International Journal of Multidisciplinary Research and Development

International Journal of
Multidisciplinary
Research and Development

Volume: 2, Issue: 10, 75-79
Oct 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 5.742

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Rhizosphere properties of three wetland macrophytes under conditions of varying organic load in the microcosm

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Abstract
The rhizospheric properties of three wetland plants viz. *Canna indica, Typha latifolia* and *Eichhornia crassipes* were analyzed in relation to low and high strength post methanation distillery effluent (PMDE). The experiments were conducted in lab scale microcosms for 15 days in small pots having soil and gravel as substrate in order to mimic wetland conditions. Morphological and anatomical features of root system, root aerenchyma pattern, root oxidase activity were studied along with plant growth parameters like shoot length, total biomass and leaf chlorophyll content. *Typha latifolia* and *Eichhornia crassipes* showed relatively higher root oxidase activity in response to high strength wastewater also the aerenchyma was well developed in both the plants as compared to that of *Canna indica*. All the three plant species tolerated high organic load of the PMDE indicating their suitability for use in treatment wetlands.

Keywords: Rhizosphere, root oxidase activity, aerenchyma, COD, wetland microcosm.

1. Introduction

Constructed wetlands (CWs) are viewed as a cost effective and environment friendly alternative for wastewater treatment to conventional treatment technologies especially in developing countries. Macrophytic plants are a major component of the CW and their roots play an important role in nutrient removal and wastewater treatment (Yang *et al*., 2007) [19]. Plant roots create a favorable habitat for attachment of microorganism, their growth, and decomposition activities (Sinha *et al*., 2007; Muench *et al*., 2007) [13, 11]. Wetland or semi-aquatic species have been reported to have adapted to water logging conditions by either rooting superficially or by developing extensive aerenchyma in the roots (Laan *et al*., 1989a; Visser, Blom & Voesenek 1996a) [9, 17]. The development of aerenchymatous tissues in roots also helps root respiration in root zone hypoxia by transporting atmospheric oxygen to rhizosphere. Yang *et al*., (1994) [20] suggested that roots with strong oxidizing power have broader oxidizing zones around it which eventually affects the redox potential of the wetland substrate (Kludze *et al*., 1994; Vartapetian and Jackson, 1997) [8, 16], resulting in enhanced chemical and microbial processes (Kirk *et al*., 1993; Wang and Peverly, 1999) [7, 18], which favour other processes like nitrification, denitrification and reduction of organic compounds that eventually lead to treatment of wastewater. According to Zhang (1990) [22], the root respiration rates can be reflected by the root oxidation activity that can be analyzed by α-naphthylamine method. Moreover, root characteristics not only influence nutrient uptake but may also have a relationship with photosynthesis of the wetland plants (Tam *et al*., 2003) [14]. All the three plants viz. *Canna indica, Typha latifolia* and *Eichhornia crassipes,* selected for this study are well known wetland species and have extensively been used in CWs and studies on their root characteristics need to be carried out to understand the root zone wastewater treatment in wetlands. In the present study, therefore, root morphology, root anatomy, aerenchyma, root length, shoot length, leaf chlorophyll, biomass and root oxidation activity (ROA) of three wetland plants was investigated and their response with respect to two strengths of wastewater was studied.

2. Materials and Methods

2.1 Plant material and Experimental Set-up: Three wetland plant species viz. *Canna indica, Typha latifolia* and *Eichhornia crassipes* were selected for the experiment. *Canna indica,* which is an ornamental plant, but also grows in wetlands was taken from the university garden, *Typha latifolia*, from a nearby water logged sewage area and *Eichhornia crassipes,* a floating plant was collected from a natural eutrophicated pond. Plants of all the three species were transplanted in circular reactors having soil and gravel as substrate and acclimatized in a greenhouse under conditions of waterlogging for two weeks using tap water, serving as constructed wetland microcosm (CWM). The water was drained out and then the plants were fed with 1L of simulated post methanated distillery (PMDE) wastewater of two strengths, S1 (5000 mg/l COD) and S2 (10,000 mg/l COD) along with a reactor fed with tap water that served as control. Each pot was planted with young plants of approx. same age and size (about 3 week old, 18-20cm height and 8-10 g wt.) with single species in each treatment which was in triplicate. Irrigation was done to maintain waterlogged conditions like wetland and measurements were taken before and at the end of experiment to see the changes in root anatomy, plant growth parameters and root activity.

2.2 Root characteristics Destructive sampling was done 15 days after the treatment and excavated roots were studied visually for their morphological features and maximum length attained by roots was measured.

2.3 Anatomical features Free hand transverse sections of fresh roots of all three plant species before and 15 days after giving wastewater treatment in the microcosm CW were studied. The sections were double stained using fast green and saffranine dyes to stain both hard and soft tissues and were examined under trinoucular microscope equipped with a camera especially to examine development for the aerenchyma.

2.4 Root oxidation activity: Measurement of root activity was done using α-Naphthylamine following Ando *et al.* (1983). After taking out the plants from each CW, roots were washed thoroughly with tap water on a sieve and sponged to remove excess water before weighing. Approx. 1g of root sample was taken for analysis from each plant and immersed in a flask containing 10ml of 20 mg/l α-naphthylamine (α-NA) and 10ml of phosphate buffer (pH 7). The flask containing the roots was air plugged and kept at room temperature in total dark condition, since α-naphthylamine is light sensitive. After 10min., 2 ml of solution was taken out and initial concentration (A1)of α-NA solution was determined after adding 1ml of 1% sulphanilic acid and 1ml of 100mg/l $NaNo_2$ to the sample aliquot and absorbance was read at 530 nm using spectrophotometer. After an incubation period of 2h at room temperature, the final concentration (A2) of α-NA was determined. The root activity was calculated as the amount of the α -NA oxidized by the roots using the equation given by Chen *et al*. (2011) [3]:

Root oxidation activity (mg g^{-1}dry weight $2h^{-1}$) = A1-A2-A0

where, A1 and A2 are the initial and final values of α-NA, respectively, while A0 is the difference in the initial and final values of α-NA sample without root that served as control.

2.5 Plant growth: Maximum shoot length, fresh plant biomass and total chlorophyll content of leaves (estimated by colorimetry following Arnon, 1949) [2] were studied as growth parameters of the three plant species in microcosm wetlands.

3. Results

3.1 Morphological and anatomical features of roots: All the three plant species used in the study are wetland plants but *Canna indica* is supposed to be a facultative upland type of plant. Because it is usually found in non-wetland areas but can also survive in wetlands in natural habitat form (1-33% probability). Whereas, *Eichhornia crassipes* and *Typha latifolia* are both obligate wetland plants because they seem to occur in wetlands almost always (> 99% probability), as described by Tiner R. (1991) [15]. On examining the root system of the three species, it was found that *Canna indica* and *Typha latifolia* possess rhizomatous root system whereas *Eichhornia crassipes* has fibrous root system (Table 1).

Table 1: Morphological and root anatomical features of *Canna indica, Typha latifolia* and *Eichhornia crassipes*

	Canna indica	**Typha latifolia**	**Eichhornia crassipes**
Wetland habitat type	Facultative upland type	Obligate wetland type	Obligate wetland type
Root Type	Rhizomatous root system with fine roots attached	Rhizomatous root system with fine roots attached	Fibrous roots
Aerenchyma (waterlogged condition)	Radial lysigenic	Radial lysigenic	Radial lysigenic

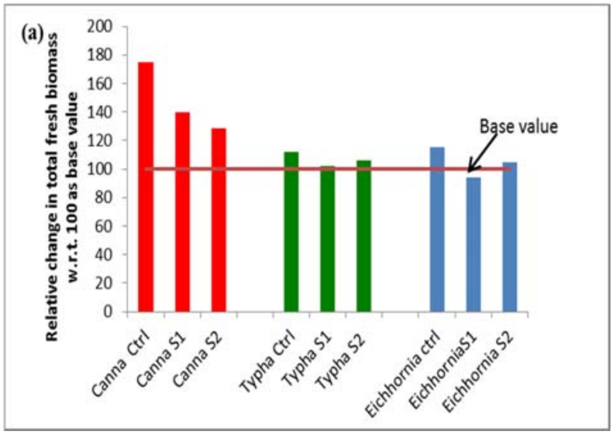
However, since *Canna indica* and *Typha latifolia* also possess many fibrous roots attached to their rhizome hence we may consider them as rhizomatous- fibrous rooted plants. Chen *et al.* (2011) [3] also suggested that there are few rhizomatic plants which are on the transitional line between rhizomatous and fibrous rooted species, considering the large number of thin fibrous roots attached they may be classified under fibrous rooted plant category.

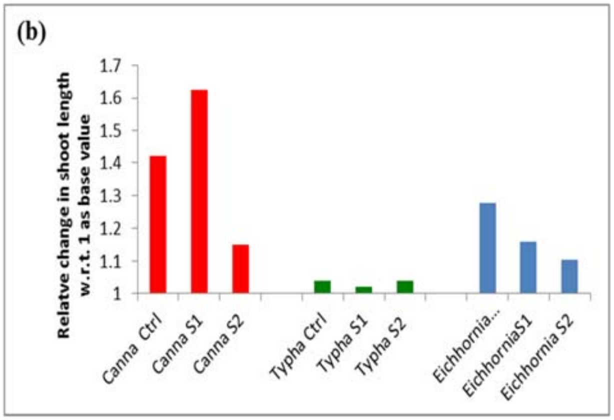
The aerenchyma was observed in all the plant species after they were waterlogged for 15 days. Aerenchyma formation helps in the maintenance of root aerobic respiration for continued energy production, nutrient absorption (Jackson and Armstrong, 1999) [5] and rhizosphere activities (Vartapetian and Jackson, 1997) [16] by providing less resistant pathway for internal atmospheric O_2 diffusion to the root tips (Justin and Armstrong, 1987; Colmer, 2003) [6, 4]. It was found that all the experimental plants possessed radial lysigenic type of aerenchyma, which is again a characteristic feature of fibrous rooted plants (Chen *et al*., 2011) [3]. The aerenchyma observed in these plants on exposure to different strengths of wastewater were not much distinctly different, therefore, the anatomical features of only control plants showing aerenchyma have been shown here. It was observed that both *Eichhornia crassipes* and *Typha latifolia* had fully developed and distinct radial lysigenic type aerenchyma. But in *Canna indica,* aerenchyma development was in limited regions and was not so distinct. This may be because of the reason, that *Canna sp*. was transplanted from a non-wetland area to the water logged experimental CWs, therefore, it was in a transitional stage of developing its aerenchyma. However, radial type cell lysis can be seen forming already (Fig 1). In case of both other plant species, they were transplanted from already water logged condition, hence they show well developed prominent aerenchyma.

Canna indica *Typha latifolia* *Eichhornia crassipes*

Fig 1: Root aerenchyma in the three plant species in waterlogged conditions

3.2 Plant Growth parameters: Length measurements of above and below ground plant parts, total biomass and total leaf chlorophyll content were taken into consideration as plant growth parameters. Fresh plant biomass seemed to increase in all plant species by the end of experiment except for *Eichhornia sp.* which experienced a slight dip in its biomaas at low strength treatment. The relative change in biomass w.r.t. initial biomass is shown in Fig 2 (a). An increase in shoot length was noted in case of all plant species (at both treatments S1, S2 and control) by the termination of experiment. *Canna sp*. was found to have increased in length more when compared to other two plants, while *Typha sp.* increased just about 3-4% (average) by the end of experiment (Fig. 2(b)). A slight increase in root length was also observed for these plant species, except for *Typha,* which recorded a slight decrease in length of the longest root that may be explained by the reason suggested by Marschner (1995) [10] that after oxygen depletion, many old roots die and numerous new roots with well-developed aerenchyma can be formed closer to the soil surface or at the stem. Change in chlorophyll content by the end of experiment was very minor in case of *Canna sp.* while a marginal increase was noted in two other species, at both treatments (Fig 2 (c)). These observations were in accordance to the findings of Pezeshki *et al.* (1993) [12] who worked on marshhay cordgrass (*Typha sp.*) and suggested that chlorophyll content may not get largely affected by hypoxia.





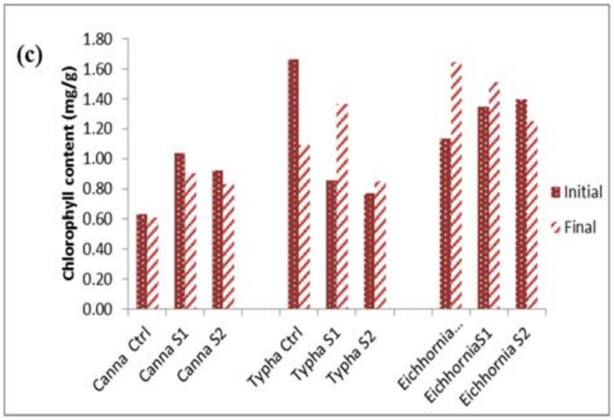


Fig 2: Change in plant growth parameters: a) Total fresh biomass b) Longest shoot length c) Total Chlorophyll content

3.3 Root oxidation activity (ROA) It was observed that ROA of all three species in CW microcosms was high than in natural conditions. At higher strength of effluent, *Eichhornia sp*. was found to have highest root activity followed by *Typha sp.* and *Canna sp.* In case of *Canna sp.* it was noted that root activity was higher in control than in S1 and S, while in *Typha sp.* root activity did not vary significantly in S1 and control. In both *Eichhornia sp*. and *Typha sp.*, ROA increased when higher wastewater strength (with greater COD) was given in the microcosm CW.

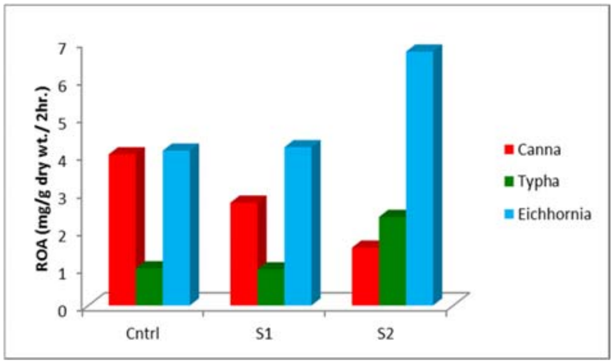


Fig 3: Root oxidase activity of plants in control, S1(COD= 5000mg/L) and S2(COD= 10,000mg/L) reactors.

4. Discussion

Many researchers have indicated that functional characteristics of plants especially in regard to their roots play a very significant role in their nutrient absorption and eventually in the overall efficiency of a constructed wetland. In the present study it was observed that all three selected plant species tolerate both the effluent strengths of 5000 and 10,000mg/L organic load. *Eichhornia sp.* was found to have well developed aerenchyma and higher root oxidation activity than the other two species. Moreover, with increasing wastewater strength, its root activity increased.

The aerenchyma in roots helps to reduce root zone hypoxia by transporting oxygen to rhizosphere. Roots with strong oxidizing power have better oxidizing zones around it which eventually affects the redox potential of the wetland substrate (Kludze *et al*., 1994; Vartapetian and Jackson, 1997) [8, 16], facilitating enhanced microbial degradation of pollutants and mineralization (Wang and Peverly, 1999) [18], and lead to reduction of organic load of wastewater.At higher organic load, there is increase in nutrient concentration, which increases the root oxidation activity in *Eichhornia*. Similar trend was observed by Yuan and Chang (1978) [21] in lowland rice roots. *Typha sp.* also showed greater ROA values in response to high organic load in the feed water, though to slightly less extent. However, contrastingly in case of *Canna,* root oxidation activity decreased with increasing wastewater strengths. This could be due to the fact that *Canna* had much less developed aerenchyma than the other two species.

However, *Canna* showed an increase in biomass and shoot length, suggesting that it has efficiently tolerated both the strengths of wastewater. A dip in chlorophyll content in control pot of *Typha sp.* was observed, whereas its total biomass and shoot length seemed to increase. This suggests that photosynthetic pigments alone may not necessarily result in increase in the overall growth of waterlogged plants (Tam *et al*., 2003) [14]. However, good plant growth and biomass in the presence of distillery effluent seem to be better indicators of their tolerance to organic pollution, whereas root zone activity of the plants determine their usefulness in treatment wetlands.

5. Conclusion

Root oxidation activity, one of the important physiological aspects of plant roots is related to the aerenchyma formation in roots. *Typha latifolia* and *Eichhornia crassipes* displayed higher ROA and well developed aerenchyma at both high and low concentrations of PMDE. *Eichhornia crassipes*, which generally has been used as floating plant also shows possibility of being used as a rooted plant, as indicated by successful establishment and growth of the plant in waterlogged soil. Suitability of using these plants in treatment wetlands is shown by their rhizosphere characteristics favoring oxidizing environment in the root zone to facilitate degradation of organic matter, good plant growth and tolerance in the microcosm wetlands fed with the distillery effluent.

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