Pak. J. Bot., 44: 97-100, Special Issue March 2012.

EFFECTS OF DIFFERENT DILUTIONS OF MUNICIPAL WASTE WATER ON SOME PHYSIOLOGICAL ASPECTS OF *EICHORNIA CRASSIPES* SOLMS

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Abstract

In the present study effects of different dilutions of municipal waste water were investigated on the physiological aspects of *Eichornia crassipes* Solms. There were five different concentrations of municipal waste water (0%, 25%, 50%, 75% and 100%) which were labeled as T_0, T_1, T_2, T_3 and T_4 respectively. Vegetative growth effected by the waste water was monitored at the end of the experiment. Vegetative reproduction of the plants was also studied by observing number of baby plants per plant. Rate of photosynthesis and rate of transpiration of the plants growing in different concentrations of municipal waste water were studied as physiological parameters. Before start of experiment and after harvesting the plants waste water samples were also analyzed. T_4 showed highest rate of physiological parameters among all the treatments while T_0 had the lowest rate of physiological parameters among all the treatments. These physiological activities were responsible for higher root shoot length in T_4.

Introduction

Environmental pollution is a worldwide problem. Today, the attention of the whole world is mainly focused on the problems which result in undesirable changes in the physical, chemical and biological characteristics of air, water and soil, ultimately affecting human life and the lives of animals and plants (Misra & Dinesh, 1991). As sewage water is really harmful for the plants as well as human being so, treatment of sewage water is very necessary. Living organisms have been more widely used in detecting the complex effects of environmental pollution (Javed, 2002; Mushtaq & Khan, 2010).

Phytoremediation, also referred as botanical bioremediation (Chaney *et al.,* 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt *et al.*, 1998). The ability of aquatic plants to accumulate pollutants from water is of great importance (Sinha *et al.*, 1996).Aquatic plants growing in polluted water absorb pollutants which enter into the food chain, posing a serious threat to human health (Anon., 1978).

Water hyacinth, *Eichornia crassipes* Solms, has drawn attention as a plant of rapid growth and high biomass production, (Knipling *et al.*, 1970) and capable of removing pollutants from domestic and industrial waste effluents. It has the ability to remove pollutants and can be used for phytoremediation purpose. While it is considered an aquatic weed in Pakistan (Marwat *et al.*, 2010; Khan *et al.,* 2010).

Nutrient availability in water is often hypothesized to be an important factor in controlling decomposition rate as well as the photosynthetic rate of plants (Enrı´quez *et al*., 1993). The impacts of nitrogen (N) and phosphorus (P) fertilization on the decomposition of aquatic plants, but the response observed is sometimes positive (Peterson *et al*., 1993) and sometimes neutral (Villar *et al*., 2001*).*Decomposition by plants is a rich source of nutrients in nutrient poor environment if nutrient contents of water are lowered than *E .crassipes* have the ability to decompose organic matter present in the waste water. *E. crassipes*, free-floating species, can often be found in nutrient-rich waters; as it obtain its nutrient from water so it is used for the treatment of sewage water.

The objectives of present study were to check the physiological parameters such as the rate of photosynthesis, and transpiration rate of *E. crassipes* while growing in different concentrations of municipal waste water and also to evaluate the potential of the *E. crassipes* as a phytoremediation plant for the treatment of municipal waste water.

Materials and Methods

The experiment was carried out in the Botanic Garden, GCU Lahore. Plant samples (visibly of same size) and water samples were collected from GCU Botanical Garden and the sewage channel (Lawrence Road, Lahore) respectively. There were total 5 treatments (Table 1) and two replicates for each treatment were used so, total 10 pots were used. After the duration of four weeks the plants were harvested. Growth parameters were studied as Root and Shoot length, No. of fresh leaves, No. of necrotic leaves and No. of baby plants per plant. Physiological parameters like photosynthesis rate ($\mu Mm^{-2}s^{-1}$), transpiration rate ($mMm^{-2}s^{-1}$) were studied with the help of "Infra Red Gas Analyzer (IRGA)" LCA4.

Table 1. Different dilutions of waste water.

Treatments	Sewage water % age	Fresh water (ml)	Sewage water (ml)
T0	0	100	0
T1	25	75	25
T2	50	50	50
T3	75	25	75
T4	100	0	100

After the harvesting of plants each dilution of municipal waste water was carried into the SDSC laboratory of GCU Lahore and stored at 4^0C in cooling machine (CAMLAB). The pH meter (INOLAB, Level1-WTW) was used to determine pH. The electrical conductivity (EC) was determined by conductivity meter (CYBERSCAN con10). Total suspended solids (TSS), total organic matter (TOM) and total dissolved solids

(TDS) were estimated gravimetrically. Carbonates, Bicarbonates, Chloride, Calcium and Magnesium were calculated by titration method. Potassium was determined by the Flame photometer (AFP 100 Flame Photometer). Chemical Oxygen Demand (COD) was determined by COD reactor (Lovibond ET 108). Biological Oxygen Demand (BOD) was determined with the help of BOD sensor (Lovibond). Nitrogen and phosphorus in different samples were determined according to Jones (1991) and Harwood *et al*., (1969), respectively. Statistical analysis of the data was carried out by using software package Co-stat version 3.03.

Results and Discussion

Municipal waste water comprises of different organic and inorganic nutrients which were absorbed by the plants. Analysis of water samples were done on two stages; first before the introduction of the plants and second after introduction of plants into the waste water, by doing so the total amount absorbed by the plants were obtained. According to the table 2 there is a comparison among the municipal waste water, tube-well water and NEQS values. pH of sewage water is about 7.41 and pH of tube-well water is about 6.99, when these values were compared with the standards which ranges 6-9 it become clear that pH values of both the treatment came into the permissible limits. In other words these were suitable for the growth of plants. Likewise when other parameters TSS, TDS and TOM were compared with the standard values it became evident that the values of these parameters were lower than that of standard values so these were also in the range of permissible limit.

Table 2. Analysis of municipal waste water and tube well water and their comparison with NEQS.

Parameters	Waste water	Tub-well water	NEQ'S values
pH	7.41 ± 0.11	6.99 ± 0.12	6-9
EC (µs)	86.0 ± 0.52	79.3 ± 0.40	<1.25ds/m
TDS (mg/l)	0.0019 ± 0.001	0.0012 ± 0.002	3500
TSS (mg/l)	0.0016 ± 0.004	0.0001 ± 0.00001	150
TOM (mg/l)	0.0002 ± 0.0001	0.0001 ± 0.00001	-
BOD (mg/l)	227.41 ± 0.43	-	80
COD (mg/l)	350 ± 0.52	2.3 ± 0.0001	150
Cl^-1 (mg/l)	326 ± 0.60	159.75 ± 0.34	1000
Ca^{+2} (mg/l)	147.4 ± 0.23	42.0 ± 0.02	-
Mg^{+2} (mg/l)	64.8 ± 0.31	28.8 ± 0.002	-
HCO_3^{-1} (mg/l)	317.2 ± 0.23	213.5 ± 0.34	-
N (%)	1.62 ± 0.002	0.5 ± 0.001	2.6
P (ppm)	400.0 ± 0.60	159.0 ± 0.12	1.6
K (ppm)	660.0 ± 0.70	135.0 ± 0.30	0.2

Cl^{-1} contents were present in excess amount in the waste water at the start of experiment and were low than that of standard value. Ca^{+2} and Mg^{+2} were also present in the waste water and also in the tube-well water. CO_3^{-2} were absent from the waste water and as well as tube-well water. While HCO_3^{-1} were present in the both solutions. The amount of $HCO_3^{-1}$was reduced after 4 week duration as is absorbed by the plants (Table 3).

NPK analysis of water sample as well as of plant sample was done. At the start of the experiment the amount of NPK were a little bit high in the water solution but at end of experiment these were reduced to much extent because of absorbance of the plant body. COD and BOD were also observed it was analyzed that BOD was much higher in the Municipal waste water and above the permissible limit. COD was also very high in the municipal waste.

Table 3. Reduction in pollution load after 4-weeks treated with *E. crassipes* in different concentrations of sewage water.

Parameters	Sewage water	Tube-well water	T_O	T_1	T_2	T_3	T_4
pH	7.41 ± 0.02	6.99 ± 0.1	7.02 ± 0.02	7.06 ± 0.01	7.09 ± 0.2	7.20 ± 0.3	7.39 ± 0.32
EC µS	86.0 ± 0.98	79.3 ± 0.4	84.3 ± 0.3	83.1 ± 0.8	83.3 ± 0.55	78.6 ± 0.65	59.3 ± 0.6
HCO_3^{-1} mg/l	317.2 ± 0.8	143.5 ± 0.5	92.2 ± 0.3	106 ± 0.9	117 ± 0.9	198.5 ± 0.9	201 ± 0.99
Ca^{+2} mg/l	147.4 ± 0.32	42.00 ± 0.08	44 ± 0.001	99.9 ± 0.001	103.7 ± 0.2	106.7 ± 0.01	116 ± 0.5
Mg^{+2} mg/l	64.8 ± 0.3	28.8 ± 0.001	23.3 ± 0.001	37 ± 0.01	46 ± 0.1	56±0.2	58±0.2
Cl^{-1} mg/l	326 ± 0.03	34.75 ± 0.001	45.1 ± 0.02	98.3 ± 0.01	102.2 ± 0.07	106.4 ± 0.43	123.5 ± 0.4
TSS mg/l	0.0016	0.0001	0.0012	0.0005	0.0007	0.00092	0.0002
TDS mg/l	0.0019	0.0012	0.0001	0.0001	0.0001	0.0002	0.0003
TOM mg/l	0.0002	0.0001	0.000042	0.00005	0.00006	0.0001	0.0002

According to table 4 the amount of NPK present in the plants treated water at the start of experiment was much higher but at the end of experiment these values were reduced to considerable amount while the amount present in the sewage water without plant did not show much difference. The reason for the reduction was that plants when introduced into these polluted water they got absorb nutrients from water and stored them in their organs in the form of biomass.

Effect of waste water was also observed on the plants parameters like No. of fresh leaves no. of necrotic leaves, Root and Shoot length of the plants and vegetative reproduction of the plant growing in sewage water dilutions results are in Table 5.

Table 4. N, K, P, BOD and COD values after 4-weeks treated with *E. crassipes* in different dilutions of sewage water.

Parameters	T_0	T_1	T_2	T_3	T_4
N%	0.7	0.14	0.28	0.42	0.59
P(ppm)	178	196	236	290	289
K (ppm)	139	245	303	290	475
BOD (mg/l)	37.7	99.8	112	105.5	120.9
COD (mg/l)	5.3	30.2	58	76.5	103

Table 5. Effect of sewage water on root and shoot length of *E. crassipes*.

Treatments	Root length (cm)	Shoot length (cm)
T_0	27.70a	15.50c
	± 4.34	± 4.04
T_1	28.50a	22.20bc
	± 4.20	± 7.13
T_2	30.5a	29.70ab
	± 3.69	± 8.80
T_3	31.25a	29.00ab
	± 3.69	± 3.74
T_4	32.20a	37.00a
	± 3.77	± 7.34
LSD	**6.02**	**9.83**

Mean followed by different letters in the same column differ significantly at p=0.05 according to the Duncan's Multiple Range Test

Root and Shoot length of plants of different treatments was compared, according to table 5 overall increase pattern was in the order of (T_0) to (T_4). The reason was again that plant showed increase in root shoot length under high nutrient concentration. The difference between (T_0) and (T_4) is significant while difference between (T_0) and (T_1) is none significant.

Table 6 shows the comparison among no. of fresh leaves, no. of necrotic leaves and reproductive behavior of plants per treatment. According to this table no. of fresh leaves in treatment (T_0) was lower among other treatments. While making a comparison among all the five treatments it is evident from table that increase in the no. of fresh leaves are in the order of T_0 to T_4. Necrotic leaves are basically yellow leaves of plants from which had suffered from chlorophyll deficiency. When rate of necrotic leaves are compared, it is reversed and is in the order of T_4 to T_0 the maximum no. of necrotic leaves found in treatment (T_0) while the minimum no. found in the treatment (T_4). In the case of reproductive behavior no. of baby plants increase from T_0 to T_4 as the most significant mean value is observed for the treatment (T_4) while in the case of T_0 no. baby plant was observed.

Table 6. Effect of waste water on the no. of fresh leaves, no. of necrotic leaves and vegetative reproduction of *E. crassipes*.

Treatments	Fresh leaves	Necrotic leaves	Vegetative reproduction (no. of baby plants)
T_0	6.7b	4.0a	0.0c
	± 2.5	± 1.1	± 0
T_1	8.7b	3.5a	0.25c
	± 1.7	± 0.5	± 0.5
T_2	10.7b	3.0a	1bc
	± 4.3	± 1.1	± 0.8
T_3	18.7a	2.7a	1.75b
	± 2.6	± 0.5	± 0.9
T_4	19.2a	2.3a	2.75a
	± 6.2	± 0.9	± 0.5
LSD	**6.64**	**1.38**	**0.98**

Mean followed by different letters in the same column differ significantly at p=0.05 according to the Duncan's Multiple Range Test

Table 7 shows changes occur in the physiological aspects of the plant when introduced in different concentrations waste water. Analysis of first week shows rate of photosynthesis and rate of transpiration were high at the start as shown by their mean significant values. From the table it is evident that high value of the photosynthetic rate was observed for the treatment (T4) and in the case of transpiration rate it is repeated and highest value observed for the treatment (T4). The reason for this was that at the first week of experiment the municipal waste water contains more nutrients which were useful for plant growth and the rate of photosynthesis and rate of transpiration becomes greater because of the presence essential nutrients like nitrogen, phosphorus and potassium. But the treatment (T0) shows little increase in photosynthetic as well as transpiration rate because there were not enough nutrients in the water. The pattern of the increase in photosynthetic rate and transpiration rate is same to the increase of concentration of sewage water. But as the days passed and plants used nutrients there were shortage of nutrients in the medium, So at the last week of experiment there were plenty of nutrients in the water that's why plant showed less photosynthetic rate and as well as transpiration rate. But again the rate of increase in the last week is same that was noted at the start (T0) to (T4). When we compare the mean significant value for first week and last week we came to know that first week values of parameters were very high as compared to the last week value (Zhu *et al*., 1999).

According to the table 8 Nitrogen, Phosphorus and Potassium were observed in the plant samples. Municipal water already had considerable amount of NPK before its application to the plants. NPK are important nutrients for the plant growth and as well as the rate of photosynthesis and rate of transpiration were based on them, so in excess amount of these nutrients plant showed excellent growth. Analysis of plants showed that maximum amount of these nutrients was found in T4 plants and very less amount was found in T0 plants. This was the reason why T4 plants showed maximum growth rate. The rate of the NPK increases from T0 to T4 (Prasad and Freitas, 2003).

E. crassipes showed excellent growth in the sewage water and also cut down considerable pollutants load in it (Gadallah, 1996). Results of this study are also confirmed by the several other workers (Zhu *et al*., 1999).

Table 7. Effect of sewage water on the physiological parameters of *E. crassipes*.

Treatments	1st Week		Last weeks	
	A ($\mu Mm^{-2}s^{-1}$)	E ($mMm^{-2}s^{-1}$)	A ($\mu Mm^{-2}s^{-1}$)	E ($mMm^{-2}s^{-1}$)
T_O	18.68c ± 3.23	1.52c ± 0.84	15.68b ± 2.25	3.28b ± 0.08
T_1	20.94bc ± 6.37	2.37bc ± 0.28	16.47b ± 1.4	3.47b ± 0.66
T_2	27.5b ± 0.45	2.87b ± 0.1o	17.7b ± 2.15	4.31ab ± 0.70
T_3	26.84b ± 3.70	3.19b ± 0.14	25.74a ± 2.9	4.39ab ± 0.41
T_4	33.63a ± 2.34	4.41a ± 0.141	26.23a ± 3.8	5.32a ± 0.65
LSD	**7.20**	**1.12**	**4.38**	**1.37**

Mean followed by different letters in the same column differ significantly at p=0.05 according to the Duncan's Multiple Range Test A= Rate of Photosynthesis E= Rate of Transpiration

Table 8. NPK uptake by *E. crassipes* from sewage water.

Treatments	Nitrogen %	Phosphorus (ppm)	Potassium (ppm)
T0	0.85c ± 0.021	2.17e ± 0.212	444.5b ± 10.6
T1	0.96c ± 0.028	2.82d ± 0.049	448.5b ± 4.94
T2	1.23b ± 0.35	3.5c ± 0.141	612a ± 16.97
T3	1.32b ± 0.141	3.8b ± 0.070	585a ± 93.3
T4	1.47a ± 0.091	4.9a ± 0.141	665a ± 7.07
LSD	**0.126**	**0.302**	**121.41**

Mean followed by different letters in the same column differ significantly at p=0.05 according to the Duncan's Multiple Range Test

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(Received for publication 12 February 2011)