TREATMENT OF LABORATORY WASTEWATER USING CONSTRUCTED WETLAND

NAME- SWATI KUMARI MISHRA
ROLL NUMBER- 22PO470021
MASTER OF BIOTECHNOLOGY
GUIDE- Dr. MEGHANATH PRABHU

TREATMENT OF LABORATORY WASTEWATER USING CONSTRUCTED WETLAND

Dissertation Report
Course Code and Course Title: GBT 651 Dissertation
Credit: 16
Submitted in partial fulfilment of the requirement for the degree of
Master Of Science in Biotechnology

by
SWATI KUMARI MISHRA

Roll no – 22PO470021

ABC ID-: 175-473-898-879

PR No- 202208790

Under the supervision of

Dr. MEGHANATH PRABHU

School of Biological Science and Biotechnology

M.sc Biotechnology

Goa University

April 2024

Examined by:

Seal of the school

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Treatment of Laboratory Wastewater using Constructed Wetland" is based on the results of investigations carried out by me in the M.Sc. in Biotechnology at the, Goa University SBSB under the Supervision of Dr.- Meghnath Prabhu and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/experimental or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Swati Kumari Mishra

Signature and Name of the student
Roll No: 22P047021

Name of the discipline: M.Sc. in Biotechnology
Name of the school: School of Biological Science and Biotechnology

Date: 08/04/2024
Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report **'TREATMENT OF LABORATORY WASTEWATER USING CONSTRUCTED WETLAND'** is a Bonafede work carried out by **MISS** SWATI KUMARI MISHRA under my supervision in partial fulfilment of the requirements for the award of the degree of **MASTERS of SCIENCE** in the Discipline **BIOTECHNOLOGY** at the **SCHOOL OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY**, Goa University.

Signature and Name of Supervising Teacher

Date-: 08/04/2024

Signature of Dean of the School/HoD of Dept

Dean of School of Biological Sciences
& Biotechnology
Goa University, Goa-40[illegible]

Date: 8/4/24

Place: Goa University/Name of college

SBSB: Biotechnology ★ GOA UNIVERSITY ★ Taleigao Plateau, Goa 403206

School/Dept Stamp

Contents

PREFACE

The sight of chemicals and detergents swirling down the sink after each glassware wash troubled me deeply. In the midst of my biotechnology studies, I couldn't shake a simple question: why couldn't we do something about the chemicals and detergents we washed down the sink in our labs?

During an internship at BITS Pilani, I stumbled upon a simple yet powerful idea: constructed wetlands. Talking to Dr. Rajayashree Yarangal opened my eyes to the possibility of treating our own water sustainably.

Excited, I shared this idea with Dr. Meghanath Prabhu, my dissertation guide, who was equally enthusiastic. With his support, I plunged into this journey.

This research is about our exploration of constructed wetlands—a journey fuelled by curiosity and a desire to make a difference. It's my hope that our findings inspire others to think creatively about environmental solutions.

SWATI KUMARI MISHRA

ACKONWLEDGEMENT

Standing here, at the culmination of my dissertation journey, I find myself overwhelmed with a profound sense of gratitude towards those who have been instrumental in shaping my journey directly or indirectly.

First and foremost, I owe a huge debt of thanks to my dissertation guide, Dr. Meghanath Prabhu. Dr. Prabhu isn't just a mentor to me; he's been like a guiding light, always there to offer support, encouragement, and even a bit of humour when things got tough. His positivity and approachability turned what could have been a nightmare into a truly enjoyable learning experience.

I also want to express my heartfelt appreciation to Dr. Rajashree Yaragal for her inspiration and encouragement in helping me choose my dissertation topic. Her passion for the subject matter ignited my own curiosity and set me on the path towards meaningful research.

Gratitude is also extended to Birla Institute of Technology, with special appreciation for Professor Srikanth Mutnuri, whose generosity in granting access to the laboratory facilities has facilitated the smooth execution and safety of my analytical pursuits. Without his generosity and willingness to share resources, my journey would have been far more challenging. Additionally, I am thankful to Goa University for providing the necessary platform for the realization of my dissertation goals. Their support and resources were instrumental in bringing my research to life.

I am deeply thankful to Paresh Gaonkar for his invaluable assistance in analyzing the samples throughout my dissertation. His down-to-earth nature has truly inspired me and made this journey easier.

A big shoutout to the amazing non-teaching staff! Serrao sir, your constant support and guidance have meant a lot to me. Samir sir and Ashish sir, thanks a ton for making sure proper distribution of the glassware and chemicals. Your help kept things running smoothly and made all the difference!

I am thankful my friend, Akash Rajwar. He was more than just a study buddy; he was a pillar of emotional support during those moments of doubt and frustration. His friendship made the journey not only bearable but memorable.

Of course, none of this would have been possible without the unwavering belief and support of my parents. They've been my biggest cheerleaders from day one, always encouraging me to pursue my dreams and believing in my ability to succeed.

And finally, I want to acknowledge the challenges and obstacles along the way. While they may have tested my patience at times, they ultimately made me stronger and more resilient.

So, here's to the incredible individuals and institutions who have helped shape my dissertation journey. I am truly grateful for each and every one of you.

FIGURES AND TABLES

ABBREVIATION USED

HSSF CW	Horizontal Subsurface Flow Constructed Wetland
VSSF CW	Vertical Subsurface Flow Constructed Wetland
FWSF CW	Free Water Surface Flow Constructed Wetland
SFCW	Surface Flow Constructed Wetland
SSFCW	Subsurface Flow Constructed Wetland
mm	Millimeter
cm	Centimeter
m	Meter
m^3	Meter cube
m^2	Meter square
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
TSS	Total Suspended Solid
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TC	Total Nitrogen
TOC	Total Organic Carbon
TNC	Total Inorganic Carbon
Cd	Cadmium
Zn	Zinc
Ag	Silver

ABSTRACT

Constructed wetland have emerged as promising budget friendly solution of the treatment of the wastewater exhibiting notable efficacy despite initial challenges. We constructed 2 stage vertical flow wetland for the treatment of the laboratory wastewater. The collected water sample from tank 1 and tank 2 along with inlet sample was further subjected to analysis to check the efficiency of the setup.

Instances of clogging necessitated pivotal adjustments, including the installation of a mesh-inlet tank to alleviate the accumulation of agar media, ensuring smoother operation. Notably, the system achieved a COD removal efficiency of 92.24%, phosphorus removal efficiency of 72.31%, Total Carbon (TC) removal efficiency of 36.44%, and Total Nitrogen (TN) removal efficiency of 91.04%. Moreover, the wetland maintained stable pH levels around 7.41, further demonstrating its effectiveness in stabilizing wastewater pH. Despite challenges, the constructed wetland system shows promise as a sustainable and efficient approach to wastewater treatment, with potential to mitigate environmental pollution and ensure water quality. Continued research and refinement are crucial for optimizing performance and advancing wastewater treatment technologies.

CHAPTER 1

INTRODUCTION

1.1 Background

Constructed wetlands have emerged as a sustainable and effective solution for wastewater treatment over the years. It mimics the natural purification processes found in wetland ecosystems (Kadlec & Wallace, 2009).

This organic system incorporates water, aquatic flora such as reeds and duckweed, microorganisms, and filter beds composed of sand, soils, or gravel. Its adaptability in design, materials, and technology makes it a versatile choice, suitable to local conditions and available land (Vymazal, 2010). There are different forms or designs of constructed wetland based on the ease of operation, treatment efficacy and construction. In the surface flow wetland, the flow of the water is above the soil level and is sealed from below to retain the water and subsurface flow which can be vertical or horizontal (Barbeau et al., 2003).

The substrate includes soil, pebbles, zeolite, anthracite, forsterite, manganese sand, granite, volcanic rocks, quartz, soil, charcoal, laterite stone, recycled clay bricks, coconut or palm kernel shells, hollow bricks, ceramic, artificial ecological substrates, steel slag, activated carbon, sponge iron to help plants anchor to specific dept (H. X. Wang et al., 2018). The surface of the wetland has high oxygen content compared to the bottom supporting aerobic purification (nitrification). Both surface and subsurface flow comes with their pros and cons. The advantage of the surface flow system is that it is relatively cheaper and easy to operate compared to subsurface flow. While the disadvantages include foul smell, breeding ground for organisms like mosquitos and lager area compared to other remediation processes (Taha et al., 2023; H. X. Wang et al., 2018). One of the key components of constructed wetlands is the choice of suitable wetland plants, such as *Canna indica*, which can thrive in waterlogged conditions and help remove pollutants from the water (Cui et al., 2010). These plants play a crucial role in pollutant uptake, oxygenation, and microbial support, making them an integral part of the treatment process.

Proper design and sizing are essential for the success of constructed wetlands. The site selection should consider factors such as proximity to pollution sources and regulatory requirements (Vymazal, 2011). Multi-cell systems are often employed to optimize treatment efficiency and ensure adequate hydraulic retention times (Mitsch & Gosselink, 2015). Monitoring and control are crucial aspects of constructed wetland operation (Vymazal, 2011). Regular assessment of influent and effluent water quality parameters, including pH, turbidity, BOD, COD, nutrient concentrations, salt and metal concentration (NaCl, NaOH, manganese, Zn, Copper, nickel sulphate, toluene, phenol, magnesium sulphate, mercury) allows for adjustments in flow rates to ensure efficient treatment (Kadlec & Knight, 1996).

Maintenance of constructed wetlands includes removing accumulated solids, managing plant growth, and ensuring that inlet and outlet structures are in good condition (Liu et al., 2007). Adequate maintenance helps preserve the wetland's treatment capacity over time. Also, Data collection and analysis are fundamental for evaluating the performance of constructed wetlands (Vymazal, 2011). Analysis of collected data can identify trends, issues, and areas for improvement in the treatment process. In addition to wastewater treatment, constructed wetlands offer multiple benefits, including habitat creation, water purification, and carbon sequestration (Hsu et al., 2011). They are environmentally friendly and sustainable solutions for mitigating the impact of various types of pollution.

1.2 Aim and objective

Following the simplicity and effectiveness of the design the aim of this research was to treat the laboratory wastewater using a constructed wetland, while objective included, Design and construction of the wetland for the treatment of laboratory wastewater.

1.3 Hypothesis/ Research question

Constructed wetland is simple and effective in treatment of wastewater. In this study it is hypothesize that constructed wetland system is able to effectively treat the laboratory wastewater. This study will address several key inquiries regarding the efficacy and implications of constructed wetlands in laboratory wastewater treatment. Firstly, what are the removal efficiencies of pollutants in wastewater treated by constructed wetland systems? Secondly, how do design factors, vegetation type, and substrate composition, influence the performance of constructed wetlands in treating wastewater? Thirdly, what are the environmental advantages and constraints associated with employing constructed wetlands for wastewater treatment? Additionally, how does the integration of supplementary treatment technologies, such as aeration or filtration, contribute to the overall effectiveness of constructed wetlands in wastewater treatment? Lastly, what are the economic considerations and feasibility aspects regarding the adoption of constructed wetlands as sustainable alternatives for wastewater treatment in comparison to conventional methods? These research questions provide a comprehensive framework for investigating the potential and challenges of constructed wetlands in wastewater treatment applications.

1.4 Scope

Laboratory wastewater is classified as hazardous waste and requires special and careful treatment. It typically contains high levels of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Nitrogen (TN), Total Carbon (TC), ammonia, and often has fluctuating pH levels (*Understanding Laboratory Wastewater Tests*, 2022.). Laboratory waste water may also contains trace amount of heavy metals ("Removal Methods of Heavy Metals from Laboratory Wastewater," 2019). This wastewater can find its way into water bodies, entering the food chain and potentially contaminating groundwater tables. Consuming water contaminated with chemicals can have adverse effects on health. Therefore, it's crucial to treat this wastewater before discharging it to ensure environmental and public health protection. There's an urgent need to explore alternative methods that are not only environmentally friendly but also cost-effective.

This research focuses on the detailed examination of constructed wetlands, including their design, operational principles, and efficacy in treating laboratory wastewater. It investigates the range of materials and substrates employed in their construction and location choices. Furthermore, the study looks into the economic aspects of utilizing constructed wetlands as an eco-friendly wastewater treatment option compared to traditional methods. Key research questions address pollutant removal rates, and both the strengths and limitations of constructed wetlands in wastewater treatment.

CHAPTER 2

LITERATURE REVIEW

Wastewater from various sources like research laboratories and pathological laboratories, tanning, leather, food and chemical industries and households, find their way into water bodies polluting them. Anthropogenic activities are a major cause of surface and groundwater pollution. Elevated concentrations of heavy metals, ammonia, phosphate, or other harmful chemicals can hold serious health implications for the human body (Witkowska et al., 2021).

Consuming polluted water that contains harmful chemical toxins has serious implications on health. People in Flint Michigan suffered skin irritations, hair loss and high levels of lead in the bloodstream. Other extreme scenarios include, damage to the immune, reproductive, cardiovascular and renal systems, cancer and diminishing brain function (*Flint Water Crisis*, 2018; Wani et al., 2015).

On the other hand, laboratory wastewater management presents unique challenges due to its varied composition and potential hazards associated with chemicals, reagents, and biological materials. Effectual treatment methods are indispensable to mitigate environmental pollution and ensure compliance with regulatory standards.

The World Health Organization has highlighted a concerning reality: over one-third of the global population faces challenges in accessing fundamental sanitation and clean drinking water. This predicament exposes individuals to water containing sewage, bacteria, and hazardous elements like heavy metals, posing significant health risks. For instance, contaminated water harboring bacteria from sewage can instigate severe illnesses such as cholera, diarrhoea, dysentery, hepatitis A, polio, and typhoid. Shockingly, an estimated 297,000 children under the age of five succumb annually due to exposure to polluted water sources, with a significant majority of these cases occurring in developing nations. Consequently, it becomes imperative to prioritize the adoption of effective and cost-efficient treatment processes to address this pressing global issue
(1 in 3 people globally do not have access to safe drinking water – UNICEF, WHO, 2019).

The emergence of microplastics, defined as plastic particles smaller than 5mm, represents a pressing concern in the realm of water pollution, warranting considerable attention and apprehension. Despite being a relatively recent focus compared to other forms of water contamination, the pervasiveness of microplastics is alarming. Recent research demonstrates their presence in the most secluded regions of the Earth, spanning from the depths of the Mariana Trench to the peaks of Mount Everest. Furthermore, these minute particles have been identified within the human body, underscoring the urgent need for action. ("EnviroTech Online",Khan et al., 2023). The direct consequences of consuming polluted water may not be immediately evident, but there are indirect effects on living organisms, including humans. This is primarily due to the fact that over two-thirds of the world's freshwater reserves are allocated for agricultural purposes. As a result, this allocation leads to diminished resources, ultimately resulting in reduced crop yields and lower-quality produce (Osman et al., 2023). Water pollution poses a significant threat to the food chain, potentially leading to a shortage of food for humanity. With the global population expected to reach around 10 billion by 2050, agricultural output must increase by roughly 50% to meet demand. However, if water pollution obstructs this vital expansion, it could trigger widespread famine and hunger, especially in developing countries (*Microplastics*, 2017) (Wiener, National Institute of Environmental Health Sciences, 2023).

Polluted water not only affects humans but also the environment in which we live. Some common environmental problems which occur due to pollution includes, eutrophication, plastic ingestion, bioaccumulation, acidification, loss of species etc.

The decline in the quality and accessibility of freshwater, particularly for household and industrial purposes, is attributed to urbanization and the limitations of conventional Wastewater Treatment (WWT) methods. While traditional WWT techniques have achieved moderate success in treating wastewater to meet standard discharge regulations, there's a pressing need for advancements in WWT to convert treated wastewater into a reusable resource across industrial, agricultural, and domestic sectors. Emerging technologies such as membrane technology, microbial fuel cells and microalgae present promising alternatives to conventional WWT processes and distribution systems. These innovations aim to effectively reduce contaminants to acceptable levels, thereby addressing the challenges posed by declining freshwater resources (Kwaku Armah et al., 2021; Paucar & Sato, 2021).

Conventional wastewater treatment processes typically involve several sequential stages. The initial step, known as preliminary treatment, employs physical methods such as screening and grit chambers to eliminate large debris and heavy solids from the wastewater. Subsequently, primary treatment utilizes sedimentation to separate solids from the wastewater, resulting in the formation of sludge and scum. Following primary treatment, the wastewater undergoes secondary treatment, which involves biological processes where aerobic bacteria degrade organic contaminants. If necessary, tertiary treatment may be applied to further refine the water by targeting remaining impurities such as nutrients and pathogens through processes like filtration or disinfection.

To achieve treatment goals of laboratory wastewater, chemical treatment approaches like coagulation, flocculation, oxidation, and disinfection are commonly utilized for the removal of organic and inorganic pollutants from laboratory wastewater. Studies have shown the efficacy of such methods in reducing pollutant concentrations and achieving compliance with discharge regulations. However, challenges such as chemical cost, sludge disposal, and potential formation of harmful by-products necessitate careful optimization and management of chemical treatment (Xiaomin Tang, 2014).

Despite the widespread application of conventional or chemical method, the method of wastewater treatment methods has inherent limitations. They often fail to address emerging contaminants, consume significant energy, generate sludge that requires careful management, inadequately remove nutrients, and necessitate substantial space and infrastructure. These drawbacks have spurred the exploration of alternative technologies aimed at achieving greater efficiency, reduced energy consumption, enhanced contaminant removal, and sustainable wastewater management (Kwaku Armah et al., 2021). To overcome the disadvantages of the conventional wastewater treatment method new emerging technologies are taking over.

Membrane System: Reverse osmosis, ultrafiltration, and nanofiltration are advanced water treatment technologies that utilize semi-permeable membranes to filter pollutants from water sources. These membranes remove particles, microorganisms, and undesirable substances, yielding clean water suitable for various applications. The membrane systems are versatile, adaptable from small-scale setups to large treatment plants. However, these membrane technologies have certain disadvantages. They are prone to fouling, which necessitates frequent cleaning and replacement of the membranes. Additionally, their efficiency in removing specific ions from water depends on the nature of the water pollutant, making them ion-specific in their effectiveness. Despite these drawbacks, these membrane technologies remain valuable tools in the realm of water treatment, offering effective solutions for purifying water from various sources (Hophmayer-Tokich, 2006.; Kwaku Armah et al., 2021).

Open Pond and Microalgae: Open Pond systems utilize microalgae to treat wastewater by harnessing the natural process of photosynthesis. Microalgal cells within these systems effectively remove nutrients like nitrogen and phosphorus from wastewater, thereby purifying it. This method is particularly advantageous due to its environmental friendliness, as it requires minimal energy input and can yield biomass suitable for various applications, including biofuel production and other fields. However, open pond systems encounter challenges that affect their productivity. Fluctuations in temperature and sunlight levels, as well as variations in wastewater composition, can significantly impact algal growth and system performance. Managing algal growth and ensuring the stability of open pond systems are key challenges that need to be addressed to optimize their effectiveness in wastewater treatment. Despite these challenges, open pond systems remain a promising and sustainable approach to wastewater treatment, offering potential solutions to environmental and energy-related concerns (Armah et al., 2021)

Microbial Treatment and Microbial Fuel Cells (MFCs): Microbial treatment utilizes microbial communities to degrade organic matter present in wastewater. One notable application of this approach is seen in microbial fuel cells (MFCs), where microorganisms generate electricity as they decompose organic substances. MFCs present a dual advantage of wastewater treatment and electricity generation, offering versatility in operating across diverse conditions. Their potential applications extend to remote areas or regions with limited access to electricity. Nonetheless, challenges include limitations in power output and difficulties in scaling up for larger applications. Additionally, ongoing research focuses on optimizing microbial communities and ensuring stable performance of MFC systems (Armah et al., 2021).

Constructed wetlands have emerged as a favoured option for wastewater treatment due to their ease of operation, low cost, minimal maintenance requirements, and versatility. The origins of this technology trace back to the early 1950s when Dr. Käthe Seidel conducted pioneering experiments using wetland plants for wastewater treatment in Germany. By the late 1960s, full-scale systems were operational. In Europe, subsurface systems gained prominence, while North America and Australia predominantly favoured free water surface systems. Although dissemination of information about constructed wetland technology was gradual during the 1970s and 1980s, the 1990s marked a significant turning point. During this period, increased global scientific exchange accelerated its international adoption. To enhance the removal efficiency of pollutants such as ammonia and total nitrogen, a combination of vertical and horizontal flow constructed wetlands emerged during the 1990s and 2000s. These combined systems synergize to achieve heightened treatment efficacy, further solidifying the appeal and effectiveness of constructed wetlands for wastewater treatment (Vymazal, 2011a; Waly et al., 2022). Currently, wetlands have garnered widespread recognition as a reliable wastewater treatment technology, offering a versatile solution for various wastewater treatment requirements. Dr. Kaethe Seidel played a pivotal role in the advancement of subsurface flow constructed wetlands (SFCWs) during the 1950s in Germany. Introducing Horizontal Flow Constructed Wetlands (HFCWs), Seidel utilized coarse materials as a rooting medium. Reinhold Kickuth furthered this innovation in the 1960s by experimenting with soil media rich in clay content, coining the term "Root Zone Method." In the early 1980s, HFCWs made their debut in Denmark, with nearly 100 soil-based systems operational by 1987. The late 1980s saw the proliferation of HFCWs to countries such as Austria and the UK, followed by widespread adoption in the 1990s across Europe, North America, Australia, Asia, and Africa. During this period, there was a notable shift towards the use of coarser materials replacing soil or sand in the systems, signifying a continuous evolution and refinement of constructed wetland technology (Vymazal, 2010).

Despite their potential for adoption in developing countries, especially by small rural communities due to their affordability and ease of maintenance, Constructed Wetlands (CWs) have not gained widespread traction in India. This limited adoption is primarily attributed to a lack of awareness and local expertise in developing this technology within the country. India's initial CW, spanning an area of 2,700 m^2 at Sainik School in Bhubaneshwar, Orissa, is planted with Typha latifolia and Phragmites karka. Currently, it treats 180-200 m^3 of wastewater and has demonstrated effective removal rates for biochemical oxygen demand (BOD) ranging from 67% to 90% and nitrogen removal ranging from 58% to 63%. Despite this successful implementation, broader awareness and capacity building efforts are needed to promote the wider adoption of CW technology in India (Juwarkar et al., 1995; Ruiz-Ocampo et al., 2022).

Additional Constructed Wetland (CW) initiatives in India include a Horizontal Flow (HF) demonstration unit at Ekant Park in Bhopal. This unit treats 70 m^3 day using a 700 m^2 HF system filled with gravel and Phragmites karka. Monitoring results from April 2002 to September 2003 revealed significant removal rates for Chemical Oxygen Demand (COD) (77%), Total Suspended Solids (TSS) (79%), and Coliform bacteria (99%). Furthermore, a field-scale HF system at Ujjain Charitable Trust Hospital in Madhya Pradesh treats 8 m^3/day, demonstrating favorable removal rates for Biochemical Oxygen Demand (BOD) (75%), TSS (78%), and Ammonium (NH4) (68%) with a surface area of 80 m^2. Similarly, in Ravindra Nagar Township, Ujjain, an HF system utilizing zeolite with particle sizes of 3-9 mm achieved approximately 70% ammonia removal between 2006 and 2008 (Parashar et al., 2022).

Limited pilot studies have been undertaken in the past decade, with notable examples including projects in Mahendragiri (Tamil Nadu) focusing on domestic wastewater and at Mother Dairy in Delhi addressing dairy wastewater, conducted by the Central Pollution Control Board (CPCB) and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ). Another significant pilot study was conducted in Ujjain, Central India, where a 42 m^2 horizontal flow system planted with Phragmites karka was utilized. Over a five-month period, this system demonstrated average removal efficiencies of 78% for Ammonium Nitrogen (NH4-N) and Total Suspended Solids (TSS), and 58-65% for Phosphorus (P), Biochemical Oxygen Demand (BOD), and Total Kjeldahl Nitrogen (TKN). (Ruiz-Ocampo et al., 2022;)

In the year 2000, a Horizontal Flow (HF) system was implemented in Ujjain, utilizing the deserted grounds of the Education College to manage sewage from the residential colony of Ravindra Nagar. Covering an area of 300 square meters and designed with a hydraulic loading rate of 40 cubic meters per day, the system was vegetated with Phragmites karka. This initiative showcased significant removal rates, achieving an 86% reduction in organic nitrogen and a 40% decrease in ammonium nitrogen.(Ruiz-Ocampo et al., 2022)

The CDD Society, a non-governmental organization based in India, has been actively advocating for the adoption of Decentralized Wastewater Treatment Systems (DEWATS) and has successfully implemented more than 350 projects across South Asia, including India (CDD 2013). These systems are characterized by a modular, energy-independent design comprising four treatment phases: a septic tank or Upflow Anaerobic Sludge Blanket (UASB), an anaerobic filter or baffled reactor, a planted gravel filter (Horizontal Flow), and, in certain instances, polishing ponds (free water system). Approximately thirty DEWATS systems have been established in India, demonstrating remarkable reductions in Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) by 97% to 99%, and treating wastewater volumes ranging from 1.5 to 615 cubic meters per day. On average, the size of the Horizontal

Flow Constructed Wetland (HF CW) per cubic meter of wastewater treated is 5.7 square meter (Gutterer, 2009).

In wetland system one pivotal aspect influencing its applicability is the design variation—horizontal and vertical wetlands. Horizontal layouts utilise gravity and topography, reducing energy needs, while vertical designs, despite needing less land, demand increased energy for operations such as pumping or syphoning. Nevertheless, constructed wetlands are considered highly cost-effective, especially in regions with accessible and affordable land (Wu et al., 2023).

The process operates through the synergistic interplay of vegetation, microorganisms, and substrates, functioning as a filtration and purification mechanism. Initially, solids settle as water enters the wetland, with larger particles being intercepted and filtered out by plant roots and substrates. Then, through natural processes facilitated by bacteria and plants, pollutants and nutrients are broken down, leading to water purification. Factors such as retention time, exposure to UV radiation, and the secretion of antibiotics by plants contribute to the effective removal of pathogens present in wastewater (Ji et al., 2022).

One of the remarkable traits of constructed wetlands is their multifaceted functionality. Not only do they efficiently treat various wastewater types—from human waste to agricultural runoff and industrial pollutants—they also pave the way for water reuse, maintain groundwater and surface water levels, and contribute to environmental conservation by providing habitats for diverse flora and fauna. Additionally, they serve as a means of water storage and enhance aesthetic appeal with their naturalistic beauty.

There are a lot of factors that affect the function and treatment efficiency of the constructed wetland; physical factors, the plant used and the bed packing material.

Physical factors affecting CW

Temperature -: The efficacy of constructed wetlands in treating wastewater is significantly impacted by water temperature. Temperature influences various processes occurring within these wetlands, including microbial reactions and the degradation of organic matter. Lower temperatures, typically below 15°C, can markedly decelerate these reactions compared to the optimal range of 20 to 35°C. Moreover, temperature fluctuations affect nitrogen cycling reactions such as mineralization, nitrification, and denitrification, although phosphorus sorption reactions appear to be less sensitive to temperature variations. While the physical removal of carbon, nitrogen, and phosphorus in particulate form is not substantially affected by temperature, overall removal rates in wetlands may vary with temperature. Design models, while useful, may oversimplify dynamics due to limited calibration data, underscoring the importance of comprehensively understanding ecosystem data to accurately interpret temperature effects. Interestingly, biochemical oxygen demand and phosphorus removal exhibit limited sensitivity to temperature, whereas nitrogen removal demonstrates a stronger dependence on it. In colder climates, seasonal temperature changes can impede treatment processes, potentially diminishing the efficiency of constructed wetlands (Allen et al., 2002; Stein & Hook, 2005).

Hydraulic Loading Rate-: The treatment efficiency of constructed wetlands heavily depends on the hydraulic residence time (HRT). HRT denotes the average duration water remains within the wetland system, directly impacting the duration of interaction between wastewater and treatment components, including plants, microbes, and substrates. A prolonged hydraulic residence time fosters extensive interaction between wastewater and treatment components, providing ample opportunities for pollutant removal. This prolonged contact facilitates processes like sedimentation, filtration, adsorption, and biological degradation, leading to enhanced removal of contaminants like organic matter, nutrients (e.g., nitrogen and phosphorus), pathogens, and heavy metals. Conversely, shorter hydraulic residence times may curtail treatment effectiveness by limiting the duration for pollutants to undergo adequate transformation or removal. Insufficient HRT can lead to incomplete treatment, potentially compromising the overall efficiency of the constructed wetland system. Optimizing the hydraulic residence time is pivotal for maximizing treatment efficiency in constructed wetlands, considering factors such as flow rate, wetland design, and specific treatment objectives (Kadlec & Wallace, 2009; Wu et al., 2023).

Pollutant Loading Rate-: The treatment efficiency of constructed wetlands is directly impacted by the rate at which pollutants are loaded into the system, as it influences the equilibrium between pollutant introduction and removal mechanisms. Pollutant loading rate denotes the quantity of pollutants introduced into the wetland system within a specified timeframe, usually expressed in terms of mass or concentration per unit time. Elevated pollutant loading rates can overwhelm the capacity of the wetland's treatment components, potentially diminishing treatment efficiency. Excessive loading may saturate available adsorption sites, exceed microbial degradation capabilities, or surpass the nutrient uptake capacity of plants, resulting in incomplete pollutant removal. Conversely, lower pollutant loading rates facilitate more efficient treatment by allowing the wetland system to effectively process and eliminate pollutants within its operational capacity. This ensures that treatment processes such as sedimentation, filtration, adsorption, and biological degradation function optimally, leading to improved pollutant removal efficiency. It is crucial to optimize pollutant loading rates to maximize treatment efficiency in constructed wetlands while avoiding conditions of overloading that could compromise performance (Kadlec & Wallace, 2008; Wu et al., 2023).

Various plants are utilized in constructed wetlands (CWs) to enhance treatment efficiency through different mechanisms. Some commonly used plants include *Phragmites australis* (Common reed), *Typha spp.* (Cattails), *Iris spp.* (Irises), *Carex spp.* (Sedges), *Canna indica* (Indian shot), *Phalaris arundinacea* (Reed canarygrass), *Acorus spp.* (Sweet flag) etc. The choice of vegetation in a constructed wetland is influenced by factors such as climate, water quality, treatment goals, and available area. Each plant species plays a unique role in treatment efficiency, influenced by its growth traits, root structure, and interactions with microbial populations. In general, the presence of vegetation in constructed wetlands boosts treatment effectiveness by fostering habitats for beneficial microbes, facilitating oxygen transfer, soil stabilization, and aiding in pollutant removal mechanisms (Bianchi et al., 2021).

Phragmites australis (Common reed): This plant is recognized for its vigorous growth and expansive root system, which aids in soil stabilization and enhances nutrient absorption. It facilitates oxygen transfer and microbial activity in the root zone, thereby enhancing the decomposition of organic matter and the removal of nutrients (D. Wang et al., 2022).

Typha spp. (Cattails): Cattails exhibit high adaptability and can thrive in diverse environmental conditions. They efficiently capture suspended solids and provide a habitat for beneficial microbes, contributing to the removal of pollutants (Lavrova & Koumanova, 2008).

Iris spp. (Irises): Irises possess dense root systems that encourage the sedimentation and filtration of pollutants. Additionally, they release oxygen into the root zone, stimulating aerobic microbial activity and bolstering the degradation of pollutants (Gao et al., 2014)

Phalaris arundinacea (Reed canarygrass): Reed canarygrass is notable for its tolerance to fluctuating water levels and resilience to harsh environmental conditions. It contributes to the removal of pollutants through processes such as sedimentation, filtration, and microbial degradation (D. Wang et al., 2022)

***Canna indica*-**: *Canna indica* is favored for wastewater treatment in Constructed Wetlands (CW) owing to its rapid growth rate, prolific biomass generation, and extensive root system. These attributes foster favorable aerobic conditions and facilitate efficient pollutant removal. Unlike other prevalent plants such as *Phragmites australis* and *Cyperus papyrus*, Canna indica distinguishes itself by its effectiveness in reducing pollution and greenhouse gas emissions. Its ability to utilize greater quantities of water, maintain extended root cycles, and stimulate nitrification processes through specialized tissue further enhance its efficacy in wastewater treatment.. (Cui et al., 2010)

Apart from its practical applications, recent studies have revealed the medicinal attributes of *Canna indica*. It has been discovered to alleviate menstrual pains and effectively treat conditions such as gonorrhea and amenorrhea. In Nigeria, the roots are ground into a powder and ingested to alleviate diarrhea and dysentery, while the flowers are utilized medicinally to address malaria. (*Canna (Canna Indica) — UIC Heritage Garden*).

Recent research has uncovered that vertical Constructed Wetlands (CWs) featuring *Canna indica* demonstrate remarkable removal efficiencies for various pollutants. These wetlands achieved an 85% elimination of total suspended solids (TSS) and significantly decreased levels of total nitrogen (TN), nitrate nitrogen (NO_3–N), ammonium nitrogen (NH_4^+–N), and nitrite nitrogen (NO_2–N) by over 95%. Moreover, these vegetated CWs exhibited notably superior capabilities in removing heavy metals compared to control conditions. Analysis of various components of *Canna indica*, including roots, rhizomes, leaves, and stems, revealed the accumulation and distribution of toxic elements. Scanning electron microscopy (SEM–EDX) analysis confirmed the adsorption of these elements onto plant tissues, their concentration in roots, and partial translocation to above-ground parts of the plant. These findings hold significant promise for the design of CWs aimed at efficiently treating industrial wastewater. Such systems offer a cost-effective and low-maintenance solution, particularly advantageous in developing countries. They facilitate the discharge of environmentally acceptable water, contributing to sustainable wastewater management practices (Ghezali et al., 2022).

Packing material for constructed wetland

Coal -: Coal has a long history dating back to 3000 BC, originating in China and Wales. It continues to be extensively utilized globally as a dependable and economical energy source, boasting an estimated reserve of 861 billion tonnes. Primarily derived from ancient plant matter, coal consists predominantly of carbon but also contains varying amounts of hydrogen, oxygen, sulfur, and nitrogen. Approximately 85–95% of dry coal comprises organic material. Beyond its traditional role as fuel, coal has diversified applications; its affordability and capacity to absorb pollutants render it valuable in water and wastewater treatment processes (Simate et al., 2016). Using coal for wastewater remediation is a strategic choice. It not only proves effective but also offers cost-efficiency and global accessibility. This versatility makes it an excellent choice for large-scale projects, particularly when alternative materials may be scarce or expensive to procure (*Coal 2021 – Analysis - IEA*, 2001). Coal-based cleaning agents may not exhibit the same level of pollutant absorption as certain alternatives, but they remain effective in addressing various contaminants present in water and wastewater. Available in diverse forms such as activated carbon and coal fly ash products, they offer versatility in targeting specific types of pollutants. This adaptability enables tailored approaches for tackling different contaminants encountered in water treatment processes (Pudasainee et al., 2020). The surface properties of coal feature areas that effectively capture pollutants, including heavy metals, thereby aiding in efficient water purification. This characteristic renders coal-based cleaning agents suitable for a wide array of water treatment applications, ranging from small-scale projects to large industrial endeavors. Their versatility and user-friendliness make them an excellent option for combatting water pollution across various settings (*Coal 2021 – Analysis - IEA*, n.d.; Pudasainee et al., 2020).

Biochar-: Biochar, a solid material derived from biomass, is produced via pyrolysis, a process that decomposes biomass under controlled oxygen levels. In contrast to activated carbon, biochar exhibits higher retention of carbon, hydrogen, and oxygen. They excel in extracting heavy metals from wastewater, surpassing other economical alternatives. Thanks to their unique structure and minimal ash content, biochar demonstrates exceptional pollutant absorption capabilities. Moreover, their production from materials such as rice husks, corn husks, tea waste, and digested sludge facilitates effective treatment of both water and wastewater (Cui et al., 2010).

Laterite-: In Northern Ireland, laterite—a material abundant in iron and aluminum—serves as a crucial agent for wastewater treatment. Particularly effective in acidic solutions, laterite demonstrates remarkable efficacy in removing phosphorus and heavy metals, including aluminum, iron, cadmium, chromium, and lead. Its effectiveness is striking, with the capacity to eliminate up to 99% of phosphorus and significantly reduce the concentrations of these metals. Additionally, laterite aids in pH regulation, moving it closer to neutral levels. Utilized as a locally sourced material, laterite has revolutionized the treatment of landfill wastewater in constructed wetlands. Achieving impressive results, it can remove up to 96% of phosphorus while decreasing levels of aluminum and iron. This cost-effective solution proves ideal for rural areas with dispersed pollution, offering a discreet yet potent means of wastewater purification. Laterite emerges as a valuable asset in the battle against pollution, showcasing its versatility in addressing various contaminants and making tangible strides in pollution control. The utilization of laterite, known for its high iron and aluminum content, has demonstrated remarkable effectiveness in achieving notable removal rates. Specifically, it has been shown to eliminate phosphorus by up to 99% and to achieve significant reductions in the concentrations of heavy metals (Kadam et al., 2009; Priyadarshani Bandara, 2022; Wood & McAtamney, 1996).

Sand-: Sand Filtration serves as a widely adopted method across various industries, serving purposes ranging from producing drinking water to treating wastewater. It represents a cost-effective means of eliminating contaminants, although considerations must be made regarding waste disposal and potential chemical additives. Among the popular filtration methods is sand filtration, wherein water traverses through a bed of sand or gravel, effectively capturing particles and contaminants. There exist two primary types: continuous and discontinuous filters. Continuous filters operate continuously by cleaning and reusing the sand, while discontinuous filters periodically pause to rinse the sand. By passing water through different sizes of sand beds, this process proves effective in removing suspended solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), and certain amounts of phosphorus and nitrogen. Research indicates that this system operates more efficiently during summer months compared to winter, with performance improvements of approximately 5% (Gunes & Tuncsiper, 2009).

Sand filtration finds widespread application across various industries, encompassing tasks such as producing drinking water and treating wastewater. It offers a simple yet versatile solution, although sometimes requiring additional chemical treatments for enhanced efficacy, alongside addressing the management of rinse water post-treatment. In the treatment of Palm Oil Mill Effluent (POME), the utilization of a combination of gravel and sand proves more effective than using either material alone. This blend demonstrates impressive removal rates, including 66% of Chemical Oxygen Demand (COD), 97% of suspended solids, 98% of turbidity, 98% of ammonia-nitrogen, 99% of Total Nitrogen (TN), and 74% of Total Phosphorus (TP). Employing gravel and sand together within sub-surface flow constructed wetlands for POME treatment shows considerable potential (Sa'at et al., 2021).

Pebbles -: In the pursuit of ensuring clean drinking water, addressing elevated turbidity levels during floods and ongoing concerns regarding natural organic matter (NOM) present significant challenges. Pebbles as matrix filtration (PMF) emerge as a straightforward, dependable, and cost-efficient solution to clarify such complex water samples. Researchers conducted experiments using a laboratory-scale PMF column with 2013 Brisbane River floodwater, demonstrating substantial turbidity reduction—exceeding 50% without the need for chemical coagulants, with even greater efficacy observed in harder water conditions. This potential for significant cost savings for water treatment plants and decreased environmental impact through reduced sludge production is noteworthy. Furthermore, PMFs exhibited notable reductions in NOM levels, ranging from 35% to 47%, and UV absorbance by 24% to 38%. Beyond addressing flood-related turbidity, the year-round NOM management capabilities of PMFs could contribute to mitigating disinfection by-products and lowering coagulant requirements in water treatment facilities. Critically, PMFs maintained minimal head losses, ensuring efficient filtration processes (Rajapakse et al., 2016).

Sea shells-: Coastal regions are grappling with a significant issue: the accumulation of vast quantities of seashells, resulting in waste disposal challenges. In China alone, approximately 15 million tons of discarded shells are generated annually, contributing to land occupation and pollution concerns. Seashells encompass a variety of types, including oysters, clams, scallops, mussels, and others. Primarily composed of calcium carbonate ($CaCO_3$), they also contain organic matter and trace amounts of salts such as potassium, sodium, magnesium, iron, zinc, selenium, and various other elements (Li et al., 2023; Wan Mohammad et al., 2017)

Seashells consist of three primary components: a protein outer layer, a calcite middle layer, and an inner layer composed of calcium carbonate crystals. Characterized by a loose structure with large pore diameters and uniformly distributed pores, seashell materials exhibit significant potential for various applications, particularly in building materials requiring excellent adsorption capabilities (Hamada et al., 2023; Li et al., 2023)

Presently, seashells are primarily employed for capturing metal ions, yet there exists limited research on their capacity to absorb oil pollution. Seashell materials possess pores, and the arrangement of these pores significantly impacts their effectiveness in absorbing oil(Li et al., 2023).

Key features that make constructed wetlands appealing include their cost efficiency in construction, operations, and maintenance, minimal energy consumption, and the ability to prepare water for reuse, making them a natural and sustainable choice in wastewater treatment. In conclusion, constructed wetlands offer a holistic, eco-friendly approach to wastewater treatment, addressing environmental concerns while providing an economically viable and versatile solution adaptable to diverse geographical and socioeconomic contexts.

CHAPTER 3

METHODOLOGY

3.1 Site selection and designing of the constructed wetland

The in-situ 2 stage wetland system was installed to check the efficiency of constructed wetland in treating the laboratory wastewater within the premises of Goa University. The study adopted a systematic approach, utilizing plastic drum modules with dimensions of 50 x 50 cm (height x diameter) as the fundamental building blocks of the wetland structure. Diverse bed media materials (Table 1) were explored and selected for their efficacy in promoting optimal filtration and purification processes within the wetland environment.

The performance was monitored for the period of 3 months by analysis of different parameters such as COD, TN, TC, Phosphorus, Ammonia, pH and metal concentration with maxiumm effluent loading rate of 98 litters of water per day (table-: 2). Tank 1 was at a height of 115 cm from ground, was filled with a combination of course and fine sand, pebbles, gravels, and seashells, while tank 2, was at a height of 65 cm from ground, employed similar media, with the substitution of seashells for biochar. The system also incorporated two aeration pipes, each with a diameter of 4.8 cm, situated within the tanks. These pipes housed 12 pores each, with 6 on either side of a 2.4 cm diameter. Positioned approximately 7 cm above the gravel, these pipes were included to ensure aerobic conditions for bacterial activity within the tanks. Four saplings of *Canna Indica* (fig.-: 2) were planted within the system 10 cm from one another. The main features of the CW are listed in the table 2.

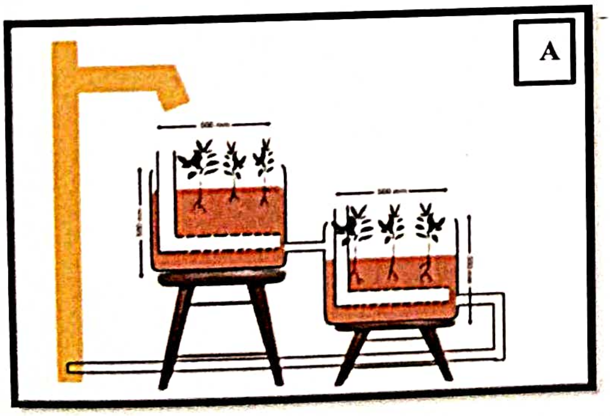

Fig.-: 1 A) Diagrammatic representation of constructed wetland design & B) Constructed wetland

Fig.-: 2) *Canna indica* sapling

Fig.-: 3) Aeration pipe placed above the bottom layer of the bed media

Table no. 1-: Various bed material used for Wetland construction.

Bed components (From top to bottom)	Height (in cm) of each material in tank 1	Bed components (From top to bottom)	Height (in cm) of each material in tank 2
Sand	7	Sand	7
Pebbles	7	Pebbles	7
Sea shells	6	Biochar	6
Pebbles + gravels	10	Pebbles + gravels	10
Gravels	10	Gravels	10

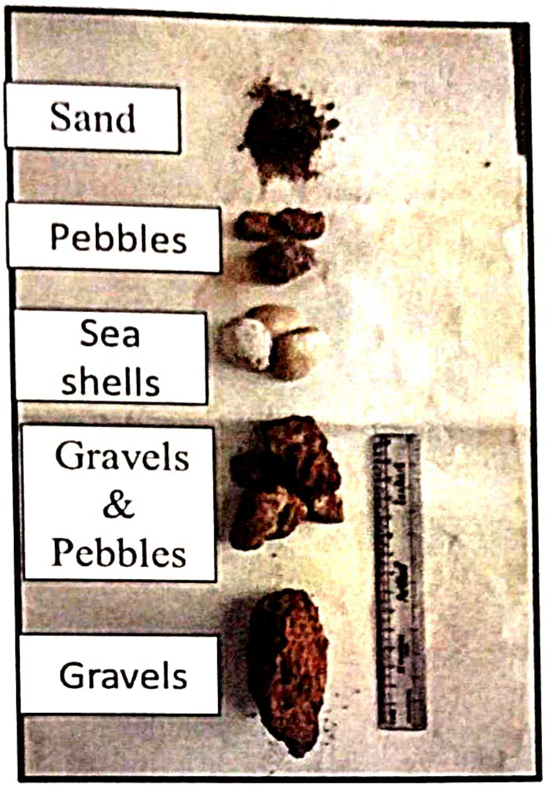

Fig.-: 4 Representative of Tank 1 bed media

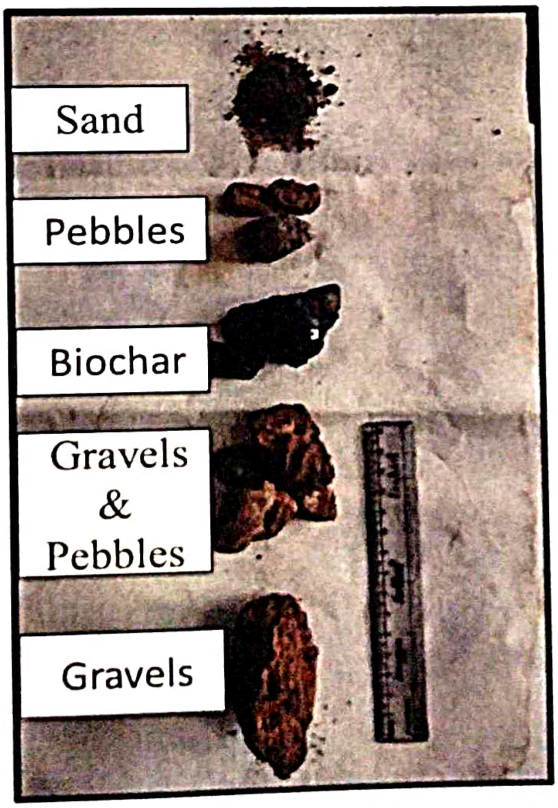

Fig.-: 5 Representative of Tank 2 bed media

Table No. 2 -: Main feature and design characteristics of constructed wetland (Yaragal et al., 2023)

No. of tanks	Dimensions (D/H) (m)	Maximum capacity (m^3)	Surface area (m^2)	Hydraulic loading rate (m^3/day/(m^2)	Volume of input wastewater (m^3/day)
Tank 1	0.5/0.5	0.098	8.25	0.01	0.098
Tank 2	0.5/0.5	0.098	8.25	0.01	0.098

3.2. Commissioning of wetland and treatment of laboratory wastewater. Collection of the samples

After filling the bed components and planting the saplings, the wetland was left to stabilize for 14 days. During this period, regular watering was carried out to prevent the saplings from drying out. After the 14-day stabilization period, the wetland was operated at full capacity. Approximately 98 litters of water were pumped per day at a constant flow rate for 5 to 6 hours each day.

(NOTE-: The capacity of the wetland was calculated using the formula height x π x radius^{2})

After commissioning and stabilization of the wetland for 14 days, each day, 1 liter of water sample was systematically collected from three distinct sources: the inlet water (influent), tank 1 effluent water, and tank 2 outlet water. At the culmination of each week, all inlet water samples collected over the course of the one week were pooled and homogenized to create a composite sample representative of the inlet water for that week. Similarly, composite samples were prepared for tank 1 and tank 2 water sources by combining the respective daily samples obtained throughout the week.

Subsequently, 1 liter of the composite samples from each source (inlet, tank 1, and tank 2) was taken for analysis of various parameters including Chemical Oxygen Demand (COD), phosphorus content, total carbon (TC), and total nitrogen (TN). This systematic sampling and analysis approach facilitated comprehensive monitoring and assessment of water quality dynamics over time for each designated source.

3.4. Estimation of Chemical Oxygen Demand (COD)

3.4.1. Preparation of reagents

- For 1 L of Digestion reagent take oven dried 10.216g of Potassium Dichromate, 33.3g sulfuric acid in 167ml of sulfuric acid
- Sulfuric acid reagent: 1% silver sulfate (1g/100ml)

3.4.2. Preparation of standard and calibration of the instrument for COD estimation

Refer-: Appendix 1

3.4.3. Sample Preparation and Analysis

About 2 ml of the sample was dispensed into a Chemical Oxygen Demand (COD) tube. Subsequently, 1.2 ml of dichromate digestion reagent was added, followed by the addition of 2.8 ml of sulfuric acid (H_2SO_4) reagent. The resultant mixture was subjected to a digestion process for a duration of 2 hours at a temperature of 148°C. Following the digestion period, the sample was allowed to cool down to room temperature. Once cooled, the absorbance of the digested sample was measured at a wavelength of 600 nm using a spectrophotometer.

3.5. Estimation of Phosphorus

3.5.1. Preparation of reagents

The mixed reagent-: For a total volume of 100 ml, the reagent comprised 40 ml of reagent A, 30 ml of reagent B, 25 ml of concentrated sulfuric acid, and 5 ml of distilled water.

Preparation of reagent A involved dissolving 62.5 grams of Ammonium molybdate in 1 litre of distilled water, while reagent B was prepared by dissolving 4.1666 grams of Ammonium Vanadate in 1 litre of distilled water.

3.5.2. Preparation of the standards of phosphorus estimation

Refer -: Appendix 2

3.5.3. Preparation of sample and analysis

A volume of 3.5 ml of the sample was aliquoted into a clean test tube, to which 1 ml of freshly prepared mixed reagent was promptly added. Subsequently, 0.5 ml of distilled water was introduced to achieve a final volume of 5 ml. The resultant solution was promptly subjected to spectrophotometric analysis, with absorbance measurements recorded at a wavelength of 450 nm within a time frame of 20 minutes subsequent to the addition of the aforementioned chemicals.

3.6. Estimation of ammonia

In a kjeldahl tube12.5 ml of the sample was combined with 12.5 ml of borate buffer and 0.5 ml of 6M NaOH. Subsequently, the mixture was subjected to distillation for a duration of 6 minutes. The resultant distillate was collected in a flask containing 12.5 ml of boric acid (20g/L) indicator solution. This collected solution was titrated against 0.02 N H_2SO_4 until the appearance of a light pink colour, indicating the endpoint of the titration process.

3.7. Estimation of TC and TN

Prior to analysis using a total carbon (TC) or total nitrogen (TN) analyser, approximately 25 ml of the sample was filtered through a 0.22-micrometer filter paper. This filtration step ensures the removal of suspended particulate matter and impurities that may interfere with the accuracy and precision of the analytical measurements.

3.8. pH estimation

The pH of the sample was analysed approximately every other day using pH meter, with measurements taken 30 minutes after collection.

3.9. Metal analysis

Metal analysis was conducted utilizing Perkin-Elmer Atomic Absorption Spectroscopy (AAS). Presence of three metals silver, cadmium, and zinc was checked.

3.9.1. Standard preparation for the metal analysis (Zn, Cd & Ag)

Refer appendix 3

3.9.2. Metal analysis sample preparation and analysis

Prior to analysis, samples underwent filtration using 0.22-micron nylon filter paper to remove dirt particles. AAS analysis was then performed on the prepared standards and filtered samples to measure absorbance values, subsequently used to establish calibration curves for each metal. Additionally, the AAS instrument was calibrated using the absorbance values obtained from the standards to ensure accurate quantification of metal concentrations during subsequent analyses.

CHAPTER 4

ANALYSIS AND CONCLUSIONS

4.1 Design and commissioning of the vertical flow constructed wetland (VFCW)

During the commissioning, water clogging in the constructed wetland was experienced on two instances. The first occurred within 20 days of its construction, followed by a second clog within 13 days, both due to the accumulation of agar media on the surface. This system flaw was rectified by installing an additional tank (referred as collection tank) to collect water samples directly from the lab containing agar pieces. The inlet of the tank was equipped with a 1-millimetre pore mesh, for effectively separating particles larger than 1 millimetre. The settled water was then pumped into Tank 1.

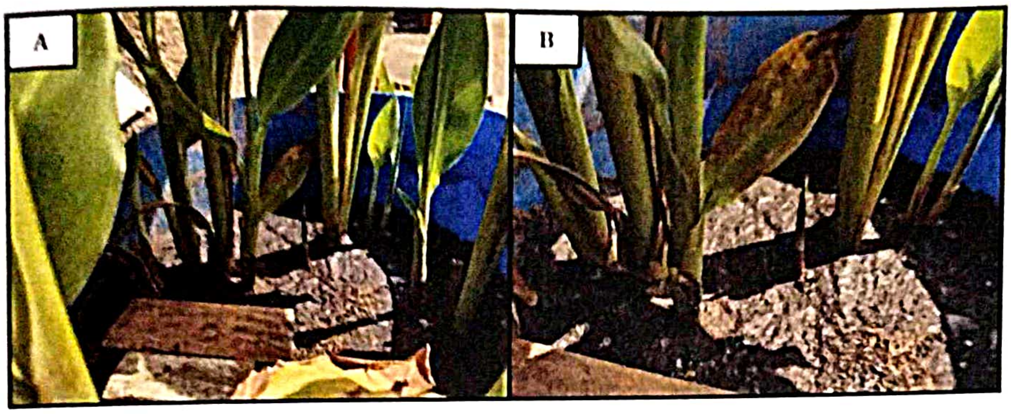

Fig. 6-: Figure showing clogging of the Tank 1 due to accumulation of the used agar media on the surface.

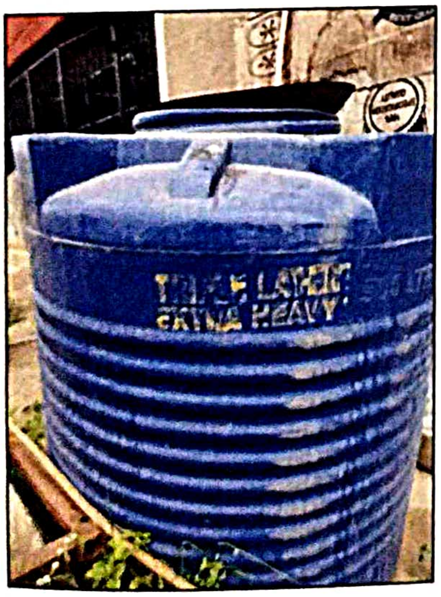

Fig. 7-: Lab water collection Tank with mesh

Fig. 8-: Mesh of 1 mm pore size used to separate agar pieces from lab. wastewater

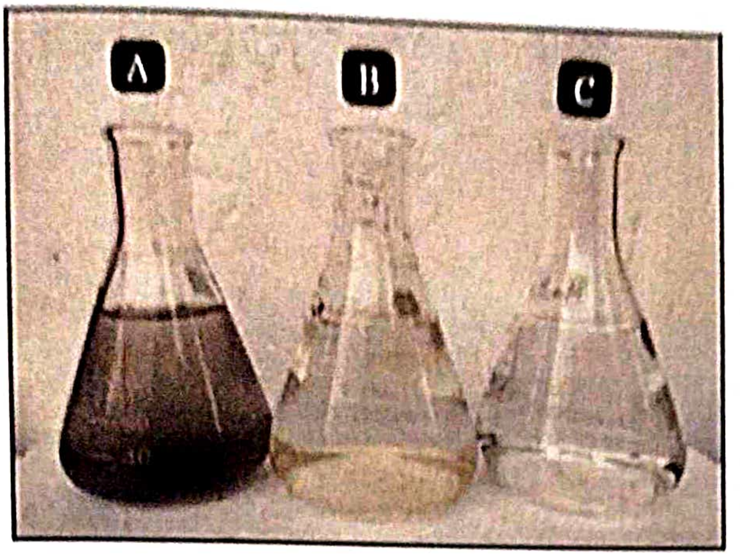

Fig. 9-: Various water sample in VFCW A) inlet sample, B) tank 1 outlet & C) tank 2 outlet

4.2. COD, TN and TC estimation:

The analysis of Chemical Oxygen Demand (COD) demonstrated a consistent decreasing trend after each stage of the constructed wetland, indicating effective pollutant removal (fig. 10). Initially, the inlet COD registered the highest levels, succeeded by tank 1 and then tank 2, affirming the progressive reduction of organic pollutants as the wastewater advanced through the system. However, on day 21, an anomaly was observed as the COD value in tank 2 surpassed that of tank 1, with the lowest value recorded in the inlet sample. This deviation can be attributed to a disturbance in the setup caused by waterlogging conditions.

Similar trends were observed in the analyses of phosphorus (fig. 11), Total Nitrogen (TN) (fig. 12) and Total Carbon (TC) (fig. 13) wherein the respective concentrations exhibited analogous patterns of reduction, highlighting the overarching effectiveness of the constructed wetland in pollutant removal.

The collective performance of the system remained commendable, exhibiting a COD removal efficiency of 64.288% subsequent to tank 1 and a notable 98.285% post-tank 2. Additionally, the constructed wetland demonstrated a phosphorus removal efficiency of 70.227% following tank 1 and a 72.316% removal efficiency after tank 2. In the case of Total Nitrogen (TN), significant results were observed with a removal efficiency of 62.92% after tank 1 and a noteworthy 91.04% removal efficiency after tank 2. However, Total Carbon (TC) removal efficiency was relatively lower, which was, 8.35%, and 36.44% after tank 2.

The data indicates a declining trend in total carbon, comprising both Inorganic Carbon (IC) and Organic Carbon (TOC) (. However, the inlet already had a lower concentration of IC. Despite this, the system demonstrated a higher reduction efficiency for TOC, with a 24.05% reduction after tank 1 and a further reduction to 43.93% after tank 2. On the other hand, the removal efficiency for IC remains unaffected as can be seen from fig. 14, 15 & 16. These findings suggest that the system is more effective at removing TOC compared to IC.

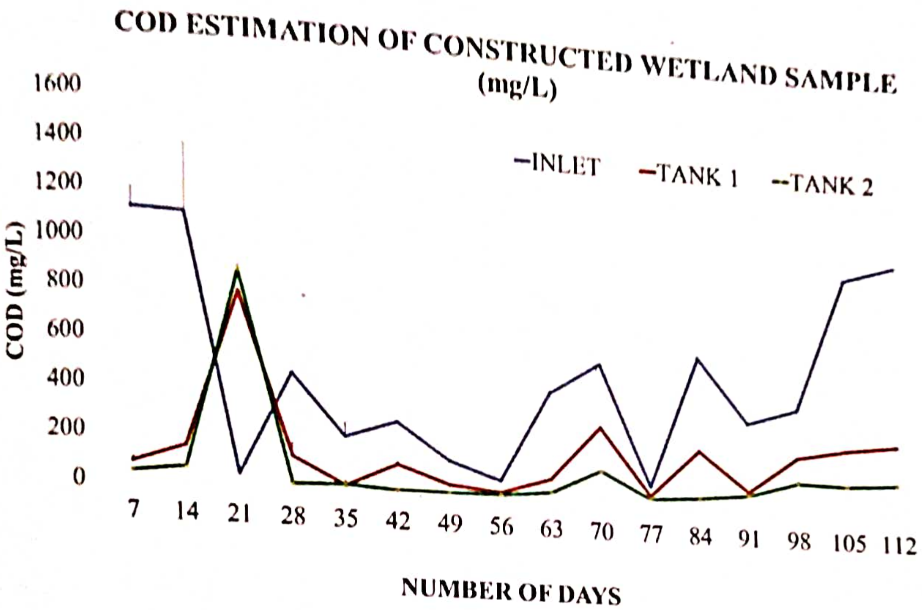

Fig. 10-: COD analysis of constructed wetland sample

The data indicates a declining trend in total carbon, comprising both Inorganic Carbon (IC) and Organic Carbon (TOC) (. However, the inlet already had a lower concentration of IC. Despite this, the system demonstrated a higher reduction efficiency for TOC, with a 24.05% reduction after tank 1 and a further reduction to 43.93% after tank 2. On the other hand, the removal efficiency for IC remains unaffected as can be seen from fig. 14, 15 & 16. These findings suggest that the system is more effective at removing TOC compared to IC.

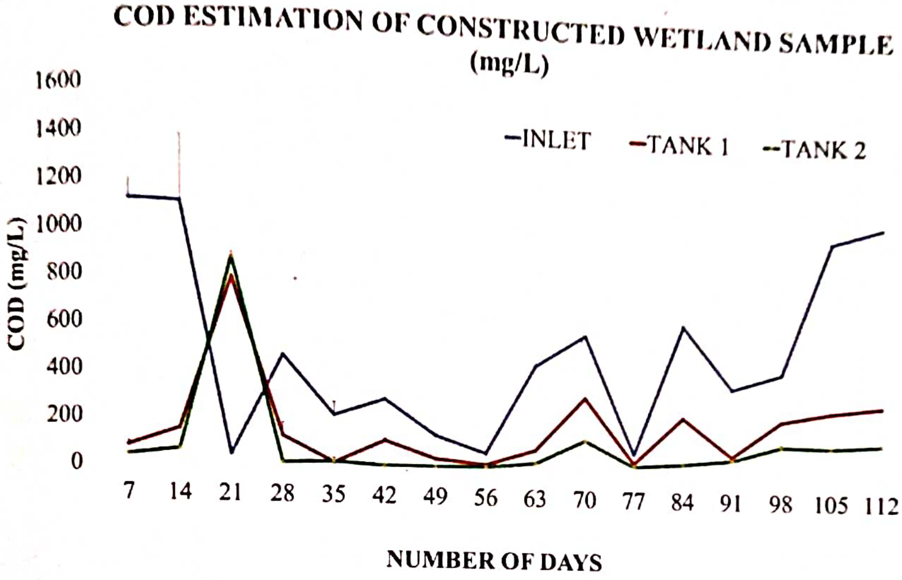

Fig. 10-: COD analysis of constructed wetland sample

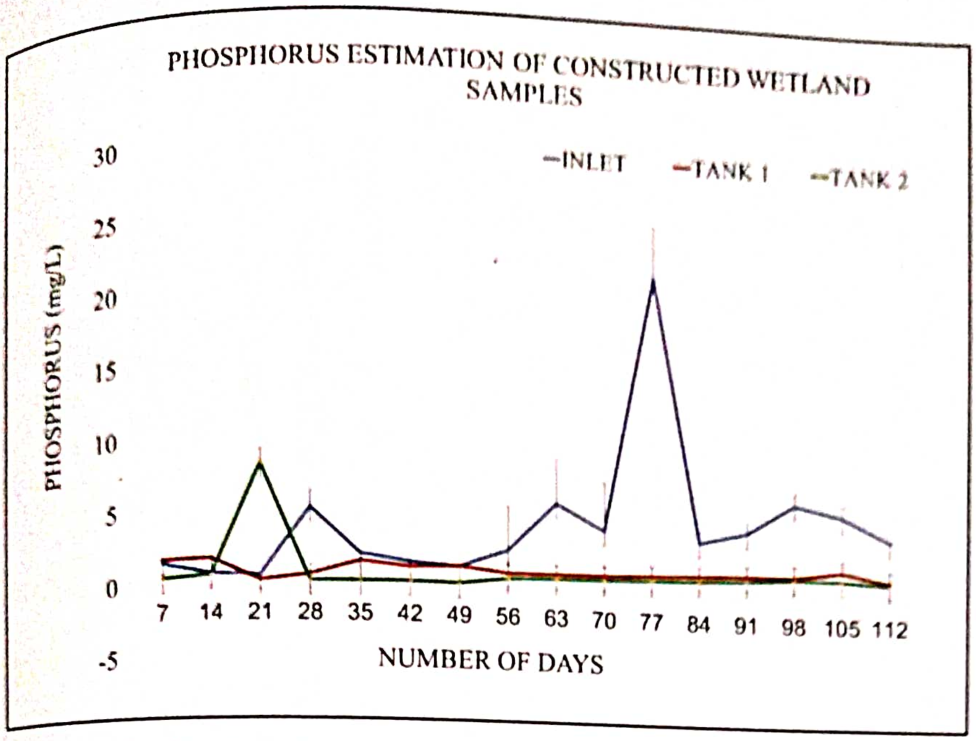

Fig. 11-: Phosphorus analysis of constructed wetland sample

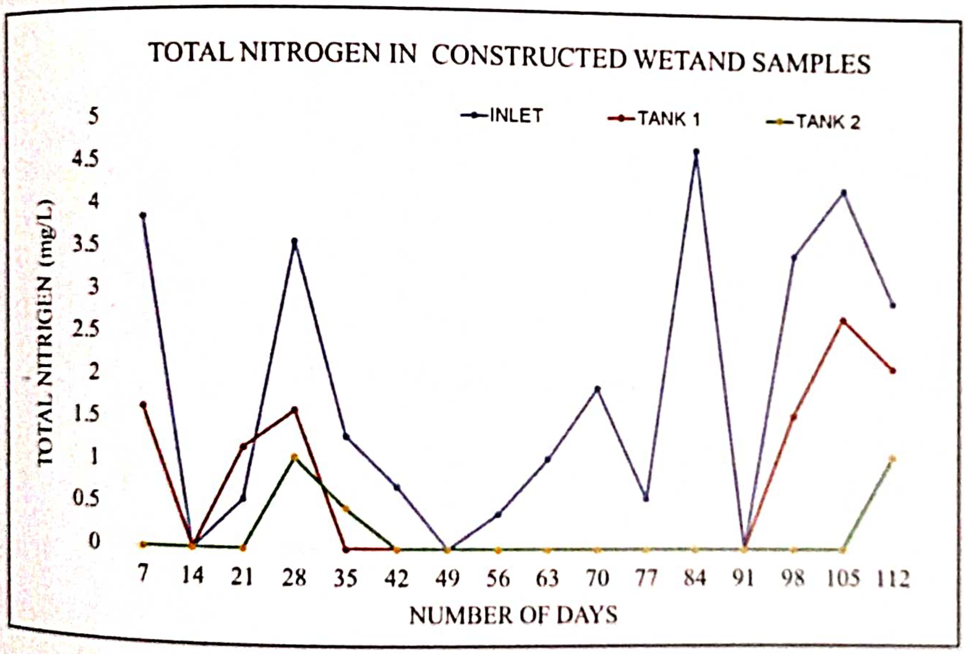

Fig. 12 -: TN analysis of the constructed wetland sample

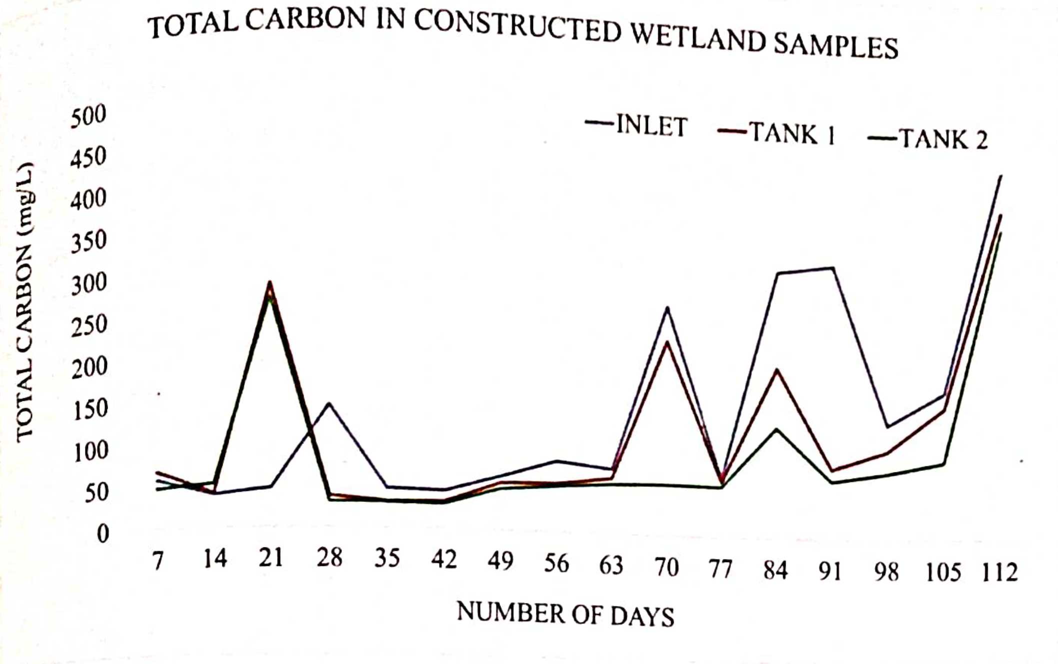

Fig.13 -: TC analysis of the constructed wetland sample

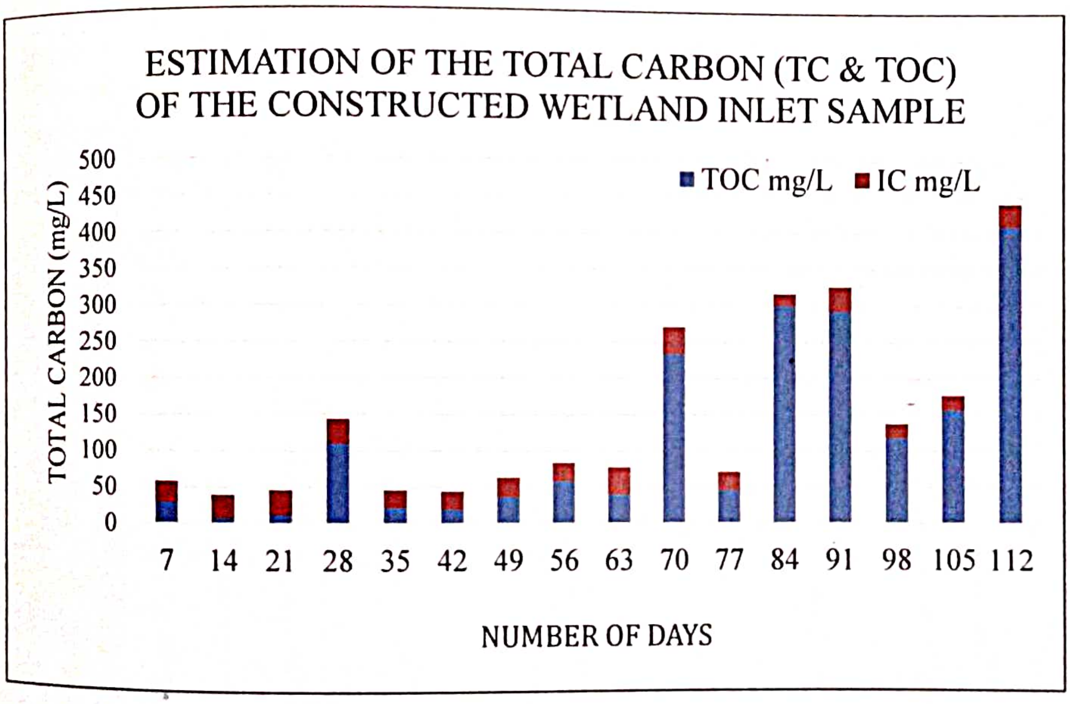

Fig.-: 14 TC analysis of the constructed wetland sample- INLET

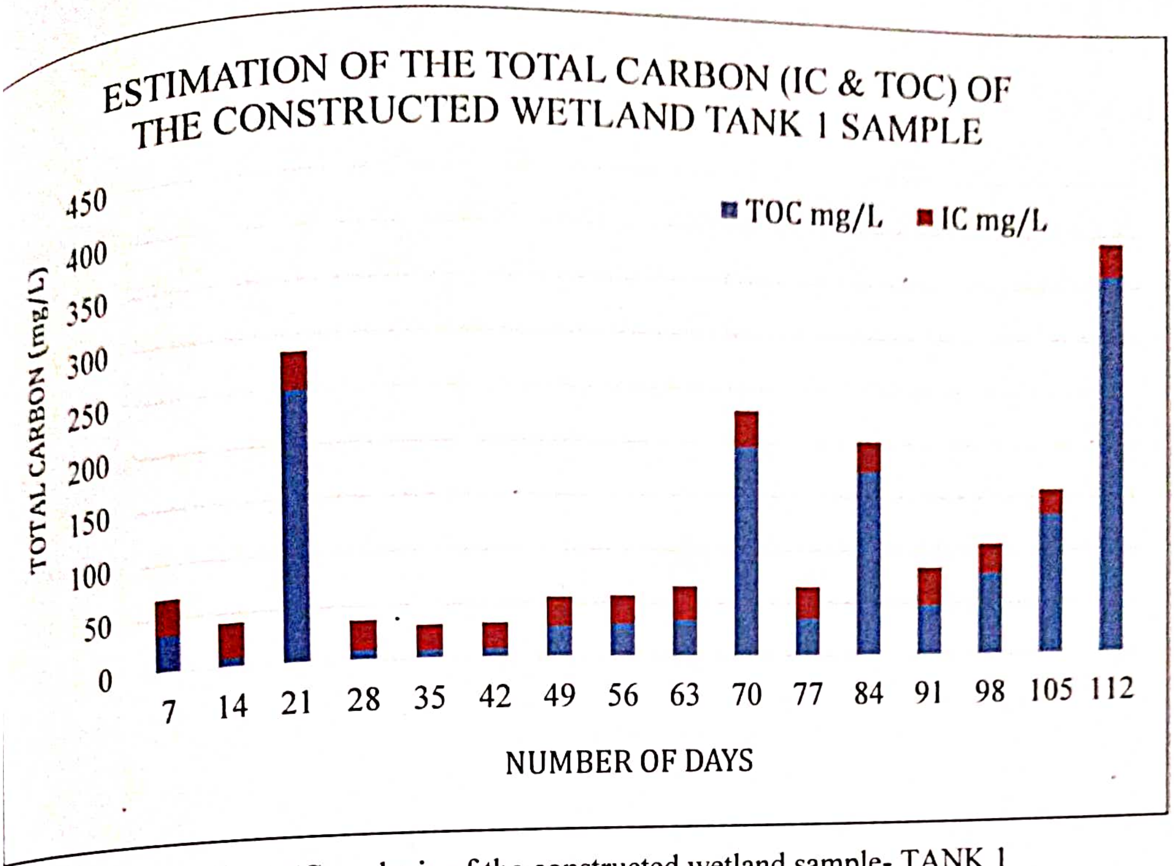

Fig.-: 15 B TC analysis of the constructed wetland sample- TANK 1

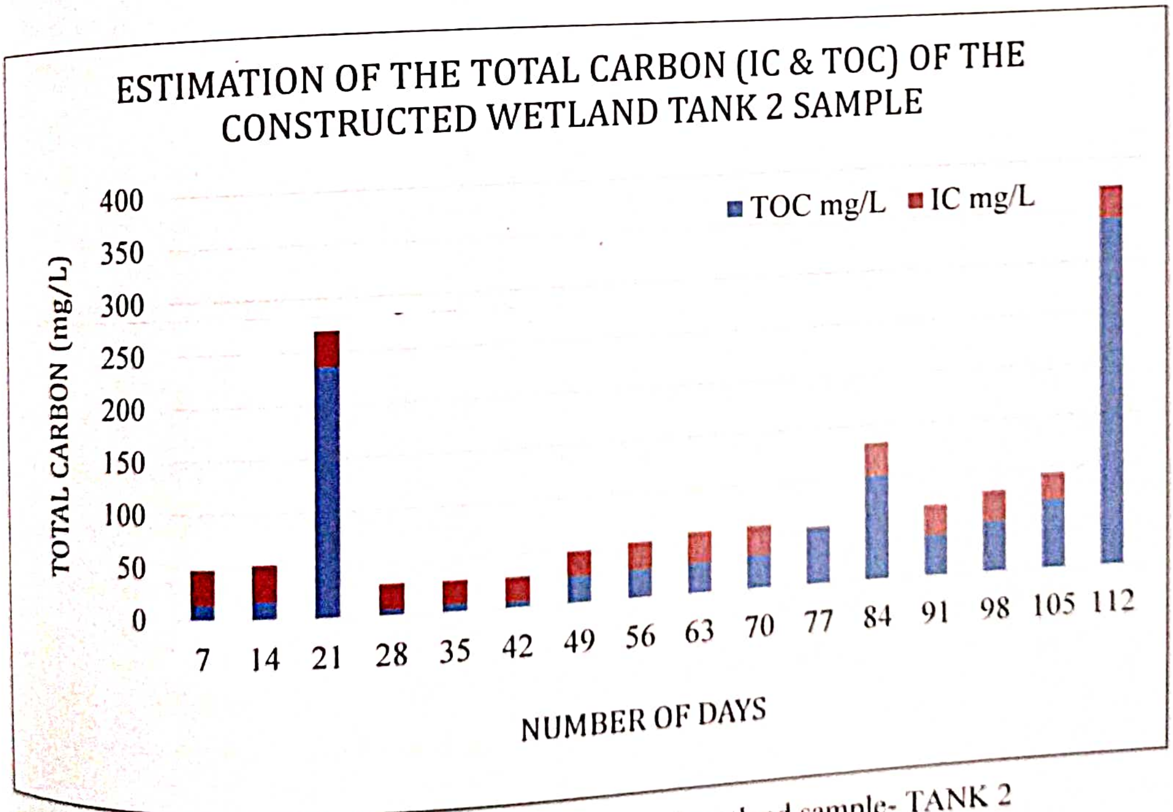

Fig.-: 16 C TC analysis of the constructed wetland sample- TANK 2

4.3. pH estimation

The pH levels in the inlet exhibited fluctuation within a wide range, spanning between 3.42 to 8.9 (fig. 15). In contrast, the pH levels within tank 1 and tank 2 remained relatively stable, hovering around 7. This consistency suggests that the constructed wetland setup effectively stabilized the pH range of the wastewater as it passed through the system. This stabilization is indicative of the wetland's efficiency in buffering and maintaining a neutral pH, thereby potentially enhancing the overall treatment process.

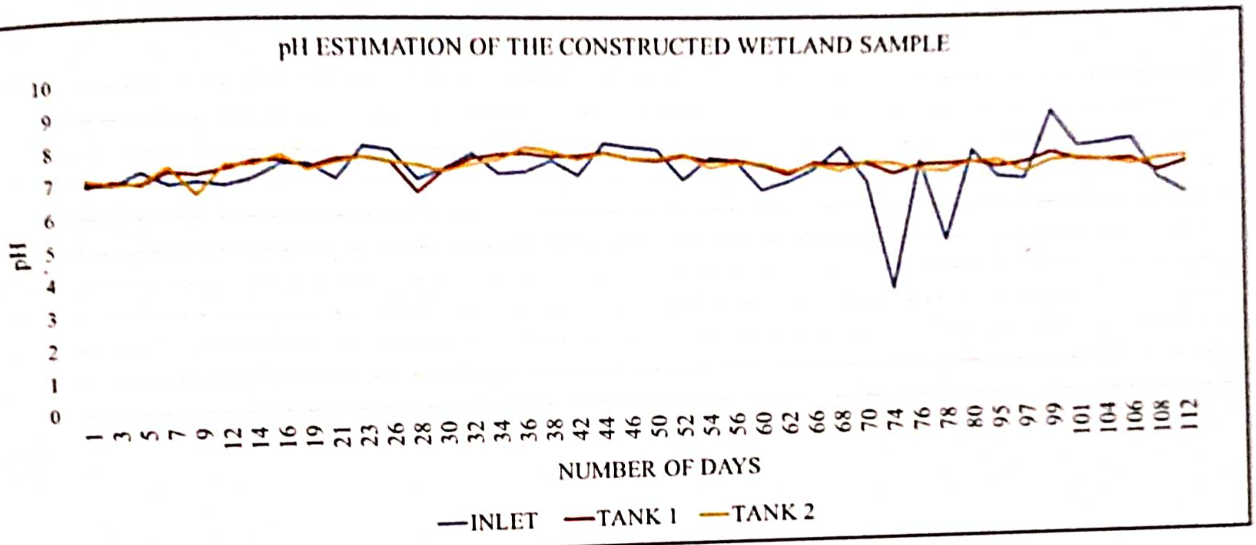

Fig. 17-: pH analysis of the constructed wetland sample

4.4. Ammonia estimation

The analysis of the inlet sample for ammonia revealed the presence of very trace amounts of ammonia, on an average 4 ppm (fig.16). Due to the limitation of the analyser in detecting concentrations below 4 ppm, the ammonia levels in the tank 1 and tank 2 samples could not be accurately determined. This limitation underscores the need for sensitive analytical techniques to assess low concentrations of pollutants in wastewater samples. Despite this challenge, the detection of ammonia in the inlet sample highlights the importance of monitoring and addressing nitrogen compounds in wastewater treatment processes.

4.3. pH estimation

The pH levels in the inlet exhibited fluctuation within a wide range, spanning between 3.42 to 8.9 (fig. 15). In contrast, the pH levels within tank 1 and tank 2 remained relatively stable, hovering around 7. This consistency suggests that the constructed wetland setup effectively stabilized the pH range of the wastewater as it passed through the system. This stabilization is indicative of the wetland's efficiency in buffering and maintaining a neutral pH, thereby potentially enhancing the overall treatment process.

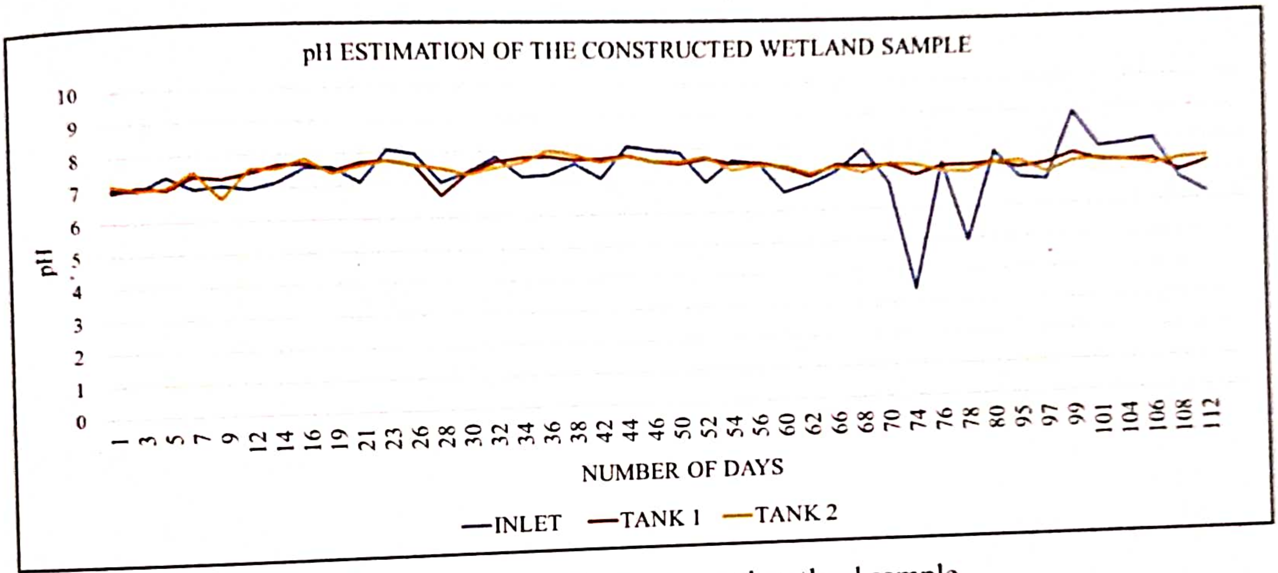

Fig. 17-: pH analysis of the constructed wetland sample

4.4. Ammonia estimation

The analysis of the inlet sample for ammonia revealed the presence of very trace amounts of ammonia, on an average 4 ppm (fig.16). Due to the limitation of the analyser in detecting concentrations below 4 ppm, the ammonia levels in the tank 1 and tank 2 samples could not be accurately determined. This limitation underscores the need for sensitive analytical techniques to assess low concentrations of pollutants in wastewater samples. Despite this challenge, the detection of ammonia in the inlet sample highlights the importance of monitoring and addressing nitrogen compounds in wastewater treatment processes.

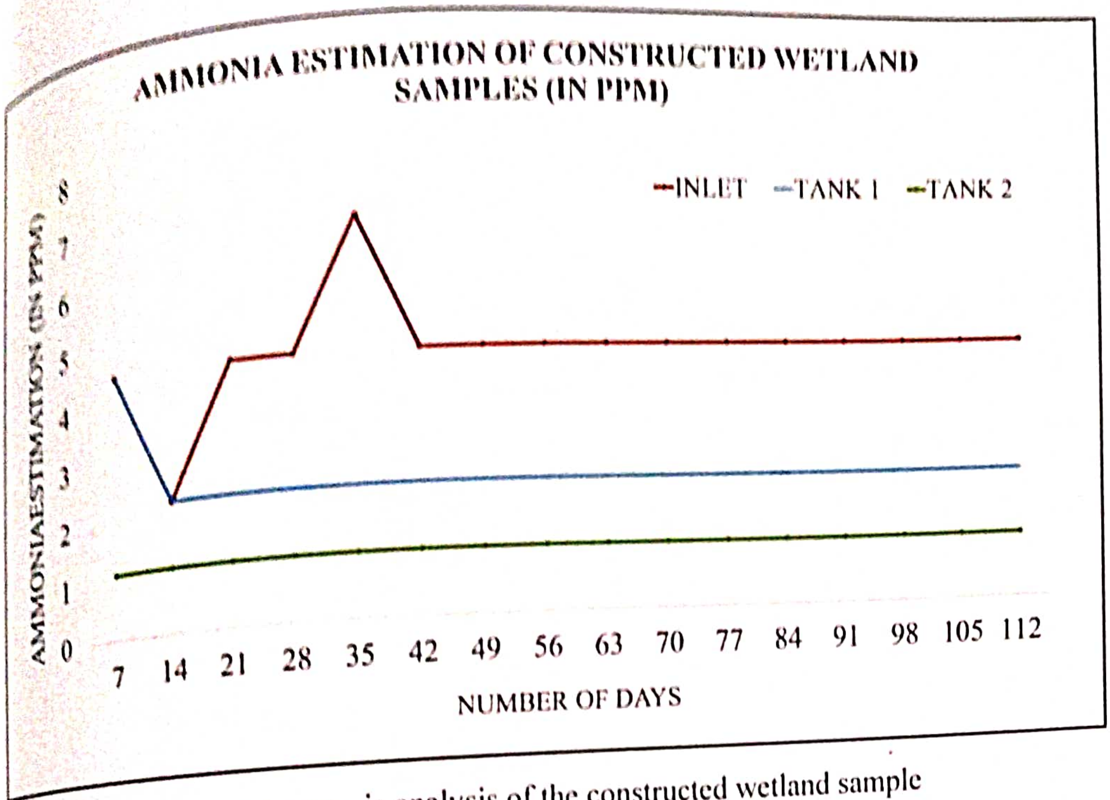

Fig. 18-: Ammonia analysis of the constructed wetland sample

4.5 Metal analysis

The analysis revealed the presence of silver (fig. 17) in the samples, with the highest concentration observed in the Inlet sample, followed by Tank 1, and then Tank 2. The overall performance of the system indicated a silver removal efficiency of 16.60% after Tank 1 and a notable increase to 41% removal efficiency after Tank 2. This suggests a progressive reduction in silver concentration as the wastewater advanced through the constructed wetland system, indicating its effectiveness in metal removal.

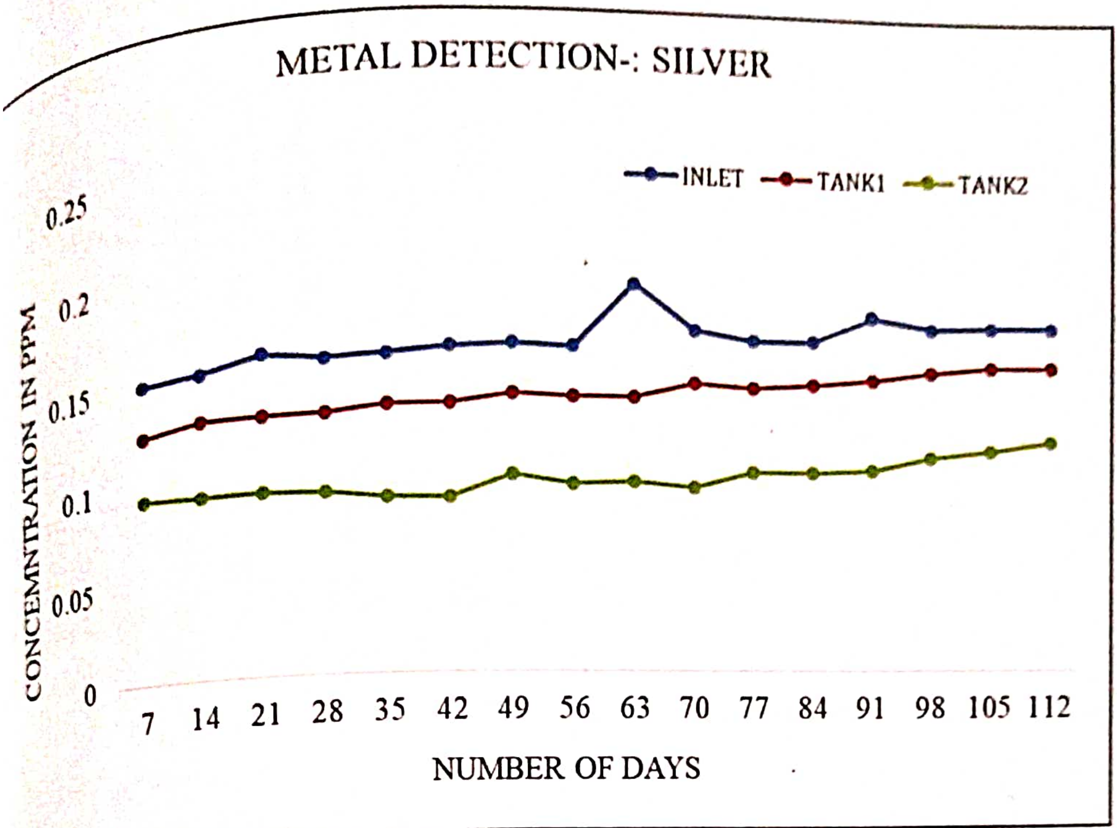

Fig. 19-: Silver metal detection in the INLET, TANK 1 AND TANK 2 outlet sample of constructed wetland sample

4.6 Discussion

The trends observed in the analysis of Chemical Oxygen Demand (COD) levels align with findings from previous studies on constructed wetlands. According to Wang et al. (2018) & Vymazal (2011), constructed wetlands have been shown to effectively reduce COD levels in wastewater due to microbial degradation of organic matter within the wetland substrate with different adsorption properties. The progressive decrease in COD concentrations from the inlet to Tank 2 corelates the sequential removal of organic pollutants as wastewater flows through the wetland system (Vymazal, 2011; Wang et al., 2018).

This system of CW showed better COD removal efficiency of 98.29 %. This is higher compared to similar study with laboratory wastewater conducted by Meutia (2001) where the author compared Surface Flow Constructed Wetland (SFCW) and Subsurface Flow Constructed Wetland (SSFCW). Meutia further observed during the dry season, the subsurface flow removed 95% of COD, slightly higher than the surface flow. In the transition period, subsurface flow decreased to 73%, while the surface flow dropped to 29%. Both flows improved to nearly 95% in the rainy season. Our result showed superior COD removal efficiency compared to all the values reported by Meutia. In a study conducted by (Yaragal, 2023) on sewage wastewater showed 78.9 % COD removal efficiency in case of mix flow Vertical Flow Constructed Wetland (MF-VFCW) and 100 % COD removal efficiency in case of Vertical Flow Constructed Wetland (VFCW). In a another report by Wang's (2019) on intertidal wetland sediment (IWS) as a novel inoculation source for saline-wastewater treatment in constructed wetlands (CWs), showed COD removal of 51.80 % which is also lower than this study.

In case of TN this system remained better compared to Meutis report showing removal efficiency of 91.04%. During the dry season, the subsurface flow removed 82% of T-N, higher than the surface flow's 41%. Transitioning to the rainy season, subsurface flow increased to 95%, while the surface flow improved to 74%. On the otherhand this system also remained superior over Yaragal system which showed only 10.4 % removal efficiency for MF-VFCW and 26.9 % removal efficiency in case of DS-VFCW.

Considering phosphorus, Meutia system showed better performance compared to this system. In his study, in the dry season, both subsurface and surface flows removed T-P with efficiencies of around 95%. The transition and rainy seasons-maintained T-P removal at approximately 93% for both flow types, with a slight drop to 76% in the subsurface flow during the rainy season. This system treatment remained superior over Yaragal system of CW which showed 66.6 %. However, Yaragal system proved to be better than this system with removal efficiency of 96.3 %. In comparison to all the studies mentioned above this system showed relatively lower phosphorus removal efficiency which is of 72.31 % after tank 2.

Regarding pH stabilization, the consistent pH levels observed in Tank 1 and Tank 2 are indicative of the buffering capacity of constructed wetlands. According to Mayes (2008), wetland vegetation and microbial processes can regulate pH levels by consuming or releasing ions, thereby maintaining a neutral pH range conducive to biological treatment processes (Mayes et al., 2008). This suggests that the pH of CW system in this study is also stabilised by healthy microbial community residing in the different layers.

Heavy metal is removed in the system via adsorption and precipitation of heavy metals in wetland substrates (Guangyi Fu, 2022). Guangyi Fu further demonstrated that constructed wetlands can effectively remove metal ions (Zn, Cr, Ni, and Pb) through interactions with organic matter and mineral components in the substrate, leading to decreased metal concentrations in effluent samples. In another study by (Saheed, 2021) on two hybrid subsurface flow constructed wetland systems (VF followed by a HF) for removal of metals. The removal efficiency of his system for Zn, Cr, Ni, and Pb ranged between 75–98%, 29–41%, 14–48%, and 23–26%, respectively.-Performance of CW in this study was in accordance with study conducted by Saheed (2021), where silver removal efficiency was of 16.60% after Tank 1 and 41% removal efficiency after Tank 2.

Constructed wetland is efficient in removing IC and TOC from wastewater is supported by existing literature. According to (Kadlec & Wallac, 2008), wetland act as sinks for carbon, and the microbial processes occurring in the wetland sediments play significant role is carbon cycling, it effectively removes IC and TOC. Research study carried out by (Yaragal, 2023) showed significant removal efficiency in mix flow vertical flow constructed wetland (MF-VFCW) of 86.2 % in case of IC and 56.3 % in case of TOC. In same study carried out using Double Stage VFCW (DS-VFCW) showed 96.4 % in case of IC and 91.8 % in case of TOC.

Yaragal study proved to be better than this system efficiency. This system showed 43.93 % TOC removal efficiency and only 5.48 % removal efficiency in case of IC.

Overall, the trends observed in the analysis of COD, phosphorus, nitrogen, and silver concentrations align with established principles of constructed wetland using substrate such as laterite, biochar, pebbles and sea shells capability in removing pollutants.

4.7 Conclusion

In conclusion, the VFCW system in this study showed promising efficacy in treating of laboratory wastewater, despite encountering initial challenges. The instances of clogging prompted crucial adjustments, notably the installation of a tank with a mesh inlet, which effectively mitigated the accumulation of agar media and ensured smoother operation of the system. The system achieved 64.288% removal efficiency after tank 1 and an impressive 98.285% removal efficiency for after tank 2 for COD. The wetland exhibited a phosphorus removal efficiency of 70.227% following tank 1 and 72.316% after tank 2. For Total Nitrogen (TN), notable findings emerged, indicating a removal efficiency of 62.92% post-tank 1 and a remarkable 91.04% removal efficiency post-tank 2. In contrast, the removal efficiency for Total Carbon (TC) was comparatively lower, (18.35% after tank 1 and 36.44% post-tank 2). The system also proved to be effective in maintaining the pH of 7.41 (average) throughout. Despite facing various challenges, the VFCW system shows great potential as a sustainable and effective method for laboratory wastewater treatment. It offers promising solutions to reduce environmental pollution and maintain water quality. The treatment efficiency met the Central Pollution Control Board (CPCP) limits for pH, phosphorus, TC, TN and COD on most of the occasions. Continued research and refinement of such systems are essential to further optimize their performance and contribute to the advancement of wastewater treatment technologies.

BIBLIOGRAPHY

1. Allen, W. C., Hook, P. B., Biederman, J. A., & Stein, O. R. (2002). Temperature and Wetland Plant Species Effects on Wastewater Treatment and Root Zone Oxidation. *Journal of Environmental Quality*, *31*(3), 1010–1016. https://doi.org/10.2134/jeq2002.1010
2. Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., & Butler, A. (2003). Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups. *Limnology and Oceanography*, *48*(3), 1069–1078. https://doi.org/10.4319/lo.2003.48.3.1069
3. Bianchi, E., Coppi, A., Nucci, S., Antal, A., Berardi, C., Coppini, E., Fibbi, D., Del Bubba, M., Gonnelli, C., & Colzi, I. (2021). Closing the loop in a constructed wetland for the improvement of metal removal: The use of Phragmites australis biomass harvested from the system as biosorbent. *Environmental Science and Pollution Research*, *28*(9), 11444–11453. https://doi.org/10.1007/s11356-020-11291-0
4. *Canna (Canna Indica)—UIC Heritage Garden*. (n.d.). Retrieved December 10, 2023, from https://heritagegarden.uic.edu/canna-indica
5. *Coal 2021 – Analysis—IEA*. (2001). Retrieved December 10, 2023, from https://www.iea.org/reports/coal-2021
6. Cui, L., Ouyang, Y., Lou, Q., Yang, F., Chen, Y., Zhu, W., & Luo, S. (2010). Removal of nutrients from wastewater with Canna indica L. under different vertical-flow constructed wetland conditions. *Ecological Engineering*, *36*(8), 1083–1088. https://doi.org/10.1016/j.ecoleng.2010.04.026
7. *Flint Water Crisis: Everything You Need to Know*. (2018, November 8). https://www.nrdc.org/stories/flint-water-crisis-everything-you-need-know

BIBLIOGRAPHY

1. Allen, W. C., Hook, P. B., Biederman, J. A., & Stein, O. R. (2002). Temperature and Wetland Plant Species Effects on Wastewater Treatment and Root Zone Oxidation. *Journal of Environmental Quality*, *31*(3), 1010–1016. https://doi.org/10.2134/jeq2002.1010
2. Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., & Butler, A. (2003). Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups. *Limnology and Oceanography*, *48*(3), 1069–1078. https://doi.org/10.4319/lo.2003.48.3.1069
3. Bianchi, E., Coppi, A., Nucci, S., Antal, A., Berardi, C., Coppini, E., Fibbi, D., Del Bubba, M., Gonnelli, C., & Colzi, I. (2021). Closing the loop in a constructed wetland for the improvement of metal removal: The use of Phragmites australis biomass harvested from the system as biosorbent. *Environmental Science and Pollution Research*, *28*(9), 11444–11453. https://doi.org/10.1007/s11356-020-11291-0
4. *Canna (Canna Indica)—UIC Heritage Garden*. (n.d.). Retrieved December 10, 2023, from https://heritagegarden.uic.edu/canna-indica
5. *Coal 2021 – Analysis—IEA*. (2001). Retrieved December 10, 2023, from https://www.iea.org/reports/coal-2021
6. Cui, L., Ouyang, Y., Lou, Q., Yang, F., Chen, Y., Zhu, W., & Luo, S. (2010). Removal of nutrients from wastewater with Canna indica L. under different vertical-flow constructed wetland conditions. *Ecological Engineering*, *36*(8), 1083–1088. https://doi.org/10.1016/j.ecoleng.2010.04.026
7. *Flint Water Crisis: Everything You Need to Know*. (2018, November 8). https://www.nrdc.org/stories/flint-water-crisis-everything-you-need-know

8. Gao, J., Wang, W., Guo, X., Zhu, S., Chen, S., & Zhang, R. (2014). Nutrient removal capability and growth characteristics of *Iris sibirica* in subsurface vertical flow constructed wetlands in winter. *Ecological Engineering, 70*, 351–361. https://doi.org/10.1016/j.ecoleng.2014.06.006

9. Gunes, K., & Tuncsiper, B. (2009). A serially connected sand filtration and constructed wetland system for small community wastewater treatment. *Ecological Engineering, 35*(8), 1208–1215. https://doi.org/10.1016/j.ecoleng.2009.03.023

10. Gutterer, B. (2009). *Decentralised Wastewater Treatment Systems (DEWATS) and sanitation in developing countries: A practical guide*. BORDA.

11. Hamada, H. M., Abed, F., Tayeh, B., Al Jawahery, M. S., Majdi, A., & Yousif, S. T. (2023). Effect of recycled seashells on concrete properties: A comprehensive review of the recent studies. *Construction and Building Materials, 376*, 131036. https://doi.org/10.1016/j.conbuildmat.2023.131036

12. *Health Effects of Microplastic Exposures: Current Issues and Perspectives in South Korea—PMC*. (2023). Retrieved December 11, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10151227/

13. Hophmayer-Tokich, S. (2006). *Wastewater Management Strategy: Centralized v. Decentralized technologies for small communities*.

14. Hsu, C.-B., Hsieh, H.-L., Yang, L., Wu, S.-H., Chang, J.-S., Hsiao, S.-C., Su, H.-C., Yeh, C.-H., Ho, Y.-S., & Lin, H.-J. (2011). Biodiversity of constructed wetlands for wastewater treatment. *Ecological Engineering, 37*(10), 1533–1545. https://doi.org/10.1016/j.ecoleng.2011.06.002

15. Ji, Z., Tang, W., & Pei, Y. (2022). Constructed wetland substrates: A review on development, function mechanisms, and application in contaminants removal. *Chemosphere, 286*, 131564. https://doi.org/10.1016/j.chemosphere.2021.131564

16. Juwarkar, A. S., Oke, B., Juwarkar, A., & Patnaik, S. M. (1995). Domestic wastewater treatment through constructed wetland in India. *Water Science and Technology, 32*(3), 291–294. https://doi.org/10.1016/0273-1223(95)00637-0

17. Kadam, A. M., Nemade, P. D., Oza, G. H., & Shankar, H. S. (2009). Treatment of municipal wastewater using laterite-based constructed soil filter. *Ecological Engineering, 35*(7), 1051–1061. https://doi.org/10.1016/j.ecoleng.2009.03.008

18. Kadlec, R. H., & Wallace, S. (2008). *Treatment Wetlands* (2nd ed.). CRC Press. https://doi.org/10.1201/9781420012514

19. Kadlec, R. H., & Wallace, S. D. (2009). *Treatment wetlands* (2nd ed). CRC press.

20. Khan, M. L., Hassan, H. U., Khan, F. U., Ghaffar, R. A., Rafiq, N., Bilal, M., Khooharo, A. R., Ullah, S., Jafari, H., Nadeem, K., Siddique, M. a. M., & Arai, T. (2023). Effects of microplastics in freshwater fishes health and the implications for human health. *Brazilian Journal of Biology = Revista Brasleira De Biologia, 84*, e272524. https://doi.org/10.1590/1519-6984.272524

21. Kwaku Armah, E., Chetty, M., Adebisi Adedeji, J., Kukwa, D. T., Mutsvene, B., Pearlman Shabangu, K., & Femi Bakare, B. (2021). Emerging Trends in Wastewater Treatment Technologies: The Current Perspective. In I. Ahmed Moujdin & J. Kevin Summers (Eds.), *Promising Techniques for Wastewater Treatment and Water Quality Assessment*. IntechOpen. https://doi.org/10.5772/intechopen.93898

22. Lavrova, S., & Koumanova, B. (2008). The role of Phragmite australis in wetlands self-purification. *Journal of Environmental Protection and Ecology, 9*, 531–539.

23. Li, T., Xin, R., Wang, D., Yuan, L., Wu, D., & Wu, X. (2023). Research Progress on the Applications of Seashell Adsorption Behaviors in Cement-Based Materials. *Buildings, 13*(5), Article 5. https://doi.org/10.3390/buildings13051289

24. Mayes, W., Batty, L., Younger, P., Jarvis, A., Kõiv-Vainik, M., Vohla, C., & Mander, U. (2008). Wetland Treatment at Extremes of Ph: A Review. *Science of The Total Environment*, *407*, 3944–3957. https://doi.org/10.1016/j.scitotenv.2008.06.045

25. *Microplastics*. (n.d.). . Retrieved December 11, 2023, from https://education.nationalgeographic.org/resource/microplastics

26. Osman, A. I., Hosny, M., Eltaweil, A. S., Omar, S., Elgarahy, A. M., Farghali, M., Yap, P.-S., Wu, Y.-S., Nagandran, S., Batumalaie, K., Gopinath, S. C. B., John, O. D., Sekar, M., Saikia, T., Karunanithi, P., Hatta, M. H. M., & Akinyede, K. A. (2023). Microplastic sources, formation, toxicity and remediation: A review. *Environmental Chemistry Letters*, 1–41. https://doi.org/10.1007/s10311-023-01593-3

27. Parashar, V., Singh, S., Purohit, M. R., Tamhankar, A. J., Singh, D., Kalyanasundaram, M., Lundborg, C. S., & Diwan, V. (2022). Utility of constructed wetlands for treatment of hospital effluent and antibiotic resistant bacteria in resource limited settings: A case study in Ujjain, India. *Water Environment Research*, *94*(9), e10783. https://doi.org/10.1002/wer.10783

28. Paucar, N. E., & Sato, C. (2021). Microbial Fuel Cell for Energy Production, Nutrient Removal and Recovery from Wastewater: A Review. *Processes*, *9*(8), 1318. https://doi.org/10.3390/pr9081318

29. Priyadarshani Bandara, A. B. (2022). LATERITE GRAINS AS A LOW-COST ADSORBENT TO TREAT HEAVY METAL-CONTAMINATED WATER: A REVIEW. *International Journal of GEOMATE*, *22*(94). https://doi.org/10.21660/2022.94.3228

30. Pudasainee, D., Kurian, V., & Gupta, R. (2020). 2 - Coal: Past, Present, and Future Sustainable Use. In T. M. Letcher (Ed.), *Future Energy (Third Edition)* (pp. 21–48). Elsevier. https://doi.org/10.1016/B978-0-08-102886-5.00002-5

31. Rajapakse, J., Millar, G., Gunawardana, C., & Roux, A. (2016). Applicability of pebble matrix filtration for the pre-treatment of surface waters containing high turbidity and NOM. *Desalination and Water Treatment*, *57*(52), 24820–24832. https://doi.org/10.1080/19443994.2016.1149741

32. Removal Methods of Heavy Metals from Laboratory Wastewater. (2019). *Journal of Natural Sciences Research*. https://doi.org/10.7176/JNSR/9-2-04

33. Removal of heavy metals in subsurface flow constructed wetlands: Application of effluent recirculation. (2021). *Environmental and Sustainability Indicators*, *12*, 100146. https://doi.org/10.1016/j.indic.2021.100146

34. Ruiz-Ocampo, H., Tondera, K., Paing, J., Molle, P., & Chazarenc, F. (2022). Long-term investigations on ammonium removal with zeolite in compact vertical flow treatment wetlands under field conditions. *Water Science and Technology*, *85*(3), 746–755. https://doi.org/10.2166/wst.2022.022

35. Sa'at, S. K. M., Yusoff, M. S., Zaman, N. Q., & Ismail, H. A. (2021). Effect of Gravel-Sand Substrate on Sub-Surface Flow Constructed Wetland for Palm Oil Mill Effluent Treatment. *IOP Conference Series: Earth and Environmental Science*, *765*(1), 012048. https://doi.org/10.1088/1755-1315/765/1/012048

36. Simate, G. S., Maledi, N., Ochieng, A., Ndlovu, S., Zhang, J., & Walubita, L. F. (2016). Coal-based adsorbents for water and wastewater treatment. *Journal of Environmental Chemical Engineering*, *4*(2), 2291–2312. https://doi.org/10.1016/j.jece.2016.03.051

37. Stein, O. R., & Hook, P. B. (2005). Temperature, Plants, and Oxygen: How Does Season Affect Constructed Wetland Performance? *Journal of Environmental Science and Health, Part A*, *40*(6–7), 1331–1342. https://doi.org/10.1081/ESE-200055840

38. Taha, S. Y., Almansoory, A. F., Al-Baldawi, I. A., Abdullah, S. R. S., Ismail, N. 'Izzati, Hazaimeh, M., & Sharuddin, S. S. N. (2023). Hybrid constructed wetland for treatment

of power plant effluent polluted with hydrocarbons. *Journal of Water Process Engineering, 56*, 104372. https://doi.org/10.1016/j.jwpc.2023.104372

39. *Understanding Laboratory Wastewater Tests: I. Organics (BOD, COD, TOC, O&G)*. (n.d.). Retrieved April .14, 2024, from https://extension.uga.edu/publications/detail.html?number=C992&title=understanding-laboratory-wastewater-tests-i-organics-bod-cod-toc-og

40. Vymazal, J. (2010). Constructed Wetlands for Wastewater Treatment. *Water, 2*(3), Article 3. https://doi.org/10.3390/w2030530

41. Vymazal, J. (2011a). Constructed Wetlands for Wastewater Treatment: Five Decades of Experience. *Environmental Science & Technology, 45*(1), 61–69. https://doi.org/10.1021/es101403q

42. Vymazal, J. (2011b). Constructed Wetlands for Wastewater Treatment: Five Decades of Experience. *Environmental Science & Technology, 45*(1), 61–69. https://doi.org/10.1021/es101403q

43. Waly, M. M., Ahmed, T., Abunada, Z., Mickovski, S. B., & Thomson, C. (2022). Constructed Wetland for Sustainable and Low-Cost Wastewater Treatment: Review Article. *Land, 11*(9), 1388. https://doi.org/10.3390/land11091388

44. Wan Mohammad, W. A. S. B., Othman, N. H., Wan Ibrahim, M. H., Rahim, M. A., Shahidan, S., & Rahman, R. A. (2017). A review on seashells ash as partial cement replacement. *IOP Conference Series: Materials Science and Engineering, 271*, 012059. https://doi.org/10.1088/1757-899X/271/1/012059

45. Wang, D., Bai, Y., & Qu, J. (2022). The *Phragmites* Root-Inhabiting Microbiome: A Critical Review on Its Composition and Environmental Application. *Engineering, 9*, 42–50. https://doi.org/10.1016/j.eng.2021.05.016

46. Wang, H. X., Xu, J. L., Sheng, L. X., & Liu, X. J. (2018). A Review of Research on Substrate Materials for Constructed Wetlands. *Materials Science Forum, 913*, 917–929. https://doi.org/10.4028/www.scientific.net/MSF.913.917

47. Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: A review. *Interdisciplinary Toxicology, 8*(2), 55–64. https://doi.org/10.1515/intox-2015-0009

48. *Water | Free Full-Text | Nitrogen Removal Ability and Characteristics of the Laboratory-Scale Tidal Flow Constructed Wetlands for Treating Ammonium-Nitrogen Contaminated Groundwater*. (2017). Retrieved December 7, 2023, from https://www.mdpi.com/2073-4441/12/5/1326

49. Witkowska, D., Słowik, J., & Chilicka, K. (2021). Heavy Metals and Human Health: Possible Exposure Pathways and the Competition for Protein Binding Sites. *Molecules, 26*(19), 6060. https://doi.org/10.3390/molecules26196060

50. Wood, R. B., & McAtamney, C. F. (1996). Constructed wetlands for waste water treatment: The use of laterite in the bed medium in phosphorus and heavy metal removal. *Hydrobiologia*, *340*(1), 323–331. https://doi.org/10.1007/BF00012776

51. Wu, H., Wang, R., Yan, P., Wu, S., Chen, Z., Zhao, Y., Cheng, C., Hu, Z., Zhuang, L., Guo, Z., Xie, H., & Zhang, J. (2023). Constructed wetlands for pollution control. *Nature Reviews Earth & Environment*, *4*(4), 218–234. https://doi.org/10.1038/s43017-023-00395-z

52. Yaragal, R. R., Shet, R., & Mutnuri, S. (2023). Comparative Study on the Performances of Modified Vertical Flow–Constructed Wetlands for the Treatment of Domestic Wastewater. *Journal of Environmental Engineering*, *149*(4), 04023008. https://doi.org/10.1061/JOEEDU.EEENG-7107

53. Mohammad, M. N. (2014). *Eutrophication: Challenges and Solutions. Tabuk, Saudi Arabia: University of Tabuk, Tabuk, Saudi Arabia.*

52. Wiens, M. (2023). Retrieved 2023, from *National Institute of Environmental Health Sciences*: https://www.niehs.nih.gov/research/programs/geh/geh_newsletter/2023/8/articles/new_research_highlights_the_problem_of_microplastic_pollution.cfm
53. Xiaomin Tang, H. Z. (2014). *Chemical coagulation process for the removal of heavy metals from water: a review.* Desalination and Water Treatment, 1-14.

APPENDIX

Appendix 1-: Calibration and standard solution preparation for the COD estimation

The calibration and standard solution were prepared as per procedure mentioned under APHA 2005 -5220C & 5220D respectively. (Closed Reflux Colorimetric Method) and standard solution of COD was prepared. The change in color of the mixed digestion sample was observed above the COD value of 300 ppm and in the graph plotting it was observed that the sample still follows the calibration curve. Hence the given equation can be used for the measurement of the COD range of 100-900 ppm (or mg/L) despite of the change in color in the sample vials.

Note-: Potassium hydrogen phthalate (KHP) standard preparation for standard COD value (APHA 2005 -5220C g): 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 COD of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 g O_2/ mL.

Molecular weight of the KHP-: 204.23 g/mol

Achieve the desired concentration of standard COD by using-:

Theoretical COD-: 1.176 mg O_2/mg

Therefore, amount of KHP needed for desired concentration

$$Weight\ of\ KHP = \frac{100\ mg/L}{1.176\ \text{mg}\ 02/\text{mg}}$$

Table 3 Standard curve preparation at COD estimation

S. NO.	COD value (mg/L)	OD 1 (600 nm)	OD 2 (600 nm)	AVG. OD
1	0	0	0	0
2	100	0.043	0.047	0.045
3	200	0.089	0.082	0.0855
4	400	0.168	0.160	0.164
5	600	0.242	0.265	0.2535
6	800	0.325	0.339	0.332
7	1000	0.396	0.405	0.4005

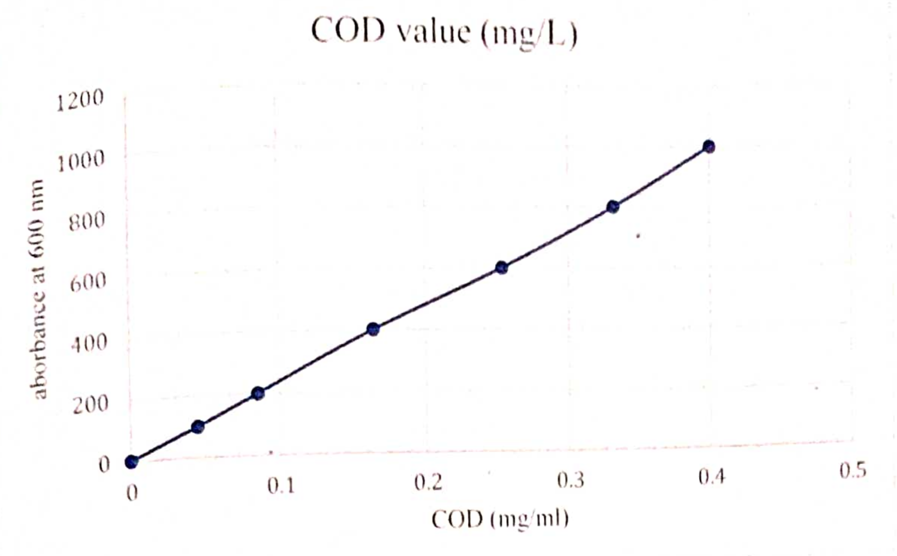

Fig. 20-: Standard curve for COD estimation

In the comparison analysis of exact volume mentioned in the procedure V/S the volumes used in lab, the data implies that the change in volume of the reaction while keeping the same ratio of reagents and sample does not variate the COD value of the sample. Comparison data table is mentioned below:

Table 4 -: Comparison data table for different volumes of reagent

Description	COD value (mg/L)	OD value 1 (600 nm)	OD value 2 (600 nm)	Average OD (600 nm)
6 ml total digestion volume (lab used)	200	0.089	0.082	0.0855
	800	0.325	0.339	0.332
7.5ml total digestion volume	200	0.083	0.078	0.0805
	800	0.326	0.344	0.335

Appendix 2-: Standard curve & standard solution preparation for the Phosphorus estimation

Stock solution of phosphorus (0.16mg/L) was prepared by dissolving 179 mg of KH_2PO_4 to give final concentration of (PO_4 -P) 0.16 mg/ml (APHA method 4500-P). Using this stock different concentration PO_4 -P prepared including blank as given in the table below-:

Table 5-: Stock solution of phosphorus

Sr. No.	Volume of stock (ml)	Volume of distilled water (ml)	Total Volume (ml)	Conc. (mg/L)
1	0	5	5	0
2	0.1	4.9	5	0.032
3	0.2	4.8	5	0.0064
4	0.3	4.7	5	0.0096
5	0.4	4.6	5	0.0128
6	0.5	4.5	5	0.016
7	0.6	4.4	5	0.0192
8	0.7	4.3	5	0.0224
9	0.8	4.2	5	0.0256
10	0.9	4.1	5	0.0288
11	1	4	5	0.032

Take 3.5 ml from each of the above tubes, and add 1 ml of mixed reagent and dilute to make final volume of 5.0 ml as given in the following table and use this diluted stock for the construction of the standard curve.

Table 6-: Conc. Vs absorbance for phosphorus estimation

Sr. no.	Volume of diluted stock sample (ml)	Volume of mixed reagent (ml)	Distilled water	Total volume	Concentration (mg/L)	OD 540 nm
1	3.5	1	0.5	5	0	0
2	3.5	1	0.5	5	0.00224	0.085

3	3.5	1	0.5	5	0.00448	0.177
4	3.5	1	0.5	5	0.00672	0.297
5	3.5	1	0.5	5	0.00896	0.372
6	3.5	1	0.5	5	0.0112	0.459
7	3.5	1	0.5	5	0.01344	0.572
8	3.5	1	0.5	5	0.01568	0.691
9	3.5	1	0.5	5	0.01792	0.751
10	3.5	1	0.5	5	0.02016	0.871
11	3.5	1	0.5	5	0.0224	0.943

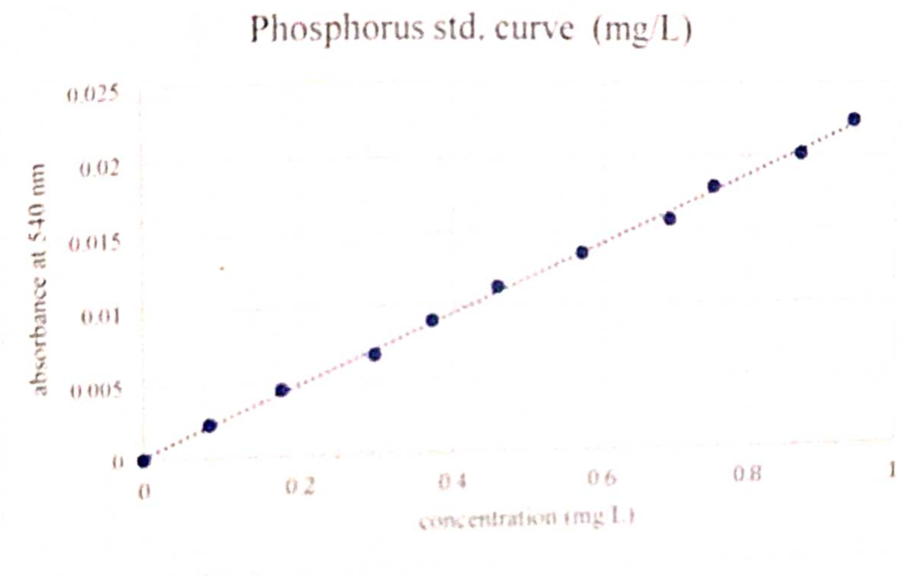

Fig. 21-: Standard curve for phosphorus

Appendix 3-: Standard preparation for the metal analysis (Zn, Cd & Ag)

For 1000 ppm stock solution of silver dissolve 15.74 mg silver nitrate in 10 ml of distilled water and for Zn and Cd prepare Subsequent dilutions using 1000 ppm ready to use standard solution. Use C1 V1= C2 V2 for subsequent dilutions (refer table 8 below)

Table 7-: Standard preparation for metal analysis (Zn, Cd & Ag)

SR. NO.	CONC. 1 mg/L	VOLUME 1	CONC 2	VOLUME 2	TOTAL VOLUME (mg/L)
1	1000	5	100	45	50
2	100	45	90	5	50
3	90	44.44	80	5.56	50
4	80	43.75	70	6.25	50
5	70	42.85	60	7.15	50
6	60	41.66	50	8.34	50
7	50	40	40	10	50
8	40	37.5	30	12.5	50
9	30	33.33	20	16.67	50
10	20	37.5	15	12.5	50
11	15	33.33	10	16.67	50
12	10	25	5	12.5	50
13	5	25	2.5	16.67	50
14	2.5	40	2	25	50
15	2	37.5	1.5	25	50
16	1.5	33.33	1	10	50
17	1	25	0.5	12.5	50
18	0.5	35	0.35	16.67	50
19	0.35	35.71	0.25	25	50

Standard for silver

Table 8

Sr. no.	Absorbance	concentration in ppm
1	0.0242	0.25
2	0.0336	0.35
3	0.066	0.5
4	0.1338	1

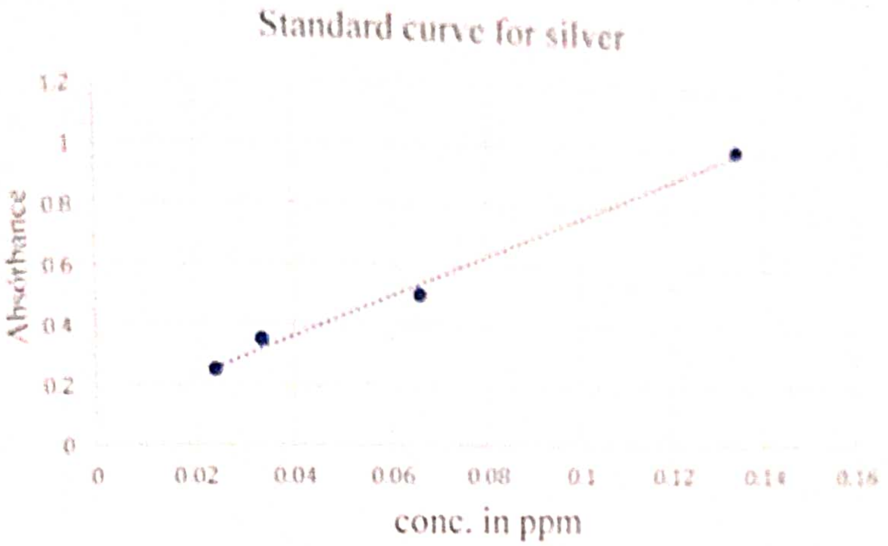

Fig. 22-: Standard curve for silver

Standard for Zn

Table 23

Sr. no.	Absorbance	concentration in ppm
1	[illegible]	[illegible]
2	[illegible]	[illegible]
3	[illegible]	[illegible]
[illegible]	[illegible]	1

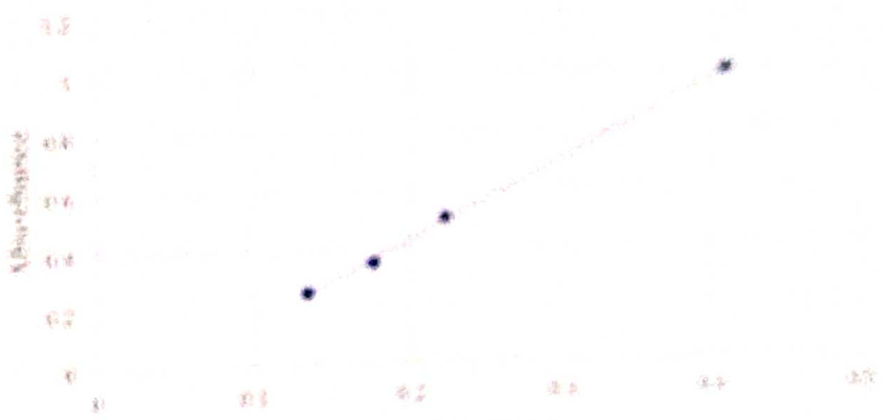

Fig. 23-: Standard curve for Zn

Standard for Cd

Table 10

Sr. no.	Absorbance	concentration in ppm
1	0.2055	0.5
2	1.0571	1
3	2.2652	2
4	4.9053	5

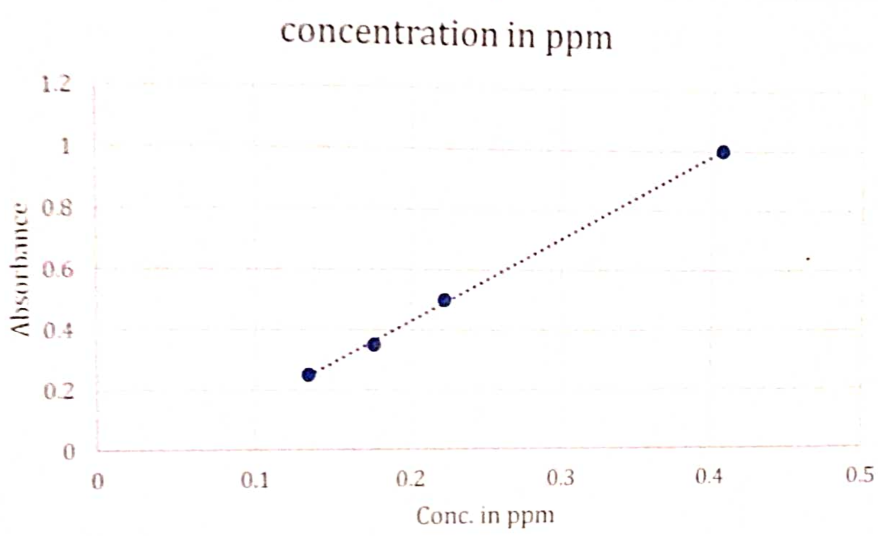

Fig. 24-: Standard curve for Cd