COMPARATIVE EFFECT OF PHENOL AND MELANOIDIN ON HEAVY METAL BIOACCUMULATION IN *TYPHA LATIFOLIA* FOR BIOREMEDIATION OF DISTILLERY EFFLUENT

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Abstract: Distillery effluent makes serious soil and water pollution because of recalcitrant water soluble compound (melanoidin), phenol and heavy metals as major pollutants present in the effluent. Wetland plant such as *Typha latifolia* was studied for its ability to accumulate heavy metals under high concentration of other pollutants. In our present study, the accumulation pattern of 8-fold heavy metal (Pb, Cu, Zn, Mn, Ni and Fe) concentration of original distillery effluent, phenol and melanoidin at variable concentrations in pot culture (constructed wetland) were studied. The physicochemical parameters in treatment pots were also analyzed before and after the pollutant treatment given to the pot culture, which revealed that *Typha latifolia* potential use for metal removal in constructed wetlands. Seasonal comparisons for heavy metal bioaccumulation in three selected plants were also studied among which *Typha latifolia* was found to be more tolerant for bioaccumulation of heavy metals in presence of other pollutants. For complete evaluation of the environmental impact of a complex industrial waste, both physicochemical and toxicological analyses were performed as physicochemical analysis only does not provide information about the toxicity of environmental sample. Usually there occurs a continuous interaction of complex mixture of compounds in the environment where toxicity is not easily detected. Therefore, *Typha latifolia* was found to be more potential and tolerant for bioaccumulation of heavy metals in presence of phenol and melanoidin, which can be potentially used for the bioremediation of distillery effluent.

Key words: Heavy metal, bioaccumulation, distillery effluent, *Typha latifolia* , constructed wetland.

Introduction: The intensification of industrial activity during the recent years is greatly contributing to the increase of the heavy metals in the environment, mainly in aquatic systems [1]. Heavy metals and their compounds are toxic to aquatic organisms even at very low concentration. They are carcinogenic and may constitute danger to human being and other lives [2]. The contamination can be remediated using a variety of technologies, whether chemical, physical or biological. Methods such as precipitation, reduction [3], artificial membranes and ion-exchange are used to remove toxic metals from industrial effluents but they are expensive, relatively inefficient and in most cases they generate a great amount of waste which is difficult to dispose of. wetland plants play an important role in metal removal via filteration, adsorption and cation exchange, and through plant-induced chemical changes in the rhizosphere [4]. Phytoremediation is a plant-based approach to remove metals from contaminated soils which requires a plant species efficient in metal accumulation and in high biomass production, while easily adapted to unfavorable environmental conditions [5]. There is evidence that wetland plants can accumulate heavy metals in their tissues such as *Typha*

latifolia [6]. Many factors influence the behavior of metal accumulation by wetland plants including pH, temperature, TDS etc. in general variations in plant species, the growth stage of the plants and element characteristics control absorption, accumulation and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots [7]. Rhizoremediation potential of spontaneously grown *T. latifolia* was also studied in polluted site [8]. Further phytoremediation of heavy metals from industrial effluent were studied in constructed wetland technology [9].

In India, there are more than 300 distilleries producing about 3.5 X 10^5 kilo litres of wastewater per annum causing severe aquatic pollution and to the near by lands. The wastewater constitutes high levels of Color (80000-90000 Copt) BOD (40000-42000 ppm), COD (82000-92000 ppm), TS (5900-6500 ppm), phenolics (300-360 ppm), sulfate (6000-6500 ppm), nitrogen (275-300 ppm) and heavy metals such as Mn^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} (45-50 ppm) as a major contents of distillery effluent [10]. Melanoidin (amino-carbonyl biopolymer), which is responsible for coloring of distillery effluent also effect directly/indirectly to the wetland plant for bioaccumulation of heavy metals by decreasing the intensity of light passing from water logged condition, increase of BOD, COD and other factors. Our preliminary investigation at effluent polluted sites found that many wetland plants could thrive in heavily metals contaminated soils/sediments, especially under water-logged conditions [11]. Wetland plant such as *T. latifolia* is reported for its ability to accumulate heavy metals under high concentration of other pollutants and its use in the amelioration of effluents [12]. Therefore, in our present study, the accumulation pattern of 8-fold heavy metal concentration of original distillery effluent such as phenol and melanoidin at variable concentrations provided separately in pot culture were studied.

The above information for the removal potential of heavy metals (Pb, Cu, Zn, Mn, Ni and Fe) by *Typha latifolia* combined with other distillery pollutants can be referred in wetland treatment systems for removal of heavy metals by wetland plants along with other pollutants.

Materials and methods:

Experimental setup and treatment:

Thirteen plastic (PVC) pots were installed in the wetland treatment system located at IITR, Gheru Campus, Lucknow (U.P.), India. Each pot measure 32 cm diameter and 35 cm depth and total volume measures 15 L. Pots were first filled with coarse gravel (4 cm), then pea gravel (1 cm) and at top fine sand (0.1 cm) was filled. Each layering was done up to 10 cm height. *Typha* twigs were planted in all the pots. Control plants were collected from a nearby natural stand, growing on fine-textured soils with a variable water table rarely above the soil surface. Rhizomes of *Typha* with small amounts of native soil attached and some shoot material were planted in all the pots at a depth of 12-15 cm, just above the free water table. During the 1-month establishment time, chemical fertilizer of nitrogen, potassium and phosphorus was added and the water level in the planted substrates maintained at or just below the rhizomes.

Treatment applications began after the establishment of potted plant. Growth conditions in the root zone were anaerobic, saturated with treatment solution (heavy metals, phenol and melonoidin) at varying concentration (Table 1 and 2) and at temperatures of 30-40ºC during may to July, the period of research reported here. Eight-fold metal concentration of original distillery effluent and around 2500 Copt of melanoidin was provided along with variable concentrations of phenol as: 0, 50, 50,100,200,300,400, mgl^{-1}in pots

1 to 7, with exception of melanoidin in pot 1 and 2. Therefore, pot1 was treated only with heavy metal solution. In remaining pots, eight-fold metal concentration of original distillery effluent and a constant concentration of phenol (100 mgl^{-1}) was provided along with melanoidin of 2600, 2000, 3700, 4800, 6000 and 7300 Copt unit of color in pots 8-13 with exception of phenol in pot

Sample collection and physicochemical analysis:

Samples were collected from all the pots at 1, 20, 40 and 60 day of treatment. Following tests were performed for physicochemical analysis such as pH, nitrate and chloride ion was determined using an Orion autoanalyser 960. Color, total soluble phosphates, sulfates, BOD, COD, Kjeldhal nitrogen and phenol were estimated by standard methods [13].

Relative growth rate (RGR):

The dried plant materials were used for the calculation of relative growth rate (RGR). The RGR was determined from equation,

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where, W1 and W2 are dry biomass at the beginning (t1) and at the end of the experimental period (t_2), respectively [14]. The RGR will facilitate the assessment of changes in above-ground biomass during the experimental period. Plant monitoring indicated the feasibility and efficiency of each planted pots for employing in treatment wetlands under tested conditions.

(iv) Heavy metal accumulation:

After acclimatization, and treatment, the plants were observed during the whole treatment period. After each 20day of interval a plant was plucked from each pot along with its roots and rhizomes, biomass estimates were made by removing all the plant parts separately. Samples were divided into roots; rhizomes and leaves washed carefully in tap water and with 10% $CaCl_2$ solution, again washed with deionized water twice. Samples were dried at 80ºC for over 72 h, and mean dry weight determined for each tissue. All samples were ground to a mesh 40 powder in a Wiley mill for chemical analysis. Water and sediment samples were acid digested, HNO_3:$HClO_4$ (3:1) by using standard methods [10] and [15] respectively. Plant tissues were ashed for 2h in quartz crucibles at 500ºC, treated with nitric acid, dried, heated to 400ºC and cooled. The residue was dissolved in 1M HCl as per AOAC international methods (2002), after cooling the digested sample was dissolved and makeup to the mark in volumetric flask (50 ml). Thereafter, tissue elements (Fe, Pb, Cu, Zn, Mn and Ni) were analyzed by using inductively couples plasma (ICP-AAS) spectroscopy.

The percent accumulation was calculated by the difference in the amount of metal accumulation between the final and initial readings. All the experiments were performed in triplicates and samples were withdrawn at regular time interval (20 days) for accumulation measurements. The percent accumulation was measured using formula:

$$\% \text{ accumulation} = \frac{(Fm^{60} - Im^{1})\ 100}{Im^{1}}$$

Where, Fm^{60} is final metal content in plant at 60 day and Im^{o} is initial metal content in plant at 1 day.

Seasonal metal accumulation in wetland plants:

To observe seasonal variations in metal accumulation pattern in three wetland plants *Typha latifolia*, *Phragmites australis* and *Cyperus papyrus*, treatments were provided as per above method described. Metal accumulations were measured in three different seasons' summer, monsoon and winter.

Acute toxicity with *Lemna minor*:

To assess the toxicity of the treatment solution

at 1 day and at 60 day (after the heavy metal accumulation in potted plants), mortality was checked with *Lemna minor* [16]. *Lemna minor* was collected from a near by aquatic body, washed 4-5 times with distilled water and culture was maintained in lab for four weeks. Healthy plants with two fronds each were allowed to grow as monocultures in tested water samples over a period of seven days. Water samples of ST1, ST3, ST7, ST9 and ST13 (treatment pots) were selected for toxicity reduction of each 1 and 60 day. The test was performed in triplicates. To quantify treatment solution effects, growth in the test solutions was compared with that of the controls. Percent mortality was checked by the given formula. Further, percent toxicity reduction was obtained by the difference between percent mortality at 1 day and 60 day sample.

% mortality =

No. of fronds in initial sample – No. of fronds in final sample X 100

No. of fronds in initial sample

Result and discussion: The physico-chemical analysis of treatment solution of metal with mixture of phenol and melanoidin at different concentration revealed that the presence of phenol with metallic solution along with melanoidin showed slight alkaline nature. Simultaneously, there is increase in the values of BOD, COD and color along with increasing the concentration of phenol (Table 1.1) at initial stage of experiment, while the contents of PO_4 and N_2 remains almost around constant in different solution mixture but there was slight increasing trend of SO_4 and Cl_2. This might be due to ionic interaction with metals and phenol (Table 1.1).

Table 1.1: Physicochemical analysis of water samples of *Typha latifolia* pots treated with heavy metals, melanoidin and increasing concentration phenol.

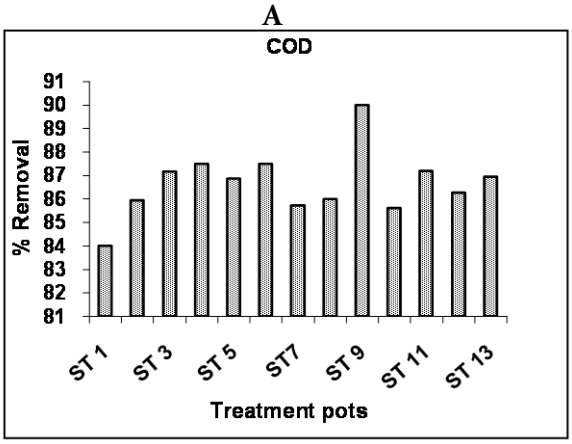
Sampling period	Parameters	Treatment pots						
		ST 1	ST 2	ST 3	ST 4	ST 5	ST 6	ST7
0 day	pH	80.1	8.10.1	8.20.1	8.30.1	8.40.1	8.50.1	8.60.1
	Color	50010	90020	240010	245020	245040	250050	255050
	COD	2500100	3200100	3500200	3600200	3800200	4000300	4200300
	BOD	1200100	1400200	1600400	1500400	1900100	2000300	2000200
	Phenol	00.0	213015	226025	301045	312050	328020	360040
	Sulfate	12.44.6	12.011.5	13.892.9	14.856.5	16.964.9	17.923.2	18.545.3
	Phosphate	00.0	2.890.4	2.660.8	2.840.5	2.550.5	2.480.8	2.990.7
	Nitrogen	823	962	925	945	799	748	654
	Nitrate	9.891.6	10.421.3	10.861.4	11.361.8	11.591.9	11.970.8	12.481.7
	Chloride	0.240.03	0.240.36	0.290.42	0.310.54	0.330.66	0.3640.21	0.380.58

20 day	**pH**	7.70.0	7.80.0	7.80.1	7.80.0	7.80.0	80.1	80.0
	Color	15020	27020	118020	155050	180060	200050	218020
	COD	160080	220040	230060	260050	280070	290030	300050
	BOD	80050	100020	120020	130040	150030	150020	160020
	Phenol	00.0	160030	164020	208020	216010	266040	284010
	Sulfate	9.090.12	10.630.11	11.260.39	14.260.27	13.660.23	14.50.54	16.980.4
	Phosphate	00.0	1.330.5	1.450.8	1.480.7	1.630.2	1.850.4	1.850.3
	Nitrogen	676	758	729	842	659	6610	6012
	Nitrate	3.530.26	4.760.19	5.470.15	5.690.24	6.150.08	6.170.2	6.540.04
	Chloride	0.220.13	0.170.18	0.20.21	0.220.22	0.230.11	0.230.16	0.230.07
40 day	**pH**	7.30.1	7.40.0	7.50.1	7.60.0	7.70.1	7.80.0	7.90.0
	Color	10010	15010	50020	65020	85030	90040	105040
	COD	100050	110040	120050	150020	160060	180080	200080
	BOD	50030	60020	60040	70050	80030	100070	110020
	Phenol	00.0	88015	116020	182020	184010	140030	248010
	Sulfate	8.740.73	10.860.33	10.910.83	11.920.25	14.160.16	14.620.19	15.180.18
	Phosphate	00.0	0.990.05	0.890.22	1.20.07	1.10.09	1.160.02	1.180.04
	Nitrogen	546	632	618	703	565	574	528
	Nitrate	3.061.2	4.151.3	1.541.2	2.031.1	2.321.4	5.651.8	5.951.3
	Chloride	0.210.22	0.20.06	0.190.12	0.170.16	0.140.13	0.120.14	0.10.09
60 day	**pH**	7.10.0	7.20.0	7.20.0	7.30.0	7.30.0	7.30.0	7.30.0
	Color	500.0	500.0	1005	1505	20010	20010	25010
	COD	40020	45010	45030	45030	50020	50010	60010
	BOD	20030	21040	22020	25020	25030	25010	30020
	Phenol	00.0	10016	18018	20012	22014	25018	30016
	Sulfate	3.742.2	3.651.8	4.842.1	7.781.6	7.991.9	8.591.1	9.631.3
	Phosphate	00.0	0.120.03	0.650.06	0.50.05	0.620.02	0.860.03	1.060.09
	Nitrogen	422	514	535	612	448	489	4312
	Nitrate	1.281.1	1.681.0	4.261.3	4.361.6	4.481.8	2.881.5	4.141.7
	Chloride	0.040.02	0.030.01	0.030.02	0.020.03	0.020.03	0.0020.04	0.010.06

Further, the incubation of wetland plant up to 20-40 days showed supportive growth of *Typha latifolia* in presence of heavy metal, melanoidin and increasing concentration of phenol while the plant in presence of increasing concentration of melanoidin along with heavy metal and

phenol showed rapid revere necrotic effect on leaves which might be due to cumulative effect of melanoidin and hyper accumulation of heavy metal and phenols in presence of higher concentration of melanoidin (Fig 1.1).

A



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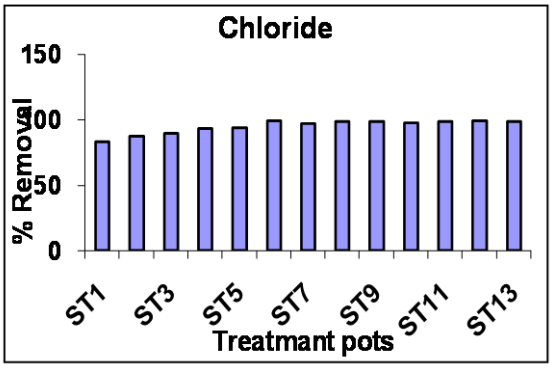

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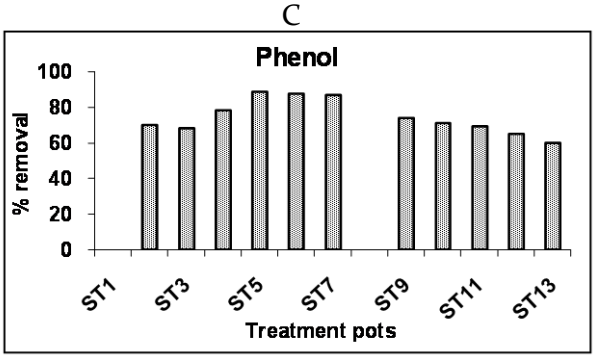

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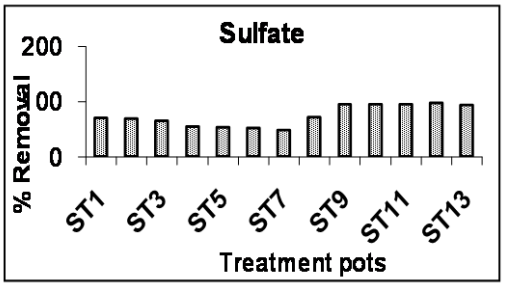

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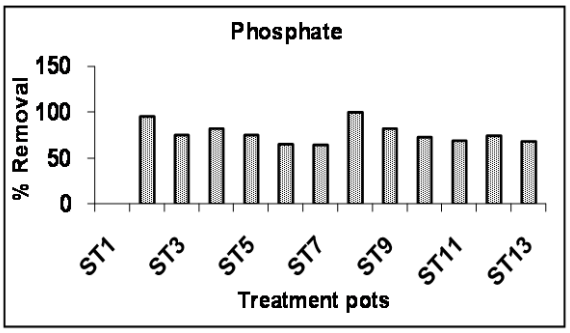

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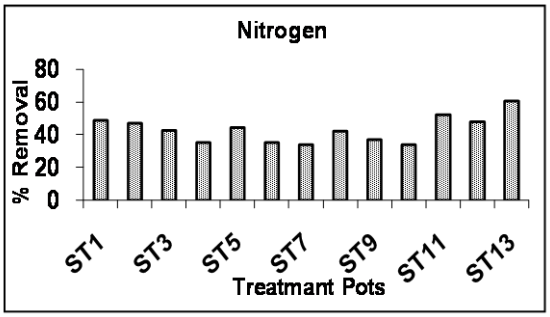

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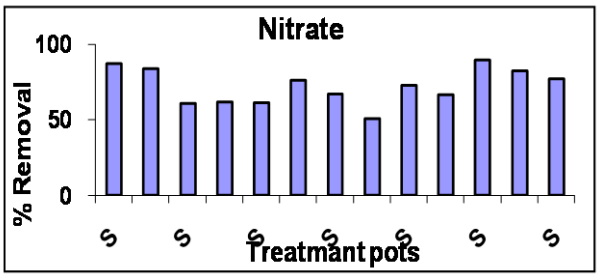

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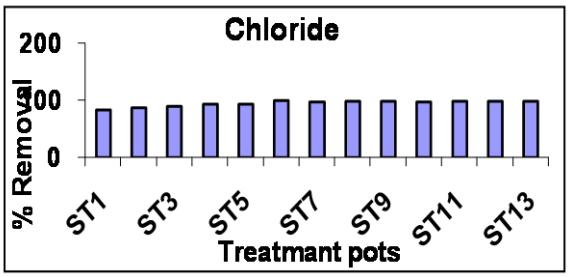


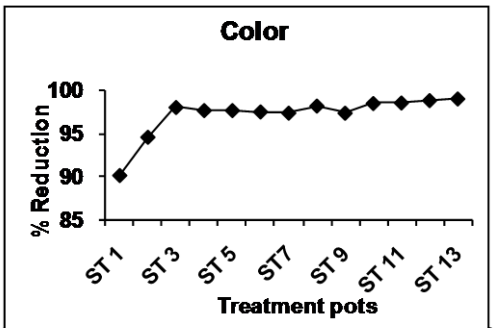


Fig.1.1: Percentage removal o[illegible]ulfate, **(E)** phosphate, **(F)** nitrogen, **(G)** nitrate, **(H)** chloride and **(I)** color in treatment pots planted with *Typha latifolia* at 60 day.

Thus the melanoidin showed more toxic effect to plant. Another factor is the low pH values in pot 1-13. Since solubility of metal ions is pH dependent, therefore, low pH values is the reason for the increase in absorption of toxic metals in roots and further, translocated to leaves which finally results in necrosis of leaves.

Further, incubation up to 60 days, plant showed emergence of new leaf and recovery of healthy plant. There was rapid reduction in pollution parameters in test solution of metal, melanoidin and variable concentration of phenol and pH at different incubation time compared with another set of experiment containing heavy metal phenol and variable concentration of melanoidin. This indicated the more toxic effect of melanoidin along with phenol for heavy metal accumulation and detoxification of test solution (Table 1.2)

and whiles the hyper accumulation of Cu and Zn was observed by the plant in all the tested concentration and there was increasing trend of heavy metal along with increasing incubation time (Fig. 1.2).

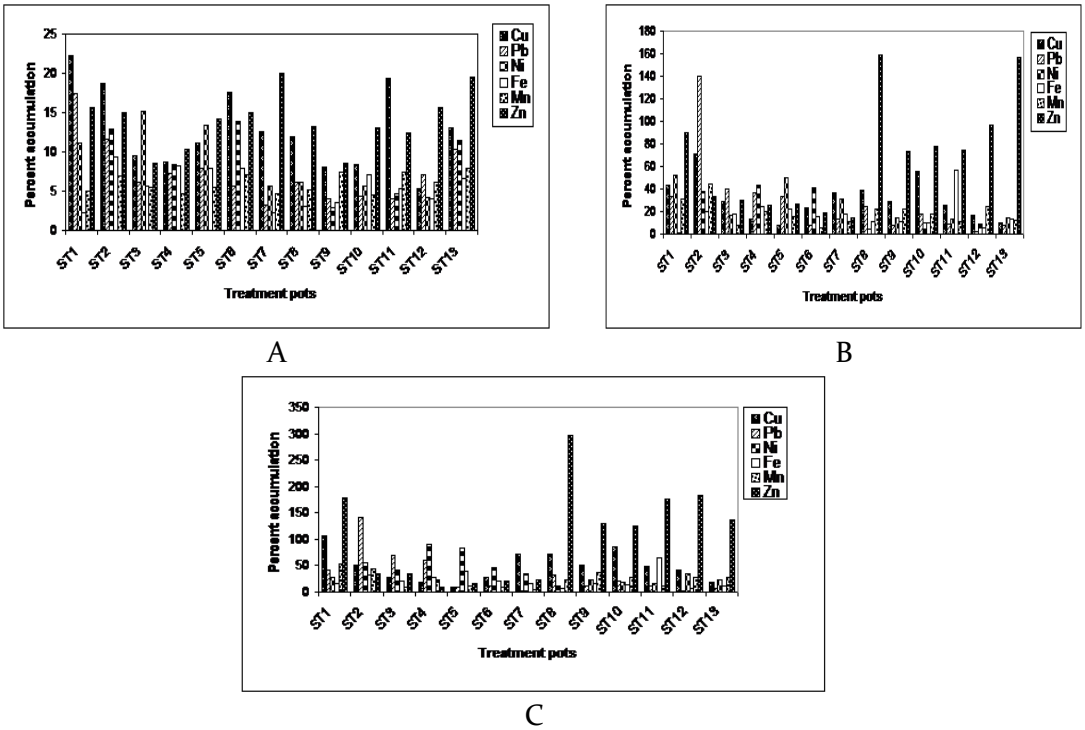


Fig. 1.2: Percent accumulation of different heavy metals in *Typha latifolia* at **(A)** 20 day, **(B)** 40 day and **(C)** 60 day after treatment.

Table 1.2: Physicochemical analysis of water samples of *Typha latifolia* pots treated with heavy metals, phenol and increasing concentration of melanoidin.

Sampling period	Parameters	Treatment pots					
		ST 8	ST 9	ST 10	ST 11	ST 12	ST 13
0 day	pH	7.20.1	7.30.1	6.60.1	6.50.1	6.50.2	6.50.2
	Color	260050	200020	370050	480030	600040	7300100
	COD	5000150	6000200	6600100	7800200	800050	9200100
	BOD	2400200	2800400	3200200	3600400	3800150	4200200
	Phenol	00	320020	320030	310040	328020	320030
	Sulfate	12.613.4	13.062.8	14.471.9	13.673.6	12.924.5	13.292.5
	Phosphate	2.951.23	2.360.98	2.870.85	2.660.96	2.511.22	2.91.1
	Nitrogen	855	684	593	715	657	884
	Nitrate	4.881.35	5.450.62	5.261.84	6.61.64	6.351.86	6.650.95
	Chloride	13.210.06	14.320.15	15.320.47	19.780.12	19.831.93	19.80.25
20 day	pH	7.10.2	7.20.1	6.70.1	6.60.2	6.60.2	6.70.2
	Color	40050	45050	500100	800150	1800200	2100100
	COD	180050	2800100	3000100	3600200	5200100	5600100
	BOD	1000100	1400200	1500250	1800300	2500200	2600200
	Phenol	00	2320	2440	3360	3160	3080
	Sulfate	12.011.36	11.521.14	11.731.05	11.170.84	11.670.69	11.460.59
	Phosphate	1.430.21	2.180.11	2.390.66	2.030.58	1.920.82	2.730.45
	Nitrogen	693	582	453	632	584	732
	Nitrate	3.350.35	4.130.21	2.160.75	1.241.26	2.790.32	3.240.15
	Chloride	10.020.16	10.030.18	10.190.19	11.960.2	8.161.36	13.720.28
40 day	pH	70.2	7.30.1	70.2	70.2	7.10.1	7.10.2
	Color	10020	18030	16040	16050	20050	40040
	COD	110050	140060	180030	180050	250020	280050
	BOD	700100	1000100	100050	1100100	120050	1200100
	Phenol	00	68020	76040	84020	92030	172030
	Sulfate	4.580.15	41.65	4.090.62	4.540.38	6.220.77	5.760.43
	Phosphate	1.260.03	1.750.08	1.490.09	1.540.12	1.890.16	2.180.02
	Nitrogen	555	514	414	546	495	623
	Nitrate	3.491.56	3.391.26	3.441.2	2.651.03	1.21.81	2.570.82
	Chloride	8.010.06	8.030.08	6.020.08	10.030.54	6.030.02	8.330.01
60 day	pH	70.2	7.30.1	7.20.1	7.20.1	7.20.0	7.20.0
	Color	5020	555	6010	755	7710	8010
	COD	70050	60060	95050	100030	110020	120050
	BOD	300100	300100	400100	50050	55050	600100
	Phenol	00	52016	32012	31215	56014	60017
	Sulfate	3.530.52	0.651.28	0.710.78	0.690.44	0.331.83	0.891.38
	Phosphate	00	1.410.09	0.760.08	0.810.19	0.630.85	1.680.26
	Nitrogen	494	433	392	345	344	354
	Nitrate	2.41.6	1.481.2	1.750.95	0.681.38	1.130.75	1.520.26
	Chloride	0.170.01	0.160.08	0.320.64	0.160.23	0.130.36	0.20.18

All values are in mg L^{-1} except pH and color is in Co-pt; ST8, treated with heavy metals and

melanoidin; ST9-13, treated with heavy metals, phenol and increasing concentration of melanoidin.

The plant showed toxic effect at colour range 4800-7300 in presence of heavy metal, phenol and increasing concentration of melanoidin (Table 1.2).

The RGR can be employed to confirm the health of plants while treatment period. Whereas, RGR values of pot 1 (treated only with heavy metal solution) is around 0.055 while the pot 2 (treated with metal and phenol) has RGR values of 0.027. This showed that pot 2 growth is slightly affected by addition of phenol. Later in pot 3 to 7 the RGR values ranges between 0.04-0.06. This revealed that the provided condition facilities the growth of plant in later stage, while in pot 8 (treated with metals and melanoidin) has RGR value of 0.006, which is comparatively low with the above values obtained i.e. melanoidin here is responsible for lowering of growth of plant due to its inhibitory action and high levels of BOD, COD and color which are interlinked with melanoidin. Similarly, while we observe pot 9 to pot 13, RGR values concomitantly decreases from 0.028 to 0.001 (Fig. 1.3).

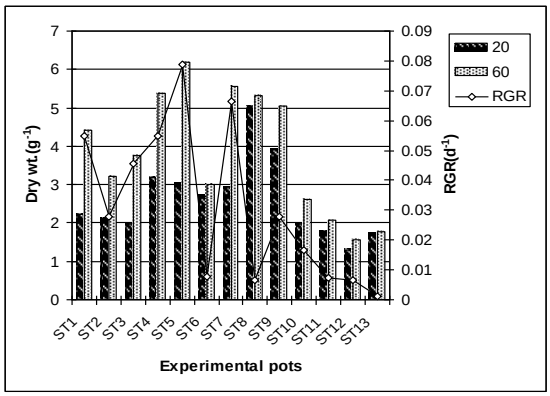


Fig.1.3: Relative growth rate and dry weight of tested pots planted with *T. latifolia*

This finally revealed that heavy metals along with phenol and increasing concentration of melanoidin effects the growth of plant by reducing the total dry biomass as thus lowering of RGR values. The RGR value of cattail was close to that reported for constructed wetlands located in tropical zones receiving municipal wastewater (9). This indicated the capability of cattail for acclimatization and growth in a treatment wetland receiving distillery pollutants (i.e. heavy metals, phenol and melanoidin) in a mixed solution. Further percent accumulation of different heavy metals in *T. latifolia*, *P. australis* and *C. papyrus* at 20 days, 40 days and 60 days were also showed in Fig. 1.2 which revealed maximum accumulation at 60 days .

The seasonal variation also affected the plant for heavy metal accumulation and color reduction of melanoidin of melanoidin solution range for color, BOD, COD and different heavy metals (Cu, Pb, Fe, Mn and Zn). The reduction rate for color in *T. latifolia*, *P. australis* and *C. papyrus* in summer, monsoon and winter is showed in (Table 1.3). The winter season showed relatively slow bioaccumulation of heavy metal compared with summer and monsoon. To check the environmental impact assessment on the treated effluent acute toxicity test was performed with *Lemna minor* in the treatment pots of *T. latifolia* and to study the effect of heavy metal accumulation at 1st and 60th day of the treatment solution (Fig. 1.4) and percent toxicity reduction of 60th day water samples of treatment pots tested with *Lemna minor* at 7 day of incubation time was also studied (Fig. 1.5).

Table 1.3: Seasonal variations of pH, BOD, COD, colour values and heavy metal accumulation in wetland plants

		% reduction			% accumulation of heavy metal in wetland plant					
	pH change	Color	BOD	COD	Cu	Pb	Ni	Fe	Mn	Zn
Typha latifolia										
Summer	8.4-7.3	92	87.5	87.5	60.51	339.37	69.36	27.71	160.76	241.84
Monsoon	8.2-7.2	90	86	85	51.46	331.68	52.28	20.92	162.9	159.42
Winter	8.1-6.9	88	82	84	45.08	343.43	61.63	15.78	236.98	116.73
Phragmites australis										
Summer	7.8-7.1	93	90	94	64.17	26.97	35.57	50.2	56	57.25
Monsoon	7.7-7.2	91	89	91	68.19	15.13	18.87	41.38	28.85	40.2
Winter	7.8-7.0	84	80	81	49.51	2.91	15	36.89	22.58	36.64
Cyperus papyrus										
Summer	8.2-7.3	90	88	86	55.84	28.96	29.28	40.67	73.65	74.54
Monsoon	7.8-7.1	86	84	85	63.88	18.25	17.4	23.52	55.24	61.06
Winter	8.1-7.0	86	85	83	43.77	11.44	11.43	6.69	47.03	37.39

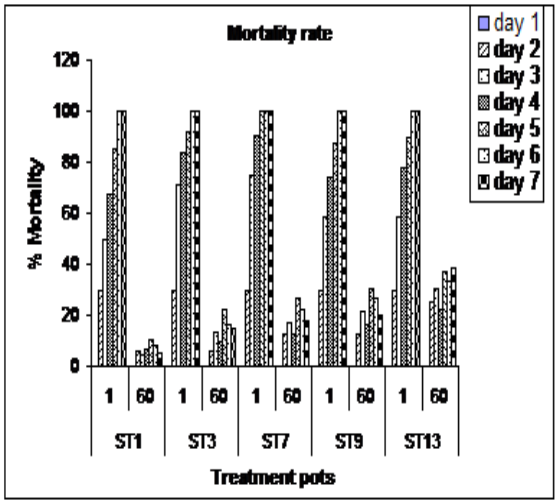
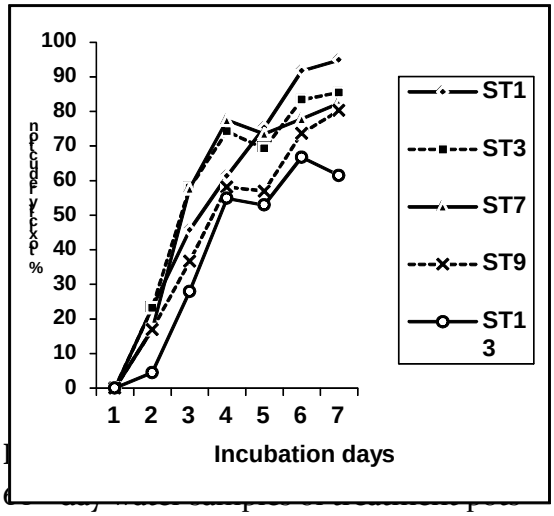


Fig. 1.4: Acute toxicity with *Lemna minor* in the treatment pots of *T. latifolia* to study the effect of heavy metal accumulation at 1st and 60th day of the treatment solution



tested with *Lemna minor* at 7 day of incubation time.

Conclusion: In the present study we observed the effect of phenol and melanoidin on heavy

metal bioaccumulation patterns in *T. latifolia*. The results revealed that there was increasing trend of metal accumulation in plants with increasing incubation time; simultaneously there was reduction in pollutants concentrations such as, BOD, COD, color, phenol and nutrient uptake like nitrogen, sulfate, phosphate along with slight fluctuations in nitrate and chloride ions. In both the sets pH change was also monitored, finally the plant maintained the pH range around 7.0-7.3. Plants with an external treatment solution of heavy metals, melanoidin (2500 Copt) and increasing concentration of phenol was much healthy and accumulated more percent of heavy metals. Therefore, the conditions are favorable for the plant growth. While plants with treatment solution of heavy metals, phenol (100 mg L^{-1}) and increasing concentration of melanoidin showed necrotic effect at initial stage but at later stage plants regain new leaves and accumulated heavy metals. But higher concentrations of melanoidin caused low bioaccumulation of heavy metals and the plants were also not much healthier at this stage. Therefore, higher range of melanoidin as external medium is toxic and lethal to plant. Further, seasonal variations were also observed in the tested plant species among which summer was the best season for the bioaccumulation of heavy metals. This study conclude that, *Typha latifolia* is also efficient for the bioaccumulation of heavy metals and concomitantly, reducing the pollutants of distillery effluent in diluted form or at low range of color concentration.

The above information for the removal potential of heavy metals (Pb, Cu, Zn, Mn, Ni and Fe) by wetland plants *Typha latifolia* can be referred in wetland treatment systems for removal of heavy metals from distillery effluent along with other pollutants. *Typha latifolia*, was chosen for the study because of their widespread availability and their proven capability to remove pollutants. Among the tested plants, *T. latifolia* showed the highest uptake of heavy metals followed by *P. australis* and *C. papyrus*. The preferential sequence of metal accumulation by the macrophytes in treatment pots were in the order of Fe > Mn > Zn > Cu > Ni > Pb. Therefore, heavy metals can be removed in the constructed wetland systems by the above wetland plants.

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