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Efficiency of Treated Domestic Wastewater to Irrigate Two Rice Cultivars PK 386 and Basmati 515 Under Hydroponic Culture System

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Abstract: Increase in human population continues to exert tension on scarceness of freshwater. Availability of freshwater for crop irrigation has become challenging. The present study was an attempt to use domestic wastewater (DWW) for irrigation of two rice cultivars after treatment with bacterial strain *Alcaligenes faecalis* MT477813 under hydroponic culture system. The first part of the study deals with bioremediation and analysis of physicochemical parameters of DWW to compare pollutants before and after treatment. Bio treatment of DWW with the bacterial isolate showed more than 90 % decolourisation along with reduction in contaminants. The next part of the study evaluates the impact of treated and untreated DWW on growth of two rice cultivars i.e., PK 386 and Basmati 515 under hydroponic culture system which provides nutrients and water to plant with equal and higher yield compare to soil. Growth parameters such as shoot and root length, fresh and dry weights of rice plants grown in treated DWW were considerably higher than the plants grown in untreated DWW. Therefore, enhanced growth of both rice cultivars grown in bio treated DWW was observed. The results supported the bioremediation efficiency of bacterial isolate and the utility of the DWW for rice crop irrigation subsequent to bio treatment.

Keywords: Domestic Wastewater; *Alcaligenes faecalis* MT477813; Irrigation; *Oryza sativa*; Hydroponic Culture System

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1. Introduction

Growing population, urbanization, industrialization, agriculture and lack of water management collectively exert stress on freshwater supply. Therefore, demand for water supply is getting increased because of rapid growth in all the aforementioned areas [1]. Pakistan as an agricultural and industrial country has been ranked third in the list of the most water scarce countries [2]. Moreover, the Pakistan Council of Research in Water Resources stated that the country would run short of clean drinkable water before 2030 [3]. These circumstances necessitate the pursuit of alternate water resources as an essential requirement to meet the rapidly increasing demand of fresh water [4].

Domestic wastewater has long been considered an important alternative source as irrigation water and is utilized for irrigation in agriculture [5-7]. The wastewater is directly utilized for agriculture in all over the country near towns/municipalities with a population more than 10,000 people [8]. Domestic wastewater typically includes organic matter and various important nutrients (N, K, P, Ca, Mn, S, Cu, Zn, Mn) that support agronomy by enhancing crop output as well as food quality [6]. Along with nutrients domestic wastewater caries hazardous toxins, which may accumulate in agricultural plants. These nutrients and heavy metals like cadmium, copper, iron, lead, nickel or zinc induce phytotoxicity and pose a menace to human beings and other animals [9]. Local communities reuse raw wastewater to irrigate crops, which can be very detrimental to animal life including humans [10-13]. Bioremediation of domestic wastewater by microbes has been considered an excellent biological activity due to metabolic activities of microorganisms such as bacteria [14-16] and their biological activity and metabolic flexibility might be tremendously advantageous in the conversion and degradation of harmful contaminants into non-toxic molecules [17]. A number of indigenous bacteria have the potential to discolour, detoxify and degrade pollutants of different types of wastewaters [16].

Rice is Pakistan's second most important cash crop that requires abundant irrigation water throughout its growing period [18-21]. Due to the issue of water scarcity, farmers are compelled to use wastewaters for crop irrigation. Out of various types of wastewaters, domestic wastewater may be utilized to irrigate these paddy fields. As the domestic wastewater contains nutrients, organic matter, high biological oxygen demand (BOD) value and a lot of contaminants [22-25], the application of this wastewater for rice irrigation without adequate bio treatment is proven damaging to rice plants [13]. After appropriate bio treatment, wastewater can be utilized for mass production of nutritious rice crop [23, 25-27].

Rice crop requires excessive amount of water which is a challenge that appears daunting for farmers in water-scarce nations. [28]. Therefore, bio treated domestic wastewater may be used in a hydroponic system as these systems assist in early maturation of seedlings, provide higher crop yield, uses 95 % lesser amount of water than usual irrigation, and cope up with late rainfall due to changing climate [29]. This kind of hydroponic systems also avoid risks associated with soil diseases and salinity and waterlogging issues [30]. Some previous studies assert that bio treated domestic wastewaters lack certain nutrients up to the desired levels of crops to grow, therefore, hydroponic system needs additional ingredients to acquire quality oriented rice production [31-39]. Researchers have also designated Hoagland solution as a promising cultures for hydroponic systems to grow different crops like wheat [31-33], tomatoes [34-35], lettuce [36-37], Arabidopsis [38] and onion [39].

Considering the aforementioned factors,, the objectives of this work were to decolourize DWW and degrade harmful contaminants in DWW using bacterial isolate *Alcaligenes faecalis* MT477813. Also, the work compared the effect of untreated and bio treated domestic wastewater on the growth of rice cultivars (PK 386 and Basmati 515) in a hydroponic culture system to obtain sustainable agricultural output.

2. Materials and Methods

2. 1 Collection and Characterisation of domestic wastewater

According to the APHA (2002) standard procedure [40], domestic wastewater was collected from the main point of discharge from Mohni Road, adjacent to Band Road, Lahore. The wastewater sample was screened for visible contaminants in the research lab, Centre of Environmental Protection Studies, Pakistan Council of Scientific and Industrial Research, Lahore. Physico-chemical parameters of domestic wastewater (in four different treatments) were analysed pre and post bio treatment such as BOD, biodegradability index (BI), chemical oxygen demand (COD), colour, dissolved oxygen (DO), electrical conductivity (EC), heavy metals quantification (Zinc-Zn, Manganese-Mn and Iron-Fe) and NPK estimation, odour, pH, salinity, temperature, total suspended solids (TSS), total dissolved solids (TDS) and turbidity according to standard methods of APHA [40] using pH meter, EC meter, DO meter, BOD and COD digester, turbidity meter and atomic absorption spectrophotometer. These parameters were then compared with the National Environmental Quality Standard values [41] to assess efficiency of treatment.

2.2 Source of Alcaligenes faecalis MT477813 culture

The bacterial strain namely *A. faecalis* MT477813 was acquired from the Plant Biotechnology Lab, Botany Department, GC University Lahore. The strain was used to decolourise DWW because it had a bioremediation efficiency of more than 90 % [15]. Initially, the strain was grown and streaked on plates containing solidified nutrient agar medium (Figure 1a). These plates were incubated at 37 °C for 24 h and colonies were prepared (Figure 1b). The LB (Lysogeny broth) agar slants were used to store the pure bacterial culture (Figure 1c, 1d) while keeping it at a temperature of 4 °C in a refrigerator [42].

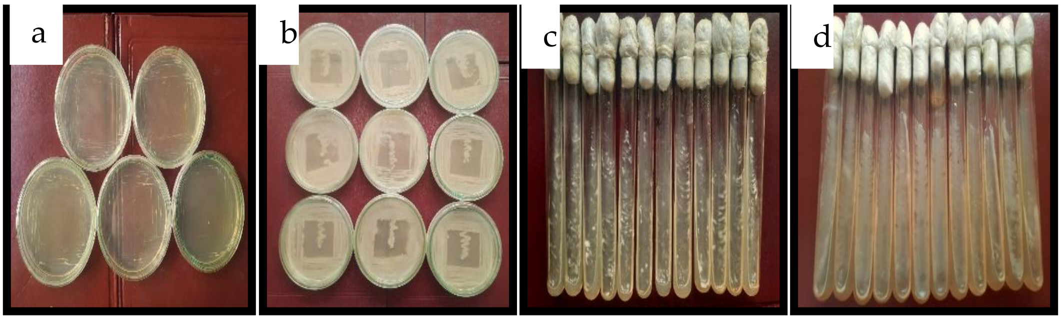


Figure 1. Culture of bacterial strain; (a) Streaking of Bacterial strain (*A. faecalis* MT477813) on NB plates, (b) Bacterial growth on plates, (c) Bacterial streaking on agar slants, (d) Bacterial growth on agar slants.

2.3 Domestic wastewater treatment

The decolourisation potential of *A. faecalis* MT477813 was tested at following optimal conditions: duration (48 h), inoculum (10 %) and temperature (37 °C) [14, 15]. Each conical flask (250 mL) was inoculated with 10 % bacterial strain solution (10 mL) in autoclaved domestic wastewater (90 mL). For bacterial strain solution preparation, a loop full of bacterial colony was taken from slant and added to distilled water (100 mL). The optical density (OD) of 1 at 545 nm was achieved (A and E Labmed, AE-S80) to ensure an equal concentration of bacterial cells in each inoculum. The flasks having wastewater after inoculation were placed in a shaking incubator (PMI Labortechnik GMBH, WIS-20R) for 48 h at 37 °C and 120 rpm [43]. The decolourisation percentage was computed by Equation (1) given below:

$$Decolourisation(\%age) = \frac{\text{Absorbance before inoculation} - \text{Absorbance after treatment}}{\text{Absorbance before inoculation}} \times 100 \quad (1)$$

All decolourisation experiments were carried out in triplicates.

2.4 Germination of rice seedlings for hydroponic cultures

Two rice cultivars under the names PK 386 and Basmati 515 were obtained from the Rice Research Centre Kala Shah Kaku. The characteristic profile of these two cultivars is provided in Table 1. Chemicals used in this experiment were procured from "Thermo Fisher Scientific USA", through "Worldwide Scientific" Syed Plaza, 30 Lahore – Kasur Rd, Jinnah Town, Lahore, Punjab Pakistan.

Table 1. Characteristic profile of rice cultivars [data taken from 44].

Characteristics	**PK386**	**Basmati 515**

Type	Fine	Fine Basmati
Class	Long grain	Extra long grain
Aroma	Yes	Yes
Chalkiness	Absent	Absent
Height of plant (cm)	117	130
Grain length (mm)	6.85	7.56
Grain width (mm)	1.78	1.64
Grain thickness (mm)	1.56	1.52
Nitrogen (N) (mg/L)	22	24
Phosphorus (P) (mg/L)	115	119
Potassium (K) (mg/L)	115	123
Iodine (I) (mg/L)	Nd	Nd
Zinc (Zn) (mg/L)	1.1	1.3
Manganese (Mn) (mg/L)	2	2.4
Iron (Fe) (mg/L)	0.8	1.1

Note: Nd= Not detected

Viable seeds of both the rice CVs were selected for germination and surface sterilization of seeds was carried out using chemical treatment. Seeds were washed with tap water and treated with 70 % ethanol solution for 40 s and with 30 % sodium hypochlorite solution (added with a few drops of Tween^{20}) for 3 min stepwise. The seeds were thoroughly washed with sterile distilled water after each chemical treatment. Under aseptic conditions, the sterile seeds were placed at equal spacing in Petri plates on filter paper (Whatman filter paper 42 having pore size of 2.5 µM) soaked with 5 mL autoclaved distilled water. Five sets of replicates were made for each cultivar. The plates were placed in growth room for seed germination under continuous fluorescent light at 26 °C ± 1 for 15 days (Figure 2).

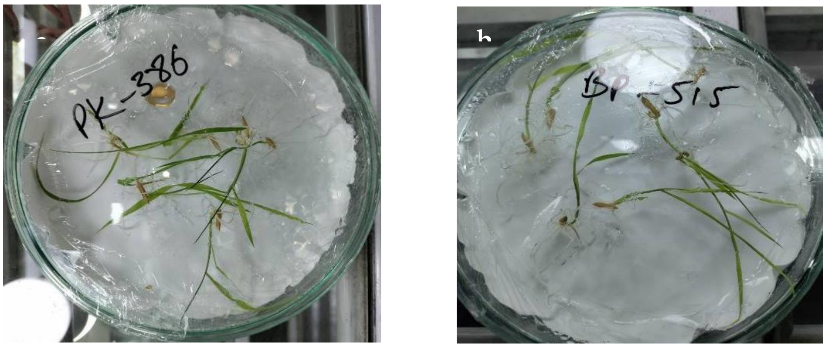


Figure 2. Germination of rice seeds on filter paper; seedlings of 15 days: (a) PK 386, (b) Basmati 515

Hoagland solution [45] was prepared as nutrient medium for hydroponic growth of rice seedlings of both the cutivars. The hydroponic culture system was designed using 1 L glass beakers. Each beaker possessed a piece of spherical recycled foam sheet one-inch-thick as floater. Five holes of equal size were made to accommodate seedlings (each hole containing one seedling). Five sets of triplicates (1 L beaker each) were prepared under aseptic conditions; each set of replicates contained 500 mL of water types presented in Table 2.

Table 2. Types of treatments and their composition.

Treatment	Composition of treatment	Water + Hoagland

Type		**Concentration**
Control	Distilled water with Hoagland Solution (DWH)	400 mL distilled wastewater + 100 mL Hoagland solution
Treatment 1	Untreated Domestic wastewater with Hoagland Solution (UTDWWH)	400 mL untreated domestic wastewater + 100 mL Hoagland solution
Treatment 2	Treated domestic wastewater with Hoagland Solution (TDWWH)	400 mL treated domestic wastewater + 100 mL Hoagland solution
Treatment 3	Untreated domestic wastewater (UTDWW)	500 mL untreated domestic wastewater
Treatment 4	Treated domestic wastewater (TDWW)	500 mL treated domestic wastewater

2.5 Transfer of seedlings to hydroponic cultures

Fifteen days old rice seedlings germinated in Petri plates were transplanted to 500 mL liquid nutrient medium in 1 L beakers under aseptic conditions. Rice seedlings were planted in the holes one seedling in each hole; roots passing through the hole were submerged in hydroponic culture medium and shoot exposed above the floater sheet (Figure 3). Seedlings of both rice cultivars were transferred in the same manner in all the five sets of replicates as mentioned.

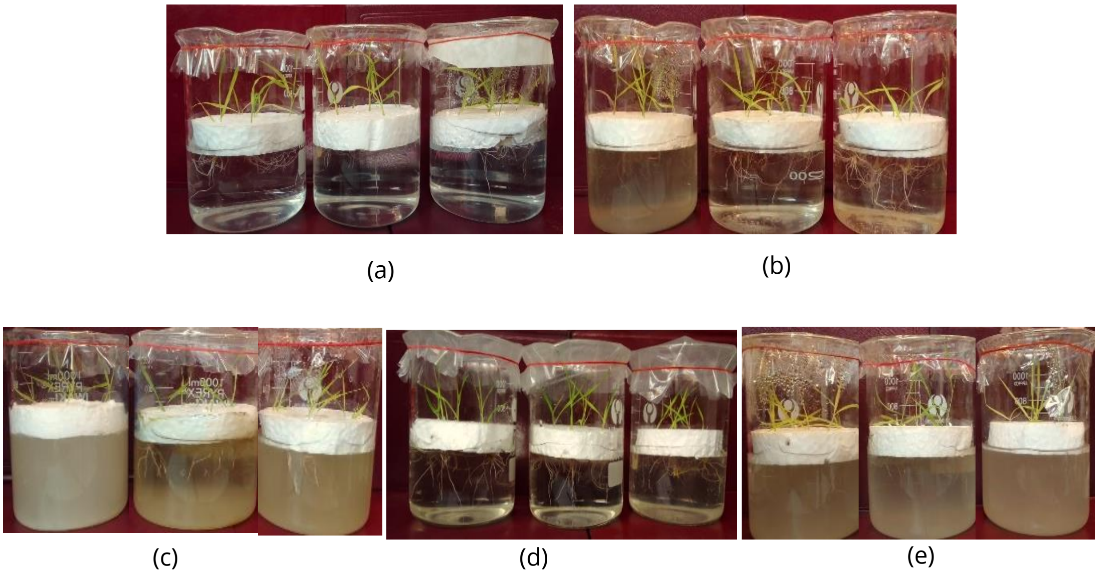


Figure 3: Rice seedlings in hydroponic culture; (a) Control (b) Treatment 1 (c) Treatment 2 (d) Treatment 3 (e) Treatment 4.

2.6 Growth of seedlings and analysis in hydroponic cultures

Beakers were wrapped in thick black polyethylene sheets with the exposed surface wrapped with a transparent PVC plastic sheet containing small holes to ensure the smooth acclimatization of seedlings. Beakers were placed in growth room under 16/8 light/dark 24 h cycle at 26 °C ± 1. The holes were increased gradually and the polythene sheet was completely removed from the beakers after 1 week. The growth experiment continued for 21 days. The plants were harvested after a growth period of 21 days and morphological growth parameters of the plants such as seedling length, shoot length, root length, fresh and dry weight of seedling, shoot and root were recorded for each treatment.

2.7 Statistical analyses

The data of results were analysed using SPSS as mean of triplicates ± SD (standard deviation) and comparisons of variance. Data values of different replicates as well as treatments were compared by One-Way ANOVA and t-test was run with two-tailed p-value to assess the magnitude of differences of corresponding means of four different treatments. These differences were only considered significant when p-values were < 0.05.

3. Results and Discussions

3.1 Physiochemical characterisation of treatments

Physiochemical characterisation of the treatments revealed that values of all parameters before treatment (UTDWW) such as EC (973 µs/cm), BOD (295.7 mg/L), COD (412.8 mg/L), pH (8.5), turbidity (38.5 NTU), TSS (383 mg/L), TDS (730 mg/L) and salinity (0.43 ppt) decreased significantly after treatment (Table 3). Results showed that the values of various physicochemical parameters were within the levels of National Environment Quality Standards after treatment with Hoagland solution. For instance, the value of TSS was 930 mg/L in untreated domestic wastewater while the 29.41. National Environment Quality Standards (NEQS) value for TSS was 500 mg/L. After bio treatment, the value was reduced to 300 mg/L and with an additional Hoagland solution, the value was reduced to 298 mg/L. Similarly, turbidity was reduced from 38.5 NTU to 18.3 NTU in bio treated domestic wastewater and then to 4.2 NTU in bio treated domestic wastewater with addition of Hoagland solution. The values of BOD and COD were also reduced from 295.7 mg/L and 412.8 mg/L to 171.5 mg/L and 140 mg/L while their NEQS ranges were 80-250 mg/L and 150-400 mg/L, respectively. The other reduced physicochemical parameters including EC, TDS, salinity, pH, N, P, K, Zn, Mn and Fe also agree well with a previous research treating DWW with *Alcaligenes faecalis* MT477813 [41] and with our previous research [14-15].

In current study, *A. faecalis* strain MT477813 led to the decolourisation percentage above 90 %. *A. faecalis* has been also reported as a biocontrol agent [46-47] that may be an answer to the question why BOD values were reduced in all the treatments. Previously, the physiochemical analysis of bio treated and untreated domestic wastewater samples (without Hoagland solution) revealed a reduction (40-70 %) in the peak intensities of many unwanted compounds which have been shown to be harmful like phenol (876 µg/L), caffeine (7 µg/L), salicylic acid (48 µg/L), naproxen (23 µg/L), diazepam (14 µg/L) and octadecene (185 µg/L) [14, 15, 48-52]. However, the present treatments with Hoagland solution showed a reduction of more than 80 % in these parameters. In light of previous work [14 - 15], it may be said that this increased %age reduction was due to Hoagland solution because in our previous works (without Hoagland solution) the reduction in values of physicochemical parameters were lower (40 - 70 %). The comparison of all four treatments show following order in terms of treatment efficiency: Treatment 2 > Treatment 4 > Treatment 1 > Treatment 3. Here, treatment 2 contains treated DWW with Hoagland solution that highlights higher treatment efficacy of *A. faecalis* with Hoagland solution. Other scientists have also designated Hoagland solution as a promising solution for hydroponic culture system to grow different crops like wheat [31-33], tomatoes [34-35], lettuce [36-37], Arabidopsis [38] and onion [39].

Table 3. The physicochemical characterisation of different treatments.

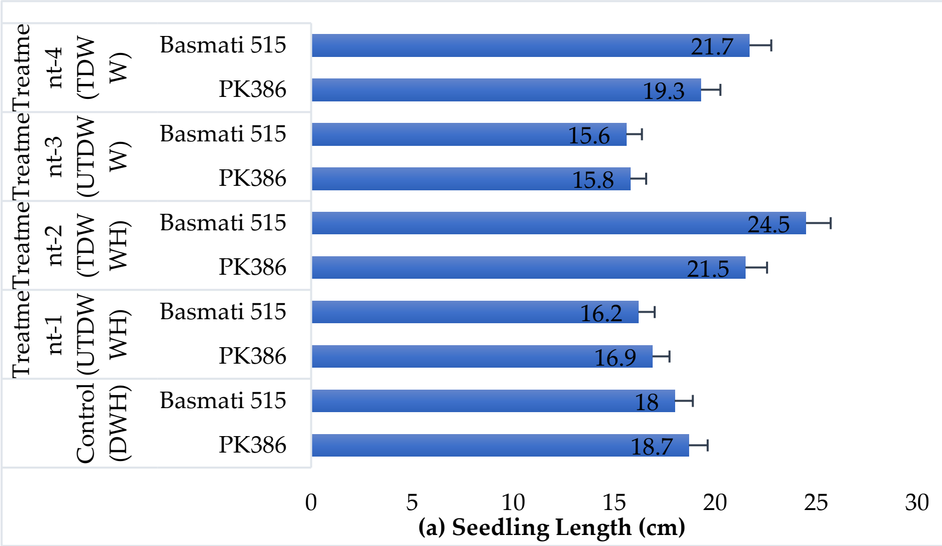
Characters	Units	NEQS [41]	Control (DWH)	Treatment-1 (UTDWWH)	Treatment-2 (TDWWH)	Treatment-3 (UTDWW)	Treament-4 (TDWW)
pH		6.6-8.5	7.6	7.8 ****	7.2 ****	8.5 ****	8.2 ****
EC	µs/cm	-	170	413.2 ***	215.4 ***	973 ***	345.9 ****
TDS	mg/L	1000	298.3	500 ****	221.3 **	730 ****	330 ****
TSS	mg/L	<500	200	930 ****	298 ****	383 ***	300 ****
Salinity	ppt	-	0.02	0.3 ****	0.12 ****	0.42 ****	0.2 ****
Turbidity	NTU	5	2.5	13.5 ****	4.2 ***	38.5 ****	18.3 ****
COD	mg/L	150-400	200	273.2 ****	140.5 ****	412.8 ****	266 ****
BOD	mg/L	80-250	140	266.9 ****	171.5 ***	295.7 ****	190 ****
N	mg/L	-	210	140.7 ****	86 ****	40.7 ****	16 ****
P	mg/L	-	31	47.5 ****	27.3 ****	17.5 ****	7.3 ****
K	mg/L	-	235	260 ****	83.6 ****	60 ****	22.6 ****
Zn	mg/L	5	0.023	2.099 ****	1.83 ****	2 ****	1.3 ****
Mn	mg/L	1.5	0.11	0.064 ****	0.094 ****	0.044 ****	0.004 ****
Fe	mg/L	2	1	3.3 ****	1.36 ****	2.3 ****	0.36 ****

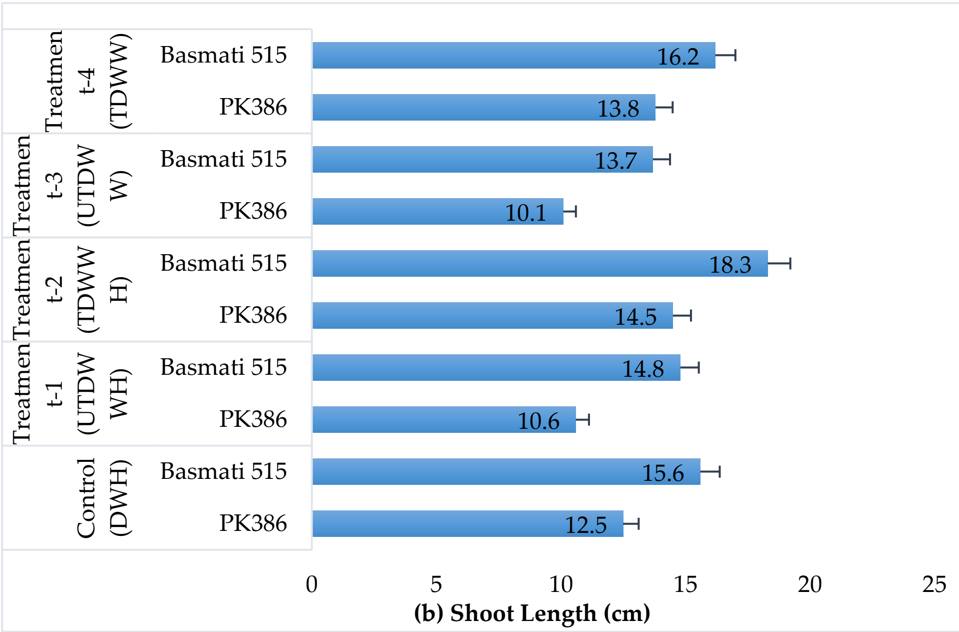
Note: NEQS= National Environment Quality Standards; Significance is indicated by $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, $p < 0.0001$****

3.2 Growth parameters of rice cultivars in different hydroponic treatments

Growth parameters were assessed under different treatments for both rice cultivars. Higher values were seen mainly in bio treated domestic wastewater with Hoagland solution (Treatment 2) and the treatment efficiencies of both rice cultivars were also compared (Figure 4 a, b and c). Shoot length of both the cultivars was observed the highest (14.5 and 18.3 cm) in Treatment 2 (TDWWH) as compared to other treatments of cultivar PK386 (13.8, 10.6 and 10.1 cm), BP515 (16.2, 14.8 and 13.7 cm) as well as control (12.5 and 15.6 cm). Moreover, it was also concluded from these readings that the treatment UTDWW has a negative impact, even with the Hoagland. This may be due to presence of toxic compounds in domestic wastewater that overshadowed the benefits of Hoagland solution [53]. Previous studies have also shown reduction in seedling length (mainly root length) as compared to control when grown in raw wastewater though mixed with Hoagland hydroponic system [31, 33, 36-39].

Comparison between Basmati 515 and PK386 under different treatments for morphological parameters, seedling length, shoot length, and root length showed that Basmati 515 performed better than PK386 in treatment 2 and 4 while PK 386 gave better performance in treatment 3 and 1 as well as in control (Figure 4). As treatment 1 and 3 are having untreated domestic wastewater, this means that PK 386 is more resilient with the wastewater, while Basmati 515 is better under treated domestic wastewater which means it is far more sensitive. This result is supported by the characteristic profile provided by Rice Research Centre Kala Shah Kaku where Basmati 515 contains more nitrogen (N), phosphorus (P), potassium (K), iodine (I), zinc (Zn) and manganese (Mn) content compared to PK 386 (see Table 1) [44]. Hence, the resilience of Basmati 515 is also attributed to presence of macro and micro nutrients like N, P, K, I, Zn and Mn. Moreover, the potential yield estimated for the Basmati rice is 75 (Maund/Acre) which is 8 % more than that of PK 386 [44] which also asserts the hardy role of Basmati 515.





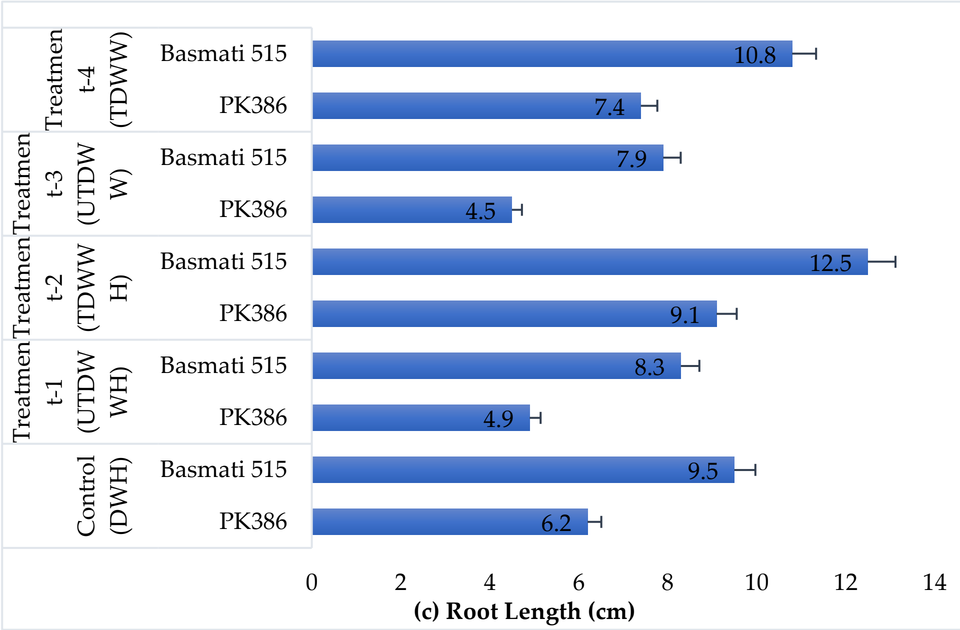


Figure 4. Comparison between Basmati 515 and PK386 under different treatments for morphological parameters: (a) Seedling length, (b) Shoot length, and (c) Root length.

In hydroponic cultures, the average shoot and root weights of PK 386 (both dry and fresh) were found to be higher in treatment 1 (UTDWWH) [Shoot (dry weight: 0.018 g; fresh weight: 0.282 g)]; [Root (dry weight: 0.009 g; fresh weight: 0.141 g)] than the untreated DWW [shoot (dry weight: 0.0114 g; fresh weight: 0.343 g)]; [Root (dry weight: 0.0057 g; fresh weight: 0.217 g)], respectively (Table 4). This indicates three possibilities: one is that the PK386 has more potential to grow well under stressed conditions [54]; second is that the weight was more due to toxic contaminants present in the untreated DWW [55]; and third is that it is due to nutrients present inside Hoagland solution that the weight has increased [56]. As far as the potential of the Hoagland solution is concerned, overall seedling fresh weight was more in treated DWW with Hoagland solution than in untreated DWW with Hoagland solution. This result is supported by a similar study carried out in the past where total fresh and dry weights were more in those hydroponic cultures with Hoagland solution [56, 57].

Table 4. Morphological parameters of PK 386 in different hydroponic cultures.

Morphological Parameters	Categories	Types of Hydroponic Cultures				
		Control (DWH)	Treatment-1 (UTDWWH)	Treatment-2 (TDWWH)	Treatment-3 (UTDWW)	Treament-4 (TDWW)
Fresh weight (g)	Shoot	0.248 (±0.034, 1.71E-3)	0.282 (±0.016, 3.86E-4) *	0.278 (±0.026, 1.15E-3) *	0.343 (±0.032, 1.56E-3) *	0.282 (±0.026, 8.86E-4) *
	Root	0.124 (±0.017, 8.56E-4)	0.141 (±0.08, 1.93E-4) *	0.139 (±0.013, 5.76E-4) **	0.217 (±0.0161, 7.83E-4) **	0.141 (±0.013, 4.43E-4) *
	Seedling	0.372 (±0.051, 2.57E-3)	0.412 (±0.024, 5.8E-4) *	0.416 (±0.039, 1.73E-3) *	0.38 (±0.0485, 2.35E-3) *	0.424 (±0.039, 1.33E-3) *

Dry weight (g)	**Shoot**	0.0128 (±0.002, 1.05E-5)	0.013 (±0.0014, 3.33E-6) *	0.0014 (±0.002, 1.35E-5) *	0.0114 (±0.0022, 6.6E-6) *	0.018 (±0.003, 1.82E-5) *
	Root	0.0064 (±0.001, 5.26E-6)	0.0063 (±0.0007, 1.66E-6) **	0.007 (±0.001, 6.76E-6) *	0.0057 (±0.0011, 3.3E-6) *	0.009 (±0.0015, 9.1E-6) *
	Seedling	0.0194 (±0.0039, 1.58E-5)	0.019 (±0.002, 5.0E-6) *	0.022 (±0.004, 2.03E-5) *	0.017 (±0.0032, 1.0E-5) *	0.028 (±0.0045, 2.73E-5) *
F-value		*0.0244*	*0.0437*	*0.0028*	*0.091*	*0.058*

Note: ± values in the table above represent standard deviation and variance. p-value indicates significance of the value of treatments in following manner: p < 0.01 = *, p<0.001 = **, p<0.0001 = ***.

In hydroponic cultures, the average shoot and root weights of Basmati 515 (both dry and fresh) were found to be higher in bio treated DWW with Hoagland solution [Shoot (dry weight: 0.012 g; fresh weight: 0.138 g)]; [Root (dry weight: 0.0061 g; fresh weight: 0.069 g)] than in the untreated DWW with hoagland (Table 5). This shows the potential of Basmati 515 cultivar for domestic treated domestic wastewater while asserted resilence of PK386 under toxic untreated domestic wastewater environment. Based upon previous research, two suppositions may be made from these observations: (1) *A. faecalis* led treated domestic wastewater with Hoagland solution is suitable for Basmati 515 rice cultivar; and (2) PK386 has more potential to grow well under stressed conditions in untreated DWW. Moreover, it is also evident that Hoagland solution is nutrient-rich and adequate for hydroponic culture systems [56, 57].

Table 5. Morphological parameters of Basmati 515 in different hydroponic cultures.

Morphological Parameters	**Categories**	**Types of Hydroponic Cultures**				
		Control (DWH)	**Treatment-1 (UTDWWH)**	**Treatment-2 (TDWWH)**	**Treatment-3 (UTDWW)**	**Treament-4 (TDWW)**
Fresh weight (g)	**Shoot**	0.138 (±0.072, 6.86E-4)	0.172 (±0.021, 7.13E-4) ***	0.204 (±0.014, 1.53E-4) ***	0.198 (±0.0264, 2.86E-4) ***	0.242 (±0.022, 1.11E-3) ***
	Root	0.069 (±0.036, 3.43E-4)	0.086 (±0.010, 3.56E-4) ***	0.102 (±0.007, 7.66E-5) ***	0.099 (±0.013, 1.43E-4) ***	0.121 (±0.011, 5.56E-4) ***
	Seedling	0.208 (±0.107, 1.03E-3)	0.258 (±0.031, 1.07E-3) ***	0.306 (±0.021, 2.3E-4) ***	0.296 (±0.0397, 4.3E-4) ***	0.364 (±0.034, 1.67E-3) ***
Dry weight (g)	**Shoot**	0.012 (±0.0028, 1.21E-5)	0.012 (±0.0016, 5.95E-3) ***	0.015 (±0.002, 1.18E-5) ***	0.094 (±0.021, 1.58E-5) ***	0.016 (±0.002, 4.1E-3) ***
	Root	0.006 (±0.0014, 6.06E-6)	0.0061 (±0.0008, 2.97E-3) ***	0.007 (±0.001, 5.9E-6) ***	0.047 (±0.0103, 7.93E-6) ***	0.008 (±0.0.001, 2.5E-3) ***
	Seedling	0.019 (±0.0043, 1.82E-5)	0.0184 (±0.0023, 8.93E-3) ***	0.022 (±0.004, 1.77E-5) ***	0.0142 (±0.031, 2.38E-5) ***	0.024 (±0.0029, 6.15E-3) ***
F-value		*0.0042*	*0.0392*	*0.0494*	*0.0589*	*0.0658*

Note: ± values in the table above represent standard deviation and variance. p-value indicates significance of the value of treatments in following manner: p < 0.01 = *, p<0.001 = **, p<0.0001 = ***.

Previous researchers have found that saplings irrigated with untreated textile wastewater had shorter length and weight [58-60]. As far as Hoagland solution is concerned, studies also endorsed its efficacy in synthesizing bacterial strains that may be helpful in treatment of different wastewaters and assist in growth of crop plants [53]. In our work, Hoagland solution fulfilled the same purpose. The results of the hydroponic experiment were surprisingly significant. The impact of UTDWW and TDWW treatments (Treatments 1, 2, 3 and 4) on growth of the two rice cultivars under study indicated that the growth parameters under TDWW with Hoagland were considerably higher compared to simple TDWW. Furthermore, UTDWW with or without Hoagland got lower values of these growth parameters (Figure 4). A similar pattern of seedling biomass (fresh and dry weight) was largely seen where TDWWH stood atop in terms of biomass (Tables 4 & 5). The possible reasons for this increased biomass are two: (1) presence of organic matter and nutrients (N, K, P, Ca, Mn, S, Cu, Zn, Mn) in domestic wastewater [6] or (2) due to the presence of Hoagland solution [31-39].

4. Conclusions

Domestic wastewater irrigation tends to have a long past, passing through many stages in both developed and under developing countries including Pakistan. Furthermore, farmers are compelled to use DWW for irrigation to combat scarcity of water. Irrigation by untreated domestic wastewater has been reported to produce a number of environmental problems. The hazardous materials in wastewater promote chemical and biological changes in the environment posing health risks to animal life. The current study was made to contribute to the approaches used to deal with ecologically hazardous pollutants in wastewater in order to make wastewater useful for irrigation. Bio treatment of DWW with *A. faecalis* MT477813 decreased the values of physicochemical parameters such as EC, BOD, COD, pH, temperature, turbidity, TSS, TDS, salinity, odour and colour. Bio treatment of DWW with the bacterial isolate showed more than 90 % decolourisation along with reduction in contaminants. Upon germination under hydroponic culture system, PK 386 and Basmati 515, growth parameters such as shoot and root length, fresh and dry weights of rice plants grown in treatment 2 (TDWWH) were considerably higher than the plants grown in untreated DWW. The bio treatment of DWW in presence of Hoagland solution showed an increase in growth parameters such as shoot length of both the cultivars. Overall, an enhanced growth of both rice cultivars grown in bio treated DWW with Hoagland solution was observed. The present work supports the school of thought that promote the use of local bacterial populations to reduce the threat by pollutants found in WW to living organisms and we recommend further work on the quality of fruit yield from these hydroponic systems.

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References

1. Khoso, S., Wagan, F. H., Tunio, A. H. and Ansari, A. A., 2015. An overview on emerging water scarcity in Pakistan, its causes, impacts and remedial measures. *Journal of Applied Engineering Science*, 13(1): 38-40.
2. Nabi, G., Ali, M., Khan, S. and Kumar, S., 2019. The crisis of water shortage and pollution in Pakistan: Risk to public health, biodiversity, and ecosystem. *Environmental science and pollution research*, 26(11): 10443-10445.
3. Shukla, V., 2018. Pakistan's Alarming Water Crisis: Country to Run out of Clean Water by 2025.
4. Chung, S. O., Rodríguez-Díaz, J. A., Weatherhead, E. K. and Knox, J. W., 2011. Climate change impacts on water for irrigating paddy rice in South Korea. *Irrigation and Drainage*, 60(2): 263-273.
5. Zhang, Y. and Shen, Y., 2019. Wastewater irrigation: past, present, and future. *Wiley Interdisciplinary Reviews: Water*, 6(3): 1230-1234.
6. Gassama, U. M., Puteh, A. B., Abd-Halim, M. R. and Kargbo, B., 2015. Influence of municipal wastewater on rice seed germination, seedling performance, nutrient uptake, and chlorophyll content. *Journal of Crop Science and Biotechnology*, 18(1): 9-19.
7. Singh, P. K., Deshbhratar, P. B. and Ramteke, D. S., 2012. Effects of sewage wastewater irrigation on soil properties, crop yield and environment. *Agricultural Water Management*, 103(7): 100-104.
8. Khalil, S. and Kakar, M. K., 2011. Agricultural use of untreated urban wastewater in Pakistan. *Asian Journal of Agriculture and Rural Development*, 1(393-2016-23907): 21-26.
9. Khawla, K., Besma, K., Enrique, M. and Mohamed, H., 2019. Accumulation of trace elements by corn (Zea mays) under irrigation with treated domestic wastewater using different irrigation methods. *Ecotoxicology and environmental safety*, 170(2019): 530-537.
10. Khan, A. and Bruce, K., 2021. How residues of deinstitutionalised practices persist over time: World Bank boundary work in development projects in Pakistan from the 1970s to the mid-2000s. *Business History*, 4(1): 1-29.
11. Contreras, J. D., Meza, R., Siebe, C., Rodríguez-Dozal, S., López-Vidal Y. A., Castillo-Rojas, G., Amieva, R. I., Solano-Gálvez, S. G., Mazari-Hiriart, M., Silva-Magaña, M. A., Vázquez-Salvador, N., Pérez, I. R., Romero, L. M., Cortez, E. V., Riojas-Rodríguez, H. and Eisenberg, J. N. S., 2017. Health risks from exposure to untreated domestic wastewater used for irrigation in the Mezquital Valley, Mexico: A 25-year update. *Water Research,* 123(2017): 834-850.
12. Keraita, B., Jimenez, B. and Drechsel, P., 2008. Extent and implications of agricultural reuse of untreated, partly treated and diluted wastewater in developing countries. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 3(58): 1-15.
13. Hamilton, A. J., Stagnitti, F., Xiong, X., Kreidl, S. L., Benke, K. K. and Maher, P., 2007. Wastewater irrigation: the state of play. *Vadose Zone Journal*, 6(4): 823-840.
14. Rashid, A., Mirza, S. A., Keating, C., Ali, S. and Campos, L. C., 2021). Hospital wastewater treated with a novel bacterial consortium (*Alcaligenes faecalis* and *Bacillus paramycoides* spp.) for phytotoxicity reduction in Berseem clover and tomato crops. *Water Science and Technology*, 83(7): 1764-1780.
15. Rashid, A., Mirza, S. A., Keating, C., Ali, S. and Campos, L. C., 2020). Indigenous *Bacillus paramycoides* spp. and *Alcaligenes faecalis*: sustainable solution for bioremediation of hospital wastewater. *Environmental Technology*, 43(12): 1903-1916.
16. Sonune, N. A. and Garode, A. M., 2015. Bioremediation potential of bacterial isolates for municipal wastewater treatment. *Current World Environment*, 10(2): 1-10.
17. Díaz, E., 2008. Genomic insights into the aerobic pathways for degradation of organic pollutants. *In:* McLeod, M.P., Eltis, L.D. (Ed.), *Microbial Biodegradation: Genomics and Molecular Biology,* ed. 1: 5-24.
18. Nirmala, B., 2018. Rice production in Asia: Key to global food security. *Proceedings of the National Academy of Sciences,* India Section B*: Biological Science,* 88(4): 1323-1328.
19. Rehman, A., Jingdong, L., Chandio, A. A., Shabbir, M., and Hussain, I., 2017. Economic outlook of rice crops in Pakistan: a time series analysis (1970–2015). *Financial innovation*, 3(1): 1-9.
20. Verma, A. K., Dash, R. R. and Bhunia, P., 2012. A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Journal of Environmental Management*, 93(1): 154-168.
21. Hai, F. I., Yamamoto, K. and Fukushi, K., 2007. Hybrid treatment systems for dye wastewater. *Critical Reviews in Environmental Science and Technology*, 37(4): 315-377.
22. Dash, A. K., 2012. Impact of domestic waste water on seed germination and physiological parameters of rice and wheat. *International Journal of Recent Research and Applied Studies*, 12(2): 280-286.
23. Li, L., Yang, Y., Tam, N. F., Yang, L., Mei, X. Q. and Yang, F. J. 2013. Growth characteristics of six wetland plants and their influences on domestic wastewater treatment efficiency. *Ecological engineering*, 60(1): 382-392.
24. Singh, R. P., Singh, P., Kothari, R., Gautam, K. and Singh, A., 2021. Application of Wastewater in Irrigation and Its Regulation with Special Reference to Agriculture Residues. In *Water Pollution and Management Practices,* Springer, Singapore. pp:177-199.
25. Tran, L. D., Phung, L. D., Pham, D. V., Pham, D. D., Nishiyama, M., Sasaki, A. and Watanabe, T., 2019. High yield and nutritional quality of rice for animal feed achieved by continuous irrigation with treated municipal wastewater. *Paddy and Water Environment*, 17(3): 507-513.

26. Fendri, I., S. Ben, R., Khemakhem, B., Ben, H. N., Gdoura, R. and Abdelkafi. S., 2013. Effect of treated and untreated domestic wastewater on seed germination, seedling growth and amylase and lipase activities in *Avena sativa* L. *Journal of Science and Food Agriculture,* 93(7): 74-156.
27. Huy, V. and Iwai, C. B., 2018. Utilisation of wastewater on seed germination and physiological parameters of rice (*Oryza sativa L.*). In *IOP Conference Series: Materials Science and Engineering*, 334(1): 012036.
28. WWF, 'Study: Growing more rice with less water', available online on <https://www.bing.com/search?q=rice+need+more+water+an+issue+to+water+scarce+countries&qs=n&form=QBRE&sp=-1&lq=0&pq=&sc=0-0&sk=&cvid=39E27064BE174AD092C4A449E8AA3B89&ghsh=0&ghacc=0&ghpl=> accessed on 28 August 2023.
29. Hydroponics rice paddy nursery: An innovative twist on growing rice in India, Rice Today, available online on <https://ricetoday.irri.org/hydroponics-rice-paddy-nursery-an-innovative-twist-on-growing-rice-in-india/#:~:text=The%20other%20advantages%20of%20hydroponics,Uses%2095%25%20less%20water> accessed on 28 August 2023.
30. Johnson, L. Why use hydroponics? available online on <https://ezgrogarden.com/hydroponics/why-use-hydroponics/#:~:text=Hydroponics%20eliminates%20soil%20and%20soil,and%20streams%2C%20harming%20fish%20populations> accessed on 28 August 2023.
31. Motuzas, J., Drobek, M., Martens, D. L., Vallicari, C., Julbe, A. & Diniz da Costa, J. C., 2017. Environmental mineralization of caffeine micro-pollutant by Fe-MFI zeolites. *Environmental Science and Pollution Research,* 25(2017): 3628-3635.
32. Gushit, J. S., Ekanem E. O., Adamu H. M. & Chindo, I. Y., 2013. Analysis of herbicide residues and organic priority pollutants in selected root and leafy vegetable crops in Plateau state, Nigeria. *World Journal of Analytical Chemistry,* 1(2): 23-28.
33. Wojcieszynska, D., Domaradzka, D., Hupert-Kocurek, K. & Guzik. U., 2014. Bacterial degradation of naproxen - Undisclosed pollutant in the environment. *Journal of Environmental Management,* 145(2014): 157-161.
34. Al Hamedi, F., Karthishwaran, K., Alyafei, M. A. M. 2021. Hydroponic wheat production using fresh water and treated domestic wastewater under the semi-arid region. *Emirates Journal of Food and Agriculture*, 33 (2): 178-186.
35. Ranjitha, K. M., Biradar, S. S., Desai, S. A., Naik, V. R., Bhat, S., Satisha, T. N., Hiremath, G., Kumar, K. J. Y., Chethana, C. K., Venkatesh, K. 2017. Media standardization for hydroponic culture to screen wheat genotypes for nitrogen use efficiency. *Int. J. Curr. Microbiol. App. Sci.* 6 (12): 2814 – 2820. https://doi.org/10.20546/ijcmas.2017.612.327.
36. Mackowiak, C. L., Owens, L. P., Hinkie, C. R., Kennedy Space Center, Florida. 1989. Continuous hydroponic wheat production using a recirculating system. NASA John F. Kennedy Space Center.
37. Prazeres, A. R., Albuquerque, A., Luz, S., Jeronimo. E., Carvalho, F. 2017. Food Biosynthesis, *In:* Hydroponic System: A Promising Biotechnology for Food Production and Wastewater Treatment. Elsevier Inc. http://dx.doi.org/10.1016/B978-0-12-811372-1.00011-7.
38. Shah, A. H., Shams Ul Munir, Noor Ul Amin, Shah, S. H. 2011. Evaluation of two nutrient solutions for growing tomatoes in a non-circulating hydroponics system. *Sarhad J. Agric.* 27 (4).
39. Jesse, S. D. 2019. Treatment of post hydrothermal liquefaction wastewater and suitability for hydroponic lettuce production. University of Illinois, Urbana-Champaign, USA.
40. APHA. 2002. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, Washington DC, USA.
41. National Environment Quality Standards (NEQS). 2000. The Gazette of Pakistan. Ministry of Environment, Local Government and Rural Development, Government of Pakistan, Islamabad, Pakistan, pp. 1289-1294.
42. Mahmood, A. and Malik, R. N., 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry,* 7(1): 91-99.
43. Ajaz, M., Rehman, A., Khan, Z., Nisar, M. A. and Hussain, S., 2019. Degradation of azo dyes by *Alcaligenes aquatilis* 3C and its potential use in the wastewater treatment. *AMB Express*, 9(1): 1-12.
44. Rice Research Institute, Kala Shah Kaku. Ayub Agricultural Research Institute, Faisalabad, accessed online from: <https://aari.punjab.gov.pk/crop_varities_rice>.
45. Hoagland, D. R. and Arnon, D. I., 1950. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station*, 347(2): 117-139.
46. Dwivedi, P. and Tomar, R.S., 2018. Bioremediation of textile effluent for degradation and decolourisation of synthetic dyes: a review. *International Journal of Current Research in Life Sciences,* 7 (4): 1948-1951.
47. Yokoyama, S. I., Adachi, Y., Asakura S. and Kohyama, E., 2013. Characterization of *Alcaligenes faecalis* strain AD15 indicating biocontrol activity against plant pathogens. *The Journal of General and Applied Microbiology,* 59(2): 89-95.
48. Rodil, R., Quintana, J. B., Concha-Grana, E., Lopez-Mahia, P., Muniategui-Lorenzo S. and Prada-Rodriguez. D., 2012. Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain). *Chemosphere,* 86(10): 1040-1049.
49. Meerbergen, K., Willems, K. A., Dewil, R., Van Impe, J., Appels, L. and Lievens, B., 2018. Isolation and screening of bacterial isolates from wastewater treatment plants to decolourise azo dyes. *Journal of bioscience and bioengineering*, 125(4): 448-456.
50. Spehia, R., Devi, M, Singh, J., Singh, S. 2018. Lettuce growth and yield in hoagland solution with an organic concoction. *International Journal of Vegetable Science*. https://doi.org/10.1080/19315260.2018.1452815.
51. Delden, S. H., Nazarideljou, M. J., Marcells, L. F. M. 2020. Nutrient solutions for Arabidopsis thaliana: a study on nutrient solution composition in hydroponics systems. *Plant Methods* 16: 72. https://doi.org/10.1186/s13007-020-00606-4.

52. Kane, C. D. 2003. Influence of nutrient solution and solution pH on onion growth and mineral content. Texas Tech University, USA.
53. Shi, X., Sun, H., Pan, H. et al. 2016. Growth and efficiency of nutrient removal by *Salix jiangsuensis* J172 for phytoremediation of urban wastewater. *Environ Sci Pollut Res.*, 23, 2715–2723.
54. Saleem, A., Zulfiqar, A., Ali, B., Naseeb, M.A., Almasaudi, A.S., Harakeh, S. 2022. Iron Sulfate ($FeSO_4$) Improved physiological attributes and antioxidant capacity by reducing oxidative stress of *Oryza sativa* L. cultivars in alkaline soil. *Sustainability 14*, 16845. https://doi.org/10.3390/su142416845.
55. Singh, R.P., Agrawal, M. 2010. Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotoxicology and Environmental Safety* 73 (4): 632-641. https://doi.org/10.1016/j.ecoenv.2010.01.020.Kang, J. G. &, Iersel, M. W. 2004. Nutrient Solution Concentration Affects Shoot: Root Ratio, Leaf Area Ratio, and Growth of Subirrigated Salvia (*Salvia splendens*). *HortScience*, 39(1), 49-54.
56. Trejo-Téllez, L. I., Gómez-Merino, F. C. 2012. Hydroponics - A Standard Methodology for Plant Biological Researches, *In:* Nutrient solutions for hydroponic systems. IntechOpen.
57. Amedi, J., Rasheed, R. and Ibrahim, D., 2021. Responce of Physico-Chemical Soil Properties to Wastewater Application and Subsequent Effects on Three Woody Species Plants. *Journal of Duhok University*, 24(2): 106-125.
58. Rana, S. & Kumar. K., 2017. Study of phytotoxic effect of textile wastewater on seed germination and seedling growth of *Triticum aestivum*. *International Journal of BioSciences and Technology,* 10(8): 58-66.
59. Magwaza, S. T., Magwaza, L. S., Odindo, A. O. and Mditshwa, A., 2020. Hydroponic technology as decentralized system for domestic wastewater treatment and vegetable production in urban agriculture: A review. *Science of the Total Environment*, 698(1): 134154.
60. Xu, B., Wang, F., Zhang, Q., Lan, Q., Liu, C., Guo, X., Cai, Q., Chen, Y., Wang, G. and Ding, J., 2018. Influence of iron plaque on the uptake and accumulation of chromium by rice (*Oryza sativa* L.) seedlings: Insights from hydroponic and soil cultivation. *Ecotoxicology and Environmental Safety*, 162(2): 51-58.