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Phytoremediation of waste water containing sewage using *Azolla pinnata* R.BR.: A case study of Centurion university of technology & management, Bhubaneswar campus in Odisha

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Abstract
Aquatic macrophytes are well known accumulators for heavy metals in contaminated water bodies. The present study aims at developing a protocol for decontamination of waste water containing sewage using a biological treatment system by employing a selected floating macrophyte - *Azolla pinnata*, occurring in the native aquatic ecosystems. The pond receiving sewage as well as the laboratory wastes from the Centurion University of Technology and Management (CUTM) campus located at Bhubaneswar belonging to Khurda district of Odisha was selected as the sampling site. High magnitude of suspended solids, BOD, nitrogen and phosphorus in the sewage of CUTM campus indicated the high pollution load. The BOD and total suspended solid values ranged from 302- 389mg/l and 419-511mg/l respectively. Sewage at different concentrations increased the fresh weight of *Azolla* by 1.5-3.6 times over those grown in tap water. The nitrogen contribution of *Azolla pinnata* R.Br. in different treatments followed almost a similar trend as that of the fresh matter yield. Besides, culture of *Azolla pinnata* R.Br. for fifteen days resulted in reduction in the total suspended solid, BOD, nitrogen and phosphate content of the effluent to minimum accepted standard value. These responses indicate the potential of *Azolla pinnata* not only as a water pollutant scavenger but also a suitable tool for phytoremediation in the aquatic environment.

Keywords: phytoremediation, sewage, *Azolla*, BOD, nitrogen and Phosphate

Introduction
Pollution of the urban areas by waste water including sewage has become a growing concern throughout the globe, and over populated towns and cities of Odisha are no exceptions. It is reported that about 70% of the surface water is polluted by sewage and industrial effluents. Its effective and economic control is one of the primary responsibilities of any industrialists, urban authorities and governments. The present experiment has been designed to provide an overview of the role of a selected native macrophyte in detoxification of waste water containing sewage of Centurion University of Technology and Management (CUTM) campus located at Bhubaneswar belonging to Khurda district of Odisha. The accumulation of heavy metals in plants and the role of plants in removing pollutants from sewage and industrial effluents are realized in order to implement phytoremediation technology, which makes exploitation of plants to extract, transfer and stabilize toxic materials from soil and water. Phytoremediation, being more cost-effective and with fewer side effects than physical and chemical approaches, has gained increasing attention in recent times, as an emerging cheaper technology which makes use of vegetation to remove and detoxify persistent pollutants.

In order to maintain good quality of soil and water and to keep them free from toxicity, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. Conventional physicochemical methods of treatment are invariably cost-intensive and cannot be employed in all industries especially in small and medium scale industries. In this situation biological treatment systems may serve as one of the alternatives. Mix cultures of algae and bacteria have long been proved to be an inexpensive process for the reclamation of waste water [1-6]. One of the recent trends is to search for new photo-autotrophic organisms with high growth rates and high utilization potential, which could be mass cultured in waste water and play a dual role of cleansing the water and serving as a source of feed and fertilizer. Free living blue-green algae, *Mazola-Anabaena* consortium may ideally be suited to perform these functions by virtue of their high growth rates and their known nutritional and fertilizer value. Though attempts in this direction using some fresh water and marine blue-green algae have been made. The idea of using any other aquatic higher plants are relatively meager [6-8]. Therefore, a new biological treatment system using a small aquatic fern, *Azolla* was tried at potted condition. The usefulness of this system with reference to the reduction of some of the pollutants in the sewage of Centurion University of Technology and Management (CUTM) campus located at Bhubaneswar reported herein.

Materials and Methods
Plant materials
The water fern *Azolla pinnata* R.Br. (Family - Azollaceae), collected from nearby water bodies of the locality was selected for the present investigation. This plant is symbiotically associated with a nitrogen fixing blue-green alga - *Anabaena azollae* rated as an efficient biofertilizer for rice.

Experimental site
Centurion University of Technology and Management (CUTM) campus, Bhubaneswar was chosen as the site for the present investigation which belongs to Khurda district of Odisha. Due to rapid expansion of the university campus the quantity of waste water including sewage increase to

manifold. Sewage water contains mostly liquids waste from toilets, baths, showers, kitchen, sinks etc. and also contains some liquid wastes from research laboratories and workshops present in the campus. For the present investigation, one of the major drains was selected, because the drain carries the waste water from the area having almost all hostels, staff quarters and laboratories of the different science and engineering departments. The studies included the assessment of various physic-chemical characteristics of drain water in different seasons of the year (2018 - 2019) and the growth performance of *Azolla*.

Pot culture

Sewage from one of the major drains of the experimental site was collected in large polythene cans (25 l) and brought to the laboratory. Before collection of samples containers were rinsed with desired drain water. Pot culture of the selected plant was done initially in tap water for 10 days and then cultured for 15 days in the waste water containing sewage of various concentrations. The effluent was diluted with tap water in order to obtain desired concentrations of 0% (control: tap water only), 20%, 40%, 60%, 80% and 100% sewage. All the treatments were in triplicates and arranged in Randomized Block Design (RBD). After 15 days of culture, the growth of *Azolla* and the status of the pollutants were determined following standard methods.

Estimation

The *Azolla* fronds were harvested and kept separately treatment-wise for the estimation of their biomass production, chlorophyll and nitrogen content. The biomass was determined by weighing the blotted fronds with the help of single pan balance (No.50T, Salter). The frond size was estimated by plotting the outline of the plant on mm graph paper. Total chlorophyll content (mg g^{-1} fresh weight) of the plant was determined following the method [9]. Samples of *Azolla* were oven dried at 70º C for 48 hours, powdered in a Willey mill and analyzed for nitrogen and phosphorous by modified Kjeldahl method and amidal blue-colouration method respectively [10].

All the collected samples were brought to the laboratory and stored at 5±1' C and analysis of various parameters such as BOD, COD, suspended solids, ammoniacal nitrogen, organic nitrogen and phosphate was done following [11]. All these parameters were also analyzed after 15 days of culturing *Azolla* in graded levels of sewage following same procedure as described earlier. Temperature and pH were measured at sampling sites with the help of digital portable kit. Temperature and pH were measured at sampling sites with the help of digital portable kit.

Results and Discussion

Visual observations have been made to generalize the physical conditions of the sewage water. Strong putrid odour existed due to the decomposition of different kinds of organic matter by microorganism. Blackish colour of the water was due to discharge of different types of pollutants into the selected drain. Formations of flocks of sewage and algae were the unique characters of the drains. Values of various physico-chemical parameters of water samples collected from water body receiving sewage discharged from the CUTM, Bhubaneswar campus in different seasons were recorded (Table 1). The pH was found always towards the alkaline side that is 7.1 to 7.5 irrespective of season of collection. The examination of BOD and COD in water indicates the real status of the extent of pollution [12]. The high BOD value (averaging 389 mg/1) in summer season indicated a high pollution load in the site receiving sewage. The high values of organic and ammoniacal nitrogen ranged between 14.7 to 17.4 mg/l and 15.8 to 20.3 mg/l respectively, which indicates high amount of nitrogenous organic matter in waste water. The nitrogen present in the organic compounds is considered as organic nitrogen. The breakdown of organic portion of the molecules by oxidation liberates nitrogen as ammonia. The increased concentration of phosphate in the waste water containing sewage also indicated the pollution status. High concentration of nitrates and phosphates are reported to be the major contributions to the deterioration of water quality in the US or elsewhere [13]. Industrial and urban waste waters which are concentrated in small areas can be treated to reduce output of these nutrients into the aquatic system. Such physico-chemical treatments, however, is expensive. Biological treatment system can be used to strip nutrients from water. Several aquatic organisms growing on shallow ponds can use dissolved ionic pollutants as nutrients and remove these ions from water. However, rooted submerged angiosperms take up nutrients from both water and sediment, and non-rooted submerged angiosperms and floating-leaved plants derive nutrients directly from water [14]. A major limitation to this approach has been the difficulty of harvesting the aquatic organisms used to strip nutrients, such as bacteria, cyanobacteria and green algae. *Azolla*, however, was found to be a good candidate for this technique. It grew rapidly, could fix molecular nitrogen for its own use and was easy to harvest. Although there is no reported instance of *Azolla* cultivation improving water quality, a New Jersey pharmaceutical manufacturer found that water drawn from a canal with a population of wild *Azolla* was easier to purify, indicating that the impurities in the canal water had been reduced[15]. Aquatic macrophytes occur in almost all fresh water bodies which are enriched by natural process or as a result of nutrient loading from urban, agricultural, mining or industrial activities. It is reported that aquatic weeds alter the water quality by accumulating various minerals and nutrients from the medium in which they thrive [16] ·Most of the elements studied were found to be accumulated by these weeds which in turn bring about characteristic elemental reduction in waste water. In Odisha, *Azolla* grows extremely well on nutrient-rich water in temporary ponds, agricultural drains as well as low-land rice fields [17,18,19].When grown in sewage generated from CUTM, Bhubaneswar campus, increased *Azolla* growth in terms of biomass, frond size, N content and chlorophyll content was observed with the decrease in mean BOD (92.4%), suspended solids (91.3%), ammoniacal nitrogen (52.8%)and phosphate (68.75%) content of the waste water (Table 2). Although most of the N came from biological nitrogen fixation, the phosphorous must have come from the sewage water. Also, if nitrates are present in the water, *Azolla* readily absorbs them even while fixing N_2 [20]. Any system used to reduce pollutants in water involves the ultimate removal of the pollutants from the system. *Azolla* is readily harvested by skimming it off the water with large porous plastic net. Mechanical procedures to harvest *Azolla* appear feasible, especially if an economic return were possible. The use of *Azolla* as a source of nitrogenous nutrient for rice cultivation is discussed in several reports [21-29]. Obviously, any organism with high N content have value

in an agronomic system, and, when incorporated with the soil would also provide other nutrients (phosphorous, sulphur, potassium etc.) for crop plants. That would compensate some of the costs of harvesting and distributing *Azolla*.

The conventional biological treatment systems are very much useful only in cases with very high BOD and COD values [30]. *Azolla* under pot trials was found to produce biomass more than 3 folds in a retention period of 15 days (Table 3). The growth of the fern in terms of its frond size, total chlorophyll and nitrogen content followed a similar trend as that of biomass production. Higher chlorophyll content (0.72 mg g^{-1}fr.wt) in *Azolla* tissues in the pots received higher sewage concentration than that of control (0.46 mg g^{-1}fr. wt.); this may be due to their higher growth and maturity under a favorable aquatic environmental conditions. There was a gradual enhancement in the growth of *Azolla* concomitant with the increase in the concentration of the sewage up to 100% (Table-3)

It was interesting to note that the concentration of the pollutants especially total suspended solids and ammoniacal nitrogen as well as BOD were decreased to a minimum accepted standard value [31], when Azolla was grown for a period of 15 days (Table 2). It is evident from the present study that the floating hydrophyte especially *Azolla pinnata* R.Br. - based treatment system proved to be a promising tool for the removal of pollutants from the liquid medium. The luxuriant growth of aquatic weeds (*Azolla*, *Lemna*, *Salvinia*, *Spirodela*, *Pistia*, *Eichhornia* sp.) in water bodies receiving waste water including municipal sewage, industrial effluent and mine waste water indicates that these can absorb and incorporate the inorganic and some organic compounds into their body. Hence the above mentioned macrophytes may have a direct role in detoxifying the waste water by reducing the level of pollutants.

Conclusion

The present study contemplates the exploitation of *Azolla pinnata* - an aquatic fern species in detoxifying the waste water from sewage. This will endow this plant specimen with a dual role as organic manure as well as contributing as waste-disposer. Thus the significance of this study lies in the implementation of a novel bioremediation technique by exploiting a selected floating plant species of native wet-land ecosystems for decontamination of waste water containing domestic liquid wastes including sewage. This procedure envisages an economically viable and sustainable technology as compared to the cost-intensive conventional physico-chemical methods being practiced by the municipal authorities. Further, the harvested biomass can not only be utilized as a source of organic nutrient source for use in crop production but also can become a suitable raw material for biogas generation as well as production of nutrient-rich vermicompost.

Table 1: Physico-chemical characteristics of sewage of CUTM, Bhubaneswar campus, Odisha

Parameters	Season			Standard for disposal into inland surface water
	Winter	Summer	Monsoon	
Temperature (°C)	17.5±0.871	36.7±1.948	26.8±1.07	-
pH	7.1±1.059	7.5±0.736	7.0±0.435	5.5-9
Suspended solids (mg/l)	474±13.228	419±14.107	511±11.135	100
BOD, 20 °C (mg/l)	302±9.643	389±11.533	303±10.148	30
Organic nitrogen (mg/l)	17.4±0.458	15.4±0.668	14.7±1.113	-
Ammoniacal nitrogen (mg/l)	18.6±0.912	20.3±0.716	15.8±1.311	50
Phosphates (mg/l)	0.17±0.043	0.35±0.132	0.22±0.09	5

Table 2: Role of *Azolla* in reducing pollutant from the sewage after 15 days of culture

Treatment	Suspended Solids (mg/l)	BOD at 20°C (mg/l)	Ammoniacal nitrogen (mg/l)	Phosphate (mg/l)
Control (No *Azolla*)	471	304	19.5	0.16
Culture of *Azolla pinnata*	41 (91.3)	23 (92.4)	9.2 (52.8)	0.05 (68.75)

Figures in parentheses represent the % of reduction from control.

Table 3: Growth of *Azolla* in graded concentration of sewage at 15thday of inoculation

Conc. of sewage (%)	*Azolla* growth in different concentrations of sewage			
	Gain in biomass (g fr. wt./pot)	Frond size (cm^2)	Chlorophyll (mg/g fr. wt.)	Nitrogen content (%) (on dry wt. basis)
0	5.25±0.376	0.9±0.136	0.46±0.132	3.1±0.364
20	7.95±0.216	1.1±0.18	0.48±0.118	3.4±0.3
40	10.75±0.437	1.1±0.186	0.5±0.081	3.8±0.07
60	13.5 ±0.242	1.2±0.219	0.55±0.087	4±0.423
80	16.5 ±0.105	1.25±0.222	0.62±0.098	4.2±0.278
100	19.0±0.366	1.3±0.158	0.72±0.05	4.5±0.218

Inoculum size: 5.0 g fresh *Azolla* /pot

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