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Root Soil (R/S) Ratio in Plants used for Phytoremediation of Different Industrial Effluents

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Abstract The study aims to find the variation of rhizhosphere microbes under various industrial effluents of organic and inorganic origin for phytoremediation. Four different types of effluents (dairy, chemical, rare earth, latex) and two types of plants (emergent, floating) were used. The root microbes were found highest in roots of emergent plants than the floating plants. The increase in biomass was highest in dairy effluent (organic) in emergent plants. The study provides a candidate species for the phytoremediation of different types of effluents.

Keywords: *rhizosphere, emergent plants, floating plants, phytoremediation, effluents*

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1. Introduction

The most applicable technology using phytoremediation strategy is constructed wetland technology (CWs). Besides water quality improvement and energy savings, CWs have other features related to the environmental protection such as promoting biodiversity, providing habitat for wetland organisms and wildlife, serving climatic [1]; hydrological functions, heavy metal bioaccumulation and biomethylation [2]. Constructed wetlands are artificial wastewater treatment systems consisting of shallow ponds or channels which have been planted with aquatic plants and which rely upon natural microbial, biological, physical and chemical process to treat wastewater. Constructed wetlands have been used to treat a variety of wastewater including urban runoff; municipal, industrial, agricultural and acid mine drainage. For some applications, they are an excellent option because they are low in cost and in maintenance requirements, offer good performance and provide a natural appearance; if not more beneficial ecological benefits.

Wetland plants are very important structural components of wetlands; their various adaptations allow competitive growth in saturated or flooded soils. These adaptations include one or more of the following traits: lenticels (small openings through leaves and stems) that allow air to flow into the plants; aerenchymous tissues that allow gaseous convection throughout the length of the plants, which provides air into plant roots; adventitious roots for absorption of gases and plant nutrients directly from the water column and extra physiological tolerance to chemical by-products resulting from growth in anaerobic soil conditions [3]. Usually respiration of rhizosphere soil is higher than respiration of bulk soil because, in addition to microbial respiration of soil organic carbon there is the contribution of root respiration and microbial decomposition of rhizo-deposition. Enzyme activity is generally higher in rhizosphere than in bulk soil, as a result of a greater microbial activity sustained by root exudates or due to the release of enzymes from roots [4].

In any wetland, the ecological food web requires microorganisms to function in all of its complex transformations of energy. In a constructed wetland, the food web is fueled by influent wastewater, which provides energy stored in organic molecules. Microbial activity is particularly important in the transformations of nitrogen into varying biological useful forms. In the various phases of the nitrogen cycle, for example, different forms of nitrogen are made available for plant metabolism, and oxygen may be either released or consumed [5]. Phosphorus uptake by plants also is dependent in part on microbial activity, which converts insoluble forms of phosphorus into soluble forms that are available to plants. Microbes also process the organic compounds and release carbon dioxide in the aerobic areas of a constructed wetland and a variety of gases (carbon dioxide, hydrogen sulfide and methane) in the anaerobic areas. Plants, plant litter and sediment provide solid surfaces where microbial activity may be concentrated.

2. Materials and Methods

2.1. Experimental setup

The constructed wetlands were created by using plastic tubs with 100 x 45 x 45 (cm) dimensions. The bottom of the tub was filled with gravel (3 cm) above which wetland soil (5 cm) is filled up. The experimental plants (*Typha latifolia* (L.), *Eichhornia crassipes* (Mart.) soloms, *Salvinia molesta* (D. Mitch), *Pistia stratiotes* (L.), *Lemna minor and Azolla pinnata*) were collected from the wetlands near the laboratory. The plants were cleaned using distilled water and kept for ten minutes in potassium permanganate solution (1%). The plants were initially subject to acclimatization in tanks containing fresh water for one month and the second generations of the plants were used for the trace metal treatment. Approximately 250g (Fresh Weight) of the second generations of the plants were collected, cleaned, blotted and planted in the constructed wetlands.

Approximately 10 liters of raw effluent from four different type of industries (Dairy, Chemical, Latex processing and rare earth processing) were collected and brought to the laboratory in plastic containers Ten liters of the different dilutions (100%, 50% and 25%) of the effluent were prepared and were then transferred to plastic tubs. For each experimental set, two controls were maintained with ten liters of well water and 10 liters of effluent respectively. The entire constructed wetlands (CWs) containing macrophytes were placed in the natural environment. Triplicate of each experimental setup and a control using fresh water was also maintained.

2.2. R/S Ratio

The R/S ratio of microbes was calculated by dividing the microbial population in the rhizosphere area by microbial population in the nonrhizosphere area.

2.3. Microbiological Analysis

For microbiological analyses, 100 ml water samples were collected from the constructed wetland treatment systems. Plate count agar (tryptone glucose yeast agar) is used for pour and spread plate methods. The plates inoculated and incubated at 350C for 48 hours. During incubation, humidity is maintained within the incubator so that plates will have no moisture weight loss greater than 15%. The colonies were counted after incubation. Microbiological parameters analysed (CFU) using standard methods [6].

$$\text{CFU / ml} = \frac{\text{colonies counted}}{\text{Actual volume of samples in dish (ml)}}$$

3. Results

3.1. R/S Ratio

In Rare Earth Factory effluent, the initial R/S ratio was highest in *Typha* sp. based CWs (4.55) than the other CWs. After the retention time of 15 days also R/S ratio was highest in *Typha* sp. based CWs (5.31).The lowest R/S ratio was in *Lemna* sp. based CWs. The final R/S ratio was higher than the initial in all plant based CWs (Table 1). In case of Latex effluent, the highest R/S ratio after retention period was in *Typha* sp. based CWs (5.96) followed by Pistia sp. based CWs (4.29).The lowest value was in *Salvinia* sp. based CWs. The initial R/S ratio was also higher in *Typha* sp. based CWs (4.40). In case of floating macrophytes, the initial value was highest in *Pistia* sp. based CWs (Table 2).

Table 1. R/S ratio of plants treated with Rare Earth Factory effluent

Plants	Microbial population Before Treatment (BT)		Microbial population After Treatment (AT)		R/S ratio (BT)	R/S ratio (AT)
	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)		
Typha sp.	764	168	988	186	4.55	5.31
Pistia sp.	286	86	386	112	3.33	3.45
Salvinia sp.	397	132	528	166	3.00	3.12
Lemna sp.	274	121	406	152	2.26	2.67
Eichhornia sp.	443	140	648	196	3.16	3.31
Azolla sp.	296	94	368	110	3.15	3.35

CFU – Colony forming units

Table 2. R/S ratio of plants treated with latex factory effluent

Plants	Microbial population Before Treatment (BT)		Microbial population After Treatment (AT)		R/S ratio (BT)	R/S ratio (AT)
	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)		
Typha sp.	784	178	1288	216	4.40	5.96
Pistia sp.	316	96	566	132	3.29	4.29
Salvinia sp.	427	162	438	156	2.64	2.81
Lemna sp.	286	141	546	172	2.03	3.17
Eichhornia sp.	443	160	768	216	2.77	3.56
Azolla sp.	336	106	398	120	3.17	3.32

CFU – Colony forming units

In dairy effluent also the initial R/S ratio was higher in *Typha* sp. based CWs (4.85). The lowest value was recorded in *Lemna* sp. based CWs (2.11). The R/S ratio after treatment was the maximum in *Typha* sp. based CWs (6.60). Among the floating macrophytes, the maximum value was in *Eichhornia* sp. based CWs (3.83) and the lowest was in *Lemna* sp. based CWs (Table 3). In Titanium Factory effluent, the highest initial and final R/S ratio was in *Typha* sp. based CWs i.e.4.48 and 9.16 respectively. In case of floating macrophytes, the highest initial R/S value was in Pistia sp. based CWs (3.51) followed by *Azolla* sp. based CWs (3.50). The highest final R/S ratio among floating macrophytes was in Lemna sp. based CWs (4.71) and the lowest in *Azolla* sp. based CWs (Table 4).

Table 3. R/S ratio of plants treated with dairy effluent

Plants	Microbial population Before Treatment (BT)		Microbial population After Treatment (AT)		R/S ratio (BT)	R/S ratio (AT)
	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)		
Typha sp.	864	178	1466	222	4.85	6.60
Pistia sp.	326	98	466	142	3.33	3.28
Salvinia sp.	467	132	648	186	3.54	3.48
Lemna sp.	298	141	516	182	2.11	2.84
Eichhornia sp.	513	162	788	206	3.17	3.83
Azolla sp.	316	102	408	120	3.10	3.40

CFU – Colony Forming Units

Table 4. R/S ratio of plants treated with Titanium Factory effluent

Plants	Microbial population Before Treatment (BT)		Microbial population After Treatment (AT)		R/S ratio (BT)	R/S ratio (AT)
	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)	Rhizosphere area (CFU)	Non-rhizosphere area (CFU)		
Typha sp.	654	146	788	86	4.48	9.16
Pistia sp.	274	78	316	82	3.51	3.85
Salvinia sp.	377	126	416	106	2.99	3.92
Lemna sp.	254	111	386	82	2.29	4.71
Eichhornia sp.	438	144	543	116	3.04	4.68
Azolla sp.	266	76	312	82	3.50	3.80

CFU – Colony forming units

3.2. Changes in plant biomass

3.2.1. Changes in Plant biomass in Control

The fresh weights of plants were taken before introduction into constructed wetland system. The increase in biomass in control was relatively lower than the treated plants. *Eichhornia* sp. showed maximum increase in biomass. The emergent species showed only 25 percentage increase in the fresh weight.

3.2.2. Changes in Plant Biomass Treated with Rare Earth Factory Effluent

Experimental plants with Rare Earth Factory effluent showed the maximum increase in biomass in the case of *Pistia* sp. with 52 per cent increase. The lowest was recorded in the case of floating macrophytes, *Lemna* sp. with 18 per cent increase.

3.2.3. Changes in Plant Biomass Treated with Latex Factory Effluent

The increases in biomass of plants were noted with latex effluent. The highest percentage increase was found in *Azolla* sp. with 56 per cent increase. The lowest was recorded in the case of *Salvinia* sp. with 28 per cent. The emergent plant species *Typha* sp. showed only 36 per cent of increase.

3.2.4. Changes in Plant Biomass Treated with Dairy Effluent

In case of dairy effluent, highest percentage increase was noted in case of *Typha* sp., the emergent plant used. *Lemna* sp. showed the least increase in biomass with 28 per cent increase.

3.2.5. Changes Plant Biomass Treated with Titanium Factory Effluent

Titanium Factory effluent showed the least increase in plants. The maximum increase in biomass was observed in *Typha* sp. with 36 per cent increase. *Lemna* sp. showed the least increase of only 10 per cent.

4. Discussion

Rhizosphere, an important interface of soil and plant, plays a significant role in phytoremediation of contaminated soil. In the present study the microbial colonies present in the wetlands were quantified by colony forming units (CFU). It was found that the emergent species (*Typha* sp.) showed maximum R/S ratio after treatment in all the effluents used for the study. The maximum R/S ratio was in the case of Titanium Factory effluent with *Typha* sp. (9.16). The importance of the microbial biomass for plant phosphorus uptake was shown to differ among plant families. Microbial biomass phosphorus in the rhizosphere was positively correlated with phosphorus uptake by three Poaceae, but not with phosphorus uptake by three Brassicas [7], although the concentration of microbial biomass phosphorus in the rhizosphere of the species from two plant families was similar.

Among the floating macrophytes, *Pistia* sp. based CWs showed maximum R/S ratio in Rare Earth Factory effluent and the lowest was in Lemna sp. based CWs. The increase in numbers of microorganisms is dependent on plant species, plant age, and soil type. In case of dairy effluent, maximum R/S ratio was with *Eichhornia* sp. based CWs and the least was with *Lemna* sp. based CWs. In the case of latex effluent, *Pistia* sp. based CWs possess the highest rate of R/S ratio and the lowest was in *Salvinia* sp. based CWs. In the case of acidic Titanium Factory effluent, among floating macrophytes the R/S ratio was highest in *Lemna* sp. based CWs. The high R/S ratio showed by the emergent plant might be due to rooted nature of the plant, which influences the nutrient content in soil, thus enhancing microbial growth. It is well established that microbial life only occupies a minor volume of soil being localized in hot spots such as the rhizosphere soil [8], where microflora has a continuous access to a flow of low and high molecular weight organic substrates derived from roots. This flow, together with specific physical, chemical and biological factors, can markedly affect microbial activity and community structure of the rhizosphere soil. It is well established that the number of microorganisms is higher in rhizosphere than bulk soil [9].

The microbial population is also confirmed by the dehydrogenase activity of soil planted with *Typha* sp. Plants sustain large microbial population in the rhizosphere by rhizodeposition, root cap cells, which protect the root from abrasion. In addition, root cells excrete mucigel, a gelatinous substance that is a lubricant for root penetration through the soil during growth [10, 11]. In return for receiving exudates, microbes in the root zone can help to solublise insoluble nutrients and recycle organically bound nutritive elements. The types of plants growing in the contaminated area influence the amount, diversity, and activity of microbial populations [12]. The emergent plant Typha latifolia showed maximum R/S ratio than the floating plants (Figure. 1). This may be due to the extensive fibrous root and tap root system present in *Typha* sp. Fibrous root structures of wetland plants provide a larger surface area for colonization than tap root systems [13]. Large microbial populations in the rhizosphere are sustained by exudation of carbohydrates and amino acids from the root [14].

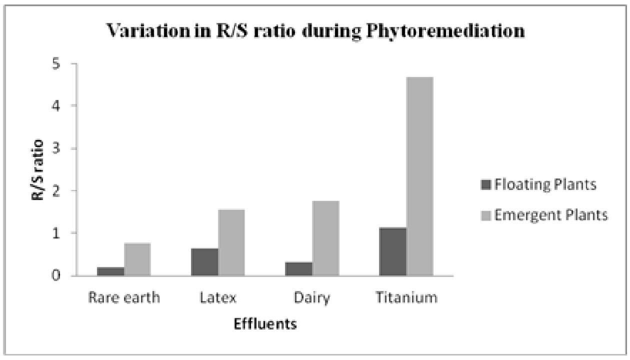

Figure 1. Variation in R/S ratio of plants treated with different effluents

The biomass of the treated plants increased after the retention time of 15 days. The plants utilized the nutrients present in the effluent for their growth. In control, the increase in biomass was less that of the treated plants. The wetland plants to increase their growth effectively used the utilization of nutrients present in the effluents. Increase of biomass is directly proportional to the amount of nutrients utilized by the plants [15]. In control CWs the maximum increase in biomass was in *Eichhornia* sp. and the lowest was in *Pistia* sp. In dairy effluent, the maximum increase was in *Typha* sp. In case of floating macrophytes, *Azolla* sp. showed maximum biomass followed by *Pistia* sp. The lowest increase in biomass was in *Salvinia* sp. Pilon-Smits [16] and Yang et al. [17] reported that the production of biomass, a deep root system, high growth rate, capacity to grow in soils poor in nutrients and to concentrate metals, associated with the characteristics of resistance to metals, are important factors for the use of plant species in soil decontamination. In latex effluent, *Typha* sp. had only 36 percentage increase in biomass but *Azolla* sp. had 56 per cent increase in biomass. In Rare Earth Factory effluent, maximum increase in biomass was in *Pistia* sp. with 52 per cent increase followed by *Eichhornia* sp. The emergent plant *Typha* sp. had only 29 per cent increase. In Titanium Factory effluent that was acidic in nature, *Typha* sp. was the only plant, which increased its biomass by 36%. Dickerman and Wetzel [18] reported that fast-growing, high-biomass crop Typha latifolia has proved to grow quickly and to produce a large biomass when compared to natural hyper accumulators. The biomass increase was highest in Dairy effluent followed by latex effluent. The biomass increase was low with rare earth effluent and chemical industry effluent (Figure 2). Study by Rajeswari et al. [19] in the effects of dye effluents on wetland plant growth had deleterious effects at higher concentration, which affected the growth of biomass.

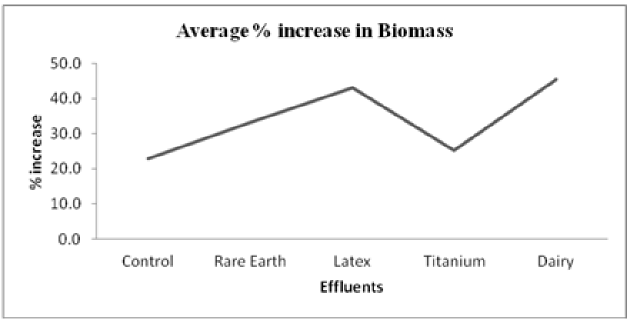

Figure 2. Average Percentage increase in biomass treated with different effluents

5. Conclusion

The microbes associated with roots of wetland plants used for phytoremediation have symbiotic association with the plants. The plants with extensive root system (*Typha latifolia*) promoted the growth of the microbes and in turn the biomass increase was high in the plant. The floating plants have low R/S ratio compared to the emergent plant. The emergent plant which has high R/S ratio and high biomass increase can be used as suitable candidate for phytoremediation of all types of industrial effluents.

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Statement of Competing Interests

The authors have no competing interests.

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