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Increasing Nitrogen Uptake and Removal Efficiency of *Eichhornia crassipes* from Domestic Sewage through Dilution Culture Study

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Abstract: Effluent from an urban pond receiving domestic sewage for many decades was investigated. The chemical analysis of sewage of the pond revealed very high concentrations of BOD, COD, total N, NH_4^+–N at alkaline pH with very low concentration of DO and caused stunted growth of *Eichhornia crasspes.* Hence, biomass productions by *E. crassipes* in different rate of diluted cultures, sewage with tap water were studied. The plant recorded significantly ($p < 0.01$) increased biomass production, N uptake efficiency as well as N removal efficiency in two times dilution than undiluted sewage culture. Analysis of variance revealed significant variation among different rate of dilutions for net biomass production ($F = 58.13$), N uptake efficiency ($F = 89.80$) and N removal efficiency ($F = 18.61$) by *E. crassipes* at the end of two months culture study.

Key words: Domestic sewage, *E. crassipes* biomass, N uptake, N removal.

Introduction

Laxmital pond in Jhansi city is heavily polluted by unrestricted discharge of domestic sewage from communities, since many decades. It is largely infested with few selected macrophytes specially *Eichhornia crassipes* **(**Mart.) Solms, being dominant. Aquatic plants growing in wastewater treatment ponds assimilate nutrients and thus subsequent harvest of the plant biomass recovers the nutrients from the waste water (Giri et al., 2012). Phytoremediation of waste water is a function of several factors (Olguin et al., 2003), among the most relevant are the nutrient removal potentiality of the plant employed (Boyd, 1970; Tripathi and Upadhya, 2003), growth and productivity of the plants (Wolverton and McDonald, 1979; Singhal and Rai, 2003) and environmental conditions of the growth medium (Imaoka and Teranishi, 1988; Caisedo et al., 2000). Water hyacinth (*E. crassipes*) due to its rapid growth (Abbasi and Ramasami, 1999) has been widely employed for treatment of a variety of waste waters. Singhal and Rai (2003) reported that *E. crassipes* exhibited inability to grow in undiluted effluent of pulp and paper mill and highly acidic distillery effluents, but grew well up to 40% effluent concentration.

Despite various studies on nitrogen removal rates by *E. crassipes,* the effect of high strength domestic sewage on N uptake and removal efficiency in particular, being an important parameter has not received due attention of the investigators. Therefore, in the present investigation in the first attempt the sewage of Laxmital pond and its aquatic vegetations were analyzed. As a second attempt, *E. crassipes* was cultured in different concentrations of sewage to asses its productivity and N uptake behaviour, and to evaluate whether the plant exhibits any potentiality to maximize productivity, N uptake and removal efficiency in domestic sewage.

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Materials and Methods

Experimental Design

First *E. crassipes* were collected from Laxmital pond and allowed to stabilize for 21 days in tap water. Before setup of experiment, representative (Laxmital pond) effluent and tap water (control) were collected separately in syntax containers followed by either immediate analysis or storing at 4 °C for analysis by next day. The details of physicochemical analysis of sewage and control are recorded in Table 1. Circular (vol. 94 L: 64 cm diameter × 38 cm depth) plastic culture tubs were used for study. Tap water of underground source, as control, was designed to support essential nutrients for minimum growth with comparison to domestic sewage. A constant water level was maintained during the study by replacing it with distilled water. In five bench-scale culture tubs (D_1, D_2, D_3, D_4 and D_5), each with three replicates, sewage was mixed with tap water in five different ratios. The dilution of sewage and tap water in culture tubs were: D_1: 90 L sewage alone; D_2: 45 L sewage + 45 L tap water; D_3: 30 L sewage + 60 L tap water; D_4: 15 L sewage + 75 L tap water; and D_5: 90 L tap water alone (control). After stabilization in tap water, healthy plants were selected, and as an inoculum, three plants were weighed (fresh weight) before being inoculated into each culture tub. Dry weight and nitrogen content were also estimated from duplicate samples for each inoculum. All the culture tubs were kept in the garden under identical ambient conditions for a period of two months. Standing fresh biomass, dry weight and nitrogen content for each culture tub was estimated separately. Fresh weight of the plant biomass was determined after removing the excess water over a filter paper for 10 minutes. After removal of standing crop, treated water in each tub was analyzed for TKN. Representative samples (whole plant) of each experimental tub was oven dried (70 °C) to constant weight separately, ground into powder and packed in airtight polyethylene bags for chemical analysis.

Nitrogen Uptake Efficiency

The nitrogen (N) uptake efficiency of *E. crassipes* in each tub was calculated separately by using the following formula:

$$E_u\,(\%) = \frac{N_1}{N_2} \times 100 \qquad (1)$$

where E_u = N uptake efficiency of the plant; N_1 = total N uptake by the plant over the period of culture and N_2 = initial total N content of the culture medium.

Table 1: Mean (± SE, *n* = 3) physicochemical properties of sewage, diluted sewage and control used in *E. crassipes* culture

Parameters	*Sewage (undiluted)*	*Diluted sewage*			*Control (tap water)*
	$(D_1)^a$	$(D_2)^b$	$(D_3)^b$	$(D_4)^b$	$(D_5)^a$
pH (0.29)#	8.8 ± 0.06	8.5±0.12	8.2 ±0.10	8.0 ±0.10	7.7 ±0.10
BOD (mgL^{-1})	126.8 ± 6.7	64.4	43.6	22.9	2.1±0.11
COD (mgL^{-1})	584.3 ±30.9	294.1	197.3	100.6	3.8 ±0.20
NH_4^+ N ($mgNL^{-1}$)	74.5 ±3.9	37.6	25.0	12.6	0.2 ±0.02
NO^-_3 ($mgNL^{-1}$)	18.3 ± 2.6	9.8	6.9	4.5	1.2 ± 0.06
Total N ($mgNL^{-1}$)	128.5 ±6.8	65.4	44.6	23.7	2.7 ±0.43
Total P	28.4 ±2.6	14.3	8.3	4.9	0.2 ± 0.03
DO (mgL^{-1})	2.1 ±0.28	ND	ND	ND	6.1±0.32

Variation of atmospheric temperature (°C) during culture experiments

	Minimum (Jan-Feb)	Maximum (Feb-March)	Mean
Temperature (°C) range	8.1	30.5	—
Mean (± 95% confidence limits, *n* = 120)	—	—	18.0 ±1.25

D_1: Sewage (undiluted); D_2: 2 times diluted sewage; D_3: 3 times diluted sewage; D_4: 6 times diluted sewage; D_5: tap water alone; [a]: Initial physicochemical properties of undiluted sewage culture and tap water (control); [b]: Initial physicochemical properties (except pH) of diluted sewage given in the table are calculated based upon the rate of sewage and tap water mixed in different cultures; #: Least Significant Difference $_{(p<0.05)}$; ND: Not detected.

Nitrogen Removal Efficiency

The nitrogen uptake efficiency of *E. crassipes* in each tub was calculated separately by using the following formula:

$$E_r\,(\%) = \frac{(I - F)}{I} \times 100 \qquad (2)$$

where E_r = N removal efficiency of the plant; I = initial total N content of the culture medium and F = final total N content of the culture medium.

Analytical Methods

All the values of chemical analysis were reported on oven dry at 70 °C (mentioned otherwise) weight basis. Methods outlined in standard methods for examination of water and waste water (APHA, 1995) were employed to determine the properties of tap water and sewage vis., pH, BOD, COD, DO, total Kjeldhal nitrogen (TKN) and total phosphorus. The steam distillation method (Allen, 1989) employing MgO with and without Devarda's alloy was used to determine NO_3^- + NO_2^--N and NH_4^+-N respectively, in the sewage and tap water. Total nitrogen in plant sample was determined by semi-micro Kjeldhal digestion technique as outlined in Allen (1989) procedure.

Statistical Analysis

All determinations were carried out in triplicates. Data obtained from this experiment was analyzed statistically using one way analysis of variance (Gomez and Gomez, 1984). Wherever significant F values were obtained, LSD (at $P < 0.05$ and $P < 0.01$) values were used to test the significance between treatment means.

Results and Discussion

Initial Physicochemical Properties of Sewage Mixed Tap Water Cultures

Table 1 summarizes the initial physicochemical properties of sewage mixed tap water culture tubs at five different dilution ratios before use in the experiment. In all treated culture tubs pH ranged between 8.8 and 7.7. The pH value as observed in D_1 culture tub was significantly ($p < 0.05$) increased than all other culture tubs, probably due to high concentration of NH^+_4-N. The observations tend to explain that the main characteristics like BOD, COD, NH_4^+-N and other constituents including TKN, and TP were very high in culture tub D_1 in comparison to the range of these values reported in the literatures for domestic wastewater. These findings were not unexpected as large quantities of these nutrients were accumulated in the pond due to uncontrolled disposal of domestic sewage since many decades. The mean DO concentrations in culture tubs were ranged between 2.1 and 6.1 mgL^{-1}. These results show that the culture was suboxic in undiluted sewage and condition became aerobic after dilution with tap water. There was a wide variation of ambient temperature during two months of culture study, from minimum 8.1 °C in first month to a maximum 30.5 °C in second month, with mean 18.0 °C. This range of temperature is adequate for efficient removal of nutrient and pathogens (Kadlec and Knight, 1996). The data on initial main characteristics (Table 1) in culture tub D_1 also tends to clarify that the medium is rich with excess nutrients whilst the characteristic properties of these parameters in culture tub D_5 depicted it to be a nutrient deficient one. However, this concentration as observed in tap water (D_5) was still higher than those of the usual values in the ground water.

Production of *E. crassipes* Biomass Over the Two Months Culture Study

The biomass productions by *E. crassipes* in five different culture tubs are presented in Table 2. An increasing trend in the final biomass of *E. crassipes* with comparison to their respective initial biomass was observed in all the culture tubs over the study period. Analysis of variance on net production of *E. crassipes* biomass revealed significant variation ($F = 58.13$; $p < 0.01$) among different rate of diluted culture tubs. The mean net biomass produced was 124.4%, 58.3% and 1.2% increase in culture tubs D_2, D_3 and D_4, respectively than culture tub D_1. Biomass was increased well up to culture tub D_3, followed by stabilization in D_4 and sharply decreased in D_5. Net biomass production as observed in culture tub D_1 was significantly reduced than D_2 and D_3, which could be related to significant adverse impact of NH_4^+-N at higher pH.

Furthermore, the presence of complex organic compounds and particularly of volatile fatty acids in high strength sewage effluent might have a strong influence on the microbial rhizospheric community (Olguin et al., 2007) inhibiting plant growth and productivity. However, dilution of high strength domestic sewage in the present study demonstrated 1.2-124.4% increase in *E. crassipes* biomass production, with the maximum production in D_2 and the minimum in D_4 culture tubs. Further, net biomass production as recorded in D_2 was significantly ($p < 0.01$) increased than other culture tubs studied, suggesting the hypothesis that two times dilution of sewage effluent with tap water supported a relatively encouraging culture medium.

Table 2: Biomass (g) production (± SE, *n* = 3) of *E. crassipes* at the end of two months cultures in undiluted sewage, diluted sewage and control (tap water)

Culture type	*Initial fresh biomass (w_1)*	*Final fresh biomass (w_2)*	*Net production (g) $w = (w_2 - w_1)\ w_3$*	*Average biomass produced (g dw day^{-1})*
D_1	118.9 ±6.3	1184.3 ±68.4	105.4±5.6	1.76±0.20
D_2	110.5 ±6.4	2815.2 ±148.9	236.5±12.5	3.94±0.23
D_3	111.0 ±10.7	2011.2 ±116.1	166.9±16.1	2.78±0.27
D_4	116.5 ±6.7	1285.4 ±68.2	106.7± 5.7	1.78±0.17
D_5	114. 2 ±6.0	532.9 ± 30.8	35.6 ±3.4	0.59 ±0.06
ANOVA				
CV(%)	11.5	10.5	13.2	15.8
$LSD_{(p<0.05)}$	ns	299.5	31.2	0.6
F	0.23	84.28	58.13	40.17

W: Net biomass production of *E. crassipes* on oven dry weight basis; dw = dry weight; W_3 : Oven dry weight of *E. crassipes* in percentage (%); CV: Coefficient of variation; LSD: Least significant difference; F: Computed F values; ns: Non-significant.

Nitrogen Uptake by *E. crassipes* Over the Two Months Culture

As presented in Table 3, in all the culture tubs (except D_1) N contents were higher in final plant tissues relative to their initial values. These culture tubs after 60 days resulted in a pronounce enhancements in the final tissue N content in comparison to their initial values. Maximum increase in tissue N content was recorded at D_1 (261.5%) followed by D_2 (237.5%), which gradually declined towards D_4 (60.8%). These values suggest that N content in plant tissue vary significantly with N content in the culture medium. With regard to total N uptake by plants in the individual culture tubs, the dilution of sewage effluent with tap water caused a striking increase (124.3%) in D_2 in comparison with D_1, but had no effect on D_3, which gradually declined towards D_5.

Statistical scrutiny of the data showed that there is a significant ($p < 0.01$) variation in the N uptake characteristics of the individual culture tub. Our hypothesis is that the shift in N values could be related to the differential uptake of N by *E. crassipes* from their respective culture medium (Table 1). It is suggested that the final tissue N content of *E. crassipes* depended on the N content present in the treated culture and the extent of uptake (Imaoka and Teranishi, 1988; Reddy et al., 1991). Furthermore, the N uptake characteristics in culture D_2 is very much in consistent with its biomass production which indirectly revealed that N is fairly effectively taken up by the plant at two times dilution of high strength domestic sewage. *E. crassipes* showed a significant difference for total N uptake and average N uptake per day performance (Table 3): total N uptake ($F = 103.12$; $p < 0.001$) and average N uptake per day ($F = 105.21$; $p < 0.001$) among different treated cultures. The total N uptake at the end, for all the five culture tubs studied shown order: $D_2 > D_1 > D_3 > D_4 > D_5$. A critical observation of the figures for N uptake in the individual culture indicated a direct correlation with their biomass production which could be explained by the fact that N is absorbed by *E. crassipes* during the period of growth and biomass production.

Table 3: Nitrogen (± SE, *n* = 3) uptake by *E. crassipes* at the end of two months cultures in undiluted sewage, diluted sewage and control (tap water)

Culture type	*Initial tissue N content (g kg^{-1})*	*Final tissue N content (g kg^{-1})*	*Total plant N (g) uptake*	*Average N uptake (mgday^{-1})*
D_1	5.2 ±0.28	18.8 ±1.0	1.98 ±0.19	33.1 ±3.2
D_2	4.8 ±0.25	16.2 ±0.9	3.83 ±0.22	63.8 ±3.4.
D_3	4.9 ±0.47	11.6 ±0.61	1.94 ±0.10	32.3 ±1.71
D_4	5.1 ±0.29	08.2 ±0.43	0.88 ±0.19	19.2 ±1.02
D_5	5.1 ± 0.67	1.2 ± 0.16	0.04 ± 0.005	0.7 ± 0.07
ANOVA				
CV(%)	14.7	10.9	14.0	13.0
$LSD_{(p<0.05)}$	ns	2.2	0.4	7.1
F	0.14	96.57	103.12	105.21

N Uptake and Removal Efficiency in *E. crassipes* over 60 Days Culture Study

E. crassipes culture process caused a sharp reduction in TKN contents in all culture tubs at the end with respect to their initial values (Table 4). The reduction in the total TKN content, both in absolute values and relative terms, at the end in culture tubs was in the order $D_1 > D_2 > D_3 > D_4 > D_5$. It is worth mentioning that total tissue N uptake in D_1 was significantly ($p > 0.001$) less than D_2 (Table 3). Thus, a high rate of reduction could not be linked to a single factor (plant uptake), but only to a combination of physicochemical and biological characteristics of the culture medium. Therefore, it is suggested that the greater reduction of N in D_1 culture could be due to higher rate of volatilisation loss of NH_3 at relatively higher alkaline pH (Olguin et al., 2003).

In view of transferring maximum percentage N from the culture medium into plant tissue, the observed N uptake efficiency at the end for culture tubs was in the order $D_2 > D_3 > D_4 > D_1 > D_5$. The N removal efficiency in different culture tubs also demonstrated similar trend: $D_2 > D_3 > D_4 > D_1 > D_5$. The examination also revealed significance difference in N uptake efficiency ($F = 89.8$; $p < 0.001$) and N removal efficiency ($F = 18.61$; $p < 0.05$) among different culture tubs (Table 4). Both N uptake efficiency and N removal efficiency in D_2 was significantly ($p < 0.001$) increased than D_1. Maximum N uptake efficiency by *E. crassipes* in culture D_2 suggested the hypothesis that ideal biochemical and environmental conditions (Imaoka and Teranishi, 1988; Reddy et al., 1991) were prevailed in the culture medium for N uptake. This clearly revealed the positive role of D_2 to maximize N removal efficiency of *E. crassipes* in domestic sewage. This hypothesis is also supported by the highest productivity at the end of culture study. Therefore, it is suggested that highest net productivity with increased N content in plant tissue and subsequent decrease in culture N content at the end of experiment revealed that N is absorbed by plants (Tripathi and Upadhya, 2003), which caused significant increase in N uptake and removal efficiency.

A critical observation of the figures on the N budget after 60 days of culture study revealed that 82.8% of the total N removal is accounted for plant uptake in culture tub D_2. Thus, a high rate of N removal could be linked to addition of more factors than N uptake by the plants. Reddy and Debusk (1985) demonstrated that N uptake by aquatic macrophytes accounted for 18 to 39% of total N removal in summer and 16 to 75% of the total N removal in winter. In the present study N uptake by *E. crassipes* accounted for 32.7% (D_1) to 82.8% (D_2) of the total N removal in early summer. These values are considerably increased in comparison with Reddy and Debusk (1985), though the studies assessing N uptake efficiency of *E. crassipes,* particularly for domestic sewage are scarce.

Conclusions

The present investigation tends to conclude that the diluted sewage cultures of Laxmital pond resulted in significant variations in the nitrogen uptake and nitrogen removal efficiency of *E. crassipes* in comparison with undiluted sewage culture. Two times diluted sewage culture showed pronounced enhancement vis., 3.8 fold and 1.3 fold in nitrogen uptake efficiency and nitrogen removal efficiency than undiluted sewage culture, respectively. However, further work with nitrogen and phosphorous under pond ecosystem is desirable before its field application.

Table 4: Nitrogen uptake and removal efficiency (%) of *E. crassipes* at the end of two months cultures in undiluted sewage, diluted sewage and control (tap water)

Culture type	*Initial culture N (mgL^{-1}) content*	*Final culture N (mgL^{-1}) content*	*N uptake efficiency (E_u)*	*N removal efficiency (E_r)*
D_1	128.5 ±6.8	61.2 ±3.24	17.1 ±0.10	52.4 ±3.03
D_2	65.6 ±3.47	14.2 ±0.75	64.9 ±3.43	78.4 ±7.55
D_3	44.6 ±2.36	17.0 ±0.90	48.3 ±2.56	61.9 ±3.57
D_4	23.7 ±1.25	10.4 ±0.55	41.3 ±2.19	56.1 ±2.97
D_5	02.7 ±0.14	01.7 ±0.09	16.5 ±0.87	37.0 ±2.35
ANOVA				
CV(%)	11.9	12.9	10.2	12.8
$LSD_{(p< 0.05)}$	11.4	4.9	7.0	17.7
F	176.8	223.49	89.8	18.61

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