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Comparative analysis of growth and survival of two Indian major carps cultured in recycled cattle wastewater

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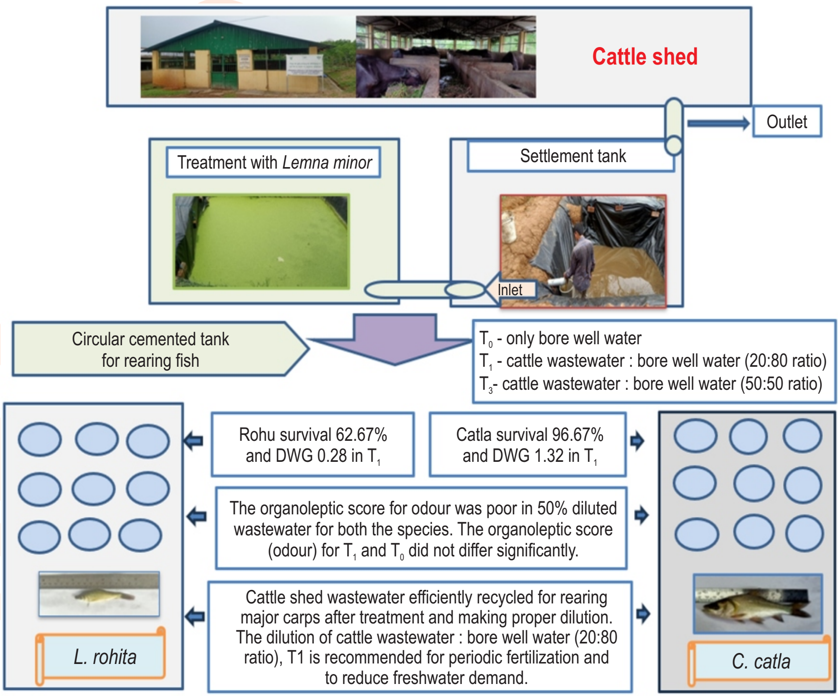
Abstract

Aim: The study aimed to evaluate the growth and survival of two Indian major carps (*Catla catla, Labeo rohita*) in diluted cattle farm wastewater to observe the probability of recycling cattle wastewater in aquaculture systems.

Methodology: A sixty days outdoor trial was conducted in cemented circular tanks.The cattle wastewater was collected and treated with lime and potassium permanganate. After primary treatment, the wastewater was diluted in the ratio of 50 (cattle wastewater): 50 (bore well water) and *Lemna minor* was inoculated for removing excess nutrients. After secondary treatment, the fish were cultured in three treatment groups viz., T_0 -only bore well water; T_1-cattle wastewater: bore well water (20:80 ratio) prepared from secondary treated dilution and T_2-cattle wastewater: bore well water (50:50 ratio) to assess the performance of two Indian major carps.

Results: *Catla* performed better than *Rohu* in terms of both survival and growth (P<0.05). *Rohu* did not survive at the end of experiments in 50% diluted cattle wastewater. The organoleptic score for odour was poor in 50% diluted wastewater for both the species. The organoleptic score (odour) for T_1 and T_0 treatments did not differ significantly (P> 0.05).

Interpretation: The results suggest that cattle shed wastewater can be recycled in aquaculture for rearing major carps after treatment and making proper dilution. The diluted wastewater efficiently enhanced the productivity, and reduced freshwater demand and eutrophication in open waterbodies by removing nutrients from wastewater.



Key words: Carp culture, Duckweed, Phytoremediation, Wastewater recycle

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Introduction

The rising global population has increased the demand for intensive farming to fulfil the growing food demand. Dairy farms generate large amount of wastewater with heavy nutrient load which causes surface water and ground water contamination. It is estimated that more than 24 billion tons of livestock wastewater is generated annually in the world (Silva-Gálvez *et al.*, 2024). High Chemical oxygen demand, Total ammonia nitrogen and Total phosphorous has been reported in cattle farm wastewater (Xia *et al.,* 2020). The cattle wastewater contains substantial amount of highly leached nitrogen, phosphorus and soluble organic matter. Therefore, the direct discharge of wastewater into the environment without treatment can cause eutrophication of open water bodies (Hu *et al.,* 2017). The organic matter present in the wastewater consumes dissolved oxygen (DO) creating a hypoxic zone in water bodies and subsequent mortality of aquatic fauna (Burkholder *et al.,* 1997). In addition, intestinal parasites, bacteria, and viruses present in livestock flushed into the wastewater can also contaminate surface water and groundwater if the water is not treated properly (Greger and Koneswaran., 2010). Water pollution caused from animal wastewater has a broad impact on the biodiversity of open water bodies. The pollutants also exert negative effects on agricultural ecosystems by destroying the food production system. Water, food and energy securities are emerging sustainable management issues worldwide. Advance technologies for recycling and reuse of livestock wastewater can be useful to increase crop yield and sustainability.

Treatment of livestock wastewater with aquatic plants can be an environment friendly, efficient option to promote sustainable development. Phytoremediation of livestock wastewater using aquatic plants such as duckweed, water lettuce, water hyacinth, and water milfoil is well documented (Hu *et al.,* 2017). In comparison to microalgae, the biomass of aquatic plants is easier to harvest after remediation, which is beneficial for the reuse of plant biomass. Duckweeds tiny aquatic plants with one or few leaves called fronds and a single root or rootlet with no stem. They have fast multiplication rate with reproduction rate about 1.4 days (Hu *et al.,* 2019), and grows throughout the year in tropical and subtropics regions.

Additionally, duckweed contains high concentrations of proteins, starches, vitamins, carotene and xanthophylls which serves a potential source of animal feed and biofuel (Ekperusi *et al.,* 2019). Xu *et al.* (2011) reported that a starch yield of 9.4×10^3 kg ha^{-1} and ethanol yield of 6.42×10^3 l ha^{-1} can be obtained from duckweed from swine effluent treatment systems, which is about 50% higher than the yield from maize. Duckweeds are efficient for removing nutrients like nitrogen and phosphorus from livestock wastewater. Appropriate low-cost technology for treating the wastewater before release into the environment and for reuse of wastewater for agricultural applications including aquaculture is required for maximum productive utilization of water. Treated wastewater can be used in aquaculture and other agricultural activities like in duckery, poultry, paddy fields and other horticultural crops for irrigation. The organic substances and nutrients from the wastewater are transformed into fertilizer to produce plant biomass.

The use of reclaimed water has been explored as a sustainable strategy to reduce freshwater consumption in a circular economy (Villamar *et al.,* 2018). Scientific and centralized options for treatment of animal refuse such as sewage and manure generated from livestock farming face technological and economic limitations. As a result, contaminants in livestock wastewater continue to pose risks to both environment and human health (Wu Xiaomei *et al.,* 2024). Successful reuse of livestock wastewater from pig farm remediated with macrophytes *Phragmites australis* and *Typha latifolia* in agricultural field as fertilizer has been reported (Dias Sofia *et al.,* 2020). Similarly, the production of food and non-food crops can be augmented by reusing the livestock wastewater as a strategy to alleviate stress on water and energy (Mukhametov *et al.*, 2022).

Indian major carp production in sewage fed aquaculture has been successfully carried out since 1930s (Calcutta model) using phytoremediated sewage, and no additional feeding and fertilization in the culture systems (Jana, 1998). However, reports on standardization of treatment protocols and incorporation of treated livestock wastewater for aquaculture are lacking. Considering the vast livestock resources in the country, and huge amount of water consumed by the sector, this is a serious lacuna. Therefore, recycling and reuse of phytoremediated wastewater from livestock farms in a high water-demanding agricultural enterprise such as aquaculture can be a sustainable strategy of climate resilient farming. Additionally, the technology will reduce the fertilization cost as it eliminates the need for intermittent fertilization. The present study aims to investigate the growth and survival of two Indian major carps in diluted cattle wastewater after proper treatment.

Materials and Methods

Study area: The experiments were conducted during 2023 at the experimental facility of the Indian Council of Agricultural Research Centre for North-eastern Hill Region Tripura Centre, Lembucherra. A dug-out plastic lined water reservoir (jalkundh) with a middle partition was constructed adjacent to the cattle shed of the Institute housing 12 milch buffaloes. The total capacity of the jalkundh was 5.0 m^3. The wastewater from the shed was designed to flow into the sedimentation unit of the jalkundh. After primary treatment and sedimentation (15 days) the supernatant water was released and diluted to 50% in the phytoremediation unit. Three treatments including control groups we included in the experiment, in circular cement cisterns of 250l capacity. All experiments were conducted following the Guidelines of the Institute Animal Ethics Committee (IAEC) of the Institute.

Experimental plant, fish and feed: Duckweed (*Lemna minor*) was used as a phytoremediator in the experiment. *Lemna minor* was collected from the Institute fish farm and maintained as pure culture on half-strength Hutner's medium (Hutner, 1953), in a growth chamber. The temperature (20°C), light intensity (40 µmol m^{-2} s^{-1}), light and dark period were maintained during the culture period. Prior to inoculation in (16 h light and 8 h dark) the experimental tanks, the pure culture of *L. minor* was multiplied in batches into polystyrene tanks. Fingerlings of *Catla catla* (5.88±0.02g) and *Labeo rohita* (4.98±0.02g) were collected from the Institute Fish Farm and acclimatized in cemented tanks at a stocking density of 25 numbers l^{-1} with constant aeration for 15 days before the experiment. The fish were fed on floating pelleted feed with 30% crude protein content @ 5% body weight into two split doses at morning and evening. Water exchange with 40% replacement was done every alternate day during this period.

Collection of Cattle wastewater and treatment: The average volume of cattle wastewater released from the cattle shed was 1500 litres per day. The solid parts from cattle shed was removed and washed. The washed-out untreated wastewater containing dung and urine was channelized into the sedimentation unit and allowed to settle for 15 days. Primary treatment of the collected wastewater was done using lime @ 5 mg l^{-1} and potassium permanganate @ 5 mg l^{-1}. The wastewater was periodically stirred manually to purge out noxious gases. Total plate count of *E. coli* was ensured to be zero at the end of primary treatment prior to release of the water for secondary treatment. At the end of 15 days, the supernatant water was released into the phytoremediation unit of the jalkundh and diluted in the ratio of 50 (cattle wastewater): 50 (bore well water) to make 50% dilution. A healthy multiplier batch of *L. minor* was inoculated in the jalkund upto 50% surface coverage area and allowed to grow for 15 days for secondary treatment to remove excess nutrients. After secondary treatment, the fish were cultured in the three treatment groups: T_0 -only bore wellwater; T_1 - cattle wastewater: bore well water (20:80 ratio) prepared from secondary treated dilution and T_2- cattle wastewater: bore well water (50:50 ratio).

Experimental design: A 3×2 factorial design with factor A (Treatment group- T_0, T_1 and T_2) and Factor B (Species- Catla and Rohu) was followed for the study. The experiment was conducted in eighteen (18) numbers circular cemented tanks (250 l capacity) in triplicate for 60 days. In total (n=180) *C. catla* and *L. rohita* were collected and distributed equally @ 10 fingerlings per tank in three different treatments. The fish were fed twice daily at 2% body weight with floating pelleted feed with 20% crude protein content during the experimental period. The water quality parameters like dissolved oxygen (DO), alkalinity, biochemical oxygen demand (BOD), Nitrate nitrogen ((NO_3-N), Nitrite-nitrogen (NO_2-N) were estimated following the standard methods by (APHA, 2023) method. The total ammonical nitrogen (NH_4-N) was estimated by phenate method and soluble phosphate (PO_4-P) by ascorbic acid method. Water quality parameters were measured fortnightly. The individual length, weight and total biomass of fish were recorded at the start of the experiment as well as after 60 days. The survival, daily weight gain (DWG), specific growth rate, etc., were measured as per (Nath *et al.,* 2021). Survival percentage was also calculated at the end of the experimental period. The organoleptic parameters of the fish (Colour, texture, odour and taste) were scored on a scale of 1 to 5 for each parameter in a blind test involving twenty volunteers. The average score across four parameters was taken as a final score.

Statistical analyses: Statistical significance of variance for fish survival, growth, organoleptic score and water quality parameters were analysed by univariate test using SPSS version (11). The LSD Duncan's Multiple Range Tests was used for post hoc comparison of means ($p < 0.05$) among the treatment group. For finding the variation between the species, t-test was performed.

Results and Discussion

The perusal of data showed that the survival of fish between the treatment and species differed significantly as presented in Table 1 and 2. The growth and survival of Catla and Rohu in T_0 treatment was statistically similar ($P>0.05$). Catla exhibited higher growth as well as survival percentage than Rohu in T_1 group ($P<0.05$). Additionally, the survival percentage of Rohu fingerlings in T_2 at the end of 60 days was zero (0%). The rate of survival of Catla was higher in T_1 treatment (96.67 %.) than T_2 treatment (90%; $P<0.05$). The daily growth rate (Table 1,2) Catla was statistically higher than Rohu in both the treatment groups T_1 (1.32 g d^{-1} against 0.28 g d^{-1}) and T_2 (5.12 gd $^{-1}$ against 0.75 g d^{-1}).

The results of the present study differ from earlier reports. Rohu reportedly demonstrated higher growth than both Catla and Mrigal reared in treated petroleum industry wastewater due to higher density of phytoplanktons (Gadhia and Gadhia,1999; Ghosh *et al.,* 1973) as well as in sewage fed fish culture (Bhakta and Jana*,* 2006). The feeding nature of two Indian major carp *L. rohita* (omniplanktivorus) and *C. catla* (zooplanktivorous) may be the reason for better survival and growth in the present work. The phyto-remediated cattle wastewater (organic manure) produces more zooplankton than phytoplankton due to less inorganic nutrients (Boyed and Tucker, 1998). Limited literature are available to support our results, however, the present work finds more zooplankton than phytoplankton and no blooms were observed. Calta shows the pelagic mode of life and Rohu as column feeder may explain better survival and growth in the study as bad water quality parameters affects column feeders more than surface feeders (Rodríguez-Mendoza *et al.,* 2011).

The size distribution pattern of Catla and Rohu fingerlings also might have contributed to the variation observed in the growth parameters, which is regulated by genetic factor rather than the environmental conditions. The ontogeny of Catla is affected by genetic makeup and reflects the environmental factors such as water quality, availability of food, swimming behavior (Swain *et al.,* 2005). Development of allometric growth patterns makes different body form to consequently suit the functional development of Catla and Rohu to support the survival and growth (Pena and Dumas, 2009). The organoleptic score was taken to check the consumer preference of fish cultured in cattle wastewater. The odour differed significantly in T_2 treatment for

Table1: Effect of treated cattle wastewater on growth and survival of *Labeo rohita* and *Catla catla*

Treatments (Factor A)	Variable	Species (Factor B)		df	F	P
		L. rohita	*C. catla*		value	
T_0	Final Biomass	54.67 ± 6.43^{aA}	88.33 ± 7.64^{aB}	17	4.654	0.01
T_1	(g)	62.67 ± 7.57^{bA}	153.00 ± 15.39^{bB}			
T_2		0 ± 0^{cA}	358.33 ± 33.29^{cB}			
T_0	Survival (%)	93.33 ± 5.77^{aA}	98.33 ± 2.89^{aB}	17	2.905	0.06
T_1		86.67 ± 5.77^{aA}	96.67 ± 2.89^{aB}			
T_2		0 ± 0^{bA}	90.0 ± 5.0^{bB}			
T_0	Daily weight	0.16 ± 0.11^{bA}	0.72 ± 0.11^{bB}	17	7.775	0.002
T_1	gain (DWG)	0.28 ± 0.12^{bA}	1.32 ± 1.01^{bB}			
T_2		0.75 ± 0.005^{aA}	5.21 ± 0.54^{aB}			
T_0	Specific growth	0.14 ± 0.08^{cA}	0.49 ± 0.05^{cB}	17	2.139	0.130
T_1	rate (SGR)	0.23 ± 0.09^{bA}	0.88 ± 0.06^{bB}			
T_2		-2.76 ± 0.01^{aA}	1.44 ± 0.04^{aB}			
T_0	Odour	3.67 ± 0.29^{bA}	3.83 ± 0.29^{bA}	17	1.354	0.308
T_1		3.83 ± 0.29^{bA}	3.83 ± 0.29^{bA}			
T_2		3.17 ± 0.76^{aA}	3.0 ± 0.50^{aA}			

Values are expressed as mean± SD (n=18). Statistically significant are presented as a,b between the treatment and A, B between the factor species

Table 2: Summary of univariate ANOVA for effects of treatment, fish species and their interaction

Variables	Source	Sum of square Type III	Errors	df	F	P
Final biomass(g)	Treatment (A)	36001.333	3004.667	2	71.891	0.000
	Fish species (B)	116322.722	3004.667	1	464.568	0.000
	A*B	90221.778	3004.667	2	180.163	0.000
Survival (%)	Treatment (A)	9558.333	216.667	2	264.692	0.000
	Fish species (B)	5512.500	216.667	1	305.308	0.000
	A*B	6825.000	216.667	2	189.000	0.000
Daily weight gain (DWG)	Treatment (A)	10.748	2.730	2	23.618	0.000
	Fish species (B)	28.558	2.730	1	125.507	0.000
	A*B	26.881	2.730	2	59.069	0.000
Specific growth rate (SGR)	Treatment (A)	4.947	0.047	2	633.455	0.000
	Fish species (B)	13.523	0.047	1	0.00346	0.000
	A*B	13.792	0.047	2	0.00176	0.000
Odour	Treatment (A)	2.028	2.333	2	5.214	0.023
	Fish species (B)	0.000	2.333	1	0.000	NS
	A*B	0.083	2.333	2	0.214	NS

both fish, however, the odour for T_0 and T_1 treatment did not differ for Rohu and Catla. Ammonia exposure causes lipid peroxidation and significantly increases malonyldialdehyde content, which is responsible for off flavor in common carp (Mirghaed *et al.,* 2019). In the present experiment the ammonia concentration was highest in T_2 treatment which could have resulted in the degradation of fatty acid and other lipids resulting in the off flavour in fish.

The chemical properties of wastewater at the end of the primary treatment were as follows: total dissolved oxygen: 0 mg l^{-1}, pH: 8.2, alkalinity: 110 mg l^{-1}, BOD- 5 day: 130 mg l^{-1}, the total ammonical nitrogen (NH_4-N): 30 mg l^{-1}, Nitrate nitrogen (NO_3-N): 10 mg l^{-1}, Nitrite nitrogen (NO_2-N): 35 mg l^{-1} and soluble phosphate (PO_4-P): 17.08 mg l^{-1}. After secondary treatment with *L. minor,* the water quality parameters improved and were as follows: total dissolved oxygen: 4 mg l^{-1}, pH: 7.5, alkalinity: 105 mg l^{-1}, BOD-5 day: 60 mg l^{-1}.Whereas, the water quality parameters for T_1 treatment were recorded in case of total ammonical nitrogen (NH_4-N) as 11 mg l^{-1}, nitrate nitrogen (NO_3-N) as 0.5 mg l^{-1}, nitrite nitrogen

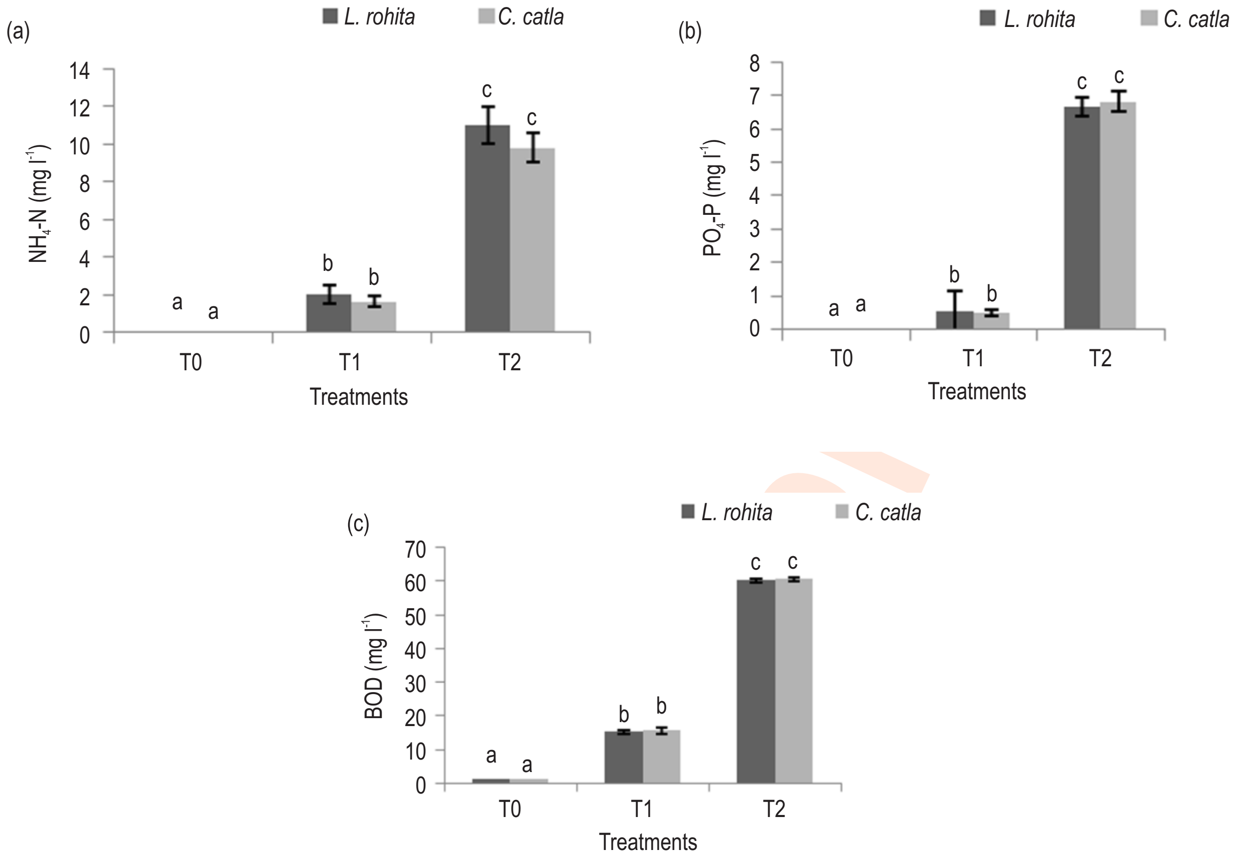

Fig. 1: Graph presenting (a) Total Ammonical Nitrogen (NH_4 -N) (b) Total Phosphorus (PO_4 -P) and (c) Biochemical oxygendemand (BOD) of different treatment groups. Values are mean ± S.D., statistically significant are presented as a, b.

(NO_2-N) as 2.0 mg l^{-1} and soluble phosphate (PO_4-P) as 6.67 mg l^{-1}.

The secondary treated wastewater considered as T_2 treatment which was diluted with bore-well water for preparing T_1 treatment for culturing two Indian Major Carp fingerlings. The water quality parameters like biochemical oxygen demand (BOD), total ammoniacal nitrogen (NH_4-N), and total phosphorus (PO_4-P) were statistically significant between the treatments (Fig. 1). All three parameters were higher in T_2 treatment than T_1 and T_0 and could be one of the reasons of less survival and growth in T_2 treatment. The other water quality parameters like pH, dissolved oxygen and alkalinity were not statistically significant between the treatment groups. The chemical contaminants are considered as sources of physiological stress to fish (Hodson, 1990). Water quality of domestic sewage affects the Succinate dehydrogen (SDH) enzyme activity in gill of Catla and Rohu which is an indicator of osmoregulatory activity. High ammonia, pH and phosphorus content effects the growth of Catla and Rohu by interfering with the respiratory activity and affecting the chloride cells of gill lamellae (Mukherjee and Jana, 2007). The results of the present study also suggest that T_2 treatment, the diluted cattle wastewater contained more ammonical nitrogen and phosphorus than T_1 and T_0, which may affect the gill lamellae and destroy chloride cells and interfere with osmoregulation to maintain homeostasis due to environmental stress, however, Catla fared better than Rohu in terms of survival and growth in poor water quality as Catla was less affected than Rohu (Beyers *et al.,* 1999).

The RNA /DNA ratio and protein content in muscle also effected in Catla and Rohu by domestic sewage water and found the Rohu was more affected than Catla due to genetic factor. It is also reported that Brown trout seemed to be more sensitive than rainbow trout to environmental stress and infectious agents (Schmidt *et al.,* 1999). The result findings suggest that diluted cattle wastewater can be used for aquaculture with Catla and Rohu as candidate fish species. Thus, the treatment T_1 [cattle wastewater: bore well water (20:80 ratio)] is recommended for good survival and growth of Indian major carps as per consumer preference.

Lemna based phytoremediation successfully reduced the nutrient load and odour of cattle shed wastewater to acceptable levels enabling its reuse for aquaculture. Indian major carps (Catla and Rohu) reared in phytoremediated wastewater diluted with 80 parts freshwater (T1) had statistically similar levels of growth, organoleptic characteristics and survival (P>0.05) as conventionally reared fish (control). The incorporation of phytoremediated wastewater eliminated the need for periodic fertilization for augmenting primary productivity during the culture duration. The results show that recycling of phytoremediated wastewater can serve triple purposes of water conservation and input cost reduction in aquaculture as well reducing uncontrolled eutrophication of open waterbodies.

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