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Utilization of *Azolla* aquatic plant as phytoremediation for treatment of effluent

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Abstract
Aquatic plants *Azolla* was grown for phytoremediation treatment in the range 0 (Control), 25, 50, 75 and 100 percent effluent. Maximum values in *Azolla* plants were observed at 50 percent effluents, for fresh matter yield and dry matter yield of 15 and 30 days growth, chlorophyll contents 25 percent level of 15 and 30 days. Overall study was concluded that aquatic plants were beneficial as bioremediation treatment of effluent before using for gardening purpose and irrigation of crop plants.

Keywords: *Azolla*, Phytoramadiation, Effluent

1. Introduction
Bioremediation is an emerging cleanup technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost. The cleanup technology is defined as the use of green plants to remove, contain, or render harmless, such environmental contaminants as heavy metals, trace elements, organic compounds and radioactive compounds in soil or water. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant. Aquatic macrophytes systems can be effectively used to reduce pollutant levels in water bodies. This is typically much less expensive than excavation followed by disposal or incineration or other *in situ* treatment strategies and reduces or eliminates the need to pump and treat a common practice at the site where hydrocarbon have contaminated ground water.

2. Material and Methods
The effluent were collected from drainage of Sachendi, Kanpur in wide mouth large plastic bottles between 7-8 AM and bottled cork immediately and all the samples were brought to settle in an open cement pond in the college garden for one week to allow microorganisms to break down solid organic waste. The waste water then filtered through an 80 µm plankton net filter and brought the laboratory and stored at 4 ^{0}C temperature in a refrigerator till before treatment analysis was completed. Care was taken to prevent under shaking of the samples and also against sun light while transporting them to the laboratory.
Sample for estimation of dissolved oxygen was collected in 250 ml bottle and fixed immediately. Physico-chemical assessment of water sample has been done as per standard procedures. Certain factors such as pH, temperatures etc. on the spot were noted and for the remaining "Standard Methods for Examination of Water and Waste Water" published by the American Public Health Association (1995) have been consulted. All chemical analysis was done in or on the following day. The details procedure of each these component were same as described in, I.B.P. Hand Book No. 8 (Gotterman *et al.* 1978); U.S.D.A. Hand Book No. 16 (Richard, 1954).
For experiments *Azolla filiculoides* plant was collected from nature in their respective season of growth. In pond the plants after removing possible contamination were cultured in to three different cemented ponds. After a week plants were transferred to three different 20 litre plastic container (Tub) filled with garden pond water.
The experiment was carried out in petridish and (3" diameter). Equal amounts (approximately 5g) of aquatic plants were inoculated. Three replicate were taken for each treatment.

Fresh matter yield was determined by weight first washing with running tap water, rinsing with distilled water and absorbing surface with clean white blotting paper.

Dry matter yield was determined by drying and finely chopped and mixed plants samples by first washing with running tap water, rinsing with distilled water and absorbing surface water with clean white blotting sheets, in a forced draught oven at 65 ^{0}C for 24 hours to constant weight. The samples were taken out from the oven and placed in a desiccators, cooled for about an hours and weighed for the determination of yield.

Chlorophyll was determined by the method of Petering *et al.* (1940). Finely chopped and weighed 200 mg of fresh matter was ground in a pestle and mortar with a little acid washed while silica sand in about 10 ml of 85% acetone. The acetone extract was filtered through Whattman No. 42 filter paper, the residue on the filter paper was thoroughly leached with 85% acetone to remain the last traces of chlorophyll and leachates were mixed. The extract was made to 25/ml and stored in dark in refrigerator till the measurement of colour intensity. The chlorophyll content was measured by estimating the absorption of the acetone extract in. Elico-CL-20A-Photo-electric-calorimeter used red filter and referring the reading to the standard calibration curve prepared by the method of Comer and Zscheile (1942).

Aquatic plant (*Azolla*) was raised in control as well as different concentrations of Effluent. The different concentrations of effluents taken for culture of plants were: Control (garden pond water), 25 percent, 50 percent, 75 percent and 100 percent.

For study, samples were drawn and estimations were made for Fresh matter yield, Dry matter yield and Chlorophyll content of aquatic plants at 15 and 30 days growth of aquatic plants.

3. Result & Discussion: (Table-1)

Fresh matter yield of 15 and 30 days growth of *Azolla* increased with the increase in effluent level into 50%. Further increase in effluent level decreased the fresh matter yield of *Azolla*.

25, 50, 75% effluent level, over control showed significantly (*P*=0.05) increase in fresh matter yield of 15 and 30 days growth of *Azolla*. 100% effluent over control showed significantly (*P*=0.05) decrease in fresh matter yield of both 15 and 30 days growth of *Azolla*.

Fresh matter yield of both 15 and 30 days growth of *Azolla* was observed at 50% effluent level.

Increase in effluent level upto 50% level increased dry matter yield of both 15 and 30 days growth of *Azolla*. Beyond 50% level further increase in the effluent level decreased the dry matter yield of both 15 and 30 days growth of *Azolla*.

As compared to control, all the level tested showed significant (P=0.05) increase in dry matter yield of both 15 and 30 days growth of *Azolla*, except 100% over control in 15 days dry matter yield of *Azolla* failed to show any significant result. Where significant (*P* = 0.05) decrease on dry matter yield of *Azolla* was observed.

The maximum dry matter yield of both 15 and 30 days growth of *Azolla* was observed at the 50 % level of effluent.

Chlorophyll content at 15 and 30 days growth of *Azolla* increased with increase in effluent level upto 25% level. Beyond 25% level further increase in the effluent level showed decrease in chlorophyll content of both 15 and 30 days growth of *Azolla*.

25% effluent level over control showed significant (P=0.05) increase in chlorophyll content of both 15 and 30 days growth of *Azolla* over control. As compared to control, 100% level in 15 days and 50, 75 and 100% level in 30 days growth showed significant (P=0.05) decrease in chlorophyll content of *Azolla*. However, 50% showed an insignificant increase and 75% level over control showed insignificant decrease in chlorophyll content of 15 days growth of *Azolla*.

Maximum chlorophyll content in both 15 and 30 days growth of *Azolla* was observed at 25% effluent level.

Table 1: Effect of effluent on Fresh matter yield, Dry matter yield and Chlorophyll content of *Azolla*

Days Growth	Percent Effluent					
	C	25	50	75	100	CD (at P=0.05)
	g fresh matter yield / treatment					
15	5.51	6.70	8.48	6.45	5.41	0.07
30	6.59	7.01	9.50	7.47	6.45	0.05
	g dry matter yield / treatment					
15	1.29	2.30	4.28	2.28	1.27	0.01
30	2.29	5.01	6.29	5.28	3.28	0.42
	mg chlorophyll/g FM					
15	0.53	0.62	0.54	0.51	0.43	0.04
30	0.57	0.66	0.43	0.41	0.36	0.04

With the increase in percent level of effluent fresh matter yield increase up to 50 percent level of 15 and 30 days growth of *Azolla* plant. The results are in agreement with result that Shakya *et al.* (2007) with *Hydrilla* in waste water, Abou El-Kheir *et al.* (2007) with *Lemna gibba* in sewage water.

A maximum dry matter yield of 15 and 30 days growth of *Azolla* was observed at 50 percent. The results are in agreement with the result that Shakya *et al.* (2008) with *Cladophora* in waste water.

Increase in Chlorophyll content was found at 25 percent level in both 15 and 30 days growth of *Azolla*. The result are agreement with the result of Deval *et al.*, (2012) with *Azolla* in zinc plating industry effluent. Chlorophyll content of plants were reduced with the pesticide and the highest damage was observed at 30 ppm has also been reported by Palanisami *et al.* (2009).

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