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Wastewater Management and Production of Livestock Feed by *Azolla pinnata*

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Abstract:
This study was undertaken to use Azolla pinnata in wastewater management and production of livestock feed. Different Physico-chemical parameters found in sewage water like, color, odor, Ph., Electric Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Chromium, Copper and Zink as heavy metals were analyzed according to Standard methods for the examination of water and wastewater before and after inoculation of Azolla pinnata. Data revealed that Azolla pinnata was very effective in treating wastewater, the resultant bio-treated sewage water appeared to have physico-chemical parameters better than the Permissible limits EPCB.
The resultant Azolla pinnata biomass was introduced as a sole feed to Wistar rats. The present study concluded that Azolla pinnata used in treating wastewater could be used as animal feed.
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I. Introduction

There is no life without water as it is the origin and source of everyone's life. The main challenge nowadays is to provide high-quality water suitable for drinking and also for everything else, wastewater treatment is the most important goal that Governments nowadays trying to gain success, so that can preserve drinking water and start use recycled or treated wastewater for agriculture and many other purposes. Since many decades, human activities create wastewater that may be destructive to the environment and encourage a loss of water (wastewater is 99% water by weight) in many places where water is insufficient. As soon as wastewater pollutes rivers and groundwater wells, water becomes useless and can't be used, so that it's mandatory to treat wastewater to make it potable or at least to be treated prior to release into the surroundings. The main goal of wastewater treatment is to lessen the pollution to much less than most permissible limits to protect the environment and human health, this could be done by allowing wastewater to be collected and treated in huge plants before releasing into the environment.

Needs for water for all life's aspects are increasing each single day, without water, no civilization. Great efforts should be done to save water and to provide potable water or Billions of people will face chronic water deficiency problem by the year 2025 according to WHO, 2005.The effects of wastewater on human health can classify into toxic, pathogenic, biological and Physio-chemical, wastewater resulting from human activities referred to as sewage which contains about 99% water and 1% organic solids [1]. Unfortunately the disposable of this sewage in many countries is done in a way that causes a severe water and land contamination. [2]. Therefore, it is so important to treat sewage water to cover the increasing demand for water [3]. Several treatment techniques were used everywhere to treat sewage water and make it less toxic, so that can be reused for agriculture or other purposes. [4].

Nowadays there is an increasing trend to use water plants like *Azolla, Algae, Fungi* and other species that can be used for the bio-remediation of wastewater. [5].

Azolla is one of these water plants; an aquatic fern scattered almost everywhere tropical and temperate in the globe. [6].It has the ability to use N_2 from the atmosphere throughout the symbiosis with *Anabaena Azolla* (a blue-green algae) which present and grow in the cavities of *Azolla* leaflets. [7]. *Azolla* species acquired a great importance in wastewater treatment [8, 9&10]. *Azolla* species has a proven results for the removal of heavy metals from wastewater. [11, 12, 13, 14, 15& 16]. Presently, *Azolla* species has been used numerously as a livestock feed and green manure in many countries across the globe. [17].

II. Materials and Methods

Collection of Sewage Wastewater:
Untreated and treated sewage wastewater was brought from a local Sewage treatment plant located in Cairo, Egypt.

Strain and Growth Conditions:

Strain: *Azolla pinnata* strain was obtained from Microbiology, Dep. Soils and Water Environment Research Institute (SWERI), Agric. Research Center, Giza, Egypt.

Growth Conditions: Yoshida medium [18] was used to grow and purify *Azolla pinnata* strain.10 grams of *Azolla pinnata* were transferred into plastic planting pots (32cm in diam. and 15 cm. in dep), filled with 1 kilogram Garden soil with 3 liters tap water; pots were then kept in the greenhouse till *Azolla pinnata* biomass covers the water surface; accumulated and involved for 1 minute in 0.01% Mercuric Chloride, rinsed thoroughly under running tap water for several minutes using fine mesh followed by air-dried for about half hour. The resulting growths were then used as a possible inoculum. [19].

Analysis of physico-chemical parameters of sewage wastewater: Different Physico-chemical parameters found in sewage water like, color, odor, Ph., Electric Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Chromium, Copper and Zink as heavy metals were analyzed according to Standard methods described by [20] before and after inoculation of *Azolla pinnata.*

Experimental set up: Batch experiments were carried out in plastic tubs (15 liters capacity) covered with black polythene papers in order to avoid excessive light. Atomic Absorption Spectrophotometer was used to estimate Physico-Chemical Parameters and heavy metals in sewage wastewater samples, according to [21]. *Azolla pinnata* inoculum was carefully introduced to the tubs, after 96 hours, tubs were opened, *Azolla pinnata* biomasses were collected for further works within this study and Physico-chemical Parameters were then estimated. Resultant *Azolla* biomasses from each wastewater (treated and untreated) were then divided into two divisions; first division was used to feed rats after drying under sunlight for three consecutive days, while the second division was then washed carefully with excessive tap water for several minutes prior to replanting under normal conditions, then dried under sunlight for three consecutive days prior to introduce to Wistar rats. Fifty Wistar rats(males), weighing between 170-180 gram/rat, left for two weeks to acclimatize with experiment's environment prior to start the experiment. After the two weeks acclimatization, rats were randomly divided into five groups, each of ten rats; Group A, defined as control with *Azolla pinnata* grown under normal conditions, group B defined as Bio-untreated, group C was defined as Bio-treated, group D was defined as re-planted *Azolla*(un-treated) and group E was defined as re-planted *Azolla*(treated). *Azolla pinnata* used in this experiment for Rats feeding was dried under sunlight for three consecutive days. All groups allowed to drink normal tap water.

Blood Collection: At the end of the experiment (15 days); blood was collected, serum obtained and kept refrigerated until it was used.

Biochemical Parameters: This study was designed to estimate the following biochemical parameters: Total Protein was done according to [22], Creatinine analysis was done according to [23], Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) analyses were done according to [24], Cholesterol was done according to Kulkarni [25], Uric acid analysis was done according to [26] and Triglycerides was done according the process described by [27], compared with University of Alabama School of Medicine laboratory (n=40,r=0.997, bias=-0.05%).

III. Results and Discussion:

Definitions:

I- Bio-untreated wastewater= *Azolla pinnata* grown on un-treated wastewater.

II- Bio-treated wastewater= *Azolla pinnata* grown on treated wastewater.

Wastewater treatment gains every single day a great importance, especially with the severe lack of suitable water for drinking and for every other aspects like, irrigation and other tasks. Many previous researches aimed to find alternative solutions so that we can use instead of wasting more water. [26]. Our study aimed first to find an alternative way to treat wastewater using *Azolla pinnata* (a water fern)for 96hrs., then evaluate *Azolla pinnata* grown on untreated and treated wastewater as a feed, this done by feeding Winstar rats on *Azolla pinnata* grown on both Bio-untreated and Bio-treated wastewater.

Data present in Table(1) showed that physico-chemical parameters of sewage samples; color, odour, Ph., TDS, TSS, EC, BOD, COD and heavy metals(Zinc, Chromium and Copper) were found to be higher than the permissible limits of EPCB (1995) before introduction of *Azolla pinnata* but after treating with *Azolla pinnata* for 96 hrs., these physico-chemical parameters were found to be decreased dramatically; TDS(Bio-untreated 42.03% and Bio-treated62.96%), TSS(Bio-untreated80.26% and Biotreated 83.33%), EC(Bio-

untreated 60.98% and Bio-treated 73.59%) BOD (Bio-untreated 75.79% and Bio-treated 80.00%), COD (Bio-untreated 70.74%and Bio-treated 72.31%) and heavy metals such as Zinc (Bio-untreated 85.05% and Bio-treated 100%), Chromium (Bio-untreated 56.97% and Bio-treated 100%) and Copper (Bio-untreated 89% and Bio-treated 100%). It's clear from these data that treated sewage water with *Azolla pinnata* (Biotreated) caused greater decreases for physico-chemical parameters, the resulted Biotreated sewage water appeared to have physico-chemical parameters lower than the Permissible limits EPCB, 1995, which indicated the great effect of *Azolla pinnata* in treating sewage water. The same finding was also reported by [28].

Table 1. Physicochemical characteristics of sewage wastewater before and after treatment using *Azolla pinnata*

S. No.	Characteristics	Permissible Limits (EPCB, 1995)	Before Treatment		After Treatment with *Azolla pinnata* for 96 hrs.		Percentage Removal	
			Untreated	Treated	Bio-untreated	Bio-treated	Bio-untreated	Bio-treated
1	Color	Colorless	Black	Brown	Colorless	Colorless	---	---
2	Odour	Odorless	Offensive	Earthy	Odorless	Odorless	---	---
3	Ph.	5.5-9.0	6.9	6.7	7.0	6.8	---	---
4	TDS(mg/L)	2100	690	810	400	300	42.03%	62.96%
5	TSS(mg/L)	100	380	30	75	5	80.26%	83.33%
6	EC(µmhos/cm)	400	1730	1420	675	375	60.98%	73.59%
7	BOD(mg/L	30	190	65	46	13	75.79%	80.00%
8	COD(mg/L)	250	475	65	139	18	70.74%	72.31%
9	Zinc(mg/L)	15	0.0823	0.074	0.0123	zero	85.05%	100%
10	Chromium(mg/L)	2	0.00495	0.00028	0.00213	zero	56.97%	100%
11	Copper(mg/L)	3	0.1632	0.00163	0.01795	zero	89%	100%

EC (Electric Conductivity) TDS (Total Dissolved Solids). TSS (Total Soluble Solids)

Table (2) showed the effect of *Azolla pinnata* as a feed on biochemical parameters; five groups, each include ten rats were examined for 15 days; first group was fed on *Azolla pinnata* grown under normal conditions; second group was fed on *Azolla pinnata* grown on un-treated sewage wastewater, third group was fed on *Azolla pinnata* grown on treated wastewater, fourth group was fed on *Azolla pinnata* that previously grown on un-treated wastewater((bio-untreated), and re-planted under normal conditions and the fifth group was fed on *Azolla pinnate* that previously grown on treated wastewater(bio-treated) and re-planted under normal conditions.

Table 2. Effect of *Azolla pinnata* on Biochemical Parameters

Groups	ALT (U/L)	AST (U/L)	Creatinine (U/L)	Uric Acid (U/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Total Protein((G/L)
Control(A)	5.00	14.00	0.85	2.27	5.39	1.75	50.55
B(Bio-untreated)	5.68	15.88	1.65	3.89	6.24	3.00	51.67
C (Bio-treated	5.57	15.40	1.48	3.45	6.18	2.67	51.49
D(re-planted *Azolla pinnata* (un-treated),	5.33	15.10	1.29	3.28	6.00	2.39	51.38
E (re-planted *Azolla pinnata* (treated).	5.19	14.60	1.00	3.00	5.88	2.17	51.10

Group A= *Azolla Pinnata* grown on Normal Conditions, Group B= *Azolla pinnata* grown on Un-treated wastewater, Group C= *Azolla pinnata* grown on Treated wastewater, Group D= re-planted *Azolla pinnata* (un-treated), Group E= re-planted *Azolla pinnata* (treated).

As shown from table (2), *Azolla pinnata* grown on un-treated wastewater(group B) showed 5.68(U/L), 15.88(U/L), 1.56(U/L), 3.89(U/L), 6.24(mmol/L), 3.00(mmol/L) and 51.67(G/L) for ALT, AST, Creatinine, Uric acid, Cholesterol, Triglycerides and Total protein respectively, comparing to 5.00(U/L), 14.00(U/L) 0.85(U/L), 2.27(U/L), 5.39(mmol/L), 1.75(mmol/l) and 50.55(G/L) for the control group(A). These values was considered in this study as the worst results followed by *Azolla pinnata* grown on treating wastewater (group C) which yielded 5.57(u/l), 15.40(u/l), 1.48(u/l), 3.45(U/L), 6.18(mmol/L), 2.67(mmol/L) and 51.49(G/L) for ALT, AST, Creatinine, Uric acid, Cholesterol, Triglycerides and Total protein respectively, then followed by *Azolla pinnata* that previously grown on un-treated wastewater and re- planted under normal conditions(Group D) which yielded 5.33(U/L), 15.10(U/L), 1.29(U/L), 3.28(U/L), 6.00(mmol/L), 2.39(mmol/L) and 51.38(G/L), followed by *Azolla pinnata* that previously grown on treated wastewater and re-planted under normal conditions(group E) which yielded 5.19(U/L), 14.60(U/L), 1.00(U/L), 3.00(U/L), 5.88(mmol/L), 2.17(mmol/L) and 51.10(G/L).

It is clear from the data that *Azolla pinnata* could be used effectively to treat wastewater [7]. The resultant *Azolla pinnata* biomass was used as a sole feed for 15 days for Wistar rats after drying under sunlight for three consecutive days, we highly recommend washing *Azolla pinnata* biomass that was used to treat wastewater under the tap water for several minutes and re- plant again under normal conditions before using as a feed, this step could build a sort of confidence to the farmers as the present study revealed that *Azolla pinnata* that used to treat wastewater and then replanted under normal conditions showed the best results, especially for *Azolla pinnata* that was previously grown in treated wastewater, the process is easy and takes only three to four days to achieve double growth of *Azolla pinnata.*

IV. Conclusions:

Azolla pinnata was very effective for wastewater treatment, it can reduce BOD and COD and remove heavy metals from wastewater, and moreover the resultant *Azolla pinnata* biomasses were safely used a sole feed to the rats without any mortalities. Further study should be done to confirm using of *Azolla pinnata* as a feed for different Animal's types.

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References:

[1]. Athalye, R.P., Vidya Mishra., Goldin Quadros, VidyaUllal. and K.S. Gokhale. Heavy metals in the abiotic and biotic components of Thane Creek, India. Poll. Res., 2001;18(3): 329-333.
[2]. Thanunathan, K., Kalyanasundaram, D., Imayavaramban, V and Singaravel, R. Growing Water Grass (Brachiariamutica) with sewage water: An effective way for Sewage Disposal and pollution free Ecosystem.J.Ecobiol. 2000; 12(3): 237-239.
[3]. Paulami,M.. Size dependent accumulation of zinc in a wastewater raised fish species, Oreochromisnilotica. Eco.Env. and Cons.2002; 8(2):161-165.
[4]. Nagarajan, K., and Sasikumar, R.. Effect of sago effluent on selected physiological aspects of the fresh water fish, Labeorohita. J. Ecotoxicol. Environ. Monit. 2002; 12(3): 233-238.
[5]. Jamuna, S. and Noorjahan, C.M. Treatment of sewage wastewater hyacinth-Eichhornia sp and its reuse for fish culture Toxico. Int., 2009; 16(2):103-106.
[6]. Kitoh, S. Shiomi, N. and Uheda, E. The growth and nitrogen fixation of Azolla filiculoides Lam. Polluted water. Aquat. Bot.1993; 46, 129-139.
[7]. Ranai Jangwattana. Using *Azolla pinnata* for Wastewater Treatment from Poultry Farm. International Journal of Environmental and Rural Development (2010) 1-2.
[8]. Watanabe, I. Roger, P.A., Ladha, J.K., and Van Hove, C. Biofertilizer germplasm collections at IRRI. International Rice Research Institute, Philippines1992.
[9]. Culley, D.D., and E.A. Epps. Use of duckweed for wastewater treatment and animal feed. WPEF.J. 173; 45: 337-347.
[10]. Forni, C., J., Chen, L. Tancioni, and C.M. Grilli. Evaluation of the fern *Azolla* for growth, nitrogen of the phosphorus removal from wastewater. Elsevier Science. 2001; 6(35):1592-1598.
[11]. Zhao, M., Duncan, J.R. Batch removal of sexivalent chromium by *Azolla filiculoides.*Biotechnol. Appl. Biochem. 1997; 26, 172–179.
[12]. Zhao, M., Duncan, J.R. Removal and recovery of nickel from aqueous solution and electroplating rinse effluent using *Azolla filiculoides*. Process Biochem. 1998; 33, 249–255.
[13]. Fogarty, R.V., Dostalek, P., Patzak, M., Votruba, J., Tel-Or,E., Tobin, J.M. Metal removal by immobilised and nonimmobilised*Azolla filiculoides*. Biotechnol. Tech. 13, 1999; 53–538.
[14]. Antunes, APM., Watkins GM, Duncan JR. Batch studies on the removal of gold (III) from aqueous solution by Azolla filiculoides. Biotechnol. Lett. 2001; 23, 249–251.
[15]. Cohen-Shoel, N., Barkay, Z., Ilzycer, D., Gilath, L., and Tel-Or, E. Bio filtration of toxic elements by *Azolla* biomass. Water, Air, Soil Pollut. 2002; 135, 93–104.
[16]. Xin Zhang, Ai-Jun Lin, Fang-JieZhao,Guo-ZhongXu,Gui-Lan Duan and Yong-Guan Zhu. Arsenic accumulation by the aquatic fern *Azolla*: Comparison of arsenate uptake, speciation and efflux by *A. caroliniana* and *A. filiculoides*. Environ.Poll 156 2008; 1149–1155.
[17]. Lumpkin, T. A. and D.L. Plucknett. Azolla: botany, physiology and use as green manure. Econom. Bot. 1980; 34:111-153.
[18]. Yoshida S, Forno DA, Corck JH, GomezKA. Laboratory manual for physiology studies of rice Laguna. International RiceRes. Inst., Los Banos, and Manila. Philippines. 1976; 61-65.
[19]. El-Shahat RM. Prospects of *Azolla* asbio-fertilizers in Egyptian conditions. MSc.Thesis, Faculty of Agric., Ain ShamsUniversity, Cairo, Egypt. 1988; 53-72.
[20]. APHA Standard methods for the examination of water and wastewater, American Public Health Association, Washington, D.C.1995; (14th ed.).
[21]. Kannagi, S. Physico-chemical characteristics and identification of Microbes of Untreated Brewery Effluent and its treatment using native fungus - Aspergillus niger, Aquatic Plant, Water Hyacinth - Eichhornia sp and Green Mussel - PernaViridis. 2007; M.Sc. Dissertation, University of Madras.
[22]. Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry Principles and Techniques, Harper and Row, 1974; New York 2nd.
[23]. Henry RJ. Clinical Chemistry. Principles and Techniques. 2nd ed. Harper and Row, New York. 1974; 882.
[24]. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J ClinPathol. 1957; 28(1):56-63.
[25]. Kulkarni KR. Cholesterol profile measurement by VAP method. Clin. Lab. Med. 2006; 26(4):787–802.

[26]. Young DS. Effects of drugs.on clinical lab. Tests. Ann.Clin. Biochem. 17; 579-581.
[27]. Lim J, Song KE, Song SH, Choi HJ, Koo SH and Kwon GC. Traceability Assessment and Performance Evaluation of Results for Measurement of Abbott Clinical Chemistry Assays on 4 Chemistry Analyzers. Arch Pathol Lab Med. 2016; 140(5):467-72.
[28]. Mamoru Kashiwaya. Current Research on Advanced Wastewater Treatment in Japan. Water Pollution Control Federation, 1980. Vol.52, number 5, 1008-1012.

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