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Treatment of Sewage Wastewater through Phytoremediation of Sheikh Maltoon Town: A Case Study of District Mardan, Khyber Pakhtunkhwa Pakistan

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Abstract

The current study was aimed to investigate three freshwater hydrophytes such as Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum*) for the treatment of sewage wastewater of Shiekh Maltoon Town, Mardan, Khyber Pakhtunkhwa, Pakistan. All experimental work was done in open containers containing sewage wastewater. The results show that all species have the capability to remove pollutants from sewage wastewater. The removal tendency of these species was in order of Common Duckweed *(Lemnoideae)* > Water Lettuce (*Pistia stratoites)* > Coontail (*Ceratophyllum demersum*). The removal efficiency of these species was checked for specific pollutants. The removal efficiency was reported greater in the initial days of the experiment and efficiency decreased with the passage of time. Statistical analysis revealed that the amount of pollutants in aqueous solutions were reduced significantly ($P \leq 0.01$) by Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum*).

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Introduction

Nowadays human activities and their basic needs are growing rapidly due to increased population in all countries and as a result our water resources have been contaminated with a greater quantity of harmful pollutants from different sectors [Raghunandan *et al*., 2018]. Certain adverse effects of urbanization such as depletion of resources, increasing in carbon amount and water pollution are reported regionally and globally that are disturbing human health and environment [Ahuti, 2015]. Industrialization has also effects on social and economic change of people and also needs innovations for improvement [Mgbemene *et al*., 2016]. Due to industrial processes, huge amount of pollutants is releasing into local water bodies and as a result causing problems to human health. Industrialization not only produces heavy metals but also producing different types of wastes that effects environment. Because of industrial revolutions, there is great discharge of chemical substances and harmful gases to the environment.

Water pollution is a serious problem that will disturb humans. However various approaches had been used to control this problem but still it is a concern for humans. Humans and environment together effected by these pollutants worldwide. All these pollutants greatly affect the function of lungs, kidney and brain and also reduced energy level in humans. Certain pollutants are carcinogenic because of their frequently exposure [Valko *et al*., 2016].

Therefore, it is essential to offer suitable method for pollutants uptake from water systems [Iram *et al*., 2013]. Several methods such as ion exchange, coagulation, adsorption, oxidation or reduction, and nanotechnology have been used to treat HMs from aqueous solutions [Jaishankar *et al*., 2014].

All these methods have their own advantages and disadvantages but almost these are expensive and time consuming. A new approach must be planned to safeguard human health and environment from the consequences of water pollution, and phytoremediation is reported one of promising and efficient techniques for pollutants removal [Tóth *et al*., 2016]. This method has developed as a relatively cost-effective procedure and can show efficiency for the removal of pollutants from water and soil and make it toxic-free compounds [Rajasulochana and Preethy; 2016]. Freshwater hydrophytes use pollutants as a food nutrient in phytoremediation method. Phytoremediation process can be made on two sites such as in-situ and ex-situ [Singh and Gupta; 2016]. Many species of hydrophytes have been used to treat pollutants from wastewater [Kumar *et al*., 2016]. Hydrophytes are frequently available and can be easily cultivated in aquatic ecosystems. Some study has proved that freshwater hydrophyte species remove pollutants, and thus enhanced water quality [Laib and Leghouchi; 2012].

In current research work, three species of freshwater hydrophytes, such as Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*, were applied because of its great availability and easy cultivation everywhere to find their treatment capability for pollutants and also to find a viable eco-friendly technique for sewage wastewater.

Materials and methods

Study area description

The study was conducted in Sheikh Maltoon Town (SMT) located in District Mardan, Pakistan Fig. 1 at 34°05' to 34°32' N latitudes and 71°48' to 72°25' E longitudes. District Mardan is the second largest District in Khyber Pakhtunkhwa (KP) Province, Pakistan with an area of 1632 km^2 and total population of 23.7 million (2017) at a density of 1448 km^2 and urban population of 19% [GoP, 2018]. SMT is a formal urban housing development with an area of 1.5 km^2 and estimated population of 17,000 inhabitants. The Town is divided into 18 sectors having average Household (HH) size ranging from 3 to 11 persons per household and house area ranging from 126 m^2 to 1012 m^2, with 253 m^2 as the most common size. Management of solid waste in SMT is the responsibility of Mardan Development Authority (MDA). Climate of Mardan region is hot semi-arid with average annual temperature and rainfall of 22.2°C and 449 mm respectively. January is the coldest and June the hottest month with average temperatures of 10°C and 33.2°C respectively whereas the highest rainfall occurs in August (122 mm) [Climate-data.org, 2018].

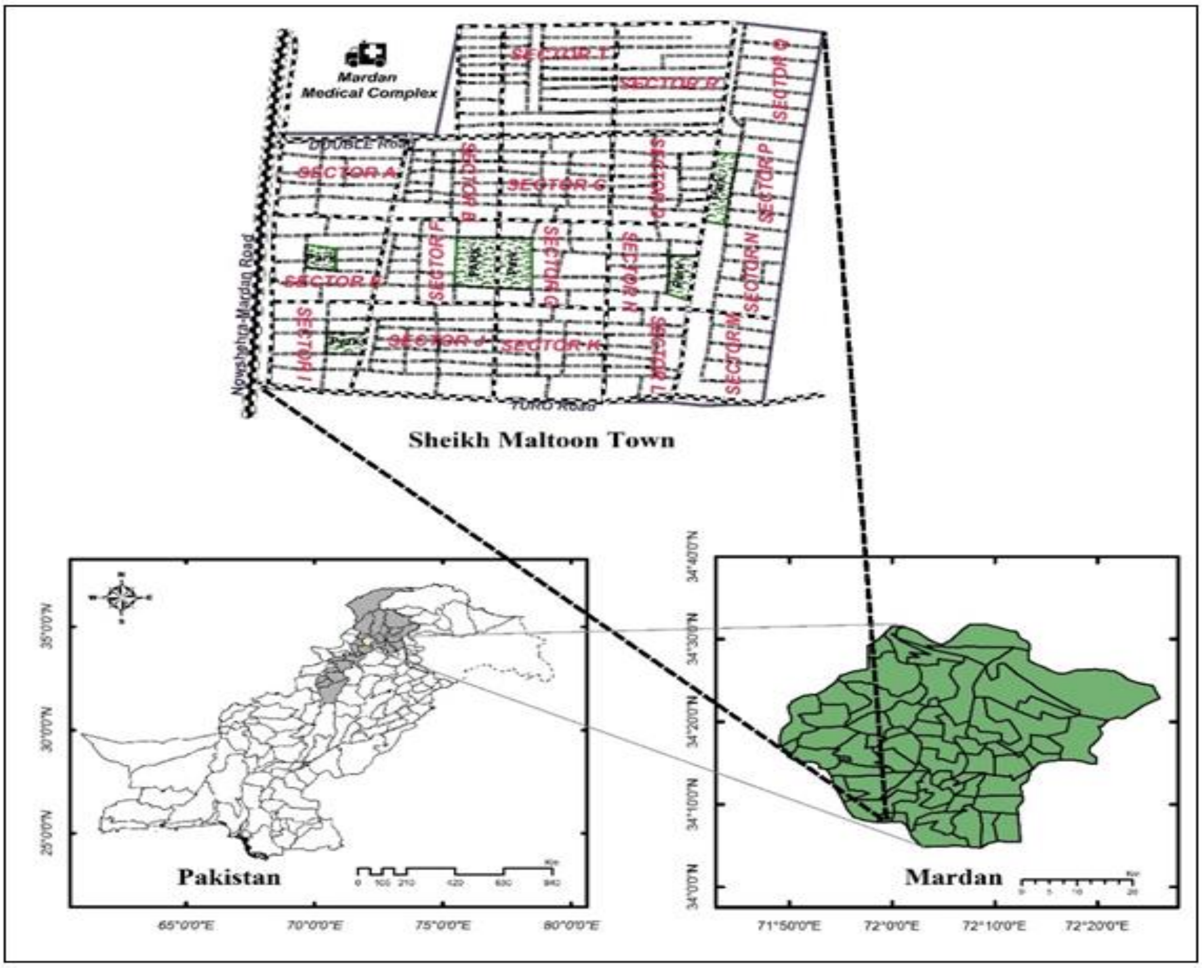

Fig. 1. Map of Pakistan showing the study area (Sheikh Maltoon Town, Mardan).

Collection of hydrophytes and cultivation

In current research, three different species of hydrophyte such as Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*, were taken from the local lake ecosystem, of National Agriculture Research Center (NARC), Islamabad, Pakistan.

The used species were selected due to distinct features like rapid growing, great resistance to pollutants and maximum uptake of pollutants. These species were planted and adapted into the open tubs at average temperature of 20 °C±0.02 with mean pH of 7.2 under sunlight for 19 d. The collected wastewater of SMT were used for irrigation of these hydrophytes. Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* were used in this study for pollutants removal as shown in Fig. 2 (a), (b) and (c).

Experimental setup

All experiments were carried out in open containers (Tubs) including 1 Litre (L) of wastewater collected from SMT, Mardan. These open containers were washed with HNO_3 (10%) to avoid contamination and then washed with distilled water. Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* of each were added to all containers. The complete experiment was carried out for 19 d in three replicates under sunlight at room temperature of 20 ±1°C.

Water quality analysis

The wastewater samples taken from Chakshahzad were analyzed using physico-chemical parameters such pH, EC, TDS, CO_3^{-2}, BCO_3^{-1}, Hardness, Ca^{+2}, Mg^{+2}, sulfates, Nitrates and BOD.

Analytical methods

After 19 d completion, hydrophytes species were collected from each container, and then washed several times with 5mM of edetic acid (EDTA) then with deionized water to remove pollutants.

The samples were dried at 100 °C for two h in oven. Pollutants was extracted from the dead biomass of hydrophytes species following the advance method of [Shamshad *et al.*, 2015]. Briefly, 3 g of powder hydrophytes was taken in beaker having 20 mL solution of HNO_3 (70 %) and H_2O_2 (30 %). Millipore filter paper (0.40 µm) was used for filtration of prepared solution into flask of 100 mL and then final solution was synthesized by using distilled water.

Statistical analysis

In this current study the data was analysed using statistical analysis. All graphs were made using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) and Microsoft Excel using individual medium of freshwater hydrophytes to present the mean values, while the map was made using ArcGIS software.

Results and discussion

Physicochemical parameters

In physicochemical analysis, different parameters (pH, EC, TDS, DO, hardness, turbidity, carbonates, bicarbonates, calcium, and magnesium) were tested as shown in Table 1. Sampling was done with five (5) days interval from 5 August to 25 August, 2021 at 32 ºC and 35 ºC. The samples were taken from SMT, Mardan. Three different species named Common Duckweed *(Lemnoideae)* Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* were added to wastewater in six (6) separate tubs (two for each specie).

All the samples were tested in the laboratory of National Institute of Bioremediation (NIB) at (NARC) Islamabad. Significant results were obtained from physicochemical analyses which are described in Table 1.

Table 1. Physicochemical parameter values of waste water samples tested on d first (1).

S.No	Parameter	Control	Duckweed	Water Lettuce	Coontail	Permissible Value
1	pH	5.51				6-10
2	EC (uS/cm)	1.121				800 µs/cm
3	Turbidity (NTU)	34.04				>5 NTU
4	Calcium (mg/L)	113.54				75 mg/L
5	Chloride (mg/L)	250.20	Same as mentioned in Control			250 mg/L
6	Total Hardness (mg/L)	500				200 mg/L
7	Magnesium	52.86				50 mg/L

pH

Control pH value of the sample was recorded as 5.51. After the introduction of plants in this wastewater sample the pH value increase and it goes from acidity towards basicity. The pH value recorded for Coontail (*Ceratophyllum demersum)* on d 19 was 7.88. The pH value recorded for Common Duckweed *(Lemnoideae)* on d 19 was recorded to be 8.02, whereas the pH value recorded for Water Lettuce (*Pistia stratoites)* on d 19 was recorded to be 7.66 as shown in Fig. 3 and Table 3.

The permissible limit for pH sets by Pakistan-NEQs is 6-10 pH (acidity/basicity). If this limit exceeds than the pH of water affects the solubility of many toxic and nutritive chemicals; as acidity increases; most metals become more water soluble and more toxic [Singh *et al.*, 2012]. The ideal pH range for soil is from 6 to 6.5 because most plant nutrients are in their most available state. Nitrogen, for example, has its greatest solubility between soil pH 4 and pH 8 and the pH value <3 and >9 cause reduced plant growth [Rybak *et al.*, 2012].

Table 2. Physicochemical parameter values of wastewater samples tested on d seven (7).

S.No	Parameter	Control	Duckweed	Water Lettuce	Coontail
1	pH	5.51	7.29	6.98	7.12
2	EC (uS/cm)	1.121	0.944	0.969	0.923
3	Turbidity (NTU)	34.04	27.38	30.13	30.42
4	Calcium (mg/L)	113.54	84.105	97.56	105.23
5	Chloride (mg/L)	250.20	172.76	135.14	221.78
6	Total Hardness (mg/L)	500	270	390.00	460.89
7	Magnesium	52.86	34.64	36.44	45.15

Electrical conductivity

Control value of EC was recorded about 1.121 µS/cm. The high EC values of the sample showed, that these effluents contain a high concentration of mobile and valence inorganic ions [Fang *et al.*, 2011]. With the addition of Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* significant changes occurred in EC value.

The EC value recorded for Coontail (*Ceratophyllum demersum* was 0.733 µS/cm,

Table 3. Physicochemical parameter values of wastewater samples tested on d thirteen (13).

S.No	Parameter	Control	Duckweed	Water Lettuce	Coontail
1	pH	5.51	7.83	7.35	7.24
2	EC (uS/cm)	1.121	0.815	0.950	0.893
3	Turbidity (NTU)	34.04	16.19	15.34	16.36
4	Calcium (mg/L)	113.54	79.80	70.51	101.79
5	Chloride (mg/L)	250.20	157.6	240.30	175.20
6	Total Hardness (mg/L)	500	260.00	195.54	430.55
7	Magnesium	52.86	11.28	15.67	32.34

Common Duckweed *(Lemnoideae)* was 0.74 µS/cm, whereas for Water Lettuce (*Pistia stratoites)* it was recorded to be 0.82 µS/cm as shown in Fig. 4. The effluent standard limits for wastewater reuse in irrigation for EC is 800 µS/cm [Tan and Ting; 2012].

Turbidity

The turbidity values showed great variations among all samples in which the hydrophytes were added. The control turbidity value for the sample was 34.04 NTU. After the addition of Common Duckweed *(Lemnoideae)* Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*, a significant change occurred in the turbidity for all samples. After completion of 19 d, the turbidity value for Coontail (*Ceratophyllum demersum)* was 21.53 NTU, Common Duckweed (*Lemnoideae*) was 5.02 NTU, while for Water Lettuce (*Pistia stratoites*), the turbidity value was recorded about 4.70 NTU see Fig. 5. These values showed that the samples after treatment through these species still contain impurities which may include finely divided inorganic and soluble colored organic compounds [Tan and Ting; 2012].

Table 4. Physicochemical parameter values of wastewater samples tested on d nineteen (19).

S.No	Parameter	Control	Duckweed	Water Lettuce	Coontail
1	pH	5.51	8.02	7.66	7.88
3	EC (uS/cm)	1.121	0.740	0.820	0.733
4	Turbidity (NTU)	34.04	5.02	8.99	21.53
5	Calcium (mg/L)	113.54	74.66	76.56	86.45
6	Chloride (mg/L)	250.20	120.91	109.2	150.40
7	Total Hardness (mg/L)	500	239.54	250.67	400
9	Magnesium	52.86	7.56	15.67	19.80

Chloride

The Chloride values show great changes among all the tested samples. For chloride, the control value was reported 120.91 mg/L. While after the addition of Duckweed *(Lemnoideae)* Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* in waste water samples significant changes were founded. By adding Common Duckweed *(Lemnoideae)*, the Chloride value after 19 d was reported 120.91 mg/L, for Water Lettuce *(Lemnoideae)*, after 19 d the values was reported 109.2 mg/L, whereas for Coontail (*Ceratophyllum demersum)* it was reported 150.15 mg/L as given in Fig. 6.

Fig. 2. (a) shows Water Lettuce (*Pistia stratoites*), (b) shows Common Duckweed *(Lemnoideae)* and (c) shows Coontail (*Ceratophyllum demersum).*

The chlorides mostly affect the soil pH ($CaCl_2$) of 5.2 to 8.0 provides optimum conditions for most agricultural plants. Microbial activity in the soil also affected by soil pH with most activity occurring in soils of pH 5.0 to 7.0. Where the extremities of acidity or alkalinity occur, various species of earth worms and nitrifying bacteria disappear [Rybak *et al.*, 2012].The standard value of Chloride for irrigation is 250 mg/L [Tan and Ting; 2012]. Chloride does not absorb to soil particles (as do ammonium and phosphorous) generally it does not form complexes with other compounds and it does not undergo biological transformation (as do nitrate and ammonium).

Additionally, chloride is one of the few ions, along with nitrate that travels at the same rate as ground water [Tastan *et al.*, 2012].

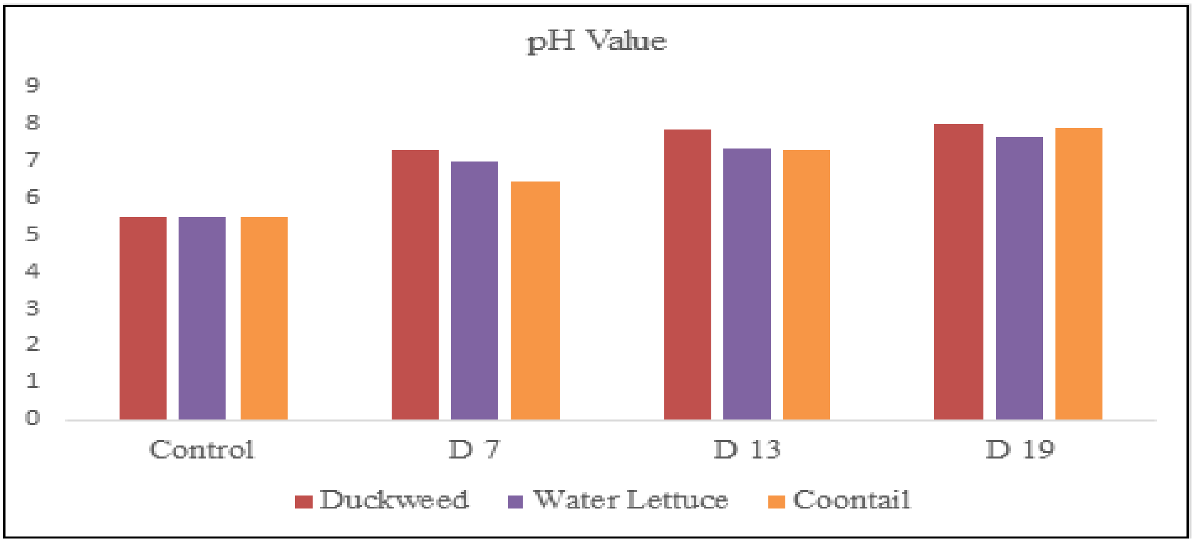

Fig. 3. Variations in pH values at five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum).*

Total hardness

The control value of the samples for its total hardness were recorded about 500 mg/L. After the addition of species to wastewater, important changes occurred. On 19 d the total hardness value was recorded 239.54 for Common Duckweed *(Lemnoideae)*, for Water Lettuce *(Pistia stratoites)* it was recorded 250.67 mg/L, while Coontail (*Ceratophyllum demersum)* it was reported 400 mg/L see in Fig. 7. The highest value recorded for the Water Lettuce *(Pistia stratoites)* which was 390 mg/L on d seven (7) as shown in Table 2. The waste water seeps through the ground and increase the hardness of ground water. The standard classifications of hard water are moderate (60-120 mg/L), hard (120-180 mg/L) and very hard more than (180 mg/L) [Karna *et al.*, 2017].

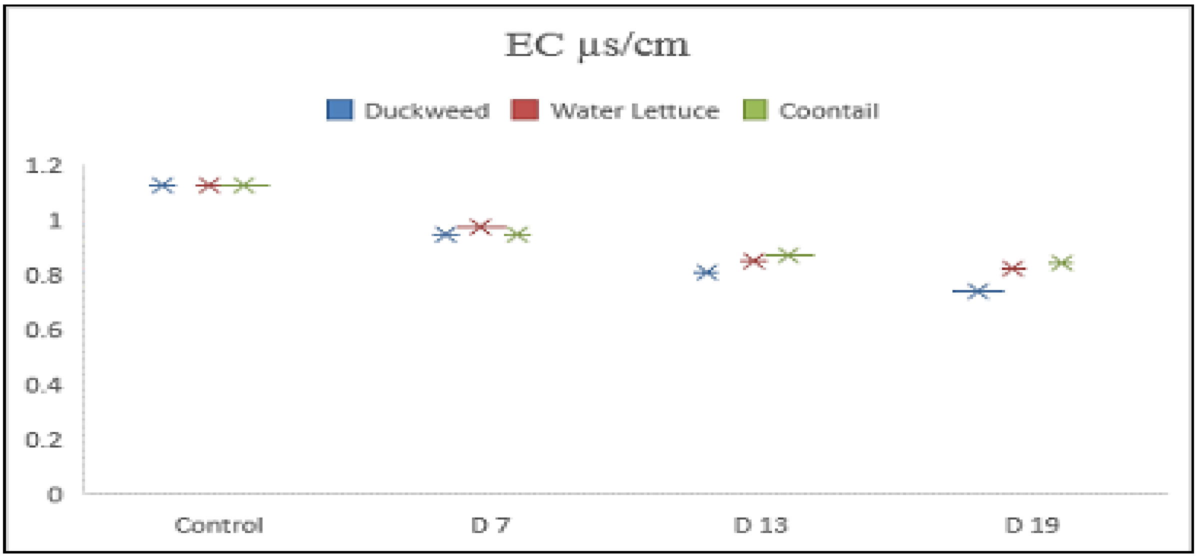

Fig. 4. Variations in EC values at five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum).*

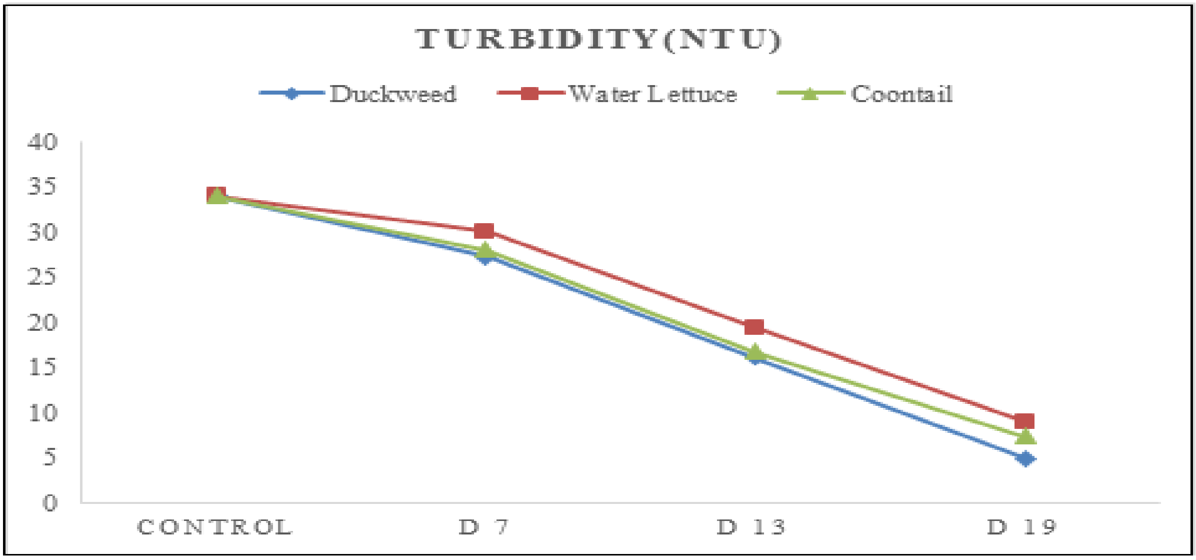

Fig. 5. Variations in turbidity values at five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum).*

Magnesium

The control value for Magnesium was recorded about 52.86 mg/L. However, after the addition of Duckweed *(Lemnoideae)* Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*, major changes were occurred.

For Common Duckweed *(Lemnoideae)* after 19 d the value was recorded 7.56 mg/L, for the Water Lettuce *(Pistia stratoites)*, the value was recorded 15.67 mg/L, while for Coontail (*Ceratophyllum demersum)* it was reported19.80 mg/L as shown in Fig 8. According to the literature Common Duckweed *(Lemnoideae)* have the greater capability to extract Magnesium from wastewater.

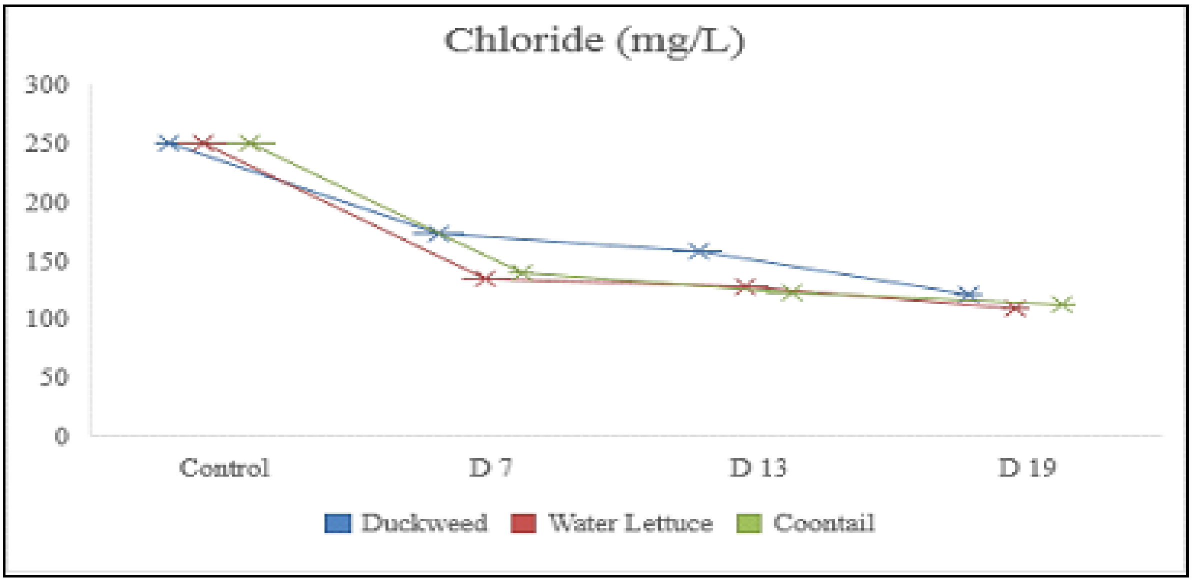

Fig. 6. Variations in Chloride values at Five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*.

Calcium

The Control value for Calcium on sampling day was recorded 113.54 mg/L. However, Common Duckweed *(Lemnoideae)* Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*, significant changes were founded. For Common Duckweed *(Lemnoideae)* after 19 d the value was reported 74.66 mg/L, for the Water Lettuce *(Pistia stratoites)*, the value was recorded 76.56 mg/L, while for Coontail (*Ceratophyllum demersum)* it was reported 168.21 mg/L as shown in Fig. 9.

Discussion

Phytoremediation has been increasingly used to clean up contaminated soil and water systems because of its lower costs and fewer negative effects than physical or chemical engineering approaches [Álvarez and Wendel; 2003]. In Pakistan, as there is plenty of abundant and agriculture-based lands, therefore, these techniques for waste water treatment can be used for safe disposal of contaminated water. Many researchers have used different plant species for treating the wastewater like Water Hyacinth, Water Lettuce, Duckweed, Bulrush, Vetiver Grass, and Common Reed for the treatment of wastewater. In many cases, especially in tropical or subtropical areas, invasive plants such as the Duckweed, Water Lettuce and Coontail were used in phytoremediation water systems [Ma *et al.*, 2016]. This is because, compared to other native plants, these plants species show a much higher nutrient removal efficiency with their high nutrient uptake capacity, fast growth rate, and big biomass production [Ma *et al.*, 2016].

In this study of phytoremediation, we have used the Common Duckweed *(Lemnoideae)*, Water Lettuce *(Pistia stratoites)* and Coontail based on their growth patterns, nutrient uptake rates and the fact that they are native to the study region.

Common Duckweed *(Lemnoideae)* also known as (water lens) are small aquatic plants belonging to the botanical family named as Lemnaceae are flowering plants which float on or just beneath the surface of still or slow-moving bodies of fresh water and wetlands.

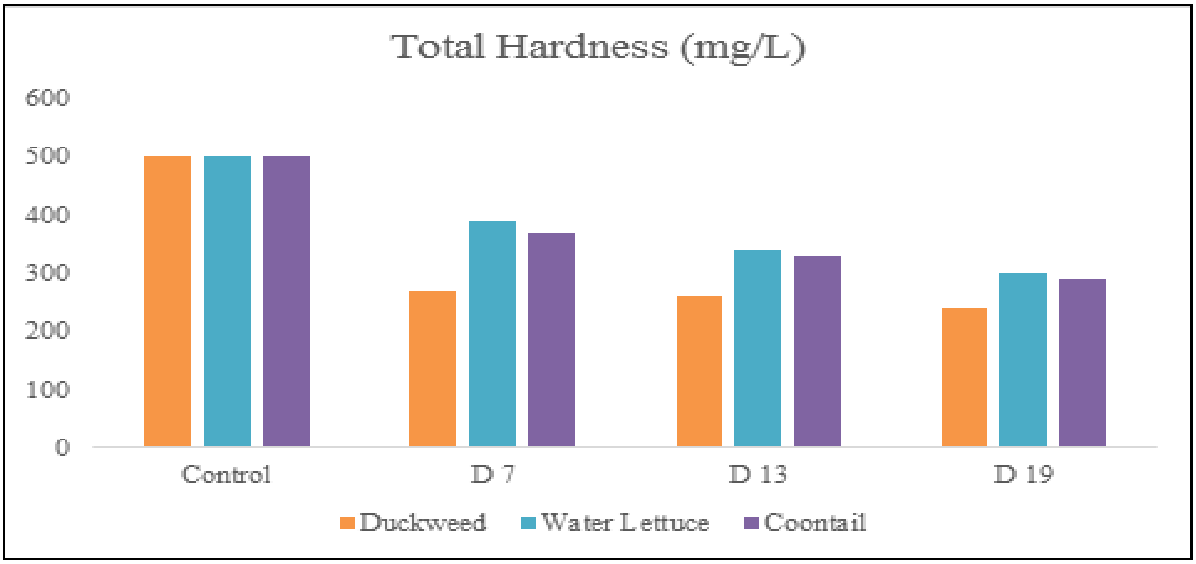

Fig. 7. Variations in Total Hardness values at five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*.

In recent years a commonly occurring aquatic plant, "Duckweed", has become prominent, because of its ability to concentrate minerals on heavily polluted water such as that arising from sewage treatment facilities. However, it has also attracted the attention of scientists because of its apparent high potential as a feed resource for livestock [Deng *et al.*, 2008]. Due its capability of rapid propagation through consuming dissolved nutrients from the waste water make Common Duckweed *(Lemnoideae)* an excellent candidate for waste water treatment. Most of the research has been done on the use of Common Duckweed *(Lemnoideae)* in waste water treatment systems because of their great potential to remove mineral contaminants from waste waters emanating from sewage works, intensive animal industries or from intensive irrigated crop production. The minimum water temperature allowing their use in waste water treatment is reported to be 7 °C [Meagher, 2000]. A second critical soil parameter is pH for the growth of Common Duckweed *(Lemnoideae)*.

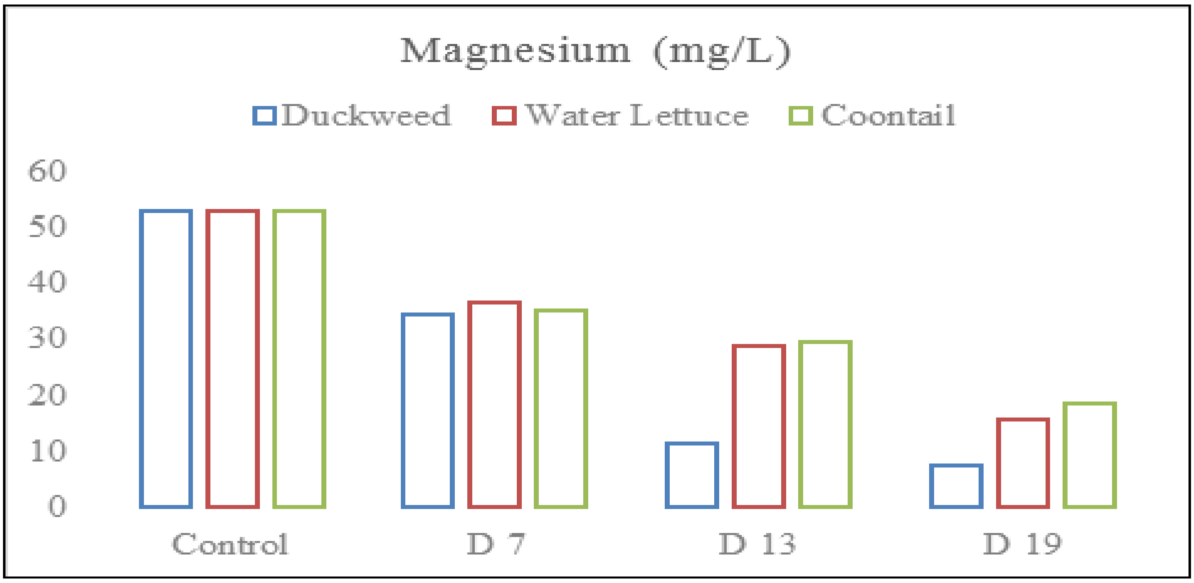

Fig. 8. Variations in Magnesium values at five (5) days interval by adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*.

The optimum pH for Common Duckweed *(Lemnoideae)* growth ranges between 4.5 and 7.5. Other authors report a narrower pH optimum ranging from 6.5 to 7.5 [Onweluzo and Akuagbazie; 2010]. Therefore, highly acid and alkaline soils are unsuitable for Common Duckweed *(Lemnoideae)* cultivation. Alkaline conditions favor, in particular, the transformation of ammonium to ammonia which is harmful to Common Duckweed *(Lemnoideae)*. As described in this study that Common Duckweed *(Lemnoideae)* increases the pH value of the selected waste water from pH 5.51 to 8.02. *Pistia stratiotes* common name of Water Lettuce is a floating perennial commonly belonging to the family Araceae. It floats on the surface of the water, and its roots hanging underwater beneath floating leaves.

Water Lettuce *(Pistia stratoites)* is non-winter-hard plant, having a minimum growth at temperature 15 °C. [Reichenauer and Germida; 2008] stated that Water Lettuce *(Pistia stratoites)* doubles its biomass in just over 5 d, triples it in 10 d, quadruples in 20 d and has its original biomass multiplied by a factor of 9 in less than one month.

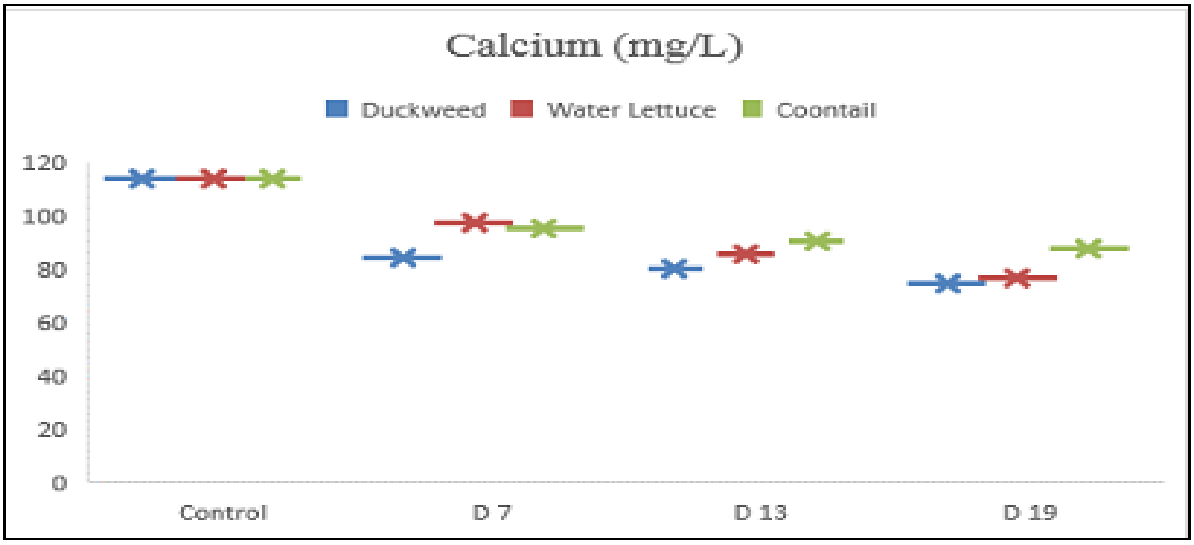

Fig. 9. Variations in Calcium values at five (5 days) interval adding Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)*.

This evolution indicates that 25 d is the maximum period to allow the plant in the system. [Khan and Mujahid Ghouri; 2011] also reported that low concentration of nutrients may reduce the performance of plant in removing nutrients. A 200-fold difference in dry weight of Water Lettuce *(Pistia stratoites)*. Most of the studies conducted on the use of Water Lettuce *(Pistia stratoites)* for the treatment of wastewater that Alkaline pH was changed to the neutral through the application of this plant. Similarly, like that of other studies this study also shows that the pH of the selected wastewater is increased from 5.51 to 7.66 which is slightly alkaline and is near to neutral through the use Water Lettuce *(Pistia stratoites)*. The reduction in pH is due to absorption of nutrients and other salts by plants or by simultaneous release of H^+ ions with the uptake of metal ions.

Preliminary study by [Kanabkaew and Puetpaiboon; 2004] revealed that Water Lettuce *(Pistia stratoites)* growth decreased the EC in the treatment plot due to salt removal from the waters by plant uptake or root adsorption and it was concluded that water quality in ponds was improved by phytoremediation with Water Lettuce *(Pistia stratoites)*. As the present study shows that that the EC value of waste water was decreased from 1.121 (uS/cm) to (0.82 uS/cm) by using Water Lettuce *(Pistia stratoites)* and show lower reduction as compared to the decrease showed by the Common Duckweed *(Lemnoideae)* which was EC 0.74 (uS/cm). From this study it was concluded that the efficiency of

Water Lettuce *(Pistia stratoites)*, Coontail (*Ceratophyllum demersum)* and Common Duckweed *(Lemnoideae)*, used for the treatment of sewage wastewater of SMT, Mardan, Pakistan.

Conclusion

Current research studied the removal efficiency of freshwater hydrophytes such as Common Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* for the treatment of sewage wastewater of SMT, Mardan. The removal efficiency of these species was affected by initial pollutants concentration in the sewage wastewater. High removal of pollutants was reported in the initial days of the experiment in water solutions. Duckweed *(Lemnoideae)*, Water Lettuce (*Pistia stratoites)* and Coontail (*Ceratophyllum demersum)* greatly remove pollutants and removal efficiency was much better for sewage wastewater of SMT, Mardan. It is important to know that these species of freshwater hydrophytes not only live for weeks but also show capabilities to treat pollutants from sewage wastewater. The great efficiencies of these species, low input, low cost for transport and easy growing made this method acceptable for the treatment of pollutants from sewage wastewater of residential areas and industries on commercial scale.

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