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The Ability of Some Aquatic and Terrestrial Plants to Purify Domestic Wastewater

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ABSTRACT

The study aimed to evaluate the ability of some terrestrial and aquatic plants for wastewater purification. Aquatic plants can remove pollutants from wastewater by consuming and accumulating various contaminants in different parts of plants. Different aquatic and terrestrial plants (*Rosa sinensis*, *Typha latifolia*, *Ocimum bacilicum*, *Azolla pinnata*, and *Salvinia molesta*) which have the ability to decrease water pollution were utilized in this study. The capability of five different species of plants was investigated by measuring chemical oxygen demand (COD), biological oxygen demand (BOD), electrical conductivity (EC), total dissolved solids (TDS), and pH of the medium. In this research, some aquatic and terrestrial plants were transplanted in wastewater plastic pots containing domestic wastewater with different ratios of 50% and 100%. Then, after 30 days, the physiological and biochemical parameters of plants were calculated to observe the effect of wastewater on plants. Results revealed higher chlorophyll and carotenoids in typha plants treated with 100% wastewater. The highest percentage of elimination in BOD (65%), COD (27%), TDS (72%), EC (83%), and pH (6.8%) was noted with the use of typha and azolla. Intriguingly, total soluble sugars, total free amino acids, and total proteins were found maximum in the hibiscus plant as compared to the other plants under 100% and 50% domestic wastewater treatment, while typha and ocimum showed lower values of these parameters irrespective of wastewater treatments. Moreover, the COD, BOD, TDS, EC, and pH trend was higher in 100% wastewater as compared to 50% wastewater. Taking into account the accumulation capacity of the tested plants especially typha can be efficiently used for the treatment of domestic wastewater.

KEYWORDS

Biological oxygen demand; chemical oxygen demand; domestic wastewater; aquatic plants; terrestrial plants

1 Introduction

The challenges of abiotic stress on plant growth and development are evident among the emerging ecological impacts of climate change [1–8]. The constraints to agriculture production are also exacerbated by the increasing human population that compete for environmental resources. Underground and surface



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water are the primary sources of fresh water; however, the rapid development of industries and an overwhelming population are continuously polluting the water all over the world and causing risk of freshwater scarcity for agricultural production, especially in developing countries including Pakistan. Farmers are using sewage and industrial water to meet water requirements [9–11], these water sources are comprised of different types of pollutants that cause harmful effects on the natural ecosystem due to the presence of inorganic, organic toxins, including pathogens, nitrates, heavy metals and salts [12,13] that causing consequent risk for human health as well as for the aquatic ecosystem. Heavy metals and nutrients are beneficial at a lower dose, but cause a critical effect on the plant life cycle at higher concentrations [14–21]. Of all the wastewater resources, domestic wastewater makes up the largest portion generated by human activities, and up to 90% of the clean water consumption is discharged as wastewater [22]. The basic access of water consumption for minimum hygiene and hydration is 20 l/c/d, while the optimum access, including bathing, laundry, food preparation, and personal hygiene requires at least 100 l/c/d [23]. The aquatic database recorded that municipal water withdrawal from 180 countries in 2017 ranged from 1 to 359 m³/c/year (3 to 978 l/c/d) with a median of 54.3 m³/c/year (149 l/c/d), which is mainly comprised of domestic consumption but may include water for urban agriculture and industrial uses [24]. Approximately 70% of domestic wastewater is generated from households or residential buildings, and the rest is generated from public facilities, commercial areas, and office buildings [25], however, the both sources share similar constituents but differ in quantity and composition [26]. Currently, conventional wastewater treatment methods are not effective for the complete removal of water contaminants [27]. A small quantity of the contaminants can cause habitat degradation due to the toxic nature of these contaminants that can interfere with many ongoing cellular processes in plant tissues [28]. However, excess sludge discharge, carbon emission, high energy equipment's and high maintenance costs pose serious challenges to the methods of wastewater removal. However, the critical analysis and wastewater treatment are still limited [29].

Phytoremediation technology is environment friendly and used for the uptake of pollutants from soil and water resources [30]. The phytoremediation technology is easy to maintain and implement and does not require highly specialized personnel and expensive equipment's [31], so the construction of wetlands as green technology can be used to reclaim various water sources. In this study, the phytoremediation potential of different aquatic and terrestrial plants such as *T. latifolia* (typha), *R. sinensis* (hibiscus), *O. bacilicum* (ocimum), *A. pinnata* (azolla) and *S. molesta* (salvinia) species were evaluated for domestic wastewater purification. Moreover, the effect of domestic wastewater was also assessed in the physiological and biochemical analysis of above-mentioned plants. A mechanistic diagram is depicted in Fig. 1.

2 Materials and Methods

2.1 Plant and Wastewater Collection for Experimental Setup

The experiment was designed to study the role of some aquatic and terrestrial plants in wastewater treatment. The experiment was conducted in the Research area of Government College Women University of Faisalabad (GCWUF). The five different plants such as typha, hibiscus, ocimum, azolla and salvinia were used in the experiment. Wastewater was collected from GCWUF. Then water was transferred to a hydroponic medium with three replicates containing 100% and 50% wastewater and control. The plants were completely washed under tap water and then transferred into hydroponic treatments. Half strength hogland solution was given during the experiment. The experiment lasted for 30 days and was designed in a completely randomized design (CRD) arranged under three factor factorial arrangement (time, plant species and wastewater treatment) for wastewater analysis, while two factor factorial (plant species and wastewater treatment) was used for plant analysis. The composition of wastewater is given in Table 1.

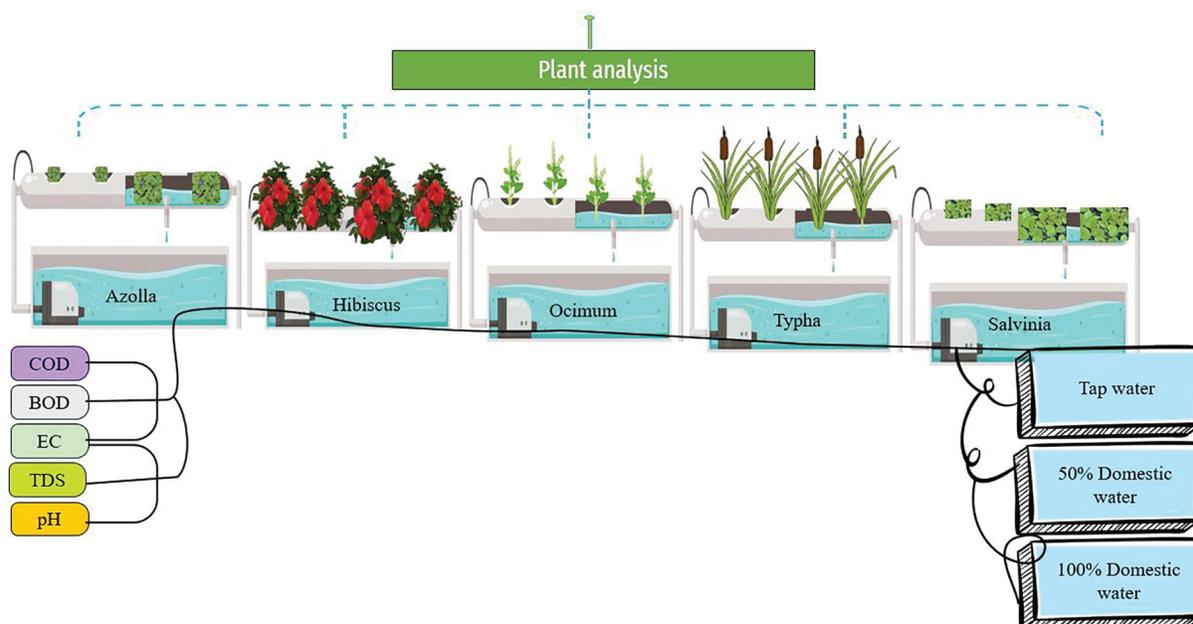


Figure 1: A graphic illustration of the effect of domestic wastewater treatment on aquatic and terrestrial plants

Table 1: Initial value of domestic wastewater

Water attributes	Tap water	Domestic wastewater (50%)	Domestic wastewater (100%)
BOD (mg/L)	100	250	450
COD (mg/L)	290	990	1100
EC (mg/L)	5.5	6	8
TSS (mg/L)	5	9	10
pH	8	8.7	9

2.2 Wastewater Analysis (TDS, pH, EC, COD, and BOD)

TDS was measured in domestic wastewater with a TDS meter. The sample was collected in plastic bottles and transferred in a beaker and the TDS meter was dipped into a beaker until a stable value of TDS was observed. PH of domestic wastewater was measured with pH meter. The sample was collected in beakers and stirrer, pH meter until stable reading was measured. EC of domestic wastewater was measured with EC meter. The sample was collected in plastic bottles and transferred into a beaker for EC detection. COD of domestic wastewater samples was measured with COD meter (Tinto meter). In addition, BOD of domestic wastewater samples was measured with a VELP BOD meter.

2.3 Physio-Biochemical Attributes

2.3.1 Chlorophyll a, b, Total Chlorophyll Contents and Carotenoids

Watada et al. [32] method was used to determine the chlorophyll contents (a, b) and total chlorophyll contents, while, Arnon [33] method was used to measure carotenoid content. Fresh leaf material (0.1 g)

of the plant was chopped and dipped in 1:1 solution of acetone (80%) and ethanol for 24 h, absorbance was measured by spectrophotometer (UV/Vis) at three different wavelengths (645, 663 and 480 nm).

2.3.2 Total Soluble Sugars

Total soluble sugars were determined by the method of Yemm et al. [34]. A 0.1 g plant leaf sample was ground in 80% ethanol solution. Extract was incubated at 60°C for 6 h. The extract was added in 6 mL of freshly prepared anthrone reagent and heated the test tubes for 10 min in a boiling water bath. Then test tubes were ice cooled for 10 min, and again incubated at room temperature (25°C) for 20 min. Optical density was observed at 625 nm.

2.3.3 Total Soluble Proteins

Total soluble protein was estimated by the method of Bonjoch et al. [35]. According to this method, 0.25 g of fresh leaves were ground with 5 mL of phosphate buffer having 7.0. Then, sample was centrifuged for 20 min at 8000 rpm. After extraction, 1 mL of supernatant was added in each test tube and then add 5 mL of Bradford dye, and mixed completely. Sample was filtered 2–3 times. The test tubes were incubated at room temperature for 5 min and then absorbance was observed at 595 nm by using a spectrophotometer.

2.3.4 Free Amino Acids

Hamilton et al. [36] method was used to determine total free amino acids. Extraction of fresh plant material was done in 0.02 M phosphate buffer (pH 7). For, 1 mL extract, 1 mL 10% pyridine and 1 mL 2% Ninhydrine solution were added to each test tube and then covered the test tube with aluminum foil. Then, heat the test tubes in a boiling water bath for 30 min. Each test tube was filled with boiling water up to the capacity of 50 mL. The absorbance was measured at 570 nm.

2.4 Statistical Analysis

The phytoremediation potential of five plant species on wastewater treatment was assessed under 100%, 50% and control conditions on physio-chemical attributes of plants and wastewater by using analysis of variance (ANOVA at 5% probability level with the help of statistical software “Statistix” (ver. 8.1, Tallahassee, FL, USA)). Two-way ANOVA (wastewater x plant species) was assessed for plant analysis, while three-way ANOVA was used for water analysis for time base studies. LSD test was performed to compare the relative means. PCA and heat map was constructed by using R. Different letters show significant differences ($p < 0.05$), while similar letters did not show significant differences.

3 Results

3.1 Water Analysis Parameters

3.1.1 pH

Statically results showed significant ($p < 0.000$) differences among aquatic plants, domestic wastewater treatments and weeks, and the interaction among these factors was also significant ($p < 0.000$) for pH of wastewater. The pH value decreased after week by week. Results showed that the pH value of 100% domestic water exposed plants decreased for typha (6.8%), hibiscus (0.2%), ocimum (6.7%), azolla (0.2), and salvinia (6.7%) after 4 weeks. The pH value of 50% wastewater decreased by using typha (2.3%), hibiscus (6.4%), ocimum (4.5%), azolla (2.5%), and salvinia (6.1%) after four weeks. the maximum decrease was observed in typha plant subjected to 100% wastewater. The maximum decrease was observed in hibiscus plant subjected to 50% wastewater and control water (Fig. 2A, Table 2).

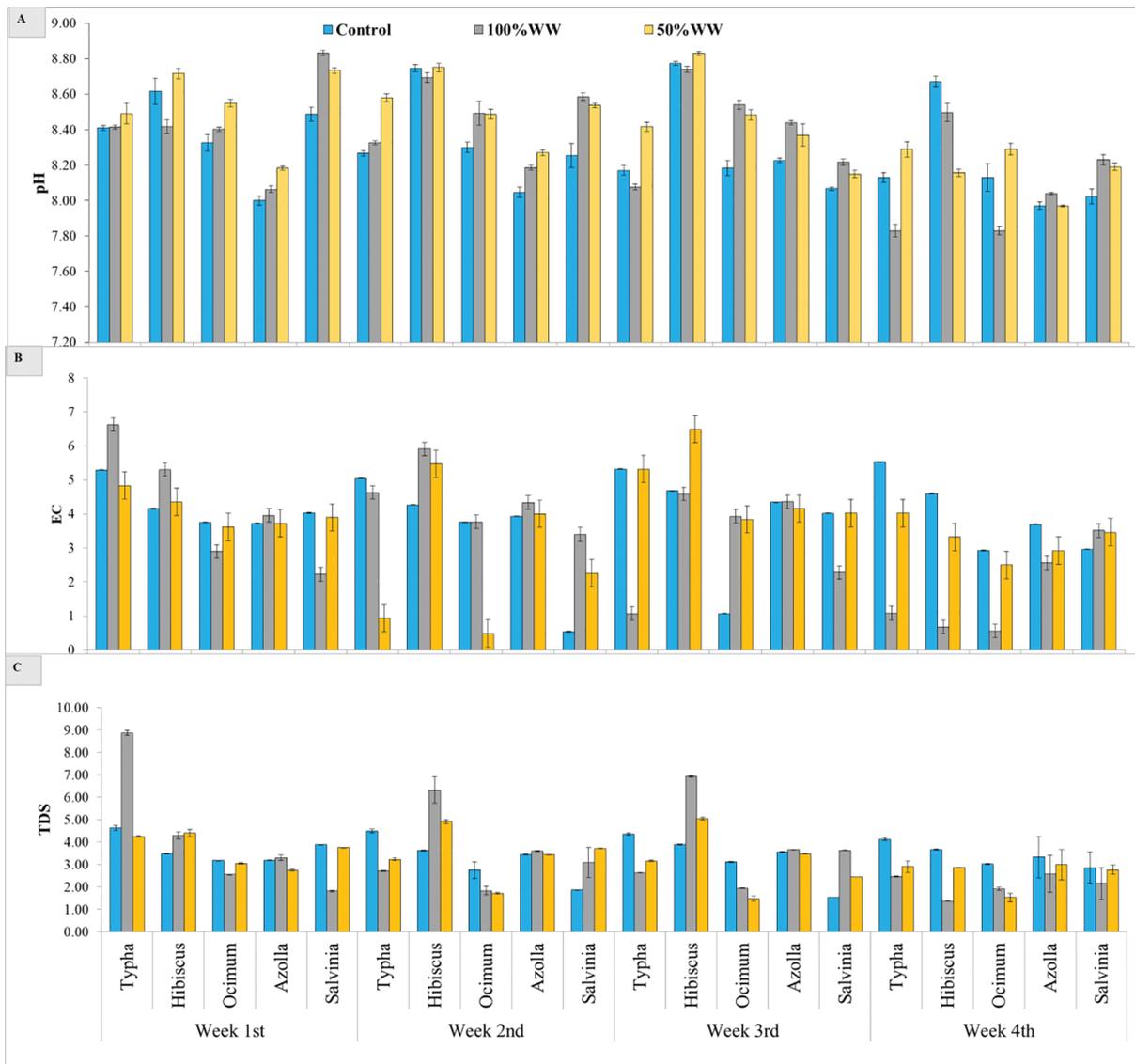


Figure 2: Effect of aquatic and terrestrial plants on the pH, EC and TDS of domestic waste water. (A) pH, (B) EC, (C) TDS

Table 2: The F-values from the three-way (plants, wastewater and weeks) ANOVA of EC, BOD, COD, pH and TDS of wastewater treatment

Source	BOD	COD	EC	pH	TDS
Plants (P)	73.06***	425.32***	355.13***	363.64***	107.37***
Wastewater treatment (T)	71644.2***	221422***	70.29***	94.58***	3.64**
Week (W)	3349.06***	5415.30***	240.11***	210.34***	45.93***
P × T	180.10***	75.99***	56.64***	43.46***	13.69***
P × W	15.13***	50.34***	77.59***	54.06***	24.76***
T × W	293.35***	139.69***	230.08***	9.15***	14.11***
P × T × W	75.76***	42.24***	85.15***	9.75***	15.43***

Note: *, **, and *** indicate significance at 0.05, 0.01, and 0.001 probability levels, respectively.

3.1.2 Electrical Conductivity (EC)

Statically results showed significant ($p < 0.000$) differences among aquatic plants, domestic wastewater treatments and weeks, and the interaction among these factors was also significant ($p < 0.000$) for EC of wastewater. The EC value decreased after week by week. Results showed that the EC value decreased under 100% domestic water by growing typha (83%), hibiscus (87%), ocimum (80%), azolla (35%), and salvinia (57%) after 4 weeks. The reduction in EC value of 50% wastewater was observed for typha (16%), hibiscus (19%), ocimum (30%), azolla (21%) and salvinia (11%) after four weeks. The maximum decrease was observed in hibiscus plant subjected to 100% wastewater, while the maximum decrease was observed in ocimum plant water subjected to 50% wastewater and control water (Fig. 2B, Table 2).

3.1.3 Total Dissolved Solids (TDS)

Statically results showed significant ($p < 0.000$) differences among aquatic plants, domestic wastewater treatments and weeks, and the interaction among these factors was also significant ($p < 0.000$) for TDS of wastewater. These values decreased week after week. Results showed that the TDS value of domestic water (100%) decreased by growing typha (72%), hibiscus (67%), ocimum (24%), azolla (22%), and salvinia (23%) after 4 weeks. The TDS value of 50% wastewater decreased were also decreased by harvesting typha (31%), hibiscus (35%), ocimum (49%), azolla (9%) and salvinia (26%) after four weeks. The maximum decrease was observed in typha plant subjected to 100% wastewater, while under 50% wastewater the maximum decrease was observed in ocimum (Fig. 2C, Table 2).

3.1.4 Biological Oxygen Demand (BOD)

Statically results showed significant ($p < 0.000$) differences among aquatic plants, domestic wastewater treatments and weeks, and the interaction among these factors was also significant ($p < 0.000$) for BOD of wastewater. Results showed that the BOD value of 100% domestic water decreased for typha (23%), hibiscus (22%), ocimum (25%), azolla (19%) and salvinia (24%) after 4 weeks. The BOD value of 50% wastewater of typha decreased (65%), hibiscus (38%), ocimum (43%), azolla (37%) and salvinia (50%) after four weeks. The maximum decrease was observed in salvinia plant subjected to 50% wastewater. The maximum decrease was observed in ocimum plant subjected to 100% wastewater and control water (Fig. 3A, Tables 2 and 3).

3.1.5 Chemical Oxygen Demand (COD)

Statically results showed significant ($p < 0.000$) differences among aquatic plants, domestic wastewater treatments and weeks, and the interaction among these factors was also significant ($P < 0.000$) for COD of wastewater. The COD value decreased after week by week. Results showed that the COD value of 100% domestic water decreased for typha (20%), hibiscus (16%), ocimum (14%), azolla (13%), and salvinia (16%) after 4 weeks. The COD value decreased at 50% wastewater for typha decreased (27%), hibiscus (18%), ocimum (22%), azolla (39%) and salvinia (19%) after four weeks. The maximum decrease was observed in typha plant subjected 100% wastewater. The maximum decrease was observed in azolla plant subjected to 50% wastewater and control water (Fig. 3B, Tables 2 and 3).

3.2 Physio-Biochemical Parameters

3.2.1 Chlorophyll a Content

The chlorophyll a content varied significantly ($p > 0.000$) in all plant species plants under all wastewater treatments. Results showed that chl a content increased up to 37%, 34%, 32%, 69% and 9% in hibiscus, typha, ocimum, azolla, and salvinia, respectively at 100% wastewater. Chl a content in plants subjected to 50% wastewater showed 60%, 31%, 22%, 6% and 11% reductions in hibiscus, typha, ocimum, azolla, and salvinia, respectively (Fig. 4A).

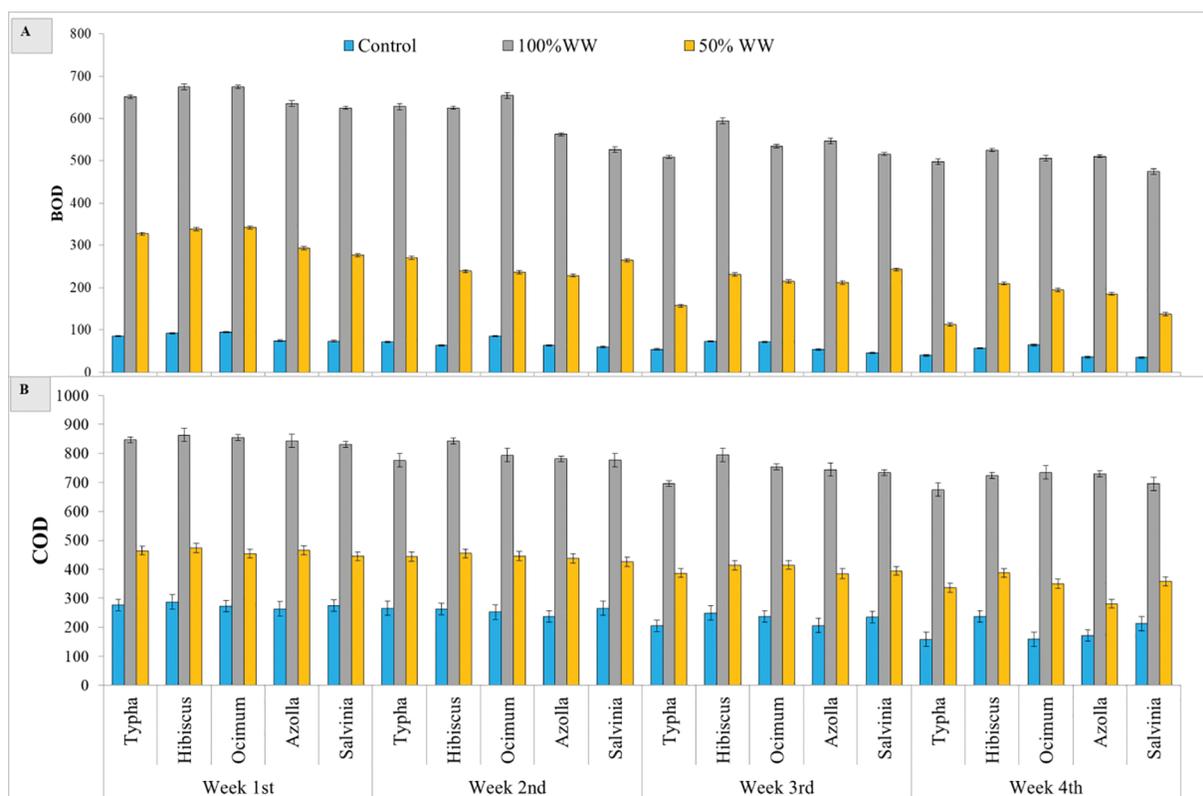


Figure 3: Effect of aquatic and terrestrial plants on the BOD and COD of domestic wastewater. (A) BOD, (B) COD

3.2.2 Chlorophyll b Content

Maximum increase in chl b contents were observed in hibiscus plant subjected to 50% wastewater. Typha, azolla, ocimum and salvinia showed maximum increase at 100% wastewater as compared to normal water (Fig. 4B).

3.2.3 Carotenoid Content

The carotenoid content varied significantly ($p > 0.000$) in all plant species plants under all wastewater treatments. Results showed that carotenoid contents in leaves increased up to 49%, 85%, 72%, 69% and 9% in hibiscus, typha, ocimum, azolla, and salvinia, respectively at 100% wastewater. Carotenoid contents in plants subjected to 50% wastewater observed were increased up to 54%, 82%, 39% 6% and 11% in hibiscus, typha, ocimum, azolla, and salvinia, respectively (Fig. 4C).

3.2.4 Free Amino Acid Content

Results showed that the amount of total amino acids increased up to 52%, 44%, 70%, 38% and 61% in hibiscus, typha, ocimum, azolla, and salvinia, respectively at 100% wastewater. Amino acid content acids in plants subjected to 50% wastewater were increased up to 35%, 55%, 97%, 9% and 37% in hibiscus, typha, ocimum, azolla, and salvinia, respectively (Fig. 4D).

Table 3: Tabular illustration of domestic wastewater analysis (EC, BOD, COD, pH and TDS). The alphabets indicate the significant ($p < 0.05$) difference among the treatments

Weeks	Plant species	EC				COD				BOD				pH				TDS		
		Control	50% waste water	100% waste water	100% waste water	Control	50% waste water	100% waste water	100% waste water	Control	50% waste water	100% waste water	100% waste water	Control	50% waste water	100% waste water	100% waste water	50% waste water	100% waste water	
1 st week	<i>Typha latifolia</i>	5.293 cd	4.84 ef	6.626a	464.3333 n	276.666 za	464.3333 n	847.6667 bc	85.333333 a	327.3333 o	651.6667 b	8.4100 k-n	8.4900 l-l	8.4133 k-n	8.4167 cd	8.4167 k-n	8.4167 k-n	4.62 c-e	4.26 d-i	8.87 a
	<i>Hibiscus rosa sinensis</i>	4.16 l-k	4.353 g-i	5.306 cd	474.00 m	287.00 y	474.00 m	863.00 a	92 a	339 n	674.6667 a	8.6167 e-g	8.7167 cd	8.4167 k-n	8.7167 cd	8.4167 k-n	8.4167 k-n	3.48 j-q	4.41 e-g	4.29 d-i
	<i>Ocimum bacilicum</i>	3.754 l-o	3.61 m-p	2.892 r-s	454.3333 o	273.0 a	454.3333 o	854.3333 b	94.66667 a	342 n	675 a	8.3267 n-q	8.5500 g-j	8.4033 l-o	8.5500 g-j	8.4033 l-o	8.4033 l-o	3.17 k-v	3.05 l-v	2.55 t-z
	<i>Azolla pinnata</i>	3.718 l-o	3.725 l-o	3.961 j-m	465 n	263.6667 b	465 n	843.6667 c	75 b	294 p	635.6667 c	8.0000 ab	8.1833 t-w	8.0633 y-a	8.1833 t-w	8.0633 y-a	8.1833 t-w	3.19 k-u	2.74 Q-w	3.30 k-t
2 nd week	<i>Sabunia molesta</i>	4.03 l-l	3.896 j-m	2.226 T	445.3333 p	275.3333 a	445.3333 p	830.3333 d	73.333333 bc	276.6667 q	625 d	8.4867 j-l	8.7333 Cd	8.8333 a	8.4867 j-l	8.8333 a	3.88 e-k	3.75 Fl	1.82 z-c	
	<i>Typha latifolia</i>	5.042 de	0.936 uv	4.633 Fh	443.6667 pq	265.6667 b	443.6667 pq	776 f	71.66667 b-d	271 Qr	628 Cd	8.2667 q-t	8.5800 Fi	8.3267 n-q	8.2667 q-t	8.3267 n-q	4.49 c-f	3.23 k-t	2.72 t-w	
	<i>Hibiscus rosa sinensis</i>	4.266 h-j	5.473 c	5.914 b	455 o	263 b	455 o	843 c	63.333333 d-f	239 St	625 d	8.7467 a-d	8.7500 a-d	8.6933 c-e	8.7467 a-d	8.6933 c-e	3.63 h-n	4.92 cd	6.32 b	
	<i>Ocimum bacilicum</i>	3.758 l-o	0.485 W	3.767 l-o	445 p	252.6667e	445 p	794 e	85 a	236.3333 s-u	654.6667 b	8.3000 c	8.4867 j-l	8.4933 i-l	8.4867 j-l	8.4933 i-l	2.75 q-w	1.72 a-c	1.83 y-c	
3 rd week	<i>Azolla pinnata</i>	3.928 j-m	4.002 i-l	4.34 g-i	437.6667 q	237.3333 d	437.6667 q	782 f	63.333333 d-f	228.6667 u	563 f	8.0467 z-b	8.2700 q-t	8.1867 t-w	8.0467 z-b	8.1867 t-w	3.44 j-r	3.44 j-r	3.60 l-o	
	<i>Sabunia molesta</i>	0.538 w	2.26 T	3.398 op	426.3333 r	265.6667 b	426.3333 r	776.6667 f	60 e-g	264.3333 r	527 h	8.2533 q-u	8.5367 g-j	8.5867 Fh	8.2533 q-u	8.5867 Fh	1.86 y-c	3.71 g-m	3.09 l-v	
	<i>Typha latifolia</i>	5.322 cd	5.322 Cd	1.071 u	386.3333 u	205.6667 f	386.3333 u	696.6667 k	54.333333 Fh	157 x	509.3333 jk	8.1700 u-w	8.4167 k-n	8.0767 x-a	8.1700 u-w	8.0767 x-a	4.36 c-h	3.17 k-v	2.63 s-x	
	<i>Hibiscus rosa sinensis</i>	4.68 e-g	6.486 a	4.586 Fh	414 s	249.3333 c	414 s	795 e	72.66667 b-d	231.3333 tu	595 e	8.7733 a-c	8.8300 ab	8.7400 b-d	8.7733 a-c	8.7400 b-d	3.89 e-k	5.05 c	6.93 B	
4 th week	<i>Ocimum bacilicum</i>	1.071 u	3.836 k-n	3.928 j-m	414.6667 s	236.6667 d	414.6667 s	754 g	71.66667 b-d	214.6667 v	534.6667 h	8.1833 t-w	8.4833 j-l	8.5400 g-j	8.1833 t-w	8.4833 j-l	3.11 l-v	1.47 b-c	1.94 x-c	
	<i>Azolla pinnata</i>	4.346 g-i	4.16 t-k	4.36 g-i	385 u	205.6667 f	385 u	744.3333 h	53.333333 gh	212.3333 v	546.6667 g	8.2267 r-v	8.3700 m-	8.4400 k-m	8.2267 r-v	8.3700 m-	3.55 i-p	3.47 j-q	3.66 h-m	
	<i>Sabunia molesta</i>	4.02 l-l	4.021 i-l	2.280 T	394.3333 t	235.3333 d	394.3333 t	733.3333 i	45.66667 hi	243 S	516 ij	8.0667 x-a	8.1500 v-y	8.2167 s-w	8.0667 x-a	8.1500 v-y	1.53 b-c	2.44 v-a	3.63 h-n	
	<i>Typha latifolia</i>	5.534 c	4.023 i-l	1.081 U	335.6667 x	158.3333 h	335.6667 x	674.3333 l	39.66667 ij	112.3333 z	497.3333 l	8.1300 w-z	8.2900 p-s	7.8300 c	8.1300 w-z	7.8300 c	4.12 e-j	2.90 n-v	2.46 u-a	
4 th week	<i>Hibiscus rosa sinensis</i>	4.6 f-h	3.317 Pq	0.674 Vw	387.3333 tu	236.3333 d	387.3333 tu	723.3333 j	56.66667 e-g	209.3333 v	525.6667 hi	8.6700 d-f	8.1567 v-x	8.4967 h-k	8.6700 d-f	8.1567 v-x	3.67 g-m	2.86 o-w	1.36 C	
	<i>Ocimum bacilicum</i>	2.930 rs	2.498 t	0.56 W	350.6667 w	159.3333 h	350.6667 w	734.3333 i	64.333333 c-e	194.3333 w	506 Kl	8.1300 w-z	8.2900 p-s	7.8300 c	8.1300 w-z	7.8300 c	3.03 l-v	1.53 bc	1.92 x-c	
	<i>Azolla pinnata</i>	3.695 l-o	2.917 rs	2.556 St	281.3333 yz	171 g	281.3333 yz	729.3333 ij	35.66667 j	185 w	510.6667 jk	7.9700 b	7.9700 b	8.040 z-b	7.9700 b	8.040 z-b	3.33 k-s	2.99 m-v	2.57 t-y	
	<i>Sabunia molesta</i>	2.962 qr	3.462 n-p	3.508 n-p	358.3333 v	213 e	358.3333 v	695.3333 k	34.333333 j	136.6667 y	474.3333 m	8.0233 ab	8.1900 t-w	8.2300 r-v	8.0233 ab	8.1900 t-w	2.85 p-w	2.77 q-w	2.15 w-b	

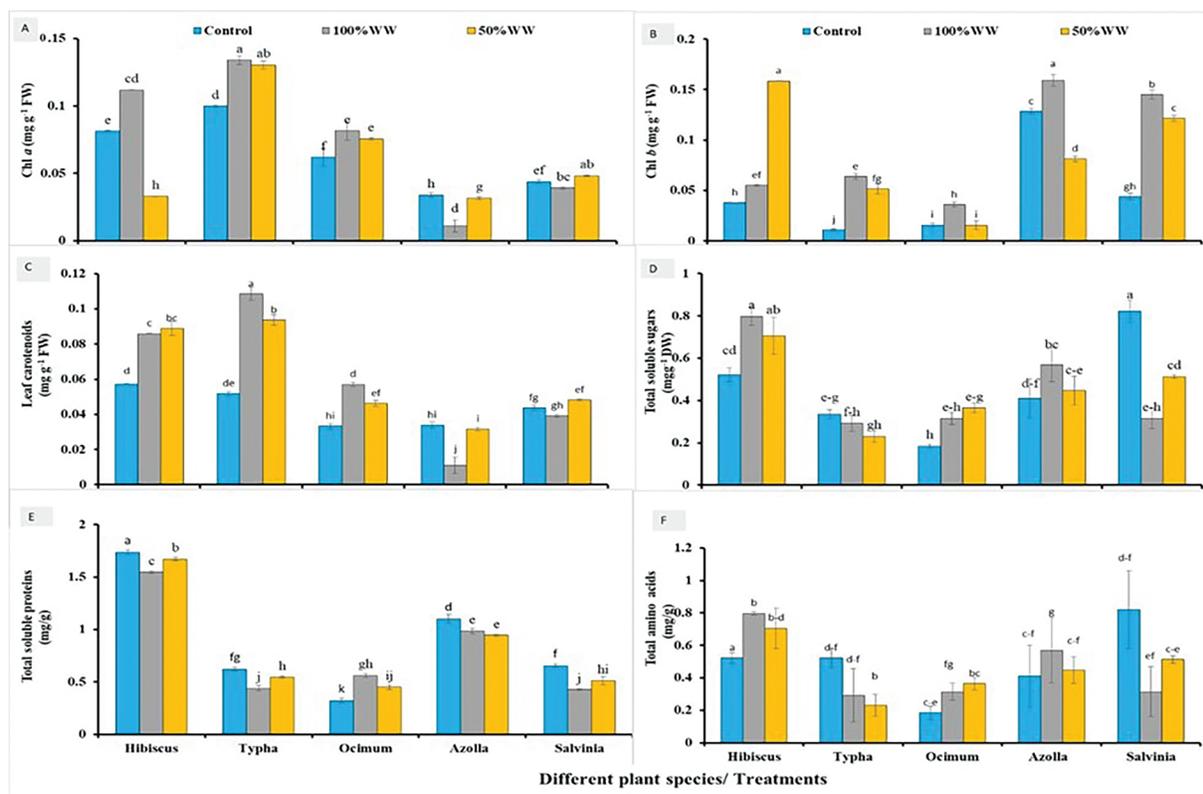


Figure 4: Effect of waste water on the physiochemical attributes of different aquatic and terrestrial plant species (A) Chl a, (B) Chl b, (C) carotenoids, (D) total soluble sugars, (E) total soluble proteins and (F) total amino acids. The alphabets indicate the significant ($p < 0.05$) difference among the treatments

3.2.5 Soluble Protein Content

The soluble protein content varied significantly ($p > 0.000$) in all plant species plants under all wastewater treatments. Results showed that the amount of total soluble proteins content decrease up to 10%, 29%, 73%, 10% and 34% in hibiscus, typha, ocimum, azolla, and salvinia, respectively at 100% wastewater. Total soluble sugar proteins in plants subjected to 50% wastewater (3.8%) decreased up to 3.8%, 12%, 39%, 14 and 21% in hibiscus, typha, ocimum, azolla, and salvinia, respectively (Fig. 5E).

3.2.6 Soluble Sugar Content

The soluble sugar content varied significantly ($p > 0.000$) in all plant species plants under all wastewater treatments. Results showed that the amount of total soluble sugars was increased up to 52%, 12%, 70%, 38% and 61% in hibiscus, typha, ocimum, azolla, and salvinia, respectively at 100% wastewater. Amount of total soluble sugars in plants subjected to 50% wastewater was decreased up to 35%, 31%, 97%, 9%, and 37% in hibiscus, typha, ocimum, azolla, and salvinia, respectively. As compared to normal water (Fig. 5F).

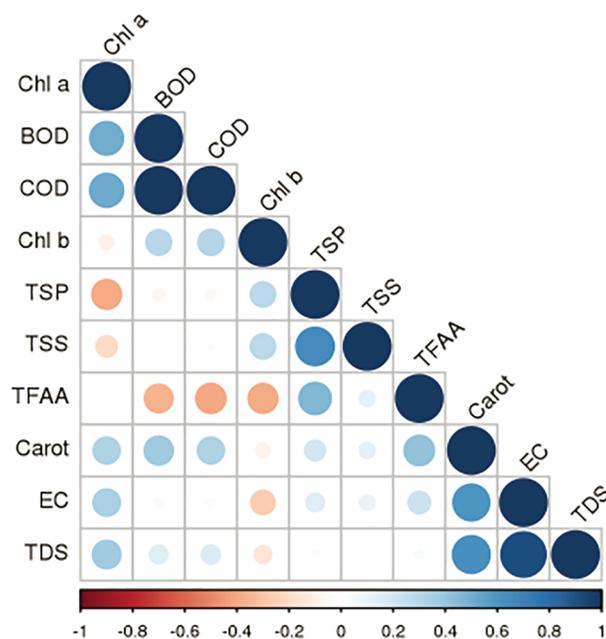


Figure 5: Pearson correlation of domestic waste parameters with plant physiochemical properties. The red color depicts negative correlation, while blue for positive correlation. TSP (total soluble proteins), Carot (Carotenoids), TSS (total soluble sugars), TFAA (total free amino acids)

3.3 Correlation and Heat Map

Pearson correlation showed a positive correlation of BOD and COD with chlorophyll a, while a negative linear correlation with total soluble sugars and total soluble proteins. Total soluble proteins and total soluble sugars showed a strong positive relationship with chlorophyll b. Moreover, total free amino acids showed a negative correlation with chlorophyll a, BOD and COD, and a positive correlation with total soluble sugars and total soluble proteins. Intriguingly, carotenoids, EC and TDS showed a positive linear relationship with all prescribed parameters, except for chlorophyll b (Fig. 5). Heat map showed that COD, BOD showed a positive relation with increasing domestic wastewater and showed maximum value for 100% domestic wastewater, while lower for control and medium for 50% domestic water irrespective for plant species and weeks variations. While pH, EC and TDS showed a medium response in almost all the treatments (Fig. 6). Heat map for plant analysis reveals that azolla and ocimum had lower chlorophyll a and chlorophyll b content, respectively irrespective of all the treatments. Similarly, lower carotenoid content was shown in azolla specie as compared to other plants. While typha, hibiscus and typha showed varying responses for photosynthetic pigments. Intriguingly, total soluble sugars, total free amino acids and total proteins were found maximum in hibiscus plant as compared to others under 100% and 50% domestic wastewater treatment, while typha and ocimum showed lower values of above-mentioned attributes under wastewater treatment. Moreover, silvinia and Azolla showed a medium response against stress (Fig. 7).

Treatment	1 st week					2 nd week					3 rd week					4 th week					
	pH	EC	TDS	BOD	COD	pH	EC	TDS	BOD	COD	pH	EC	TDS	BOD	COD	pH	EC	TDS	BOD	COD	
Hibiscus	Control	8.62	4.16	3.48	92.00	287.00	8.75	4.27	3.63	63.33	263.00	8.77	4.68	3.89	72.67	249.33	8.67	4.60	3.67	56.67	236.33
	100% WW	8.42	5.31	4.29	674.67	863.00	8.69	5.91	6.32	625.00	843.00	8.74	4.39	6.93	595.00	795.00	8.50	0.67	1.36	525.67	723.33
	50% WW	8.72	4.35	4.41	339.00	474.00	8.75	5.47	4.92	239.00	455.00	8.83	6.49	5.05	231.33	414.00	8.16	3.32	2.86	209.33	387.33
Typha	Control	8.41	5.29	4.62	85.33	276.67	8.27	5.04	4.49	71.67	265.67	8.17	5.32	4.36	54.33	205.67	8.13	5.53	4.12	39.67	158.33
	100% WW	8.41	6.63	8.87	651.67	847.67	8.33	4.63	2.72	628.00	776.00	8.08	1.07	2.63	509.33	696.67	7.83	1.08	2.46	497.33	674.33
	50% WW	8.49	4.84	4.26	327.33	464.33	8.58	0.94	3.23	271.00	443.67	8.42	5.32	3.17	157.00	386.33	8.29	4.02	2.90	112.33	335.67
Ocimum	Control	8.33	3.75	3.17	94.67	273.00	8.30	3.76	2.75	85.00	252.67	8.18	1.07	3.11	71.67	236.67	8.02	2.93	3.03	64.33	159.33
	100% WW	8.40	2.89	2.55	675.00	854.33	8.49	3.77	1.83	654.67	794.00	8.54	3.93	1.94	534.67	754.00	8.42	0.56	1.92	506.00	734.33
	50% WW	8.55	3.61	3.05	342.00	454.33	8.49	0.49	1.72	236.33	445.00	8.48	3.84	1.47	214.67	414.67	8.31	2.50	1.53	194.33	350.67
Azolla	Control	8.00	3.72	3.19	75.00	263.67	8.05	3.93	3.44	63.33	237.33	8.23	4.35	3.55	53.33	205.67	7.97	3.70	3.33	35.67	171.00
	100% WW	8.06	3.96	3.30	635.67	843.67	8.19	4.34	3.60	563.00	782.00	8.44	4.36	3.66	546.67	744.33	8.04	2.56	2.57	510.67	729.33
	50% WW	8.18	3.73	2.74	294.00	465.00	8.27	4.00	3.44	228.67	437.67	8.37	4.16	3.47	212.33	385.00	7.97	2.92	2.99	185.00	281.33
Salvinia	Control	8.49	4.03	3.88	73.33	275.33	8.25	0.54	1.86	60.00	265.67	8.07	4.02	1.33	45.67	235.33	8.02	2.96	2.85	34.33	213.00
	100% WW	8.83	2.23	1.82	625.00	830.33	8.59	3.40	3.09	527.00	776.67	8.22	2.28	3.63	516.00	733.33	8.23	3.51	2.15	474.33	695.33
	50% WW	8.73	3.90	3.75	276.67	445.33	8.54	2.26	3.71	264.33	426.33	8.15	4.02	2.44	243.00	394.33	8.19	3.46	2.77	136.67	358.33

Figure 6: Heat map of timely changes in domestic wastewater in relation to pH, EC, TDS, BOD and COD. The red color depicts lower values while, yellow and blue for medium and high values, respectively

	Treatment	Chlorophyll a			Chlorophyll b			Carotenoids			Total soluble sugars	Total soluble proteins	Total free amino acids			
		Control	100% WW	50% WW	Control	100% WW	50% WW	Control	100% WW	50% WW						
Chlorophyll a	Hibiscus	2.151	2.037	1.436	0.522	0.798	0.706	0.047	0.100	0.092	0.522	0.798	0.706	Total soluble sugars	Total soluble proteins	Total free amino acids
	Typha	1.150	2.111	2.583	0.335	0.292	0.230	0.032	0.107	0.095	0.522	0.292	0.230			
	Ocimum	3.027	2.285	3.247	0.185	0.314	0.365	0.033	0.058	0.046	0.185	0.314	0.365			
	Azolla	0.207	0.643	0.586	0.411	0.569	0.447	0.034	0.009	0.032	0.411	0.569	0.447			
	Salvinia	1.665	1.426	1.400	0.822	0.315	0.513	0.044	0.039	0.048	0.822	0.315	0.513			
Chlorophyll b	Hibiscus	0.038	0.055	0.159	1.737	1.547	1.670	0.047	0.100	0.092	0.522	0.798	0.706	Total soluble proteins	Total free amino acids	
	Typha	0.011	0.064	0.052	0.623	0.440	0.544	0.032	0.107	0.095	0.522	0.292	0.230			
	Ocimum	0.016	0.036	0.016	0.322	0.560	0.450	0.033	0.058	0.046	0.185	0.314	0.365			
	Azolla	0.129	0.159	0.081	1.103	0.985	0.945	0.034	0.009	0.032	0.411	0.569	0.447			
	Salvinia	0.044	0.145	0.122	0.656	0.428	0.511	0.044	0.039	0.048	0.822	0.315	0.513			
Carotenoids	Hibiscus	0.047	0.100	0.092	0.522	0.798	0.706	0.047	0.100	0.092	0.522	0.798	0.706	Total free amino acids	Total soluble proteins	Total free amino acids
	Typha	0.032	0.107	0.095	0.522	0.292	0.230	0.032	0.107	0.095	0.522	0.292	0.230			
	Ocimum	0.033	0.058	0.046	0.185	0.314	0.365	0.033	0.058	0.046	0.185	0.314	0.365			
	Azolla	0.034	0.009	0.032	0.411	0.569	0.447	0.034	0.009	0.032	0.411	0.569	0.447			
	Salvinia	0.044	0.039	0.048	0.822	0.315	0.513	0.044	0.039	0.048	0.822	0.315	0.513			

Figure 7: Heat map of timely changes in domestic wastewater in relation to chlorophyll a, chlorophyll b, carotenoids, total soluble sugars, total soluble proteins and total free amino acids. The red color depicts lower values while, yellow and blue for medium and high values, respectively

4 Discussion

Abiotic stresses, such as high and low temperatures, ultraviolet radiations, heavy metals, salinity and excessive and deficient water, are hostile to plant growth and production, leading to higher yield reduction across the world [7,37–44], however, these consequences were exacerbated with overwhelming population and current climate changes [43]. Anthropogenic activities increase the risk of environmental pollutants, and the major risk is freshwater resources. The use of plants to recover water and soil from

contaminants has been proven to be an effective approach and widely accepted as a cost effective and environmentally friendly technique in the last two decades for wastewater purification soil remediations [45,46]. Aquatic plants that float on the surface of water floating (azolla, and salvinia) and submerged macrophytes typha, hibiscus, and ocimum were found to be used for wastewater purification because these plants are very efficient for the elimination of heavy metals from sewage water. Aquatic plants are beneficial for improving water quality due to their higher efficiency in reducing COD, BOD, pH, TDS and EC (Sivakumar et al. [47], Nikam et al. [48], Pratik et al. [49]). In the present research, EC, TDS, BOD, COD and pH were observed with quite the same decreasing pattern in both 100% and 50% wastewater as compared to control. From, five different aquatic plants (typha, hibiscus, azolla, ocimum, and salvinia), typha showed a high reduction in BOD, COD, EC, TDS and pH (Figs. 2A–2C and 3A and 3B) (Table 2). The reason for the reduction of above-mentioned parameters in typha is that it finds near water and is a wetland specie and can grow in flooded water with water depth almost 2.6 feet. Typha has an extensive root system that acts as a source of accumulation of heavy metals and helps in wastewater purification. Our results corroborate with the findings of Ezeogu et al. [31] by using *Eichinochloa pyramidali* as the experimental plant. They found BOD and COD removal rate up to 87.68% and 95.1%, respectively. In another study, Sivakumar et al. [47] found maximum removal percentage of COD, BOD and TDS in a paper mill effluent and percent reduction was 79.1%, 88.6%, and 82.3%, respectively with the use of *Azolla caroliniana*. Moreover, according to Nikam et al. [48] *Typha lotifolia* removes 85%, 70.5% and 83.3% TDS, BOD and COD. In addition, they found pH reduction from 7.6 to 6.2 and 6.1 by using typha and *Phragmites australis* as model plants. Additionally, Pratik et al. [49] noted % reduction in NH₃-N, BOD, COD and the percent reduction was 89.42%, 89.21%, 76.90%, respectively. The reason for SOD, BOD and TDS reduction is the phytoextraction ability of these plants especially typha.

The biochemical attributes such as, amino acids, total soluble sugars, and total soluble proteins of all plants were also observed after 30 days of phytoremediation process in plants subjected to 100%, 50% wastewater and control as shown in Figs. 4A–4F. The increase in these contents was observed in 100%> 50%> wastewater, the reason is the presence of various nutrients essential for the synthesis of these biochemical attributes. It was also observed that total soluble sugars were higher in plants subjected to wastewater than control. These results are corroborated by the findings of Fan et al. [50], who noted higher photosynthetic efficiency under domestic wastewater that might be due to the higher accumulation of nutrients that assimilates into chlorophyll (as nitrogen and magnesium are the part of chlorophyll). However, Gupta et al. [51] found that total chlorophyll and total free amino acids decreased, while phenols, ascorbic acid, total protein, and soluble sugars increased in *Raphanus sativus* and *Colocasia esculentum* except for *Brassica nigra* L. for the protein under wastewater. The higher sugar concentration in this study elaborates the defense responses under wastewater treatment [52]. Plant adjusts energy metabolism to enhance acclimation demands by activating photosynthetic machinery. Moreover, tolerant plants constitutively enhanced detoxification and several stress related proteins against stress [53,54]. So, it evaluated due to stress conditions increased plant sugar and protein content. Similarly, correlation data also showed that total soluble sugars, total free amino acids and total proteins were found maximum in hibiscus plant as compared to others under 100% and 50% domestic wastewater treatment, while typha and ocimum showed lower values (Figs. 5–7). It is recommended that more concentration of soluble sugars and protein contents could provide an adaptive mechanism to maintain favorable osmotic potential under metal toxicity [55]. Moreover, more aquatic plants can be tested for their phytoremediation potential to remediate wastewater resources.

5 Conclusion

In this study, typha showed better performance concerning pollutant uptake from domestic effluents than hibiscus, azolla, ocimum, and salvinia. Interestingly, total free amino acids, total soluble sugars, and total

proteins were found higher in hibiscus plant as compared to others under 100% and 50% domestic wastewater treatment, while typha and ocimum showed lower values. A penalty scheme or incentive is required to build up to ensure the quality of domestic water treatment and to phytoremediate the wastewater resources at the community or central level. Further research on domestic wastewater treatment and long term uses for full-scale operation is urgently needed to avoid freshwater scarcity.

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