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Auxin treatment of wetland and non-wetland plant species to enhance their phytoremediation efficiency to treat municipal wastewater

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Auxin treatment of wetland and non-wetland plant species for increasing their phytoremediation efficiency to treat municipal wastewater was studied. The mesocosms were set up with gravels and polyethylene balls as the inert support media. The wetland plant species (*Alternanthera philoxeroides, Eichhornia crassipes)* and non-wetland species (*Chrysopogon zizanioides, Festuca arundinaceae)* were treated with six concentrations (0.5, 1.0, 2.0, 4.0, 8.0 and 10.0 mg/L) of natural auxins (Indole-3-acetic acid, Indole-3-butyric acid) and a synthetic auxin (1-Naphathaleneacetic acid). The optimum auxin concentration was found to be 2 mg/L of IAA, 1mg/L of IAA and 1mg/L of IBA for *Alternanthera philoxeroides*, *Festuca arundinaceae* and *Chrysopogon zizanioides,* respectively. The removal efficiencies of auxin treated *Alternanthera philoxeroides, Festuca arundinaceae* and *Chrysopogon zizanioides* for BOD, Nitrate and Phosphate was 12-15, 30-44 and 29-42 % more than the untreated plants.

Keywords: natural auxin, synthetic auxins, support media, constructed wetland.

Introduction

There are two basic types of constructed wetlands (surface flow and subsurface flow wetland systems). Surface flow wetlands are with flow of wastewater over saturated soil substrate. In subsurface flow wetlands wastewater flows vertically or horizontally through the substrate. The wastewater comes in contact with microorganisms, living on the surfaces of plant roots and the substrate[1], allowing pollutant removal from the bulk liquid.The common macrophyte species often employed in wetlands are [2] *Phragmites, Typha, Scirpus*, etc. Herouvim *et al*., (2011) reported that the presence of macrophytes is essential for wetlands in terms of improving nitrogen removal performances because they provide surfaces and oxygen for the growth of microorganisms in the rhizosphere, thereby enhancing nitrification[4]. The roots of macrophytes provide organic carbon in the form of root exudates[5,6]. The constant release of oxygen in the rhizosphere is of an important interest in connection with the exploitation of rhizosphere for treatment of wastewater. The roots of these plants help in contributing oxygen to the cells which allows some aerobic treatment to take place at the root zone.Root hairs, and lateral root development is a consequence of the need for a plant to exploit nutrient rich areas and to avoid areas with high concentrations of toxic elements. For proper development of the root Auxin is important as it is involved in mechanisms such as gravity response, branching and root hair growth [7]. New root formation is induced by auxin by breaking root apical dominance induced by cytokinins. In horticulture, auxins, especially NAA and IBA, are commonly applied to stimulate root growth when taking cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation. Exogenous IAA usually inhibits root growth[8]. Viehmannova *et al*. (2007), compared the influence of growth regulators on the root induction of the *Musa* genus plants cultivated within in vitro conditions. Different concentrations of growth regulators such as naphthaleneacetic acid, indole-3-acetic acid, 6-benzylaminopurine, 2, 4-dichlorophenoxyacetic acid were used. The study proved that most roots were created by using 1-naphthaleneacetic acid at concentration of 5.4 μM.

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Lavenus *et al*. (2013) stated that phytohormone auxin acts as a common integrator to many endogenous and environmental signals regulating lateral root formation. Studies have been reported on the use of plant hormones to enhance root growth to decontaminate soil. Liphadz *et al* (2006) determined the effect of auxin, indole-3-acetic acid (IAA), on root growth in soil with metals from sewage sludge, when the tetrasodium salt of the chelate EDTA (ethylenediamine-tetraacetic acid) was added to solubilize the metals. Root and shoot growths of metal-stressed plants were most effectively increased with 10^{-10} M IAA, and also the extraction of both metals was significantly increased at this treatment level[12].

Similarly, investigation was made by Hado *et al*. (2010) to examine the role of gibberellic acid (GA_3), indole-3-acetic acid (IAA) and EDTA in improving phytoextraction of the Pb and plant growth on Pb added soil. The efficiency of the phytoremediation system also depends on the root mass as increased root surface provides more attachment surface for the microbes involved in the cleaning process. Hence, in this study an attempt was made to increase the root mass by using auxins. The optimum concentrations of different auxins (indole-3-acetic acid, indole-3-butyric acid and 1-naphthaleneacetic acid) for root growth in wetland and non-wetland plant species was determined. Later the auxin treated plants were used in two different support media for the phytoremediation of municipal wastewater.

Methodology

Waste water and plant species used

The municipal waste water was obtained from a municipal wastewater treatment plant, Worli, Mumbai, India. The wetland plants species used in the study were *Alternanthera philoxeroides* (Alligator weed, Family: Amaranthaceae) and *Eichhornia crassipes* (Water hyacinth, Family: Pontideriaceae) and the non-wetland plant species *Chrysopogon zizanioides* (Vetiver Grass, Family: Poaceae) and *Festuca arundinacea* (Fescue grass, Family: Poaceae).

Adaptation of land plants to the water environment

Festuca arundinacea and *Chrysopogon zizanioides* being terrestrial plants needed to be adapted to the water environment. The non-wetland plants were kept in soil with water for 7 days. Then soil was partially cleaned with water and the plants were reintroduced to fresh water and maintained for another 7 days. Then the plant roots were cleaned properly and were made soil free and reintroduced to fresh water. They were maintained in fresh water for 15 days with the water being changed at intervals of 5 days.

Determination of optimum Auxin concentration

The wetland and non-wetland plant species chosen for the study were subjected to various auxin types and auxin concentrations. The auxins used were natural auxins, Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) and synthetic auxin, 1-naphthaleneacetic acid (NAA). Six concentrations (0.5, 1.0, 2.0, 4.0, 8.0 and 10.0mg/L) of each type of auxins were tested for the determination of the root initiation and root growth increment in all four plant species. The solution of the auxin was prepared in 1N NaOH as a solvent and then dissolved in water. The stock solution prepared was 100 mg/l concentration. The prepared auxin solution was stored at 4°C in opaque bottles and used within 2 days. The auxin solution of different concentration was prepared and the plants were kept in them for further root growth. The plants were observed for 10 days to check for root elongation and growth. Then each plant species showing response to auxins was treated with respective optimum auxin concentration in water. Sufficient root mass was allowed to develop.

Use of auxin treated plants in each type of mesocosm

After sufficient root mass had developed each plant was kept in a wetland mesocosm with defined number of gravels of size 2.5, 2 and 1.5 cms and plastic balls of approximate size 5 cms which had been kept in municipal wastewater for 15 days. The auxin treated plants were acclimatized in municipal wastewater for 7 days with support media. The municipal wastewater was drained from the mesocosms and fresh municipal wastewater of equal quantity was added in each mesocosm and sampling was done on 5 th day for determining treatment efficiency. 5 days of sampling time was selected as the removal of BOD, N and P was more when the treatment time was 5 days as compared to 3 days. At 5 days of treatment time for the plant species used, the BOD removal efficiency was higher by 10 to 12 %, N removal was higher by 8 to 11 % and P removal was higher by 6 to 9 % than at 3 days of treatment time. Each mesocosm (eg. Fig1) comprised of 1 plant, 150 gravels and 16 polyethylene balls separately and 600mL municipal wastewater. The control set was run simultaneously. The control set comprised of non-treated auxin plants.

Analysis of the parameters

BOD, ammonical nitrogen and phosphorous were analysed based on APHA Standard Methods (2001). The methods used for BOD, ammonical nitrogen and phosphorous were Winklers method, Titrimetric method, Stannous chloride method, respectively. The municipal waste water had BOD, ammonical nitrogen and phosphorous average values as 172, 10 and 0.24 mg/l, respectively.

Statistical Analysis

Statistical analyses were performed using the software SPSS (SPSS Inc., Chicago, IL, USA; Version 13.0).

Fig. 1—Mesocosm (with Chrysopogon zizanioides)

Results & Discussions

Determination of optimum Auxin concentration

Six auxin concentrations i.e. 0.5, 1.0, 2.0, 4.0, 8.0 and 10.0 mg/l, were studied for the determination of optimum auxin concentration for wetland and non-wetland plant species. *Alternanthera philoxeroides* showed the best response in terms of root initiation and root growth to 2 mg/L of IAA, whereas *Festuca arundinacea* responded best to 1 mg/L of IAA and *Chrysopogon zizanioides* to 1 mg/L IBA after 10 days of treatment (Table 1, Fig 2,3,4). Water hyacinth did not respond significantly to the auxins. There was no significant response to 1-naphthaleneacetic acid (NAA) by any of the plant species. Different plants respond differently to auxins. Kukavica *et al* (2007) studied changes in growth, peroxidase profiles, and hydroxyl radical formation in IAA (0.5 – 10 mg/l) treated pea plants grown hydroponically and in isolated roots in liquid in vitro culture. They found that IAA inhibited root elongation, both in hydroponically grown pea plants and in isolated roots in vitro. Kulkarni *et al* (2013) treated *Brassica juncea* lants with 2.5, 5.0 and 7.5 M auxin indole-3-butyric acid (IBA) and highest Au uptake was observed for plants treated with 5.0 M of IBA. Sevik and Guney (2013) studied the effect of of IAA, IBA, NAA, and GA3 on rooting and morphological Features of *elissa officinalis*. Stem Cuttings. They found that the auxin group of hormones (IAA, IBA, and NAA) did not have an apparent effect on rooting rate but had an effect on the morphological characteristics of newly generated plants. Root development in particular reached significantly different values in the plants that

Table 1—Root growth response of different plant species to different types of auxins and auxin concentrations

	IAA				IBA				NAA			
	AP	FA	CZ	EC	AP	FA	CZ	EC	AP	FA	CZ	EC
0.5 mg/L	+	+	-	-	+	+	+	-	-	+	-	-
1.0 mg/L	++	++	-	-	+	+	+++	-	-	+	-	-
2.0 mg/L	+++	+	+	-	+	+	+	-	-	+	-	-
4.0 mg/L	++	+	+	-	-	-	+	-	-	-	-	-
8.0 mg/L	++	-	-	-	-	-	-	-	-	-	-	-
10 mg/L	+	-	-	-	-	-	-	-	-	-	-	-

+ less growth; ++ more growth; +++ best growth
AP: *Alternanthera philoxeroides*; FA: *Festuca arundinaceae;* CZ: *Chrysopogon zizanioides;*
EC: *Eichhornia crassipes***,**
IAA: Indole-3-acetic acid
IBA: Indole-3-butyric acid
NAA: 1-Naphathaleneacetic acid)

Fig. 2—Chrysopogon zizanioides after treatment

Fig. 3—Alternanthera philoxeroides after with 1mg/L IBA treatment with 2mg/L IAA

Fig. 4—Festuca arundinaceae after treatment with 1mg/L IAA

received the auxin group of hormones. On comparing the responses of IAA and IBA it was observed that on increasing the concentration of IAA from 0.5 to 2 mg/l there was increase in rooting in *Alternanthera philoxeroides* but the same response was not observed when IBA was used on *Alternanthera philoxeroides*. In contrast to this in *Chrysopogon zizanioides,* on increasing the concentration of IBA from 0.5 to 1 mg/l there was increase in rooting. It has been reported in literature that when supplied to the rooting solution, IAA and IBA differ in their root inducing ability. IBA treatment usually increases root number dramatically while stimulating root formation along the whole stem base. IAA at the same concentration may even reduce root number and the roots formed appear in the lowest part of the cutting base [18].

Efficiency of auxin treated plant species

The BOD removal efficiencies of auxin treated Alternanthera philoxeroides, Festuca arundinaceae and Chrysopogon zizanioides in gravel support media were 8, 15 and 14 % more than in the non-treated plants. Nitrate removal was 42, 44 and 39 % more than in auxin treated plants while phosphate removal was 30, 29 and 29 % more in auxin treated plant species. The efficiency of the auxin treated plants in polyethylene balls support media was also significantly higher than the non-treated plants. In polyethylene balls support

Table 2—Removal (%) efficiency of auxin treated wetland and non-wetland plant species and non-auxin treated plants species for HRT of 5 days

	BOD	P	N
Wetland Plants Species			
Alternanthera philoxeroides (Treated with 2mg/L IAA)			
TP (G)	81±0.53	95±0.99	94±0.95
NTP (G)	73±2.71	53±0.84	64±0.81
TP (PEB)	83±3.21	97±0.41	91±0.72
NTP (PEB)	68±2.50	51±1.82	55±2.13
Non- Wetland Plant Species			
Chrysopogon zizanioides (Treated with 1mg/L IBA)			
TP (G)	83±3.9	93±0.49	93±0.94
NTP (G)	69±3.0	54±0.51	64±1.96
TP (PEB)	79±3.7	97±0.18	91±1.13
NTP (PEB)	68±2.7	53±0.59	63±1.7
Festuca arundinacea (Treated with 1mg/L IAA)			
TP (G)	85±3.2	86±0.39	95%±0.66
NTP (G)	70±2.6	52±0.52	66±1.67
TP (PEB)	81±3.2	85±0.51	94±1.00
NTP (PEB)	69±2.6	50±2.7	62±1.57

TP: Treated plants, NTP: Non-auxin treated plants, G: gravel, PEB: Polyethylene balls

Table 3—Anova table showing significant difference between auxin treated and non- treated plant species (HRT : 5 days)

	Alternanthera philoxeroides			*Chrysopogon zizanioides*			*Festuca arundinacea*		
	SS	F	SIG	SS	F	SIG	SS	F	SIG
Gravel BOD	107.6	30.09	.005	276.3	23.8	.008	351.2	37.9	.004
N	1325.9	1336.3	.001	1195.4	461.8	.000	1284.2	557.6	.001
P	2633.8	2586.3	.002	2214.5	7462.2	.000	1731.4	5780.7	.001
PEB BOD	328.9	35.9	.004	177.1	18.2	.013	204.0	22.0	.01
N	2037.3	832.9	.001	1181.8	30.2	.005	1526.9	883.2	.001
P	3127.9	1708.7	.000	2923.8	440.5	.000	1797.1	440.1	.002

PEB: Polyethylene Balls

media the BOD removal efficiencies of auxin treated Alternanthera philoxeroides, Festuca arundinaceae and Chrysopogon zizanioides were 15, 11 and 12 % more than in the non-treated plants. Nitrate removal was 46, 35 and 44 % more than in non-treated plants while phosphate removal was 36, 32 and 28 % more in auxin treated plant species (Table 2).

Table 3 shows the statistical calculations performed using SPSS 13.0. The auxin treatment proved to be beneficial in the treatment of municipal wastewater. The values of auxin treated plants in comparison to non-auxin treated plants showed significant difference. The significance value observed was less the 0.05 for the three auxin treated wetland plant species and non-wetland plants for all parameters of analysis of municipal wastewater.

Conclusions

The optimum auxin concentration was found to be 2 mg/L of IAA, 1mg/L of IAA and 1mg/L of IBA for Alternanthera philoxeroides, Festuca arundinaceae and Chrysopogon zizanioides, respectively. Eichhornia crassipes did not respond satisfactorily to the auxins. The investigation showed significantly more removal of BOD, nitrate and phosphate by the auxin treated wetland and non-wetland plant species. Therefore, auxin treatment increased the efficiency of the plants significantly to treat municipal waste water.

Nomenclature list

TP: Treated plants
NTP: Non-auxin treated plants
G: gravel
PEB: Polyethylene Balls
AP: *Alternanthera philoxeroides*
FA: *Festuca arundinaceae*
CZ: *Chrysopogon zizanioides*
EC: *Eichhornia crassipes*
BOD: Biochemical Oxygen Demand
N: Nitrogen (Ammoniacal)
P: Phophorous

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