55	1.62	1.71	1.57	2.85
8	Chloride (mg/lt)	110.07	102.89	179.46	607.79	571.89
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	03.01.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.41	7.38	7.32	7.54	7.49
2	Conductivity(µS/cm)	945.20	836.80	1224.00	1828.00	1499.33
3	COD (mg/lt)	135.90	75.63	148.91	48.31	79.24
4	BOD (mg/lt)	26.88	17.50	28.13	12.31	19.69
5	TSS (mg/lt)	118.00	44.00	40.00	54.00	158.00
6	TDS (mg/lt)	410.00	400.00	616.00	1088.00	1096.00
7	Nitrate-N (mg/lt)	1.37	1.22	1.24	1.44	1.55
8	Chloride (mg/lt)	95.72	134.00	134.00	411.57	421.15
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	08.02.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.19	7.24	7.15	7.35	7.38
2	Conductivity(µS/cm)	954.50	734.90	1175.00	2406.50	5015.00
3	COD (mg/lt)	88.00	143.00	92.60	106.90	58.90
4	BOD (mg/lt)	19.19	69.38	17.92	25.83	9.75
5	TSS (mg/lt)	100.00	150.00	58.00	88.00	286.00
6	TDS (mg/lt)	474.00	478.00	772.00	1800.00	3408.00
7	Nitrate-N (mg/lt)	0.76	1.12	2.53	2.06	1.36
8	Chloride (mg/lt)	95.72	90.93	224.93	717.86	1646.30
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	14.03.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.30	7.29	7.30	7.84	7.58
2	Conductivity(µS/cm)	830.50	911.60	1527.00	1552.50	4336.00
3	COD (mg/lt)	105.26	65.02	101.14	65.02	51.60
4	BOD (mg/lt)	24.50	14.35	21.33	14.50	10.38
5	TSS (mg/lt)	70.00	38.00	110.00	68.00	160.00
6	TDS (mg/lt)	780.00	838.00	1390.00	1410.00	4228.00
7	Nitrate-N (mg/lt)	1.70	1.87	2.01	1.83	1.98
8	Chloride (mg/lt)	129.96	119.96	259.92	399.88	649.79
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	12.04.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.10	7.19	7.31	7.84	7.64
2	Conductivity(µS/cm)	910.40	900.20	1500.00	1299.50	1915.00
3	COD (mg/lt)	92.73	58.15	121.38	95.05	74.51
4	BOD (mg/lt)	35.08	20.48	37.40	32.46	22.46
5	TSS (mg/lt)	38.00	48.00	78.00	112.00	178.00
6	TDS (mg/lt)	680.00	628.00	1262.00	1146.00	1810.00
7	Nitrate-N (mg/lt)	0.77	0.92	0.60	1.34	0.71
8	Chloride (mg/lt)	89.97	114.96	259.92	449.86	248.15
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	15.05.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	6.94	6.91	7.08	7.17	7.33
2	Conductivity(µS/cm)	902.20	935.40	1259.00	12648.00	13207.33
3	COD (mg/lt)	80.53	102.14	101.81	211.38	163.86
4	BOD (mg/lt)	30.40	32.92	32.50	72.25	29.50
5	TSS (mg/lt)	80.00	88.00	120.00	388.00	328.00
6	TDS (mg/lt)	832.00	884.00	1146.00	10540.00	9876.00
7	Nitrate-N (mg/lt)	1.30	1.44	1.27	1.57	1.34
8	Chloride (mg/lt)	113.29	129.96	204.94	3948.77	3598.88
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	13.06.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.06	7.11	7.17	7.28	7.35
2	Conductivity(µS/cm)	1000.00	1063.00	1108.60	3971.00	7190.00
3	COD (mg/lt)	150.78	72.64	66.76	72.62	52.48
4	BOD (mg/lt)	44.28	25.72	24.77	17.95	16.03
5	TSS (mg/lt)	66.00	72.00	102.00	248.00	302.00
6	TDS (mg/lt)	800.00	888.00	930.00	2674.00	6092.00
7	Nitrate-N (mg/lt)	1.31	1.36	0.63	0.55	1.36
8	Chloride (mg/lt)	116.63	129.96	194.94	1349.58	1749.46
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	11.07.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.10	7.08	7.29	7.31	7.20
2	Conductivity(µS/cm)	908.60	1067.00	1402.00	1336.00	1479.00
3	COD (mg/lt)	107.32	105.26	76.36	78.44	53.66
4	BOD (mg/lt)	34.63	29.25	37.88	12.94	12.17
5	TSS (mg/lt)	64.00	68.00	128.00	110.00	150.00
6	TDS (mg/lt)	840.00	912.00	1310.00	1308.00	1420.00
7	Nitrate-N (mg/lt)	0.76	0.77	0.92	0.77	0.98
8	Chloride (mg/lt)	107.63	127.19	225.04	215.25	264.17
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	09.08.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	6.99	6.95	6.95	7.15	7.09
2	Conductivity(µS/cm)	862.40	920.80	966.60	1050.00	1020.00
3	COD (mg/lt)	77.38	39.68	51.58	32.74	27.45
4	BOD (mg/lt)	23.00	13.59	10.92	9.20	7.78
5	TSS (mg/lt)	52.00	70.00	88.00	132.00	122.00
6	TDS (mg/lt)	818.00	906.00	926.00	988.00	972.00
7	Nitrate-N (mg/lt)	1.31	1.16	0.98	0.94	0.95
8	Chloride (mg/lt)	127.19	132.09	151.66	225.03	225.03
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	07.09.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.48	7.31	7.35	7.39	7.44
2	Conductivity(µS/cm)	931.10	757.00	1174.00	1149.00	1136.00
3	COD (mg/lt)	120.00	100.00	140.00	80.00	80.00
4	BOD (mg/lt)	31.38	25.83	33.25	20.00	19.75
5	TSS (mg/lt)	72.00	58.00	92.00	102.00	108.00
6	TDS (mg/lt)	916.00	688.00	1080.00	1104.00	1122.00
7	Nitrate-N (mg/lt)	1.53	1.07	1.37	1.06	0.97
8	Chloride (mg/lt)	69.98	154.95	184.94	189.94	199.94
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	06.10.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.29	7.14	7.32	7.26	7.06
2	Conductivity(µS/cm)	1810.20	1822.00	976.70	787.00	704.30
3	COD (mg/lt)	44.00	65.00	134.00	34.00	32.00
4	BOD (mg/lt)	10.12	15.10	26.63	7.58	6.68
5	TSS (mg/lt)	40.00	68.00	98.00	76.00	54.00
6	TDS (mg/lt)	760.00	790.00	956.00	742.00	620.00
7	Nitrate-N (mg/lt)	2.35	1.04	1.01	1.04	1.04
8	Chloride (mg/lt)	169.95	109.97	134.96	114.96	119.96
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	07.11.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.16	7.22	7.14	7.25	7.19
2	Conductivity(µS/cm)	987.00	1022.00	1178.00	1480.00	1259.33
3	COD (mg/lt)	95.24	95.24	123.81	76.19	47.62
4	BOD (mg/lt)	20.42	20.42	31.88	20.94	13.64
5	TSS (mg/lt)	80.00	98.00	114.00	158.00	134.00
6	TDS (mg/lt)	924.00	1008.00	1136.00	1392.00	1226.00
7	Nitrate-N (mg/lt)	0.86	0.93	1.03	1.01	1.20
8	Chloride (mg/lt)	112.52	117.41	190.79	244.61	225.04
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	08.12.2023				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	6.96	7.06	7.16	7.21	7.28
2	Conductivity(µS/cm)	1003.00	828.20	782.70	1495.00	1590.00
3	COD (mg/lt)	61.60	48.02	41.76	31.32	32.36
4	BOD (mg/lt)	17.06	10.56	11.71	10.61	9.14
5	TSS (mg/lt)	102.00	70.00	56.00	176.00	210.00
6	TDS (mg/lt)	994.00	790.00	742.00	1472.00	1568.00
7	Nitrate-N (mg/lt)	0.82	0.70	0.85	0.75	1.01
8	Chloride (mg/lt)	127.19	112.52	92.95	244.61	322.88
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	05.01.2024				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	6.95	7.08	7.16	7.25	7.20
2	Conductivity(µS/cm)	850.60	943.50	1094.00	1222.00	1494.67
3	COD (mg/lt)	45.08	67.62	71.54	51.94	46.39
4	BOD (mg/lt)	20.79	28.10	25.19	17.63	27.29
5	TSS (mg/lt)	88.00	98.00	110.00	126.00	162.00
6	TDS (mg/lt)	812.00	898.00	1020.00	1136.00	1424.00
7	Nitrate-N (mg/lt)	1.29	1.37	1.30	1.04	1.05
8	Chloride (mg/lt)	124.96	124.96	204.94	239.93	279.91
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	02.02.2024				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.04	7.21	7.18	7.46	7.59
2	Conductivity(µS/cm)	940.00	826.60	1218.00	1515.00	1644.33
3	COD (mg/lt)	87.78	86.86	85.01	48.97	41.58
4	BOD (mg/lt)	17.00	16.58	15.88	13.54	10.28
5	TSS (mg/lt)	92.00	74.00	118.00	170.00	192.00
6	TDS (mg/lt)	892.00	776.00	1120.00	1434.00	1562.00
7	Nitrate-N (mg/lt)	0.89	0.71	0.69	0.66	0.69
8	Chloride (mg/lt)	119.96	114.96	219.93	319.90	299.90
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	06.03.2024				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.24	7.17	7.31	7.35	7.19
2	Conductivity(µS/cm)	840.70	790.70	1475.00	1554.00	1647.00
3	COD (mg/lt)	97.76	68.43	97.76	58.66	29.33
4	BOD (mg/lt)	35.08	17.80	43.83	17.44	11.15
5	TSS (mg/lt)	96.00	68.00	166.00	182.00	226.00
6	TDS (mg/lt)	822.00	732.00	1416.00	1520.00	1618.00
7	Nitrate-N (mg/lt)	0.68	0.59	0.72	1.36	2.34
8	Chloride (mg/lt)	149.95	109.97	139.96	349.89	354.89
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

Sl. No.	Parameters	04.04.2024				
		Lalkuthi Syphonne	Bantala Lock Gate	Before CLC	DWF Channel at Ghushighata	After Confluence of DWF and SWF at Ghushighata
1	pH	7.08	7.19	7.22	7.18	7.35
2	Conductivity(µS/cm)	970.00	784.20	1327.00	1892.00	1963.00
3	COD (mg/lt)	66.10	51.52	67.07	63.18	55.40
4	BOD (mg/lt)	14.70	13.54	20.85	17.10	15.60
5	TSS (mg/lt)	108.00	74.00	136.00	278.00	298.00
6	TDS (mg/lt)	938.00	724.00	1208.00	1810.00	1934.00
7	Nitrate-N (mg/lt)	1.43	1.08	1.52	1.29	1.25
8	Chloride (mg/lt)	149.95	89.97	219.93	379.88	439.86
9	Heavy Metals, if any	BDL	BDL	BDL	BDL	BDL

5.4. Graphical Interpretation of Physicochemical characteristics

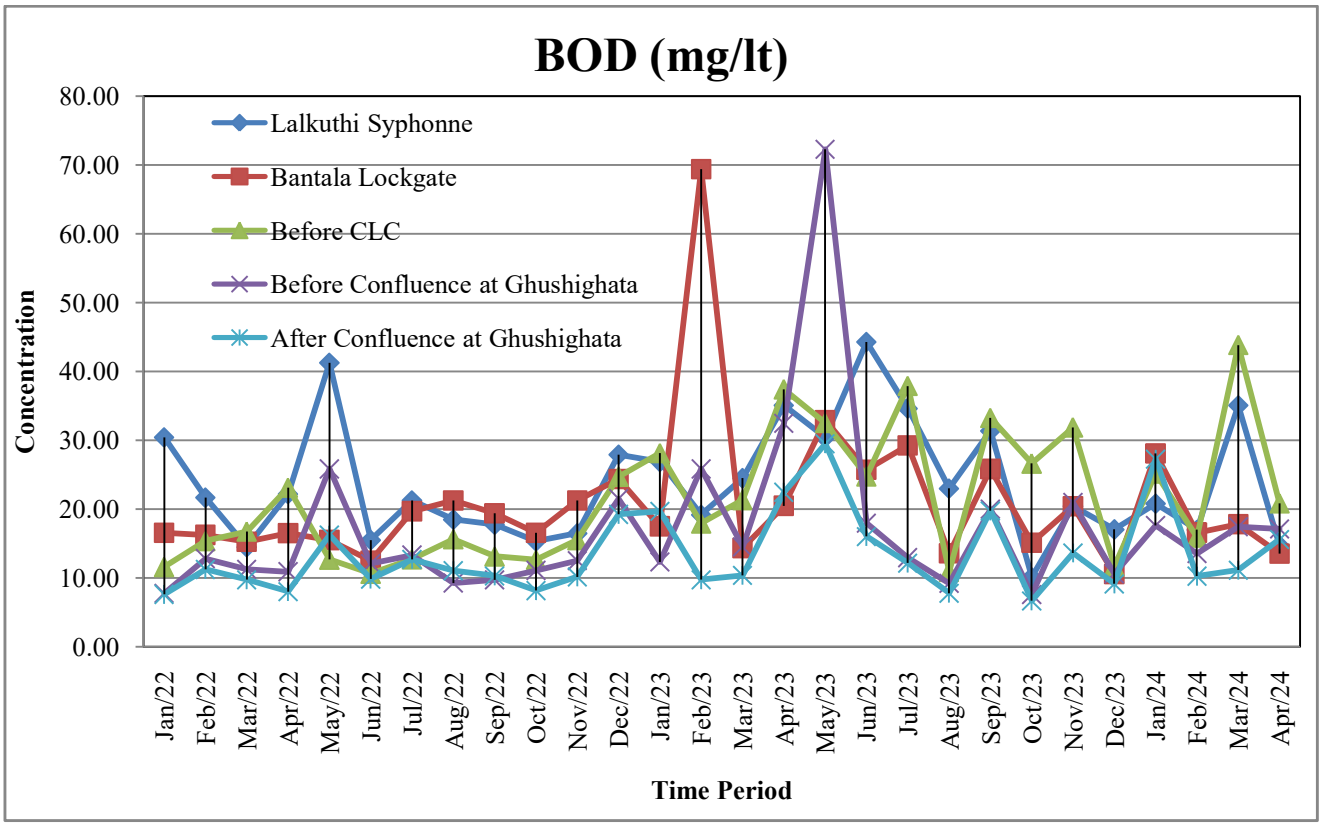

Fig. 5.1. Month wise BOD concentration along the flow towards D/S

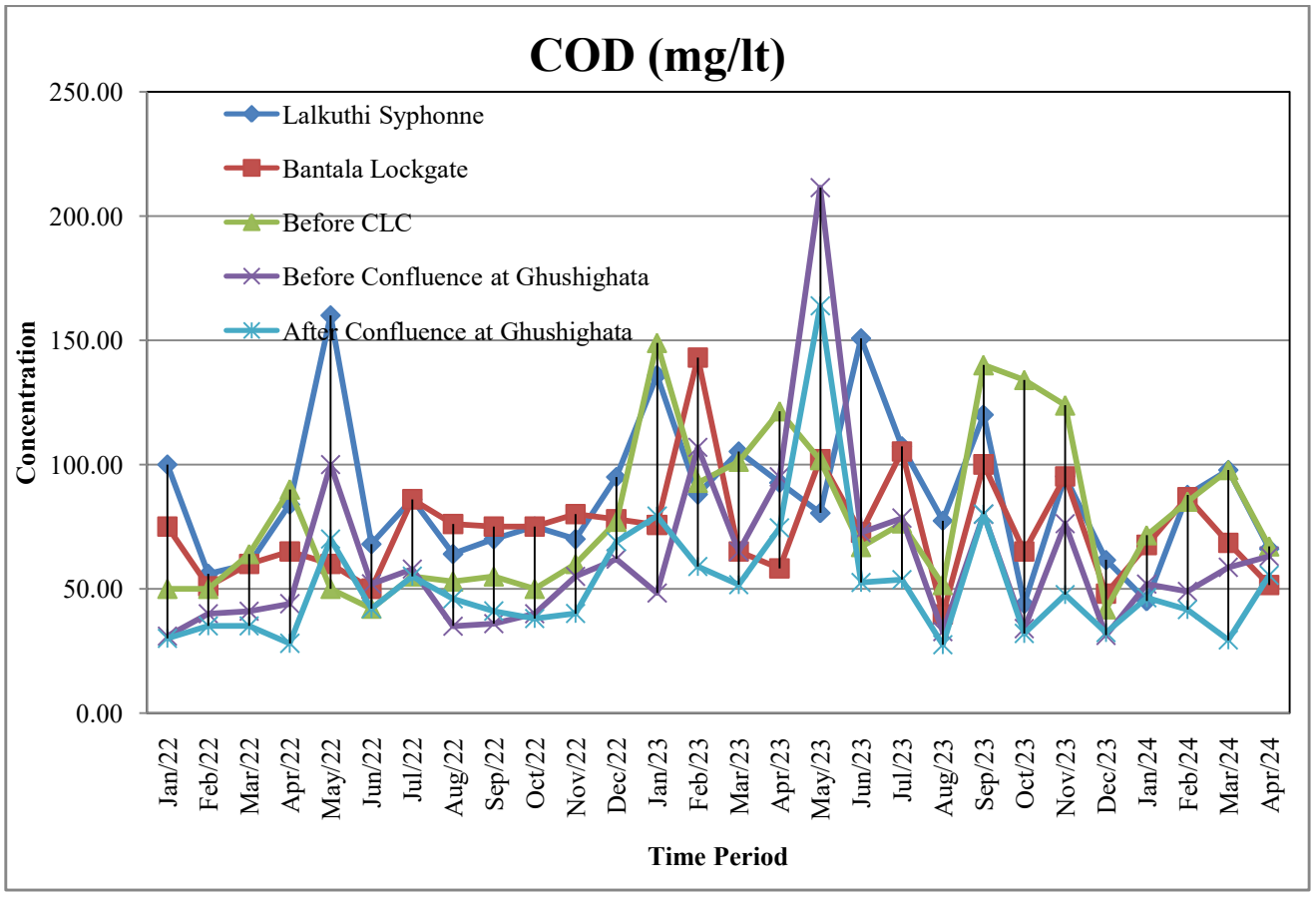

Fig. 5.2. Month wise COD concentration along the flow towards D/S

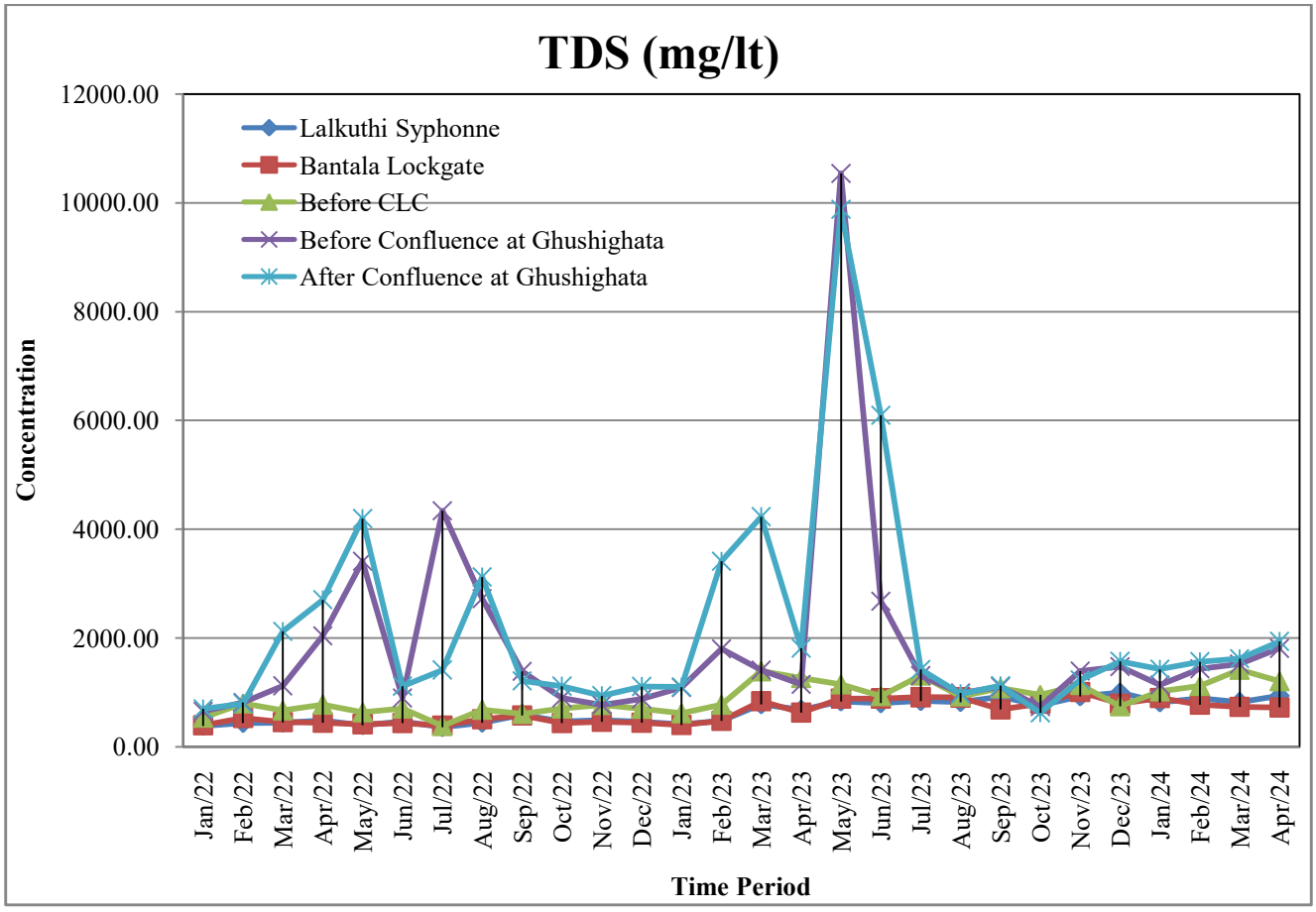

Fig. 5.3. Month wise TDS concentration along the flow towards D/S

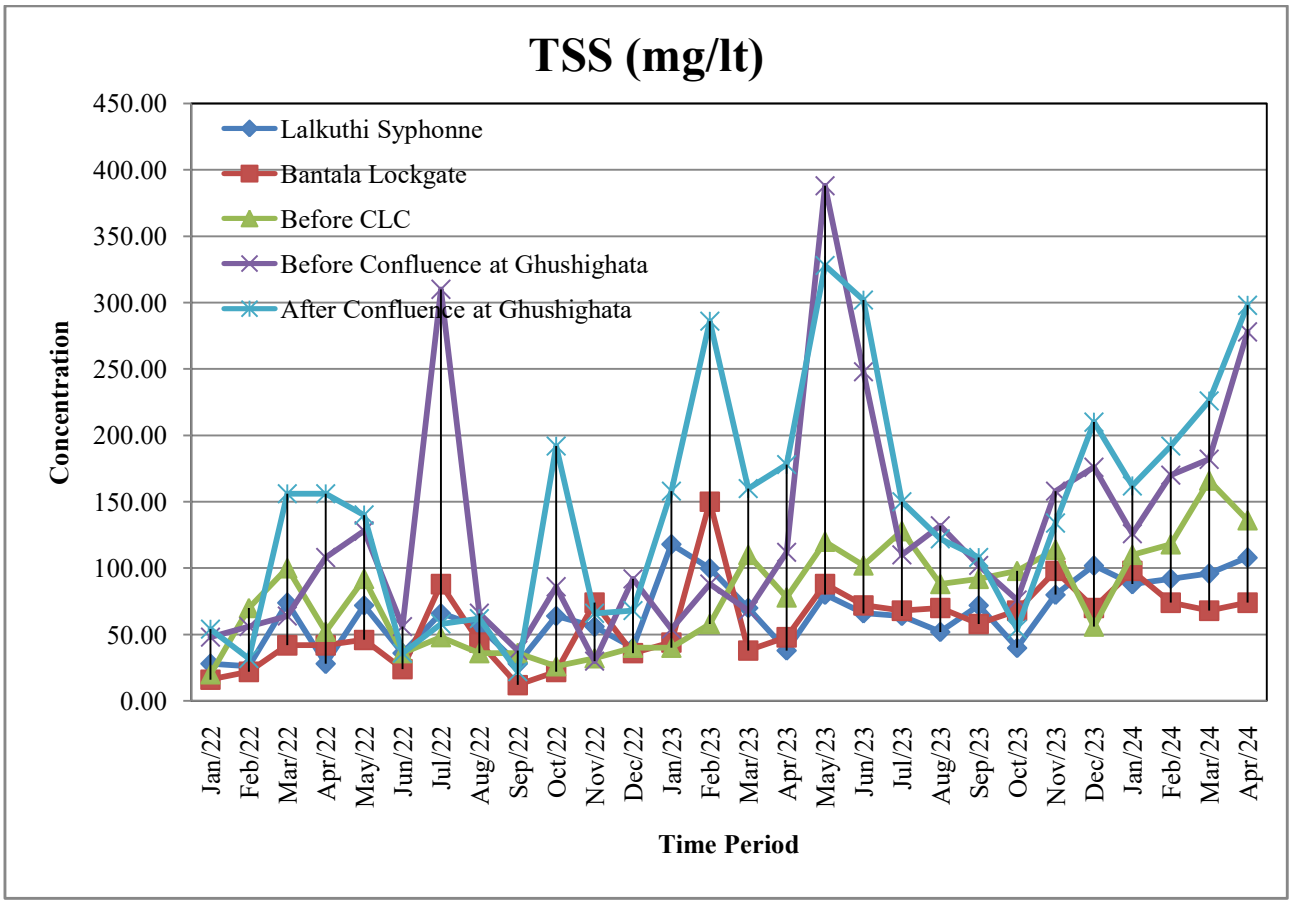

Fig. 5.4. Month wise TSS concentration along the flow towards D/S

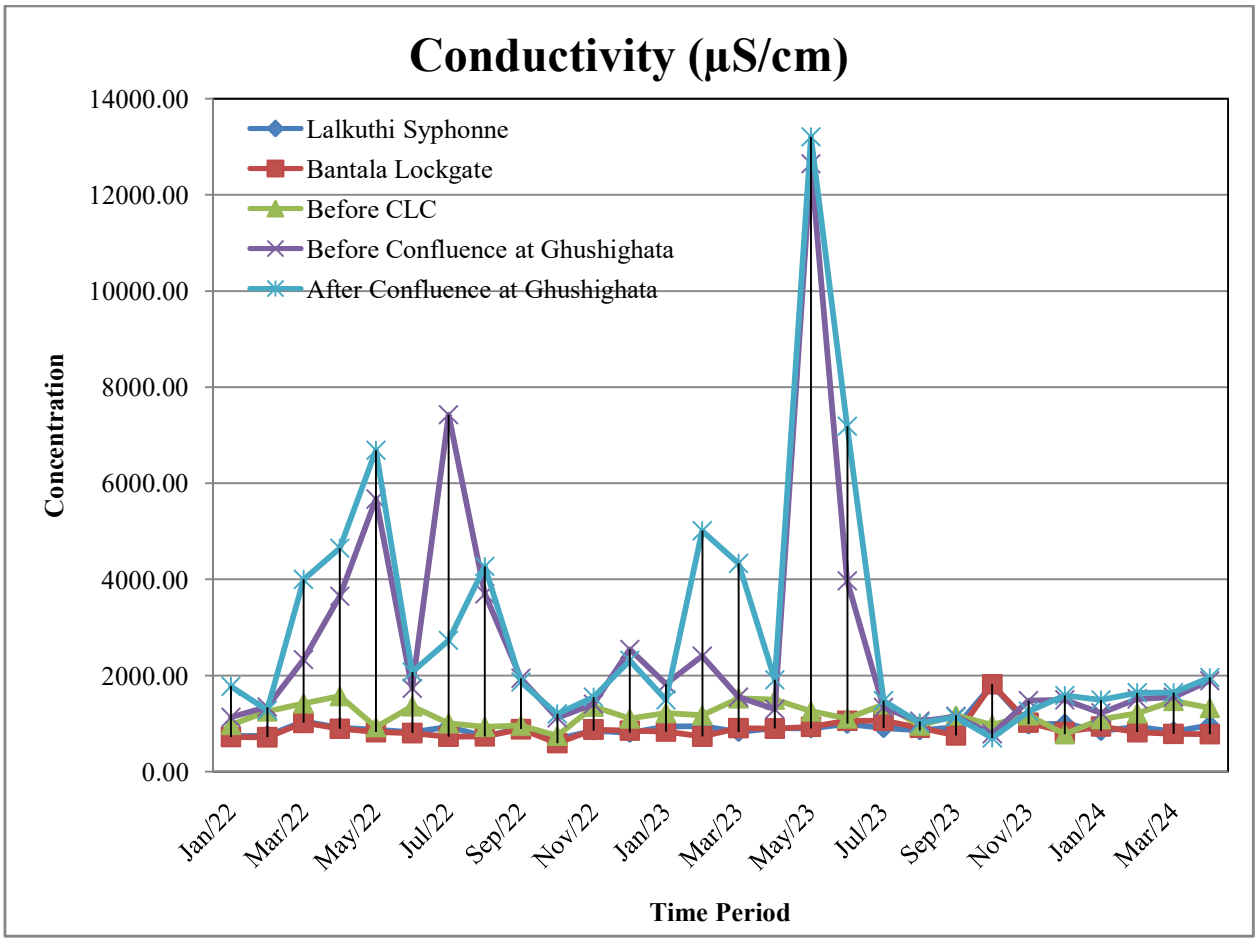

Fig. 5.5. Month wise Conductivity concentration along the flow towards D/S

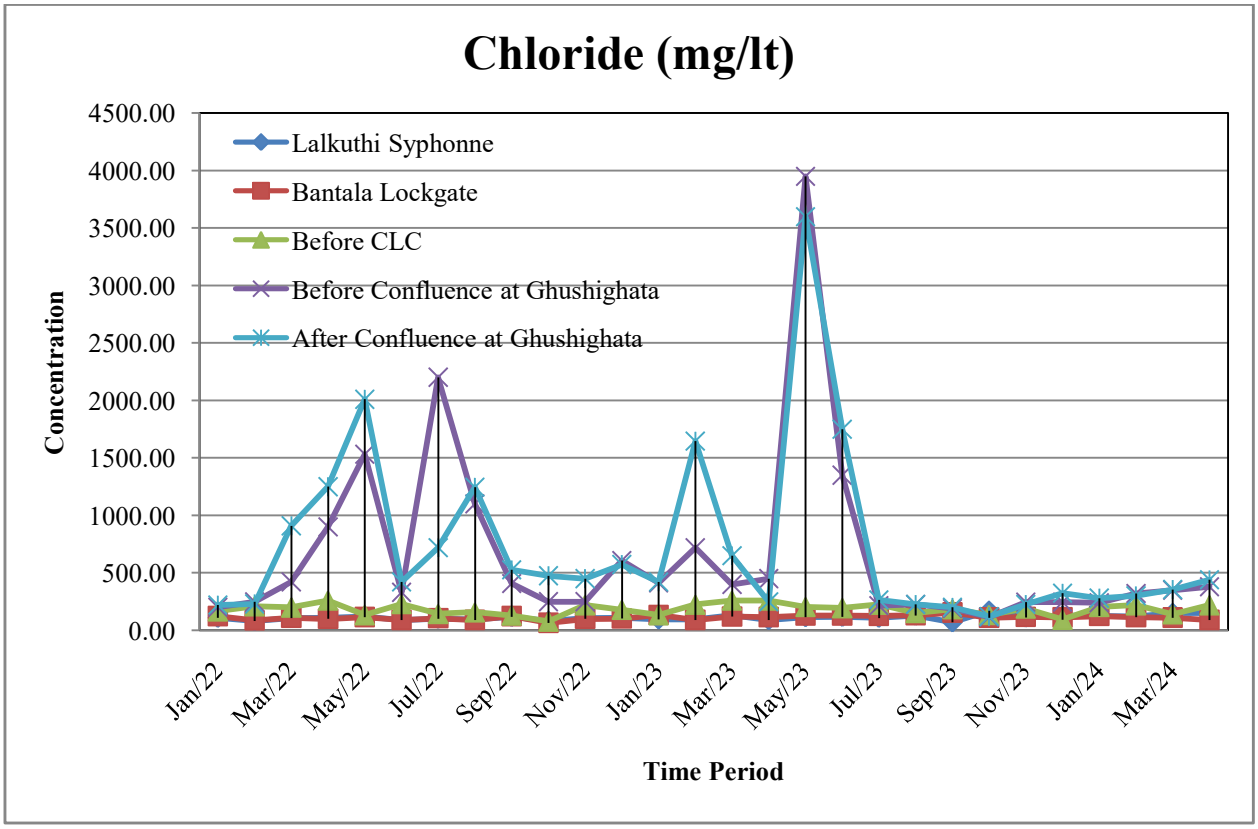

Fig. 5.6. Month wise Chloride concentration along the flow towards D/S

5.5. Observations made from the graph

1. In every sample it is observed that the COD value is many times more than the BOD value indicating presence of inorganic discharges from Industries.
2. We also observed several hikes in COD and BOD values along the flow that indicates at several locations new waste load are being introduced to the flow.
3. No distinct seasonal variation is observed in physicochemical parameters, there is no separable variation in pre-monsoon and post-monsoon data.
4. Average value of COD remains below 100 ppm and average BOD value remains below 30ppm with a increasing trend with time which indicates increase in waste generation.
5. Total dissolved solids (TDS) are the main problem compared to the suspended solids. TDS value of the sewage is much higher averaging more than 1000 ppm where TSS value of the sewage is averaging approximately 100 ppm.
6. From the graphical similarity of TDS and Conductivity we can say that the Dissolved solids are mainly of inorganic ions which are main controlling factor in Conductivity.
7. Chloride is one of the main governing pollutants which have a major contribution in Conductivity and TDS.
8. There is a hike in Chloride value as well as in Conductivity value observed from February ’22 to June’23 after the Calcutta Leather Complex Sample Point indicating high discharge of Chloride Pollutant from that Industrial Zones.
9. The graphical representation of TDS, Conductivity and Chloride values shows a parallel variation indicating high contribution of chloride component in the sewage as well as in TDS values.
10. Quantities of Heavy metals are below detection limit in the sewage.

5.6. Discussion based on Test Results and Site Visits

1. There are 3 numbers of division of Canals after Bantala Lock-Gate that is, (i) Main DWF, (ii) Secondary or, Pakka Canal and (iii) Feeding Canal.

Fig. 5.7. Canal division at Bantala Lock Gate

2. All Treatment Ponds (TP) are consuming sewage water for fisheries from Feeding Canal only and after pisciculture use discharging the same into Feeding Canal. The methods they are adopting for the sewage water intake into the TP's are,

a. By using Pipe and Pump.

b. Through a Secondary Canal or, Drain, i.e. only when the TP's are far away from the Feeding Canal.

c. Direct intake from the Feeding Canal by using underground pipes.

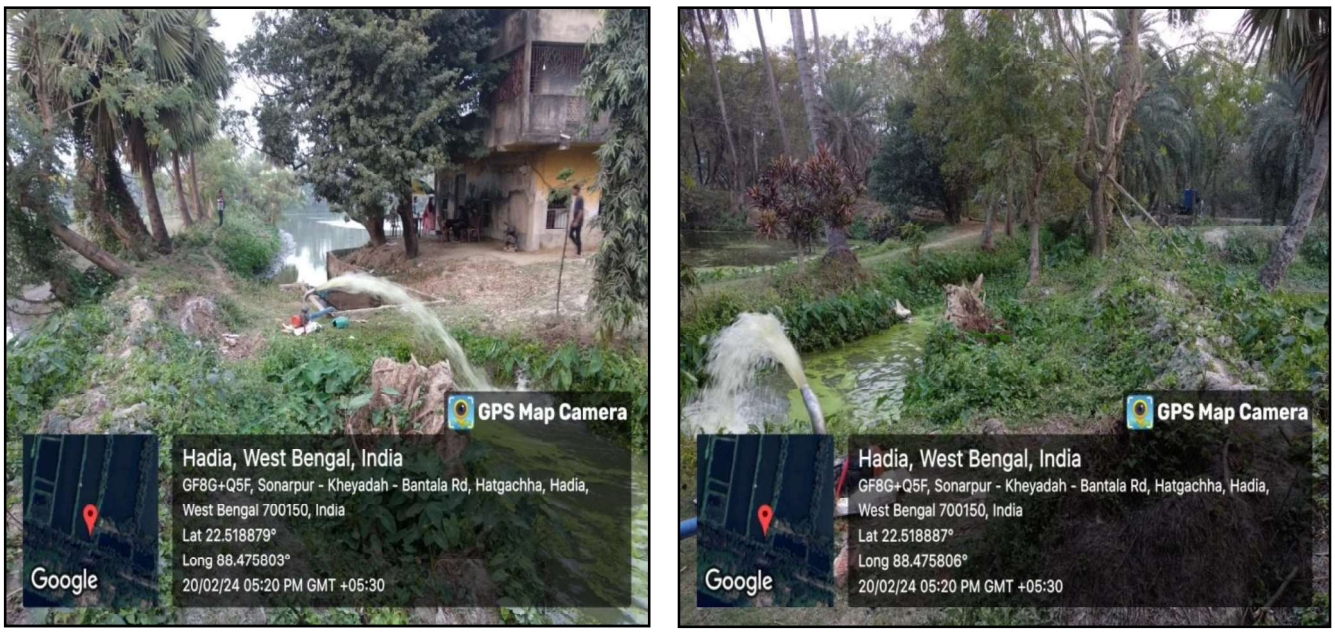

Fig. 5.8. Treated water is pumping out to the drain from Treatment Pond

3. Depths of all ponds are within 0.3-0.8 m (i.e. not exceeding 1 m Depth).

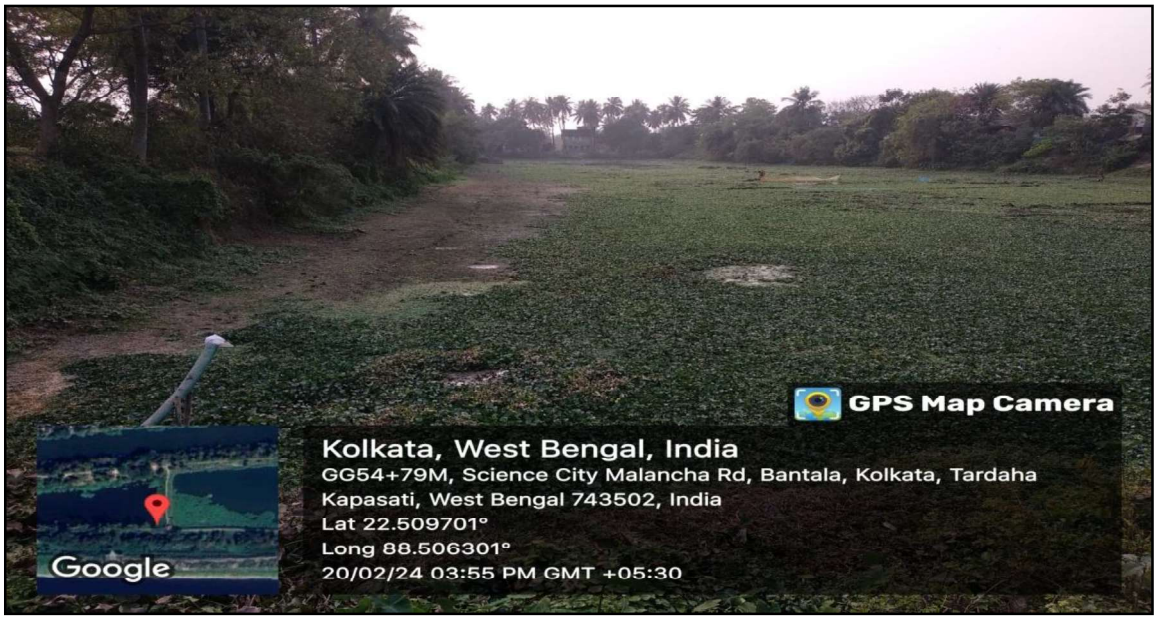

Fig. 5.9. An empty pond for getting a better idea about depth

4. At the end (22.503309, 88.509235) few pond are taking water from Feeding canal and discharging it in Kestopur Canal hence, diverting the treated water to another canal route.

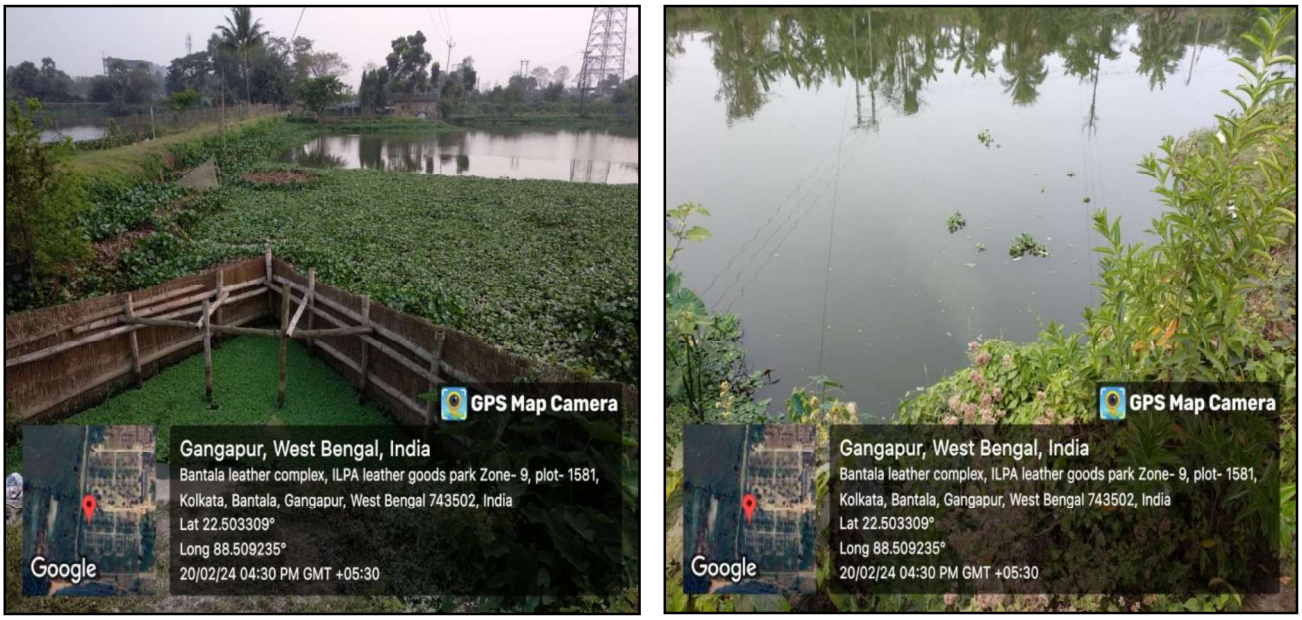

Fig. 5.10. Side of a Treatment Pond Connected to Kestopur Canal

5. As because all TPs are taking water from Feeding Canal and discharging the same also in it hence, the Water Quality is getting better and better along the D/S of the Feeding Canal and the Discharge of Canal Flow is also decreasing along the D/S and finally getting terminated near Karaidanga, Gangapur (22.507817, 88.513468).
6. At present most of the treatment ponds or, bherries are privet owned and no inter connection is found between them. The reason for no interconnectivity mentioned by the local fish farmers are as mentioned below,

a. Fishes might get relocated from one pond to another.

b. Sewage load or, fish food got reduced when water travels from one pond to next pond hence, they have to use pump to get proper sewage load as food. To avoid this they directly consume sewage water from feeding canal.

c. Nuisance or, poison might spread among the pond if there is any interconnectivity.

7. Near Bamanghata area few bherries are found with proper interconnectivity. Where the waste water being travelled from one TP to next TP by reducing the waste load.

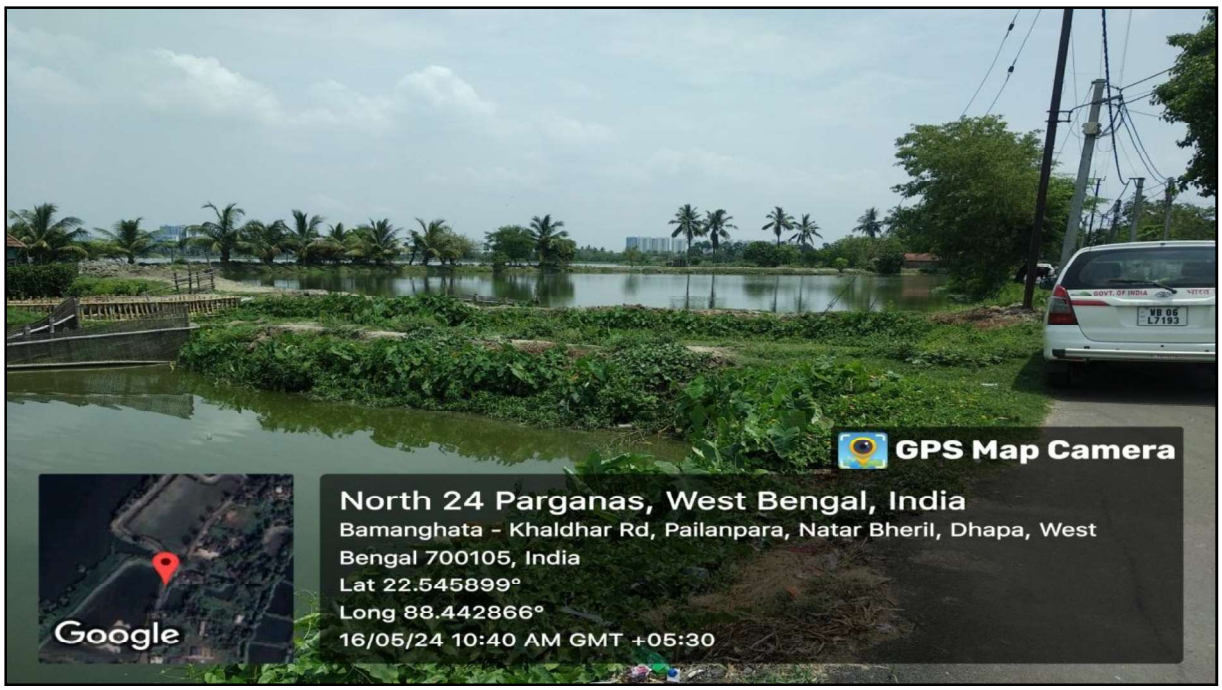

Fig. 5.11. Treatment Pond with Proper interconnectivity

Chapter-6

6. Conclusion

Referring to the thesis titled **'Assessment of Efficacy of Natural Treatment System of East Kolkata Wetland for Treatment of Municipal Sewage of Kolkata City'** where we tried to indentify the actual sewage pathway and existing natural treatment efficacy in the zone of East Kolkata Wetland. Hence we did a survey on the EKW area by reviewing literature, doing site visit, collecting samples from various ponds and canals and by communicating with locals and fishermen to understand the past and existing sewage flow pathway, conventional treatment procedure adopted by fishermen and finally we can conclude the followings,

i) Almost all ponds are receiving sewage ones a year and after that allowing the intake of sewage water as per pond's water level and fish food demand. Very few ponds are there that are receiving sewage water twice a year.

ii) There is no existing interconnection in between ponds in most of the part of EKW where effluent irrigation is being practiced.

iii) Treatment ponds are taking influent from feeder canal and after use discharging the effluent into the same feeding canal which is finally getting submitted in main DWF canal. Fishermen are using connecting drains and pumps as per their convenience for the waste water intake and disposal.

iv) BOD, COD and Nitrate-N values are on safer side as per Schedule VI, General Standards For Discharge of Environmental Pollutants Part-A: Effluents, The Environment (Protection) Rules, 1986.

v) TSS values are exceeding effluent standards as per Schedule VI, General Standards For Discharge of Environmental Pollutants Part-A: Effluents, The Environment (Protection) Rules, 1986. Indicating several source even after the EKW treatment.

vi) Chloride and Conductivity values are found also very high indicating industrial discharge. We have also observed hikes in these values during several months indicating industrial discharge without treatment.

Chapter-7

7. Future Scope of the Study

The East Kolkata Wetland covers a vast area, numerous canals and drains along with the treatment ponds are there forming a circuit in the sewage pathway. In the stipulated time period of my master degree thesis it was not possible to track and perform the quantitative and qualitative assessment of the whole sewage pathway.

- An extensive survey work along with quantitative and qualitative assessment with sufficient laboratory facility, manpower and transportation should be performed on year basis to get a proper understanding about whether the kidney of Kolkata performing as per its said natural treatment standards or not.
- A survey should be conducted to identify the locations of effluent outlets in the sewage pathway and perform quantitative and qualitative assessment of those points too.
- Various studies conducted by several researchers mentioned significant presence of heavy metals in the EKW sewage water but no such Government report has shown the presence of heavy metals. A joint survey should be conducted to get a clear idea about the present scenario of heavy metal contamination in EKW.

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