

Research article

Performance evaluation of Subsurface Flow Constructed Wetlands by treating urban Domestic wastewater using multivariate statistical analysis

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Abstract

The constructed wetland (CW) technology for eliminating contaminants from wastewater is environmentally favorable. A one-year pilot study was conducted at ICRISAT in Hyderabad (Telangana) to evaluate the efficacy of subsurface flow-constructed wetlands (SSF-CWs). To treat and evaluate their suitability for irrigation reuse, urban domestic wastewater was continuously fed into non-vegetative and vegetative wetland chambers with combination vegetation of *Typhalatifolia* & *Ageratum conyzoides* (CW-T) and *Canna indica* & *Ageratum conyzoides* (CW-C) and Control (non-vegetative). For one year, raw wastewater and treated wastewater were collected monthly and analyzed for quality. pH, BOD, COD, Total Solids, Total Suspended Solids, Total Dissolved Solids, Nitrogen, Sulphates, Ammonia, and Phosphorus were all investigated. The concentrations of all parameters are reduced by 60 to 90 percent in this constructed wetland (approx.). Water quality parameters were analyzed using statistical approaches such as principal component analysis (PCA), correlation analysis, time series analysis, and cluster analysis. In this investigation, PCA identified a reduced number of two primary components, showing that 95% of changes affect water quality. The first factor explained 51% of the variance in EC, TDS, COD, Nitrates, and Ammonia. The second factor accounted for 44% of the remaining variance in pH, BOD, Phosphates, and Sulphates. Between EC, TDS, Nitrates, Ammonia, and COD, the dendrogram of physicochemical parameter similarity revealed a maximum similarity of 76.85 percent. The SSF-CWs for the treatment of urban wastewater achieved more than 80% removal efficiency with no running expenses, minimal maintenance costs, enhances the environment, provides a natural habitat for birds, is odor-free, and can be recommended for agricultural use, according to this study.

Keywords: *Ageratum conyzoides*, *Canna indica*, Constructed wetlands, *Typhalatifolia*, Wastewater treatment.

1. Introduction

Water contamination caused by the direct discharge of untreated wastewater is a widespread environmental problem in developing countries. Environmental awareness has grown in the previous few years, and the treatment of pollution and contamination in the environment has become a top priority for concerned governmental authorities around the world. Typically, appropriate environmental remediation approaches for a specific type of waste is chosen ensure the effectiveness of the degrading activity and the technique's cost [1].

According to scientists and experts, there is no universally accepted remediation strategy that is acceptable for all types of pollutants and all sources; alternatively, an efficient remediation approach may require the use of two or more technologies in combination [2].

The rapid urbanisation and the need for water sources have necessitated improved wastewater recovery methods. Wastewater cannot be disposed of in an improper manner that endangers both persons and the environment's health [3]. India is predominantly an agriculturally oriented developing country, with agriculture being the primary source of income for the majority of the population. The global cultivated area increased by more than six times in the last century, from 40

million hectares to 260 million hectares, necessitating the need for new water sources. Every year, the amount of irrigated land is expected to grow by 1%. Though a forecast for a 13.6 percent rise in irrigated water by 2025 has been made, it must match the increased demand for water, particularly in India's irrigation system [4].

Mother Earth eliminates pollutants from water resources through natural wetlands through a variety of natural processes such as biodegradation, sorption, phyto stabilization, phyto extraction, and Rhizo filtration [5]. Constructed wetlands degrade contaminants through natural processes, making it an environmentally benign remedial option with minimal negative impact on the environment [6].

Wetlands are defined by soil saturation over long enough periods of time to allow anaerobic conditions to develop. Natural freshwater and saltwater wetlands, as well as constructed wetlands, are all types of wetlands. Wetlands built for contamination cleanup incorporate complex processes including waters, earth, vegetation, fauna, microbes, and the ecosystem. Various remediation approaches were used in constructed wetlands, including biological degradation, phytoremediation, and natural diminution [7].

Physical processes like filtration and sedimentation, chemical processes like adsorption and precipitation, and biological processes like biodegradation and plant uptake are all common in wetlands [8]. Constructed wetlands (CWs) are a cost-effective and efficient wastewater treatment option. Metals, nutrients, biological compounds, pathogens, and suspended particles are all removed by these simple to operate system [9]. Many researchers have looked into the design, development, and operation of CWs and discovered that they are an effective method for purifying polluted water [10].

The current study uses a subsurface flow (SSF) wetland to treat wastewater, with a foundation made up of permeable soil that is sealed from the bottom [6]. The water level in the SSF should always be below the soil level (see Figure 1). In the SSF wetland, the flow path is either horizontal or vertical. Low solid content wastewaters are acceptable for SSF wetlands. Deposition containers or troughs are used to remove solids from wastewater when the solid content is high [11]. The important features of SSF wetlands include colder climates endurance, fewer odour concerns than SF wetlands, more sorption and exchange sites than SF wetlands, and more efficient land use than SF wetlands. Because the water level is below the ground surface, SSF wetlands can be built beneath public parks. SSF wetlands have some shortcomings, including a greater price than SF wetlands, which are utilized for small flows, and pore clogging [12].

The main objective of this study is to evaluate the wastewater quality performance of a subsurface constructed wetland in terms of delivering an effective way of wastewater treatment. The research was conducted at ICRISAT in Hyderabad, Telangana. To treat urban domestic wastewater, a subsurface flow constructed wetland containing *Typhalatifolia*, *Ageratum conyzoids*, and *Canna indica* was planted.

2. Materials and methods

2.1 Presentation of the study site

The study was conducted in three identical subsurface flow constructed wetlands in ICRISAT, Patancheruvu station, Telangana. During the study period, a minimum temperature of 14.6(°C) and a maximum of 42.0(°C), the annual rainfall of 456.8(mm) was recorded.

2.2 Experimental Design

An elevated tank holds untreated wastewater for treatment in constructed wetlands, while a storage tank holds treated wastewater. The constructed wetlands will have a processing capacity of 30 to 50 m³/day, based on the hydraulic retention duration (5 to 3 days). Constructed wetlands are divided

into four chambers (A to D) in sequence, each measuring 1m*1.5m and 4m*1.5m in size. The following filter media were used: 1) Sand (15 to 25 cm thick), 2) Gravel (10mm size 15 to 25 cm thick), 3) Gravel (20mm size 25 cm thick), and 4) Gravel (40mm size 25 cm thick). Chambers B&C are cultivated with vegetation, while chambers A&D are completely devoid of flora. The two created wetland chambers are planted with vegetation and equipped with just filter media, whereas the control cell is not. The first CW (CW-T) has Typha and Ageratum conyzoids, while the second CW (CW-C) has Canna indica and Ageratum conyzoids. Water samples from the inlet (before treatment) and outflow (after treatment) of constructed wetland chambers were collected and analysed to determine the efficacy of the wetlands. pH, Electric conductivity (EC), Total solids (TS), Total dissolved solids (TDS), Total suspended solids (TSS), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Ammonia (NH3), Nitrates (No3-), Sulphates (So42), and Phosphates were all used to assess the removal efficiencies of CWs. For this study, CW-T and CW-C were planted alongside a control wetland compartment with no vegetation. Every month, water samples from the inlet and outlet of constructed wetland chambers are collected and analysed to determine the efficiency of constructed wetlands. (Figure1) represents the constructed wetlands.

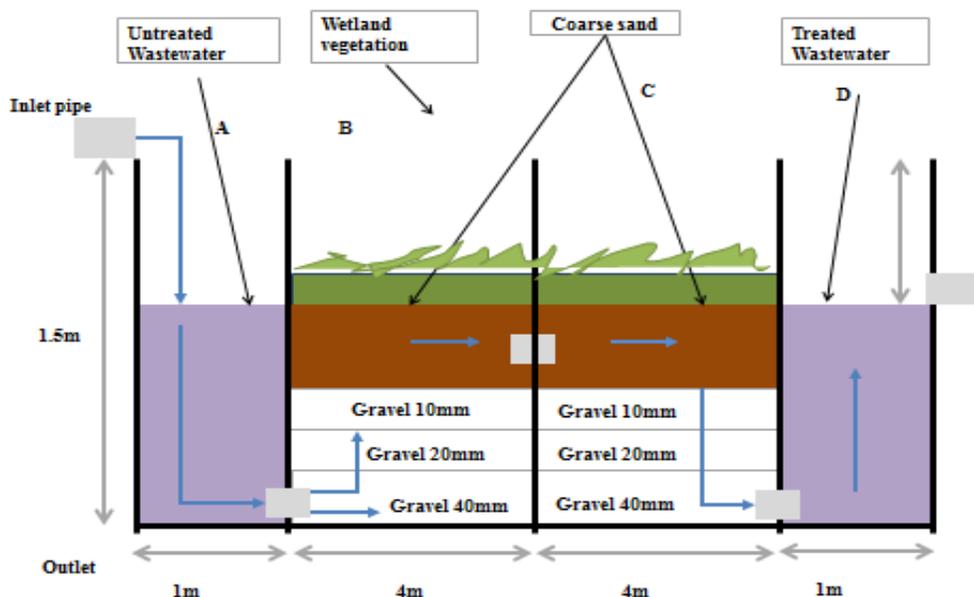


Figure 1: Subsurface flow Constructed wetland diagram

2.4 Multivariate Statistical analysis

The statistical analysis was carried out with the aim to determine substantial variation across all water quality measures using multivariate techniques like principal component analysis (PCA), correlation analysis and cluster analysis. The important changes in the varying factors during the investigation are revealed using time series analysis. Minitab 17 software was used for all statistical analysis.

3. Results and Discussion:

3.1 Water Physico-chemical parameters

For a year, inlet outlet wastewater samples from different chambers of the constructed wetland were monitored. Table 1 shows the mean values of physicochemical parameters at different

chambers and their average removable efficiencies over a 12-month experimental period (June 2015–July 2016).

3.1.1 pH: The concentration of hydrogen ions in water is measured by the pH scale, which measures the strength of acidity or alkalinity. The pH of current urban domestic wastewater ranged from 7.54 to 7.85 in the three constructed wetland chambers, indicating that the wastewater is neutral.

3.1.2 Electrical conductivity (EC) and Total Dissolved solids: The reductions in the EC in the three different constructed wetland chambers found to be 77, 83 and 81% respectively. The result shows that the average concentrations of TDS varies from 757 mg/l to 174 mg/l (77%) in CW-T, 881 mg/l to 146 mg/l (83%) in CW-C and 748 to 147 mg/l (81%) control chambers respectively.

Table 1: Mean concentrations of the main physio-chemical properties and their removable efficiencies between different constructed wetland systems (CWs).

Parameters	CW-T			CW-C			Control		
	Inlet	Out let	Avg % removal	Inlet	Out let	Avg % removal	Inlet	Out let	Avg % removal
pH	7.79	7.59	3	7.85	7.59	3	7.68	7.54	2
EC (µs/cm)	1165	268	77	1355	224	83	1151	227	81
TDS (mg/l)	757	174	77	881	146	83	748	147	81
BOD (mg/l)	52	2.67	95	48	9.62	79	16	3.40	80
COD (mg/l)	118	18	84	120	22	81	120	16	86
Nitrates (mg/l)	3.38	1.27	62	3.22	1.27	60	3.42	1.39	59
Ammonia (mg/l)	43	8.63	79	45	13	69	47	13	70
Sulphates (mg/l)	13	1.83	86	13.34	1.29	89	13	1.45	87
Phosphates (mg/l)	3.33	0.60	82	4.53	0.47	86	2.77	0.32	87

3.1.3 COD and BOD₅: Inlet COD was 118 mg/l to 120 mg/l in CW-T, CE-C, and Control, respectively, and outlet COD was 18 mg/l to 22 mg/l. In three separate constructed wetland chambers, COD was reduced by 84%, and 86 %, respectively, the similar removable efficiency in COD was reported by Choudhary et al., 2007 [13].

The amount of oxygen consumed by microorganisms during the biological reaction of oxygen with organic material is referred to as BOD. The inlet BOD in the current study ranges from 52 mg/l, 48 mg/l and 16 mg/l in the CW-T, CW-C, and Control groups, respectively. In the outlet, BOD levels in CW-T, CW-C, and Control are 2.6 mg/l, 9.6 mg/l, and 3.4 mg/l, respectively. The average percentage reduction is 84%, 81% and 86% respectively. BOD removal has a higher level of control than CW-T and CW-C. These differences could be attributable to differences in microbial bioactivity [14].

3.1.4 Nitrates and Phosphates:

Due to their function in algae growth and eutrophication of water sources, nitrogen and phosphorus are particularly important contaminating elements of domestic wastewater. The organic form of nitrogen can be found in sewage. In wastewater, the main nitrogen forms are organic nitrogen and ammonia nitrogen (NH₃-N). Under aerobic and anaerobic circumstances, organic nitrogen is normally transformed to nitrate. As a result, nitrate removal accounted for the majority of total nitrogen (TN) removal. Hydrophytes absorption, volatilization, and nitrification/denitrification were the three primary mechanisms involved in NH₃-N removal [15]. Nitrogen is required for optimum plant growth. They use their floating roots to absorb nitrogen and convert it into biomass [16]. The differences in Nitrates and Sulphates values between the input and output were investigated. The

average nitrate removal efficiencies in CW-T, CW-C, and Control were 62, 60, and 59 percent, respectively. In the CW-T, CW-C, and Control groups, sulphate removal efficiencies were 86, 89, and 87 %, respectively. The nitrate content in both the inlet and outlet during the investigation did not exceed 4 mg/L, and there was no significant difference between the inlet and outlet values.

Phosphorus is an essential nutrient for plant growth and is often a limiting factor in crop yield. A complex biogeochemical cycle transforms phosphorus in the wetland. Phosphorus removal is critical because it is a primary impediments nutrient for algal blooms in aquatic environment [17]. Adsorption, precipitation, and plant uptake levels are often the key activities accountable for phosphorus elimination in the constructed wetland. The most usual filtering elements used in subsurface flow constructed wetland have a gravel base, which is generally much better at absorption than plant roots [18]. Similarly, the average Phosphorus in the inlet in CW-T, CW-C, and Control was 3.3 mg/l, 4.6 mg/l, and 2.77 mg/l, respectively. After successfully treated, it drops to 0.60, 0.47, and 0.32 mg/l in the CW-T, CW-C, and Control chambers respectively. In all three chambers, the outlet Phosphorus removal efficiency was determined to be less than 80%.

3.1.5 Ammonia and Sulphates:

In the three chambers, the inlet and outlet Sulphates concentrations differed significantly (Table1). There was a considerable difference in the removal efficiencies of 86 % in CW-T, 89 % in CW-C, and 87% in Control. Throughout the study, ammonia concentrations were measured from 43 to 79 mg/l, with mean removal effectiveness ranging from 69 to 79 percent. Figure 2 shows the average removal percentages of physicochemical characteristics of wastewater such as pH, EC, TDS, and TSS over a 12-month period. pH removal ranged from 2 to 3 percent (on average 2.5 percent), and EC removal ranged from 77 to 83 percent (with an average reduction of 80 percent).

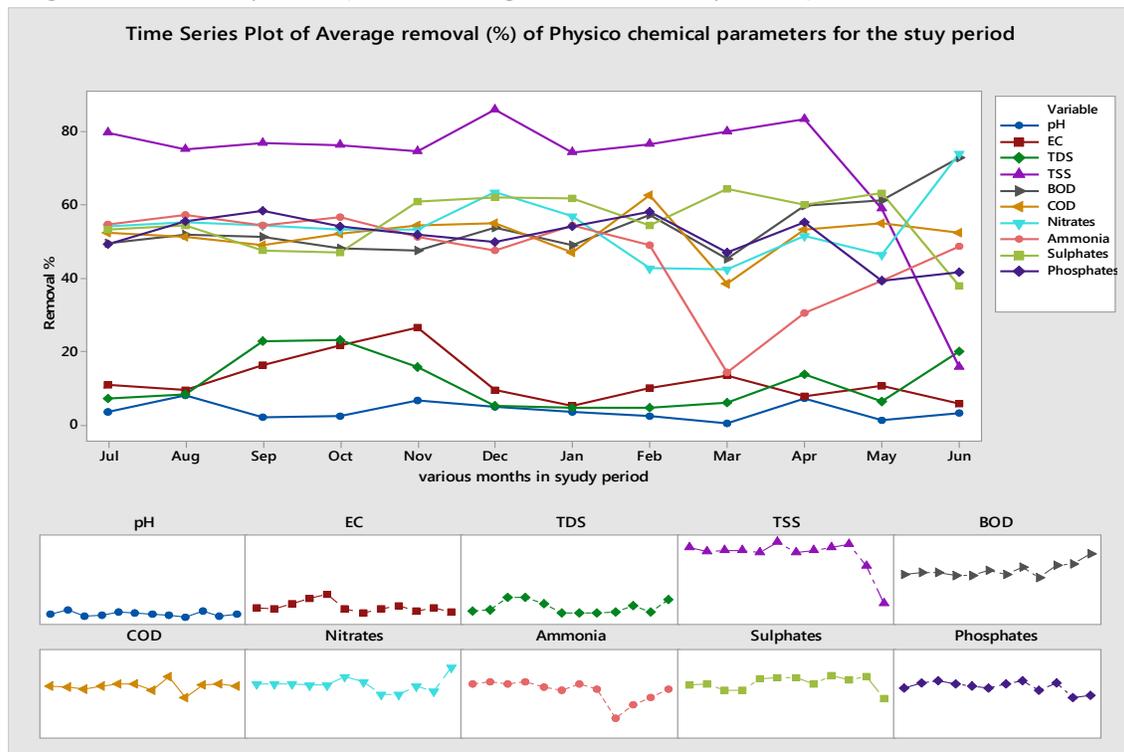


Figure 2: Time series plot for the Physiochemical concentration removal percentage for the study period.

During the study, the reduction in BOD ranged from 78 to 94 percent, with an average of 84 percent. COD reductions ranged from 81 to 86 %, with an average reduction of 83 %, while Nitrate reductions ranged from 58 to 61 percent, with an average reduction of 59 percent, Ammonia reductions ranged from 68 to 79 percent, Sulphates reductions ranged from 86 to 88 percent, and Phosphates reductions ranged from 82 to 87 percent, with an average reduction of 85 percent. During the investigation, all parameters showed considerable removal efficiency, and the output parameters were far within the TSPCB domestic wastewater discharge levels' permitted limits. As a result, the research would be a viable alternative to the revolutionary wastewater treatment technology that allows wastewater to be treated and reused for agricultural purposes.

3.2 Multivariate statistical analysis:

3.2.1 Principal Component Analysis (PCA): The most relevant factors and physicochemical characteristics influencing water quality were extracted using principal component analysis. It was hard to define conclusive results due to the complicated interconnections. Principal component analysis, on the other hand, might not only gain information to a certain extent and explain the characteristics of the data in detail, on different amplitudes by grouping similar the sample data, but it could also describe their different characteristics and help illustrate the relationship between the variables by using the variable lines. Principal component analysis was performed using Minitab 17 software to determine the main principal components from the original variables [19]. The 10 physicochemical characteristics were reduced to two primary variables (factors 1 and 2) based on the eigenvalues screen plot (Figure 3) from the screen plot's dropping off points [20].

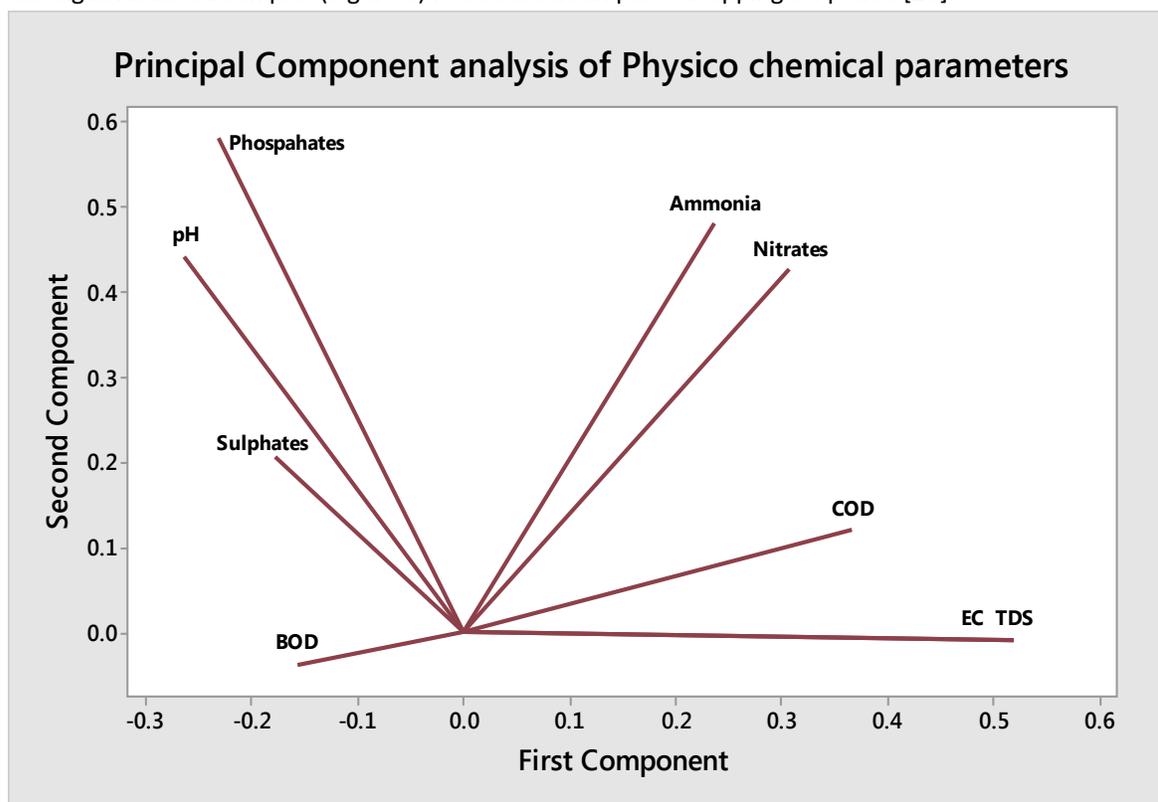


Figure 3: Principal component analysis of Physiochemical parameters of the urban domestic wastewater in subsurface flow constructed wetlands.

The first factor (3.27), which corresponds to the greatest eigenvalue, accounts for about 51.00% of the overall variance. Nearly 44.00 % variability is accounted for by the second factor, which corresponds to the second eigenvalue (1.75). To assess the nature of variation and principal trends among these variables, a correlation analysis was generated and factor loading was defined [21]. EC, TDS, COD, Nitrates, and Ammonia were the 05 major factors affecting the water quality of the constructed wetland, according to further investigation of factor loadings.

The largest factor loading value (>0.95) for factor 1 is EC, TDS, COD, Nitrates, and Ammonia, indicating that they are the most influential variables for the first factor or major component. It also represents the fact that excessive EC, TDS, COD, nitrates, and ammonia loadings are the source of the wastewater's most significant pollutants. Sulphates have the largest factor loading value for the second component, which accounted 44% of the remaining variance between pH, BOD, and Phosphates, implying that phosphate is also a major environmental contaminant in wastewaters.

3.2.2 Correlation Matrix:

The correlation index describes the relationship between two variables by describing how well one variable may relate to another, with high correlation coefficient values indicating a favourable relationship and conversely. The dependent (x) is completely controlled by the independent (y) and vice - versa in terms of variables determination [22]. In addition, if the correlation coefficient value is close to zero, and therefore has no correlation. A high positive number (r) indicates a positive link; however, if it is negative, the relationship is inverse. A statistical computation based on Pearson's correlation matrix was performed to better determine the cause of pollution, whether manmade or ecological.

Table 2: Pearson correlation coefficient between physicochemical properties of urban domestic wastewater in subsurface flow constructed wetland.

Variables	pH	TS	TDS	TSS	BOD	COD	Nitrates	Ammonia	Sulphates	Phosphates
pH	1	-0.11871	0.262663	0.567927	0.195226	0.340613	0.014833	-0.36611	0.66448	-0.0049310
TS		1	0.683226	0.306071	0.234422	0.462723	0.113036	0.371194	-0.02691	0.0105292
TDS			1	0.572169	0.53462	0.719519	0.197407	0.565964	-0.40772	-0.1031881
TSS				1	0.626277	0.702358	0.380195	-0.20864	0.70096	0.0683288
BOD					1	0.809007	0.305997	0.234319	-0.36222	0.1563337
COD						1	0.223014	0.269444	-0.34955	0.0108911
Nitrates							1	0.135624	-0.02576	0.5710363
Ammonia								1	0.161985	0.1362617
Sulphates									1	-0.4414504
Phosphates										1

Table 2 shows the correlation coefficient for all physicochemical parameters. The largest positive connection is seen between BOD and COD (80%). In table 2, the positive correlations of more than 50% between the physicochemical characteristics were bolded in colour. Ammonia, sulphates, and phosphates, like COD and BOD, indicate the source of organic contaminants in urban home wastewaters. While parameters with a negative or no relationship suggest that the source of the parameters in wastewater is not a single natural or anthropogenic input.

3.2.3 Cluster analysis:

The nearest neighbour method was used to perform cluster analysis, and the resulting dendrogram is shown in Figure 4.

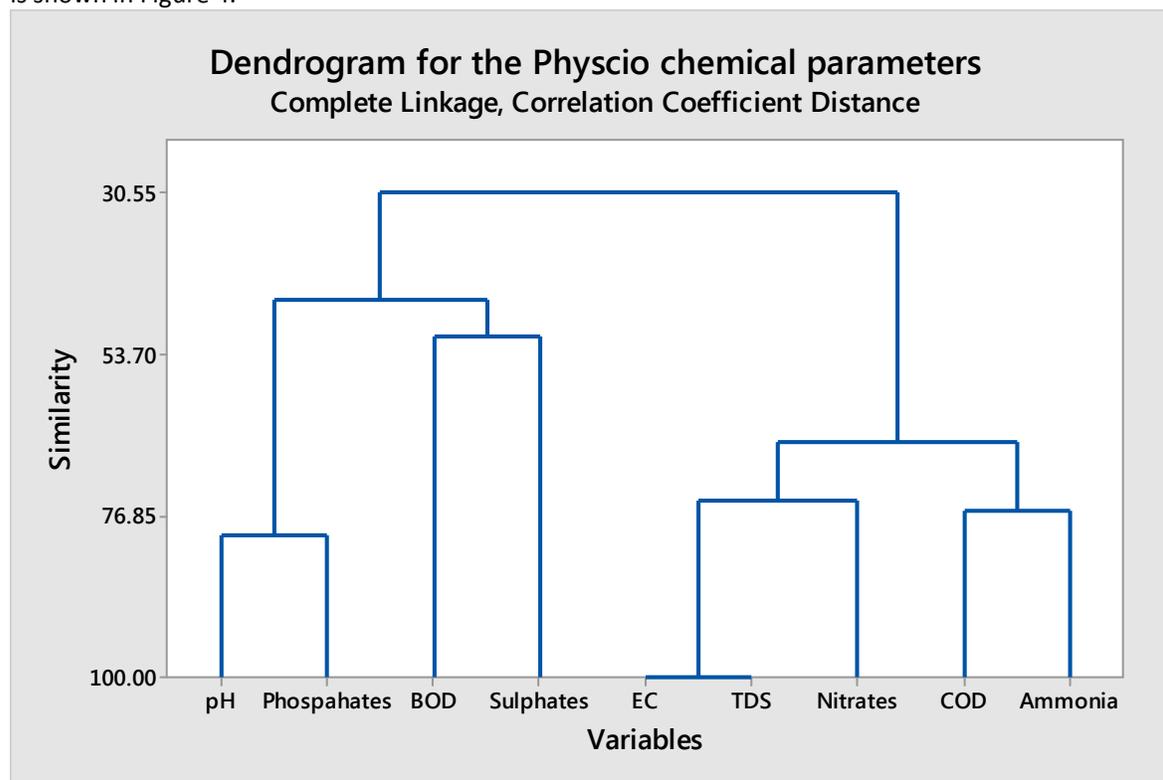


Figure 4: Hierarchical dendrogram for physicochemical parameters in wastewater using nearest neighbor, Pearson correlation.

Clusters 1 and 2 had 76.85% of EC, TDS, Nitrates, COD, and Ammonia, indicating that these pollutants were contaminated at a greater level. Cluster 3 has a BOD and sulphate content of 53.70 percent, indicating moderate pollution. Cluster 4 is made up of pH and Phosphates, and it shows the least amount of contamination.

4. Conclusions

This study found that the subsurface flow constructed wetland system at ICRISAT, Hyderabad, Telangana, performed well for urban domestic wastewater, with all main water quality metrics decreasing by at least 60 to 90 % of total average removal, resulting in improved water quality. The quality of the outlet water is significantly lower than the TSPCB's acceptable standards for domestic wastewater disposal. The constructed wetland deserves to be considered as a feasible alternative to traditional wastewater treatment. The cost - efficient constructed wetland technology can help to reduce the present wastewater management problem in developing countries of discharging untreated domestic wastewater into freshwater resources due to its low maintenance requirements, ease of operation, and good bulk pollutant removal performance. The cleansed sewage water could be used in agriculture, according to a planned study.

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References

1. Hassan, I., Chowdhury, S.R., Prihartato, P.K., Razzak, S.A., Wastewater Treatment Using Constructed Wetland: Current Trends and Future Potential Processes 2021, 9, 1917. <https://doi.org/10.3390/pr9111917>
2. Donde, Oscar & Navalía, Atalitsa. (2020). Constructed Wetlands in Wastewater Treatment and Challenges of Emerging Resistant Genes Filtration and Reloading. 10.5772/intechopen.93293. <http://dx.doi.org/10.5772/intechopen.93293>
3. Almuktar, S.A.A.N., Abed, S.N., Scholz, M., Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. Environ. Sci. Pollut. Res. 2018. 25, 23595–23623. <https://doi.org/10.1007/s11356-018-2629-3>.
4. Austin, David & Liu, Lin & Dong, Renjie. (2011). Performance of integrated household constructed wetland for domestic wastewater treatment in rural areas. Ecological Engineering. 37. 948-954. 10.1016/j.ecoleng.2011.02.002. <https://www.sciencedirect.com/science/article/abs/pii/S092585741100067X?via%3>
5. Mustafa, H.M., Hayder, G., Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: A review article. Ain Shams Eng. J. 2021, 12, 355–365. <https://www.sciencedirect.com/science/article/pii/S2090447920301131>.
6. Dan, T.H., Quang, LN., Chiem, NH., Brix, H., Treatment of high-strength wastewater in tropical constructed wetlands planted with Sesbania sesban: Horizontal subsurface flow versus vertical downflow . *Ecological Engineering*, 2011. 37 (5), 711-720. <https://doi.org/10.1016/j.ecoleng.2010.07.030>
7. Truu, J., Truu, M., Espenberg, M., Nõlvak, H., Juhanson, J., Phytoremediation and Plant-Assisted Bioremediation in Soil and Treatment Wetlands: A Review. Open Biotechnol. J. 2015, 9, 85–92. <https://openbiotechnologyjournal.com/contents/volumes/V9/TOBIOTJ-9-85/TOBIOTJ-9-85.pdf>
8. Barik, D., Energy from Toxic Organic Waste for Heat and Power Generation, 1st ed.; Woodhead Publishing: Sawston, UK, 2018. Available online: <https://www.elsevier.com/books/energy-from-toxic-organic-waste-for-heat-and-power-generation/barik/978-0-08-102528-4>.
9. Calheiros, C.S.C., Rangel, A.O.S.S., Castro, P.M.L., Constructed wetlands for tannery wastewater treatment in Portugal: ten years of experience. Int. J. Phytoremediation 16, 2014. 859–70. <https://doi.org/10.1080/15226514.2013.798622>.
10. Saeed, T., Sun, G., A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. J. Environ. Manage. 112, 2012. 429–448. <https://doi.org/10.1016/j.jenvman.2012.08.011>

11. Perdana, M.C., Sutanto, H.B., Prihatmo, G., Vertical Subsurface Flow (VSSF) constructed wetland for domestic wastewater treatment. *IOP Conf. Ser. Earth Environ. Sci.* 2018, 148, 012025. <https://iopscience.iop.org/article/10.1088/1755-1315/148/1/012025>
12. Gunter, L., Gabriela, D., Jaime, N., Anacleto, R.S., *Wetland Technology: Practical Information on the Design and Application of Treatment Wetlands*; Langergraber, IWA Publishing: London, UK, 2020. https://ri.conicet.gov.ar/bitstream/handle/11336/114556/CONICET_Digital_Nro.4fb03870-739e-4c77-a6d3-ba20c6a0a43b_A.pdf?sequence=2&isAllowed=y
13. Choudhary, Dr. Mahendra & Sharma, Dr., *Water Hyacinth: A Complete Solution to Wastewater Treatment, Reclamation and Environmental Protection*. *Journal of the Institution of Engineers (India)*. 2008. 89. 7-9. https://www.researchgate.net/publication/281612671_Water_Hyacinth_A_Complete_Solution_to_Wastewater_Treatment_Reclamation_and_Environmental_Protection
14. Steinmann, C.R., Weinhart, S., Melzer, A., A combined system of lagoon and constructed wetland for an effective wastewater treatment. *Wat. Res.*, 2003. 37:2035-2042. <https://www.sciencedirect.com/science/article/abs/pii/S0043135402004414>
15. Sommer, S.G., Olesen, J.E., Modelling ammonia volatilization from animal slurry applied with trail hoses to cereals. *Atmospheric Environment*, 2000. 34:2361-2372. <https://www.sciencedirect.com/science/article/abs/pii/S1352231099004422?via%3>
16. Baskar, G., Treatment of wastewater from Kitchen in an Institution Hostel Mess using Constructed Wetland. *International Journal of Recent Trends in engineering*, 2009. https://www.researchgate.net/publication/228647428_Treatment_of_wastewater_from_kitchen_in_an_Institution_Hostel_Mess_using_constructed_wetland.
17. Wetzel, R.G., *Limnology-Lake and River Ecosystems*, Academic Press, San Diego, CA. 2001. <https://www.journals.uchicago.edu/doi/10.1086/380040>
18. Vymazal, J., Removal of phosphorus in constructed wetlands with horizontal subsurface flow in the Czech Republic. *Water, Air, and Soil Pollution*, 2004. 4: 657-670. <https://link.springer.com/article/10.1023/B:WAFO.0000028385.63075.51>
19. Van Der Gucht K., Sabbe, K., De Meester L., Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. *Environmental Microbiology*, 2001.vol. 3, no. 11, pp. 680-690. <https://doi.org/10.1046/j.1462-2920.2001.00242.x>

20. Cattell R. B., Jaspers, J. A., A general plasmode (No. 30-10-5-2) for factor analytic exercises and research," *Multivariate Behavioral Research Monographs*, 1967. vol. 67, p. 211.
<https://psycnet.apa.org/record/1968-09684-001>
21. Aruga, R., Negro, G., and Ostacoli, G., Multivariate data analysis applied to the investigation of river pollution, *Fresenius' Journal of Analytical Chemistry*, vol. 346, no. 10-11, pp. 968–975, 1993.
<https://link.springer.com/article/10.1007/BF00322761>
22. Voudoris K., Panagopoulos, A., Koumantakis, J., Multivariate statistical analysis in the assessment of hydrochemistry of the northan korinthia prefecture alluvial aquifer system (Peloponese, Greece). *Nat Resour Res* 2000. 9(2):135-146.
<https://link.springer.com/article/10.1023%2FA%3A1010195410646>