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PHYTOREMEDIATION POTENTIAL OF AZOLLAPINNATA TO TREAT SUGAR CANE INDUSTRIAL EFFLUENT

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Abstract

Phytoremidiation technology is continuously receiving attention as an innovative, profitable substitute for the treatment of industrial effluent. The results of this experiment show *Azolla pinnata* growth in the varied concentration of effluent which reduced both organic and inorganic pollutants found in the effluent sample. The positive growth of *Azolla pinnata* sp was confirmed by the dermination of parameters such as biomass estimation, chlorophyll content, biomolecular contents , measuring growth rate. The growth of *Azolla pinnata* increased in 25% concentration of the effluent when compared to other experimental concentration and there was no growth at 100% concentration. Experimental results suggested that low concentration was found to have high potential of biomass production. The maximum removal rate was observed in 25% dilution of effluents and minimum in 50%. No response was observed in 75% and 100% effluent concentration. The rate of toxicity reduction depends on percentage of dilution factor in the presence of aquatic species. Results revealed reduction of toxic waste after treatment of effluent in the presence of *Azolla pinnata*. Considerable reduction in pollutants and harmful substances observed during the experiment proving that the plant *Azolla pinnata* fern could be considered as phytoremediating agent, also demonstrating its efficiency in wastewater treatment.

Key words: *Azolla pinnata*, effluent, *vigna radiata*, phytoremediation, irrigation

Introduction

The environmental pollution is one of the most severe problems nowadays.Cane Sugar Industry being an important role in the Indian economy as well as in the foreign exchange earnings and also plays a very vital part in polluting the environment with its waste discharge. With expansion of Sugar plants, pollution due to inadequate and it becomes threat for environment.Many of these compounds are both toxic and persistent in terrestrial and aquatic environments [1].

The contamination of soil, surface and groundwater is simply the result of the accumulation of these toxic compounds in excess of permissible levels. In the sugar industry, water is used for cleaning purposes in the different sections of the factory generates wastewater.Studies of physicochemical properties of the sugar industrial effluent which were collected and analyzed from the mill house, process house and final combined wastewater indicated that the effluent qualities and quantities are quite different

Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil[2] .

Azolla pinnata has a tremendous potential to take up heavy metals among which include Mercury and Cadmium (70–94%) may be used as a bio-accumulator to absorb heavy metals in effluent. *Azolla* is used as a food supplement fresh dried or ensiled for a variety of animals, including pigs, rabbits, chickens, ducks, and fish. Applications or remediation approaches become imperative when the buildup of these toxic substances in water andsoil is beyond permissible limits. [3] Novel Biotechnology approaches are being applied for treating wastes as a process of bioremediation. Phytoremediation of wastewater using the floating plant system is a predominant method which is economic to construct, requires little maintenance and increase the biodiversity. The present study is focused on the phytoremediation wastewater treatment and removal of sugar cane effluent pollutants based on the abilities of aquatic fern to recycle nutrients, offer an attractive solution for the bioremediation of water pollution.

In the present study has focused for the analysis of the composition of sugar industry effluent and evaluates the alternative treatment options used to remediate on the challenges impacts of effluent through *Azolla pinnata* can be considered as phytoremediation approaches.

Materials and Methods Sample collection

The sugar cane industry effluent was collected from Rajashree sugar cane industry near Theni Dt, Tamil Nadu, India. Plant materials used in this study was *Azolla pinnata* collected from *Azolla pinnata* farm and water pond which located in Theni Dt, Tamilnadu, India, the samples were collected in polythene containers (5 liters). The plants were washed thoroughly and maintained in tap water for 15 days to acclimatize to laboratory conditions.

Treatment Methods

Batch experiments were conducted in 4 plastic tubs (5litres capacity). 5 to 10 g of sample *Azolla pinnata* were introduced in the effluent made of dilution in various ranges, 25, 50, 75 and 100% up to 3 liters/tub which were kept separately for a period of 30 days. After 30 days the inoculated *Azolla pinnata* samples were harvested and used for biomass studies subsequently culture filtrates which means treated effluent subjected for furthermore experiments. Efficiency of culture filtrate was tested by using *Vigna radiate* seeds germination also other growth parameters to confirm phytoremediation potential.

Experimental set up

The *Azolla pinnata* weight of 5-10 gms were introduced in 2.5 liter of diluted in various ranges, 25,50,75 and 100% untreated, *Azolla pinnata* were inoculated samples separately for a period of 30 days. After a period of 30 days, the biochemical estimation such as Protein, Carbohydrate, chlorophyll of different samples were carried out after harvested of *Azolla pinnata* from various concentration effluent treated and the culture filtrate has used for germination of *Vigna radiata* and further growth parameters studies.

Analysis of physicochemical parameters of effluent

The physico-chemical parameters of effluent such as color, odour, pH,, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) present in the effluent samples were studied following the Standard methods outlined by [4] before and after introduction of the *Azolla pinnata* plants in the effluent samples. The initial and final level of minerals in the effluent samples was estimated in the Atomic Absorption Spectrophotometer (AAS) as per the procedure of [5].

Germination studies

These were carried out the Petri dishes kept moist by a layer of cotton and a coarse filter paper. Ten seeds per Petri dish of *Vigna radiata* . Five Petri dish are maintained for each concentration. A portion (20ml) of each concentration of the effluent was added to Petri plate on alternate days and control received the same amount of normal water. The germination was growth or not.

Treatment of plants with industrial effluent

A different set of pots of 12cm diameter was filled with soil which contained sand. The *Vigna radiata* are put in the pot after 3 days the seeds are germinated. After 15 days the plants are treated with effluent (25%, 50%, 75%, and 100%) and control plant is treated with normal water then the result is interpreted.

Minerals analysis

Azolla pinnata grown in different concentration of the effluent were used for the estimation of biomass. After one month of *Azolla pinnata* culture grown in the effluent were collected and taken fresh weight and allow it to dry under shade to remove all moisture content then dry weight of the sample weighed. Dried plant biomass were digested by wet digestion method according to [6] and analyzed by AAS to determine the metal concentrations in *Azolla pinnata* tissues. Samples of dried *Azolla pinnata* (0.5 g) were digested with 15ml of concentrated nitric acid, perchloric acid and sulphuric acid in the ratio 10: 4: 1. The digested samples were filtered through whatman No. 42 filter paper and the final volume of each sample was made up to 100 ml with distilled water. The concentration of K, Na, Mg, Ca, Mn, Zn, Mn and Fe were determined by atomic adsorption Spectrophotometry [7].

α- Naphthalene Bio assay

The *Vigna radiata* seeds were washed thoroughly in tap water and surface sterilized with 0.1% mercuric chloride. The surface sterilized seeds were washed thrice with sterile water. 25 seeds were placed in a sterile petriplate containing moistened filter paper. plant were treated with sugar cane industrial effluent treated with *Azolla pinnata* each of different concentration ranges 25%, 50%, 75%,100% and control contains only tap water which were kept for 3 days under labortory conditions. After emerged radical the length of roots and shoots of plant were measured in cm and taken from the petriplates. The roots were washed in the water cut in to 2-3 cm segment. The excess water removed by squeezing the roots with filter paper. 1-2 g of this sample was weighted and transferred to an Erlenmeyer flask containing 50ml of 20- ppm α- nepthalamine solution. The flasks were kept in a rotatory shaker for 2-3 hrs at 100-200 rpm. 20ml of aliquate of α- napthalamine solution was pipetted out after the incubation and diluted to100ml with distilled water. To this 1.0 ml of sulphanilic acid (1 g in 100 ml 30% acetic acid) and 1.0 ml with distilled water. The tubes are incubated for 30-60 minutes color development and read at 500 nm in a colorimeter by the methods of [8].

Estimation of biomolecules

The shoot portions of *Vigna radiata* were collected in each batch of experiments and homogenized with Tris buffer, the extracted contents were centrifuges. The supernatant was used for carbohydrate precipitation and the amount estimated by the method of Anthrone [9]. The leaves are subjected for the extraction of protein and the amount of protein was estimated by the method described by Lowery et al [10] using bovine serum albumin as standard. Total chlorophyll contents were estimated by the method of Arnon [11] using 80% acetone as solvent for pigments extraction procedure.

Results and Discussion

***Azolla pinnata* culture were inoculated in various dilution of the sugarcane industrial effluent as per experiment**

design. The results of physicochemical parameters analysis are represented in table 1 which reflect characterization of effluent before and after remediation in the presence of A*zolla pinnata.* The amount of reduction in each mentioned parameter shows the potential of A*zolla pinnata* in the treatment process of water pollution.

Physicochemical characteristics of the effluent revealed color of the waste water was recorded as dark brownish due to the presence of sugar residues in the waste water. The pH of the effluents is generally depends on process involved in of industry. Final effluent samples showed more acidic in nature, since it contained the acidic pH 4 the values of pH are due to the change in the manufacturing process of industry and several chemicals are used for coagulation of impurities.

Results given in the Table1 revealed effluent created warm condition at the point of discharged area in water bodies. The reported waste water temperature was changed from 70°C to 37°C due to thermal stratification which showed water temperature fluctuations. Sugarcane molasses spent wash after biological treatment results in significant removal of BOD and COD from the effluent. Oxidation process could achieve 90% decolorization for biologically treated spent wash with simultaneous COD reduction. It also resulted in improved biodegradability of the effluent.

Table 1: Characteristics of Sugarcane industrial effluent

S.No	Parameters	Before Inoculation (mg/L)	After Inoculation(mg/L)
1	Color	Thick Black	Normal
2	Odour	Bad	Normal
3	pH	4	6.5
4	Temperature	70°C	37°C
5	BOD	49000	8590
6	COD	24500	7490
7	Total Solids	950	758
8	Suspended solids	95	78.5
9	Dissolved Solids	2689	987
10	Volatile Solids	47000	600

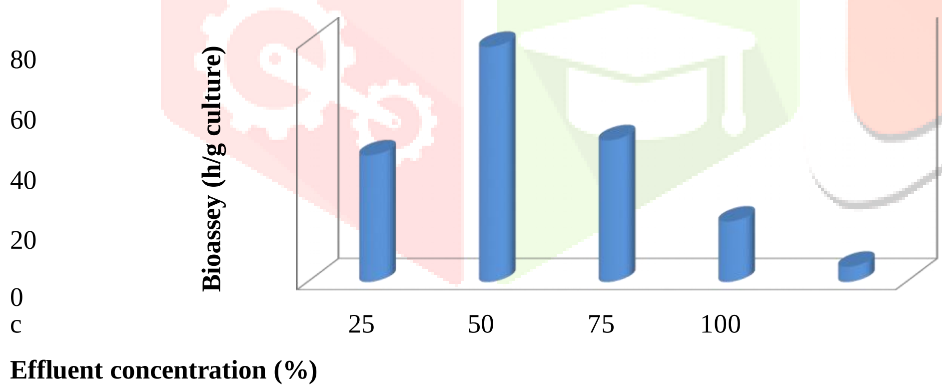
Most fertilizer on the market contains large amounts of potassium, phosphorus and nitrogen. Micronutrients such as Mg, Ca, Cu, Mn, Zn Na, Fe and macronutrients N, P, K needed for plant growth are found in the effluent. After inoculation (30 days) the aquatic plants absorb these minerals and use them as secondary nutrients.

Table 2: Analysis of minerals in the effluent

S.no	Parameters	Concentration(mg/lit)Bef o re inoculation	Concentration(mg/lit) After inoculation
1	Potassium	386.0	296.0
2	Copper	0.9	0.6
3	Magnesium	679.0	479.0
4	Sodium	487.0	278.0
5	Zinc	1.6	1.2
6	Phosphorus	432	368
7	Calcium	541.0	268
8	Lead	0.2	0.1
9	Manganese	268.0	187.0
10	Nitrogen	312	159

Azolla pinnata aquatic ferns grown in diluted effluent has consider as culture filtrate used substrate for all analysis. Totally 25 number of *Vigna radiata* seeds were placed in petri plates containing 10 ml of diluted effluent allow it for germination. Figure 1 showed the percentage of germinated seeds in the presence of leach ate which could stimulate inoculated seed to germinate. Results showed that maximum number of seeds were emerged at 25% of effluent treated with *Azolla pinnata* culture filtrate than control and other concentration used in the experiments.

Figure1: Percentage of *Vigna radiata* seed germination in effluent



Legends: X axis indicates various dilutions of the effluents; Y axis indicates of bioseedling assay in *Vigna radiata* grown in filtrate of *Azolla pinnate* inoculated in effluent as mentioned in methods; Valves are means of five replicates.

The experimental results indicated that as the dilution of the effluent increased there was an increase in the growth rate of shoot and root length of the *Vigna radiata* seeds germinated on the petriplates contained 10ml of each of different concentration such as 25,50,70 and 100% were poured into the petridishes. The percentage of seed germination and radical emerged length of shoot and roots were measured [Figure 1]. Results revealed that the growth of root length higher in 25% dilution as compared with other dilution of effluent and control. Overall observation reflect 25% concentration enhanced growth of plant that could be considered as positive finding in the case of effluent treated with aquatic fern for 30days thereby increased biomass of *Azolla pinnata* automatically which released nutrient in the culture filtrate whereas it has suggested leased out factors helpful for the growth of *Vigna radiata* in all batch of experiment. Growth of shoot length was faster than development of roots length of germinated seeds [Figure2].

Figure 2: Growth of *Vigna radiata* in culture filtrate of *Azolla pinnata*

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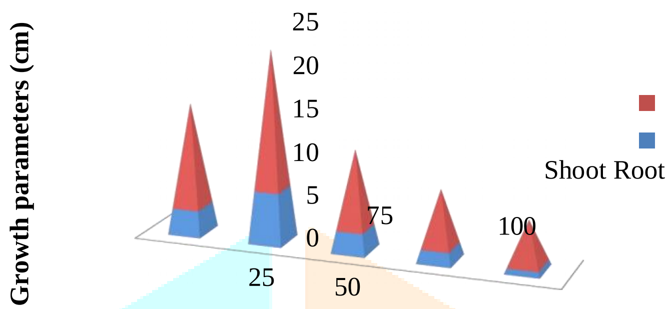


Concentration of effluent (%)

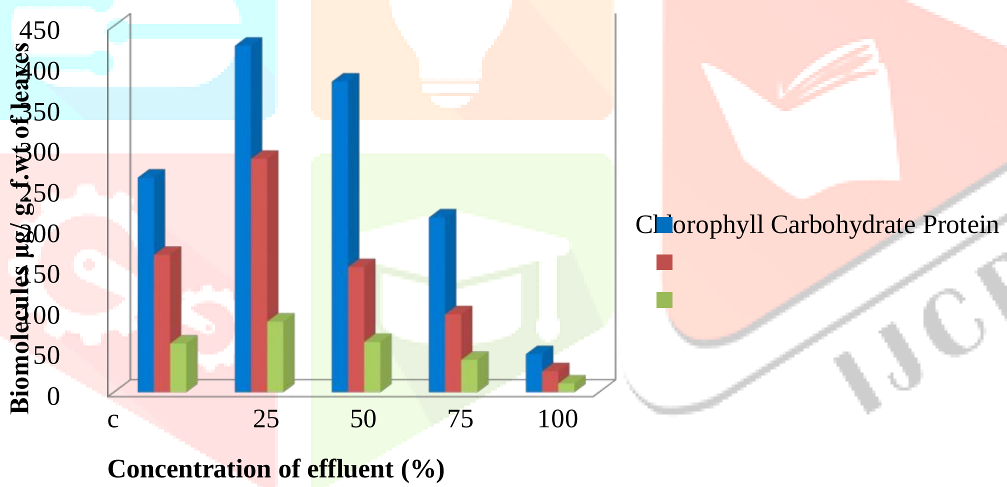
Legends: X axis indicates various dilutions of the effluents; Y axis indicates root and shoot length of *Vigna radiata* grown in filtrate of *Azolla pinnate* inoculated in effluent as mentioned in methods; Valves are means of five replicates.

Azolla pinnata fern absorbed organic and inorganic component from effluent has played significant role in the development of *Vigna radiata* also more specifically increased shoot portion length than root length. *Azolla pinnata* acts as promising agent to remove pollutant from waste water also biomass of *Azolla pinnata* positively enhanced thereby effluent treated by phytoremediation. The estimation of primary product aquatic ecosystems is essential for the valuation of biological potential of these ecosystems. Studies based on primary production are also very important in the understanding of pollution effect on the system efficiency as all organic productions in the ecosystem depend on photosynthetic organisms. Biomass of *Azolla pinnata* has enriches soils with organic matter could be considered as eco friendly bio-fertilizer. *Azolla pinnata* plants healthy and matured were harvested then rinsed with distilled water and blotted on filter papers to remove adherent water then dried and used for digestion for determination of minerals contents. Various environmental factors affect the rate of net primary productivity on land and the total storage of organic carbon in plant tissues as biomass. The result indicates that *Azolla pinnata* can increase biomass due the accumulation of organic contents of waste from diluted effluent.

The weight of *Azolla pinnata* was recorded after keeping on a filter paper to remove excess water. Physiochemical analysis of effluent revealed the presences of phosphorous mg/g d.wt and nitrogen etc [Figure3]. The photosynthetic activities of *Azolla pinnata* plant enhanced also elements in the biomass which could be used as bio fertilizer for paddy field using *Azolla pinnata* subsequently culture filtrate applied as substrate to induced germination of seeds also growth rate of *Vigna radiata* plant. According to the present findings, phytoremediation is an emerging technology using selected plants to clean up the contaminated environment from hazardous contaminant to improve the environment quality.

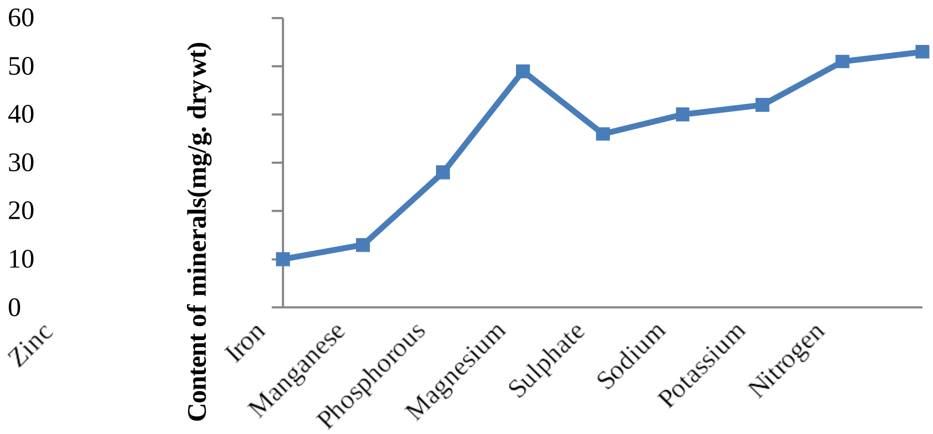
Vigna radiata plants productivity was increased which has been explained by the presences of biomolecules such as chlorolophyll, protein and carbohydrate contents. The healthy growth of plants were determined by synthesis of biomolecules in plants which could be confirmed in the experimental results given in Figure 3. The data revealed that chlorophyll contents were increased in 25% effluent dilution treated plants than control and other concentration mentioned in the experimental design. In general, crop plants provide almost everything that man need and the nature has been influencing to make use of available resources. Phytoremidiation efficacy showed by plants is based on the biomolecule compound that is present in the plants which are known as macromolecules of plants or secondary metabolites, stored in various plant parts but are essential for humans to get plant constituents'. Bioactive chemical constituents are the important plant metabolites which show remarkable health related benefits. Further, screening of phytoconstituents are the primary steps for development of new drug.

Figure 3- Analysis biomolecules in *Vigna radiata* germinated in culture filtrate



Legends: X axis indicates various dilutions of the effluents; Y axis indicates biomolecules content such total chlorophyll, carbohydrate, and protein) *Vigna radiata* grown in filtrate of *Azolla pinnate* inoculated in effluent as mentioned in methods; Valves are means of five replicates.

Figure 4: Analysis of minerals in *Vigna radiata* germinated in culture filtrate



Minerals

Legends: Shows the concentration of minerals accumulated in the biomass of *Azolla pinnata* which has grown in various concentration of industrial effluent analyzed by AAS. X axis indicates various minerals parameters; Y axis indicates concentration of minerals of *Azolla pinnata* inoculated in effluent as mentioned in methods. Valves are Means of five replicates.

Phytoremediation efficiency of A*zolla pinnata* has been confirmed by the determination of reduction in the level BOD, COD, pH, Temperature, Total Solids, Suspended solids, Dissolved Solids, Volatile Solids of treated effluent. This phytotoxicity studies aims to effectively support in water management, suggesting a way to re-use wastewater for agricultural purposes by reducing contaminants from waste water.

Conclusion

The application of biodegradation of the industrial wastewater treatments could be a viable and low-cost alternative to conventional physical and chemical processes. Phytoremidiation, the biological treatment method is effective for highly polluted agro-industrial waste waters from the sugar industries. *Azolla pinnata* plants have been adopted and used for phytoremediation purposes. After the application of phytoremediation utilizing *Azolla pinnata* demonstrates impressive ability of improving wastewater quality and thereby reducing or eliminating the negative effects on the environment during wastewater discharge. According to results the use of treated wastewater in agricultural field might be supporting of disposal and would sustain agriculture in non irrigated areas where the availability of fresh water is scarce. It reduces fertilizer and irrigation water cost as it mineral content in the treated effluent could be used for enhancing the growth of agricultural crops.

Reference

1. K.Sanjay "Environmental Pollution and its Management in Sugar Industry in India: An Appraisal.Jor. Sugar Tech.7 (1)2005; pp. 77-8.
2. APHA"Standard Methods for the Examination of water and waste water" 19th Edition ,APHA, WPCF, AWWA, 1995; New York,USA.
3. Newete, S.W. and Byrne, M.J. (2016). The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. Environmental Science and Pollution Research, 23(11): https://doi.org/10.1007
4. F. Abraham Samuel, V. Mohan,, and L. Jeyanthi Rebecca (2014). Physicochemical and heavy metal analysis of sugar mill effluent. Journal of Chemical and Pharmaceutical Research, 2014, 6(4):585-587
5. R.P.Athalye, K.S. Vidya Mishra,, Q.Goldin, U.Vidya. and S. Gokhale,. Heavy metals in the abiotic and biotic components of Thane Creek, India. Poll. Res., 18(3): 2001; 329- 333.
6. B.N. Paul, S. Chandra, S. Das, P. Singh,, B.K Pandey (2014). Mineral assay in atomic absorption spectroscopy, The Books of Natural sciences,4(1)pg.
7. Vinod kumar, Jogendra Singh,, and Pankaj kumar (2018), adding benefits to phytoremediation of sugar mill effluent by growing water hyacinth (Einchhornia crassipes): Evaluation of biomass for biogas production. environ. sci.3(3): 275-288.
8. G.Renuga. Tannery effluent induced alteration in the biochemical parameters of blue green algae. Poll res. 24(1): 2005; pp.113-117.
9. J.E. Hedge and B.T Hofreiter (1962). In: Carbohydrate Chemistry 17 (Eds Whistler RL and Be Miller, JN) .Academic Press, New York.
10. Protein assay by Lowry,OH,NJ Rosbrough , AL Farr,, and RJ Randall.J.Biol. chem.. 193: 265. 1951.
11. D.I Arnon, Copper enzymes in isolated chloroplasts, polyphenol oxidase in beta valgaris. Plant physiol., 24, 1949; pp.1-15.