The Pharma Innovation Journal 2022; SP-11(7): 1190-1193

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 1190-1193

www.thepharmajournal.com
Received: 07-05-2022
Accepted: 10-06-2022

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Waste water treatment from dairy farm by using Azolla (*Azolla pinnata*)

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Abstract
The present study was carried to test the Azolla (*Azolla pinnata*) as bioremediation agent for dairy farm liquid waste and to find out the effect of feeding Azolla obtained from bioremediation on growth performance of Nellore lambs. Eighteen Nellore lambs having uniform body weights were selected and randomly divided into 3 groups T0 (0% Azolla), T1 (10% Conventional Azolla), and T2 (10% Phytoremediation Azolla obtained from dairy farm liquid waste) of 6 lambs in each group in a completely randomized design. The experimental diets were offered to the lambs for a period of 90 days. Bioremediation of dairy farm liquid waste with Azolla decreased the pH from 7.96 to 7.38 and the colour of the liquid waste was changed from greyish black to brown colour. The turbidity, COD, BOD, Nitrogen and Phosphorous content was decreased to 48.14, 0.74, 0.43, 30.76 and 7.44%, respectively. The present study concluded that Azolla can be used for bioremediation of dairy farm liquid waste in an eco-friendly manner.

Keywords: Azolla, pH, COD, BOD, nitrogen, phosphorous, colour, turbidity

Introduction
Intensive livestock production operations generating huge quantities of farm effluent which contains a mixture of dung, residual feed material etc. The major contaminants in the effluent are nitrogen and phosphorus usually arises from breakdown of proteins in the dung and feed residues. The farm effluent without any treatment now is being discharged on land or into different water bodies, resulting into contamination of drinking water bodies and environment. Therefore, treatment of the effluent in an eco-friendly manner to convert the pollutants into useful biomass is essential to the environment. The available conventional technologies like ultrafiltration, chemical oxidation and reduction, electrochemical treatment, reverse osmosis, coagulation-flocculation, and ion exchange etc. are highly expensive and not practically feasible for adaption by the livestock producers.

Bioremediation technology is one of the novel methods in the field of waste and environment management because plants are solar driven and is presently gaining immense credibility for being eco-compatible. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site (Arora *et al*., 2006). Azolla is an Aquatic macrophyte floating freely on water and rich in protein, essential amino acids, vitamin A, B12, and b-carotene and minerals like calcium, phosphorous, potassium, iron, copper, manganese, etc (Pillai *et al*., 2005). Azolla also used for waste water treatment and is more efficient than terrestrial plants because of their faster growth and larger biomass production, relative higher capability of pollutant uptake and better purification effects due to direct contact with contaminated water (Sood *et al*., 2012). Azolla can fix atmospheric nitrogen with help of blue green algae, Anabaena azollae, which makes the azolla tend to contain relatively high level of nitrogen and can be a protein source for animal feeding. *Azolla pinnata* was used to reduce the BOD of waste water (Ranaijangwattana *et al*., 2010) [17]. Azolla removed ammonia, total organic carbon, nitrite, phosphorous from waste water and prevents eutrophication in water bodies (Divya *et al*., 2018) [7]. The present research was carried to find out the effect of azolla on dairy farm liquid waste as a bioremediation agent.

Materials and Methods
The dairy farm liquid waste bioremediation using Azolla was tested in 20 litre plastic tubs with dimensions of 60X30X30 cm. A total of 7 clean plastic tubs were taken and 6 tubs were filled with dairy farm liquid waste obtained from a buffalo farm unit (50 numbers) located at

Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram and the remaining tub with plain water for about 10 cm height. About 10g fresh Azolla was inoculated for each tub. All the tubs were kept under shade net. The temperature and pH in the tubs were monitored throughout the experiment. After 10 days, the Azolla covered the tub in a complete mat was harvested and washed with distilled water and dried in oven at 60 ^{0}C for further analysis. The dairy farm liquid waste samples were collected before and after Azolla growth in sterile plastic bottles, corked tightly and stored at a temperature below 4 °C to avoid any physico-chemical changes in the effluent for further analysis.

Estimation of Physico- Chemical Parameters

The physico-chemical parameters of dairy farm liquid waste such as temperature, pH, color, turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD), Nitrogen and Phosphorous were estimated (APHA 1998) [2] in dairy farm liquid waste before and after bioremediation using azolla.

Temperature

The temperature of the dairy farm liquid waste samples before and after bioremediation using Azolla was measured using thermometer. The bulb of the thermometer dipped into liquid waste sample and the temperature was measured and recorded.

pH

The pH of the dairy farm liquid waste samples before and after bioremediation using Azolla was estimated using pH meter (OAKTON pH 700). The sample was mixed well and dipped the pH electrode in liquid waste sample and drawn for pH measurements. Rinsed the pH electrode and blot the electrode with soft tissue paper and dipped the pH electrode into next sample.

Turbidity

The turbidity of the dairy farm liquid waste samples before and after bioremediation using Azolla was estimated using Spectrophotometer (919-100 Nano spectrophotometer) and the difference in turbidity was recorded.

Colour

The colour of the dairy farm liquid waste samples before and after bioremediation using Azolla was noticed by visible observation.

Biological oxygen demand (B.O.D)

The Biological Oxygen Demand (BOD) of the dairy farm liquid waste samples before and after bioremediation using Azolla was estimated using BOD incubator (Devi *et al.*, 2014) [5]. The pH of the dairy farm liquid waste sample was neutralized and the samples were filled in 6BOD bottles without bubbling. To each bottle, 1ml of allylthiourea was added. The dissolved oxygen content was determined in three of the 6 BOD bottles by titration method and taken the mean of the three readings (D1). The rest of the three BOD bottles were incubated at 27degrees in a BOD incubator for 3days and estimated the oxygen concentration in all the three incubated samples. The mean of the three incubated samples were calculated (D2).

Calculation BOD (mg/L) = D1-D2

D1= Initial DO in sample (mg/L)
D2= DO after 3days incubation (mg/L)

Chemical oxygen demand (C.O.D)

The Chemical Oxygen Demand (COD) of the of the dairy farm liquid waste samples before and after bioremediation using Azolla was estimated using COD incubator (AOAC, 2007) and the changes in COD were recorded.

Dairy farm liquid waste sample of 100 ml was taken in conical flask and added 10 ml of Potassium permanganate, and 10 ml of concentrate Sulphuric acid. In another conical flask 100 ml of distilled water was taken which was taken as blank and added the above reagents in same quantity. The two conical flaks were kept in water bath at 100 degrees for 30 minutes. Then removed the conical flasks from water bath and cooled. Then 1ml of Potassium Iodide was added and shaken well. To this few drops of starch was added and titrated with N/80 Hypo until the colour disappears.

$$COD = \frac{10x\ (V_1-V_2)}{V}$$

V_1 = Volume of Hypo for Blank
V_2 = Volume of Hypo for sample

Nitrogen

The Nitrogen content of the dairy farm liquid waste samples before and after bioremediation using Azolla was measured by Kjeldhal method (AOAC, 2007) and the changes in Nitrogen was recorded.

$$\text{Nitrogen (mgN/l)} = \frac{V \text{ X } 0.00014 \text{ X } 100}{A}$$

1ml of N/100 H2SO4 = 0.00014mgN
V- Volume of digested sample diluted
A – Aliquot (ml) taken for distillation

Phosphorous

The phosphorous content of the dairy farm liquid waste samples before and after bioremediation using Azolla was estimated by Ammonium molybdate method (Eibl and Lands 1969) and the difference in phosphorous content was recorded.

$$\text{Phosphorous (mg/dl)} = \frac{\text{Absorbance of test x Concentration of Standard}}{\text{Absorbance of Standard}}$$

Results and Discussion

Table 1: Effect of bioremediation with Azolla on physico-chemical properties of dairy farm Liquid waste

Parameters	Dairy farm liquid waste		Percentage decrease
	Before treatment	After treatment	
pH	7.96±0.02	7.38±0.04	-
Colour	Grayish black	Brown	-
Turbidity	0.27±0.05	0.14±0.02	48.14
COD	27±0.60	26.8±0.48	0.74
BOD	1202.2±4.48	1197±4.90	0.43
N	0.13±0.003	0.09±0.008	30.76
P	5.37±0.19	4.97±0.04	7.44

Azolla (*Azolla pinnata*) cultivation on dairy farm liquid waste for 10 days decreased the pH from 7.96 to 7.38 and the colour of the waste water was changed from Grayish black to brown colour. The turbidity, COD, BOD, Nitrogen and Phosphorous content was decreased to 48.14, 0.74, 0.43, 30.76 and 7.44%, respectively.

Effect on pH

Dairy farm liquid waste bioremediation using Azolla (*Azolla pinnata*) decreased the pH from 7.96 to 7.38. This might be due to absorption of the ions and other organic pollutants present in the dairy farm liquid waste by Azolla. Similar decrease in pH from 7.89 to 7.4 was observed by Devi *et al.* (2014) [5]. while treating the river water with Azolla. Parallel to the present findings Nilesh *et al.* (2015) [15]. also observed changes in pH from alkaline to neutralby Azolla treatment of waste water. Several research workers also observed the decline in pH while treating waste water with Azolla (Rizwana *et al.*, 2014; Nair and Kani 2016 and Bhavsar *et al.*, 2012) [18, 13, 4] is consistent with present findings.

Effect on colour

Treatment of dairy farm liquid waste with Azollain the present study changed the colour of the liquid waste from greyish black to brown colour. This might be due to absorption of the pollutants present in the dairy farm liquid waste by Azolla. Divya Soman *et al.* (2018) [7] also observed the change in colour of the municipal waste water from brown to colourless after treatment with Azolla.

Effect on turbidity

Dairy farm liquid waste bioremediation using Azolla (*Azolla pinnata*) decreased the turbidity of the liquid waste from 0.27 to 0.14 (48.14%) in the present study may be due to the reduction in suspended particulates, particularly the organic matter, resulted an increase in transparency of the liquid waste. The decrease in turbidity of waste water after treating with Azolla was observed by Vigneshwaran *et al.* (2017) [24]. Parallel to the present findings, turbidity reduction in the waste water with Azolla treatment was also reported by Gopal and Ghosh (2008).

Effect on COD

The decrease in COD of the dairy farm liquid waste in the present study was observed from 27 to 26.8 (0.74%) after bioremediation using Azolla. The reduction in concentration of COD due to bioremediation may be attributed to the biological processes particularly microbial actions enhanced due to the favorable environment created by the Azolla by the provision of oxygen and surface area through the root system (Amare *et al*., 2018). Similar results were reported by Sudani *et al.* (2014) [20] in treating the sewage water with hyacinth and *Azolla pinnata* but the reduction of COD was higher (65-85%) compared to the results observed in the present study. Similar findings of reduction in COD was also observed by Vigneshwaran *et al.* (2017) [24], Anil kumar (2015) [1], Rizwana *et al.* (2014) [18], Swati *et al.* (2017) [21], and El-Din *et al.* (2018) while treating the waste water with Azolla and macrophytes.

Effect on BOD

The present study observed decrease in BOD from 1202.2 to 1197 (0.43%) in dairy farm liquid waste after bioremediation using Azolla which may be due to biological processes particularly microbial actions that enhanced conducive environment created by the Azolla by the provision of oxygen and surface area through the root system. Similar findings were reported by Devi *et al.* (2014) [5] while treating the industrial effluent and sewage water with Azolla. Higher per cent (41) in reduction of BOD compared to the present study was observed by Ranaijangwattana (2010) [17] while treating the waste water from slaughter house with *Azolla pinnata.* Tokhun *et al*. (2011) [22] and Devi *et al.* (2014) [5] also reported reduction of BOD in waste water and sewage water, respectively with Azolla treatment.

Effect on Nitrogen

The present study detected decrease in Nitrogen content from 0.13 to of 0.09(30.76%) in dairy farm liquid waste after bioremediation using Azolla. This may be due to uptake of Nitrogen by the Azolla and attached microorganisms for their growth in the liquid waste (Marimon *et al*., 2013 and Korner *et al*., 2003). The findings of Divya *et al.* (2018) [7] who reported 54.8% ammonia reduction in waste water after Azolla treatment corroborated the present findings. Vigneshwaran *et al*. (2017) [24], Forni *et al.* (2001) and Tokhun *et al*. (2011) [22] also observed decrease of nitrogen content in waste and sewage water, respectively after Azolla treatment.

Effect on Phosphorous

Bioremediation of dairy farm liquid waste using Azolla decreased the Phosphorous content from 5.37 to 4.97 (7.44%) in the present study may be due to uptake of Phosphorous from the liquid waste for the growth of Azolla. Similar finding of decrease in Phosphorous from waste water was earlier reported by Divya *et al.* (2018) [7] is consistent with the present results. Vigneshwaran *et al*. (2017) [24], Forni *et al.* (2001) [11] and Tokhun *et al*. (2011) [22] also observed decrease of Phosphorous content in waste and sewage water, respectively after Azolla treatment.

However, compared to the present findings, the differences in per cent removal of turbidity, COD, BOD, Nitrogen and Phosphorous reported by several research workers may be due to difference in substrate used for Azolla growth.

Conclusion

Azolla can be used for reducing the pollutants in dairy farm liquid waste in eco-friendly manner. Bioremediation of dairy farm liquid waste using azolla saves water and environment.

Acknowledgements

The authors are thankful to Sri Venkateswara Veterinary University, Tirupati for providing facilities to carry out the present study.

Conflict Of Interest

The authors declare no conflict of interest regarding the submission of this manuscript.

References

1. Anil Kumar P. Waste water management through aquatic macrophytes. International. Research Journal of Environmental Sciences. 2015;4(3):41-46.
2. APHA. Standard Methods for the Examination of Water and Wastewater (20thEdition) American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, 1998

3. Bennicelli R, Stepniewska Z, Banach A, Szajnocha K, Ostrowski J. The ability of *Azolla caroliniana* to remove heavy metal (Hg (II),Cr (III), Cr (VII) from municipal waste water. Chemosphere. 2004;55(1):141-146.
4. Bhavsar SR, Pujari VR, Diwan VV. Potential of Phytoremediation for dairy wastewater treatment. IOSR Journal of Mechanical and Civil Engineering (IOSRJMCE), 2012, 16-23.
5. Devi MK, Wahengbam Singh N, Wahengbam Singh RC, Heigrujam Singh B, Nongmaithem Singh M. Determination of the ability of azolla of bioremediation. European Journal of Experimental Biology. 2014;4(4):52-56.
6. Dipu S, Anju A, Kumar V, Thanga SG. Phytoremediation of dairy effluent by constructed wetland technology using wetland macrophytes. Global Journal of Environmental Research. 2010;4(2):90-100.
7. Divya Soman, Anita V, Anju Arora. Bioremediation of municipal sewage water with *Azolla microphylla* International Journal of Advanced Research. 2018;6(5):101-108.
8. El-Din SMB, Abdel-Aziz RA. Potential uses of aquatic plants for wastewater treatment. Journal of Microbiology and Biotechnology Reports, 2018, 2(3).
9. Eribo O, Kadiri MO. Growth performance and phytoremediation ability of *Azolla pinnata* in produced water. Journal of Applied Sciences and Environmental Management. 2016;20(4):1053-1057.
10. Forni C, Chen J, Tancioni L, Caiola MG. Evaluation of the fern Azolla for growth, nitrogen and phosphorus removal from wastewater. Water research, 2001;35(6):1592-1598.
11. Lakshmi KS, Sailaja VH, Reddy MA. Phytoremediation-a promising technique in waste water treatment. International Journal of Scientific Research and Management, 2017, 5(6).
12. Mithraja MJ, Marimuthu J, Mahesh M, Paul ZM, Jeeva S. Phytochemical studies on *Azolla pinnata* R. Br., *Marsilea minuta* L. and Salvinia molesta Mitch Asian Pacific journal of tropical Biomedicine. 2011;1(1):S26-S29.
13. Nair CS, Kani KM. Phytoremediation of dairy effluent using aquatic macrophytes. International Journal of Scientific and Engineering Research. 2016;7(4):253-259
14. Naghipour D, Ashrafi SD, Gholamzadeh M, Taghavi K, Naimi-Joubani M. Phytoremediation of heavy metals (Ni, Cd, Pb) by *Azolla filiculoides* from aqueous solution: A dataset. Data in brief. 2018;21:1409-1414.
15. Nilesh BD, Jadhav MV, Rahane R. Use of phytoremediation for treatment of dairy industry waste water for analysis of COD and BOD. International Journal of of Engineering and Technical Research. 2015, 3(2)
16. Noorjahan CM, Jamuna S. Biodegradation of Sewage Waste Water Using *Azolla microphylla* and Its Reuse for Aquaculture of Fish Tilapia Mossambica. IOSR Journal of Environmental Science. 2015;9:75-80.
17. Ranaijangwattana. Using *Azolla pinnata* for waste water treatment in slaughter house. International Journal of Environment and Rural Development. 2010;1(2):23-17.
18. Rizwana M, Darshan M, Nilesh D. Phytoremediation of textile waste water using potential wetland plant: Eco sustainable approach international journal of interdisciplinary and multidisciplinary studies (IJIMS), 2014;1(4):130-138.
19. Sheel R, Anand M, Nisha K. Phytoremediation of heavy metals (Zn and Pb) and its toxicity on *Azolla filiculoides*. int J Sci Res. 2015;4(7):1238-1241.
20. Sudani BR, Thummar DM, Sojitra KM, Gajera AD, Bavriya SS. Lab Scale Study of Water Hyacinth for Bioremediation of Waste Water. International Journal of Research in Advent Technology, 2014, 2(5).
21. Swati Zingade A. An assessment of Phytoremediation method for dairy effluent. International Journal of Applied and Pure Science and Agriculture. 2017;3:2394-5532.
22. Tokhun N, IWAI CB, Ta-oun M. Wastewater treatment from piggery farm by usingAzolla (*Azolla microphylla*). Khon Kaen Agr. J.39 Supplement, 2011, 269-273.
23. Veerabahu C, Radhika D, Mohaideen A, Indrani S, Priya R. Phytochemical and biochemical profiles of *Azolla microphylla* cultured with organic manure. Int. J Curr. Agric. Res. 2015;4(8):131-133.
24. Vigneshwaran R, Chandu S, Hareesh.U, George JP, Thomas J. Sewage water treatment using azolla algae. International Journal of Advance Engineering and Research Development. 2017;4(3):2348-4470.