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Sugarcane bagasse biochar-amended sediment improves water quality, growth, and physiology of *Pangasianodon hypophthalmus*

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Abstract

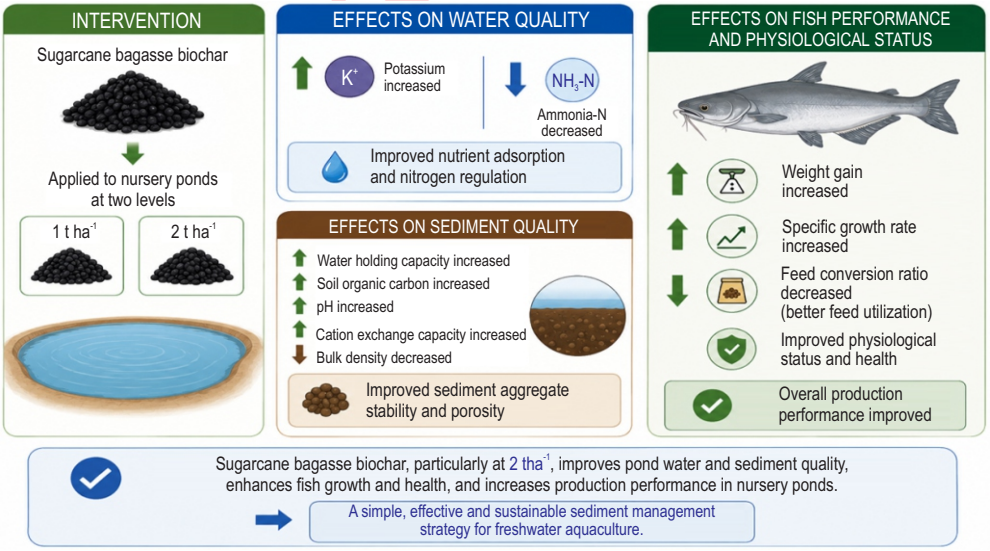
Aim: To evaluate the effects of sugarcane bagasse biochar as a sediment amendment on water quality, sediment properties, growth performance and physiological responses of *Pangasianodon hypophthalmus* in freshwater aquaculture systems.

Methodology: Sugarcane bagasse was pyrolyzed to produce biochar, crushed and sieved. Pond sediments were amended with biochar @ 1 t ha^{-1} and 2 t ha^{-1} and control with no biochar. Three treatments were tested for 90 days. Water quality parameters, sediment properties, growth performance were evaluated.

Results: Biochar significantly improved the water quality, reduced ammonia-nitrogen levels, enhanced sediment organic carbon and cation exchange capacity, and improved fish growth performance.

Interpretation: Sugarcane bagasse biochar improved the freshwater aquaculture system and fish growth performance, demonstrating its potential as a sustainable sediment amendment strategy.

Key words: Nutrient dynamics, Sediment amendment, Soil organic carbon, Sugarcane bagasse biochar, Water quality



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Introduction

Freshwater aquaculture has emerged as one of the fastest-growing food production sectors globally, playing a pivotal role in enhancing food security, generating employment, and supporting rural livelihoods (FAO, 2022). With increasing pressure on capture fisheries and a rising demand for affordable animal protein, inland aquaculture system has become an important contributors to sustainable fish production (Boyd *et al.,* 2022). Freshwater environments including ponds, tanks, reservoirs, and rivers provide favorable conditions for the culture of commercially important species such as *Pangasianodon hypophthalmus, Oreochromis niloticus, Labeo rohita* and *Catlacattla* (Rathod, 2021; Sidiq *et al.,* 2024; Singh, 2025). The intensification of freshwater aquaculture has significantly increased the productivity per unit area through improved seed quality, formulated feeds, aeration systems, and better pond management practices (Laktuka *et al.,* 2023).

Production systems range from extensive earthen ponds relying on natural productivity to highly controlled recirculating aquaculture systems (RAS) designed for efficient water use and biosecurity. However, intensification also present challenges, including deterioration of water quality, accumulation of organic waste, sediment nutrient imbalances, and increased susceptibility to disease outbreaks (Ruben *et al.,* 2025). Water quality remains a primary determinant of fish health and growth performance (Menon *et al.,* 2023). Water quality parameters such as, pH, ammonia-nitrogen, alkalinity and hardness directly influence the metabolic processes and feed utilization efficiency (Yang *et al.,* 2024). To address these challenges, recent researcher have focused on sustainable sediment and water management strategies, including the application of organic amendments such as biochar.

Biochar, a carbon-rich material produced through the pyrolysis of biomass, has been recognized for its potential to improve soil structure, enhance nutrient retention, increase cation exchange capacity, and adsorb toxic compounds (Amal *et al.*, 2023; Kara *et al*., 2024; Konduri *et al*., 2026a). Its integration into aquaculture pond sediments may offer a promising approach to improving water quality, fish growth performance, and physiological health while enhancing environmental resilience (Kara *et al*., 2025, 2026). Organic amendments, such as biochar, have gained attention as sustainable tools to improve sediment properties, enhance nutrient retention, and mitigate the accumulation of harmful compounds (Konduri *et al.,* 2024; Tachangliu *et al*., 2025; Konduri *et al*., 2026b). Biochar can increase cation exchange capacity, organic carbon content, and water holding capacity in pond sediments, thereby improving both the physico-chemical environment and the physiological performance of cultured fish. Therefore, entensive studies related to application of biochar for improving fish growth, sediment quality, and water quality in aquaculture systems have been conducted (Najmudeen *et al.,* 2019; Abakari *et al.,* 2021; Mehmood *et al.,* 2023;Konduri *et al.,* 2026a). However, these studies primarily focus on shrimp, tilapia and carp culture whereas information on the freshwater pond culture of *Pangasianodon hypophthalmus* remains scarce. Although Pangasius has emerged as one of the most commercially important freshwater species worldwide, contributing substantially to global whitefish production and aquaculture expansion (Anderson *et al.,* 2018), the effects of sediment amendment with biochar on its growth performance, physiological responses, and culture environment has not been adequately investigated. Therefore, the present study was planned with the aim to evaluate the effects of sugarcane bagasse biochar amended sediment on freshwater pond ecosystems, with a focus on water quality, growth performance and physiological profiles of *Pangasianodon hypophthalmus* juveniles cultured in outdoor freshwater ponds.

Materials and Methods

Experimental site: The experiment was conducted at the ICAR-Central Institute of Fisheries Education (CIFE) regional center Powarkheda, Narmadapuram, Itarsi, Madhya Pradesh, India. The study was conducted in unidimensional six square earthen ponds, each encompassing a surface area of 100 sq m (10m x10 m) and a depth of 2 m in replicate. The research design comprised of three distinct experimental groups viz. C (Control group without biochar in sediments); T_1 and T_2 (1 and 2 tons of biochar ha^{-1}).

Preparation of sugarcane bagasse biochar and characterization: The sugarcane bagasse raw material was procured from Betul district, Madhya Pradesh, India. The raw material sample was air-dreid until the moisture content reached 10-15%. Biochar was prepared by using Drum kiln (400°C) for a duration of 1 hr to produce sugarcane biochar. The resulting biochar yield was calculated as a percentage of the initial dried biomass. (Srinivasan *et al.,* 2015).

After preparation, the sugarcane bagasse biochar was characterized for its physico-chemical properties. The pH and electrical conductivity (EC) were measured using a pH electrode and an EC meter, respectively, following the method described by Rajkovich *et al.* (2012). The cation exchange capacity (CEC) of the biochar was determined according to the modified method of Hendershot *et al.* (1993). The moisture and ash contents of the biochar were measured using a gravimetric method (Novak *et al.,* 2009). The water holding capacity and available potassium content was estimated by following ASTM D1762-84 standards (ASTM, 2021).

The bulk density, and porosity of the biochar were estimated by weighing bottle method. Characterization of elemental composition of sugarcane bagasse biochar was conducted by following the diacid digestion of 0.5 g of biochar. Total potassium content was estimated by flame photometer. Total phosphorus content was estimated by spectrophotometer (Double-beam UV-visible, MOTRAS Scientific). Ash and moisture contents were determined following the standard methods of

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ASTM (1990).

Pond preparation and stocking of fingerlings: The ponds were dried for 10 days to facilitate sediment conditioning and reduce potential pathogenic loads.Sugarcane bagasse biochar was applied beforehand to water filling. Biochar slurry was prepared for T_1 and T_2, with T_1 (20 kg in 400 L) and T_2 (40 kg in 800 L) maintained at a 1:20 biochar-to-water ratio, the mixtures were soaked for 24 hr and applied uniformly to the pond bottoms of T_1 and T_2 treatment, while the control group contained only freshwater without biochar. Fingerlings of uniform size were stocked @ 30 nos. m^{-2} with an average weight of 15.60±0.16 g and a length ranging 6 to 8 cm. Thereafter, the fish were fed twice in a day with commercial feed.

Physico-chemical parameters of water: The physico-chemical parameters of the water including pH, total alkalinity, ammonium-N, were periodically measured on the 1st, 15th, 30th, 45th, 60th, 75th and 90th days following the standard methods of APHA (2017). The concentration of potassium ions in the water were estimated by flame photometer (India-Electronics, India).

Soil parameters: The soil physico-chemical parameters such as pH and water holding capacity were analyzed following the standard protocol of ASTM (2008). The bulk density of sediment was determined following the method of Toková *et al.* (2022). Soil organic carbon (SOC) was estimated by Walkley-Black method (1934), and sediment organic matter was calculated by multiplying SOC by 1.724. Cation exchange capacity (CEC) was measured using the method described by Song and Guo (2012), available potassium (K) was determined following Estefan *et al*. (2013), and available phosphorus (P) was analyzed according to FAO (2021).

Estimation of growth parameters of fish: At the end of the experimental period, growth performance parameters, including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival (%), were determined according to the method of Konduri *et al.* (2024).

Fish sampling procedure: After 90 days, *Pangasianodon hypophthalmus* juveniles (n=20) were randomly sampled from each pond, anesthetized with clove weighed and dissected. Blood samples (0.5–1 ml) were collected from the caudal vein in sterile syringes: EDTA-anticoagulated for hematological analysis; clot-formed, centrifuged (3000 × g, 10 min, RT), and serum stored at −20°C for biochemical assays. Liver tissue (~0.3 g) was rinsed in saline, homogenized in 3 ml Tris buffer (10 mM, pH 7.5, 0.25 M sucrose, 0.01 M EDTA), sequentially centrifuged (10,000 rpm, 4°C, 10 min; then 5000 rpm, 10 min), and thereafter, the supernatants were stored at −20°C.

Estimation of enzyme activities: SOD and CAT activities were analyzed in liver tissues. SOD activity was measured by inhibition of epinephrine auto-oxidation at 480 nm (Misra and Fridovich, 1972) and expressed as U mg^{-1} protein. Catalase activity was determined by decomposition of H_2O_2 using a colorimetric method (Claiborne, 1985) and expressed as µmol H_2O_2 decomposed min^{-1} mg $protein^{-1}$ at 25°C.

Statistical analyses: Data were analyzed using IBM SPSS Statistics (version 22.0). Differences among the experimental groups were evaluated by One-way ANOVA, followed by DMRT for post hoc comparisons. Statistical significance was considered at $p < 0.05$. Results were presented as mean ± SE.

Results and Discussion

The physical and chemical properties are very important parameters for biochar as they alter with change in the contents of raw materials, also with change in the pyrolysis temperature and types of modifications (Table 1). The temporal variation was exhibited in physico-chemical parameters of water under different treatments during 90 days of experimental period (Table 2). There was increase in the pH of T_1 and T_2 treatments due to alkaline nature of the biochar (Lehmann and Joseph, 2009; Zhang *et al.,* 2013). Biochar was found to increase the water alkalinity in T_1 and T_2, thereby improving the buffering capacity of the water (Table 2). Ammonia was significantly reduced in the treatment groups as compared to control at the end of 90 days. Adsorption of ammonium ions onto biochar surface reduces the concentration of toxic un-ionized ammonia in water (Atkinson *et al.,* 2010; Kara *et al.,* 2025). The treatment groups showed higher potassium concentrations than control due to high potassium content of sugarcane bagasse biochar (34.1 g kg^{-1}).

The relatively higher nitrate levels in biochar-treated groups indicate enhanced microbial activity, likely due to the provision of favorable microhabitats within the porous biochar matrix (Lehmann and Joseph, 2009). This transformation of

Table 1: Physical and chemical characteristics of sugarcane bagasse biochar pyrolyzed at 400°C

Physical properties	Values
Moisture (%)	3.21±0.15
Water holding capacity (%)	218±4
Bulk density (g cm^{-3})	0.63±0.2
Particle density (g cm^{-3})	1.31±0.2
Porosity (%)	81.5±3
Ash (%)	35.1 ±0.4
Total dissolved solids (mg l^{-1})	3072±128
Yield (%)	25 ± 0.21
Chemical properties	**Values**
pH	8.5±0.05
Electrical conductivity (dSm^{-1}),	1.61±0.04
Cation exchange capacity (cmol (+) kg^{-1})	88.40±1.46
Total potassium (gk g^{-1})	34.1±0.42
Total phosphorus (gk g^{-1})	2.19±0.4

Values are mean of three replicates ± S.E.

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Table 2: Physico-chemical properties of water during the experimental period (days)

Parameters	Treatments	1	15	30	45	60	75	90
pH	C	7.62±0.03^{a}	7.64±0.03^{a}	7.63±0.05^{a}	7.65±0.04^{a}	7.63±0.02^{a}	7.64±0.02^{a}	7.66±0.03^{a}
	T_1	7.64±0.01^{a}	7.65±0.02^{a}	7.66±0.02^{a}	7.74±0.02^{b}	7.86±0.04^{b}	8.17±0.02^{b}	8.22±0.05^{b}
	T_2	7.65±0.02^{a}	7.66±0.01^{a}	7.67±0.01^{a}	7.76±0.02^{b}	7.95±0.01^{c}	8.24±0.06^{b}	8.25±0.02^{b}
Total alkalinity	C	60.0±3.0^{a}	62.0±2.0^{a}	65.0±3.0^{a}	65.5±1.5^{a}	68.5±1.5^{a}	70.0±6.0^{a}	72.0±5.0^{a}
(ppm)	T_1	61.0±2.0^{a}	65.5±2.5^{b}	68.5±1.5^{b}	70.0±2.0^{b}	95.0±2.5^{b}	100.0±2.0^{b}	98.0±2.5^{b}
	T_2	60.5±2.5^{a}	65.0±2.0^{b}	68.0±2.0^{b}	73.0±1.0^{c}	100.0±1.5^{c}	106.5±1.5^{c}	110.0±1.5^{c}
Ammonia-N	C	0.05±0.00^{c}	0.13±0.01^{c}	0.23±0.00^{c}	0.30±0.01^{c}	0.34±0.01^{c}	0.27±0.01^{c}	0.28±0.01^{c}
(ppm)	T_1	0.04±0.00^{b}	0.10±0.00^{b}	0.18±0.01^{b}	0.25±0.02^{b}	0.24±0.01^{b}	0.22±0.01^{b}	0.23±0.01^{b}
	T_2	0.02±0.00^{a}	0.07±0.01^{a}	0.14±0.01^{a}	0.17±0.00^{a}	0.20±0.01^{a}	0.14±0.01^{a}	0.15±0.01^{a}
Potassium (ppm)	C	5.01±0.30^{a}	6.71±0.32^{a}	6.63±0.14^{a}	6.80±0.53^{a}	6.80±0.04^{a}	6.99±0.35^{a}	7.05±0.30^{a}
	T_1	5.08±0.23^{a}	6.69±0.35^{a}	7.39±0.55^{b}	6.98±0.40^{a}	7.26±0.36^{b}	7.66±0.40^{b}	7.99±0.35^{b}
	T_2	5.07±0.24^{a}	6.74±0.23^{a}	7.14±0.27^{b}	7.72±0.52^{b}	7.88±1.20^{c}	7.98±0.28^{c}	8.30±0.25^{c}
Nitrate-N (ppm)	C	1.50±0.04^{a}	2.50±0.01^{b}	2.40±0.00^{a}	2.55±0.00^{a}	2.60±0.00^{a}	2.65±0.00^{a}	2.70±0.08^{a}
	T_1	1.52±0.02^{a}	2.40±0.00^{a}	2.92±0.00^{b}	2.90±0.00^{b}	2.80±0.00^{b}	2.85±0.00^{b}	2.85±0.03^{b}
	T_2	1.53±0.01^{a}	2.55±0.01^{c}	3.12±0.00^{c}	3.10±0.00^{c}	3.00±0.01^{c}	3.05±0.01^{c}	3.08±0.10^{c}

Table 3: Physico-chemical parameters of sediment during the experimental period

Parameters	Control	T_1	T_2
pH	6.5 ± 0.2^{a}	7.1 ± 0.1^{b}	7.6 ± 0.2^{c}
Organic carbon (%)	0.82 ± 0.06^{a}	1.15 ± 0.02^{b}	1.30 ± 0.10^{c}
Available nitrogen (mg kg^{-1})	210 ± 12^{a}	245 ± 15^{b}	280 ± 18^{c}
Available phosphorus (mg kg^{-1})	18 ± 2^{a}	26 ± 3^{b}	34 ± 4^{c}
Available potassium (mg kg^{-1})	145 ± 10^{a}	190 ± 12^{b}	230 ± 15^{c}
Cation exchange capacity (cmol kg^{-1})	10.5 ± 0.8^{a}	14.2 ± 1.0^{b}	18.6 ± 1.2^{c}
Bulk density (g cm^{-3})	1.42 ± 0.05^{c}	1.30 ± 0.04^{b}	1.18 ± 0.03^{a}
Moisture content (%)	18 ± 2^{a}	24 ± 2^{b}	29 ± 3^{c}
Total organic matter (%)	1.4 ± 0.1^{a}	2.2 ± 0.2^{b}	3.1 ± 0.3^{c}

Values are mean of three replicates ± S.E. Values with various superscripts in the same column differ significantly ($p < 0.05$). C (control pond), T_1 (pond with 1 tons biochar ha^{-1} and T_2 (pond with 2 tons biochar ha^{-1})

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ammonia into nitrate further contributes to the reduction of ammonia toxicity. Application of sugarcane-derived biochar altered the physico-chemical properties of freshwater pond soil across treatments (Control, T_1 and T_2) (Table 3). Soil pH showed a progressive increase from slightly acidic conditions in the control (6.5) to near neutral in T_1 (7.1) and moderately alkaline in T_2 (7.6) treatments, the increase in pH was likely due to alkaline nature of the biochar (Table 1). There was significant difference in the organic carbon content in treatment group as compared to the control group. The increase in organic carbon might be due to high carbon contents of biochar, as biochar produced at low temperature has higher organic carbon contents (Demisie *et al.,* 2014).

Available nitrogen increased from 210 mg kg^{-1} (control) to 280 mg kg^{-1} (T_2), while available phosphorus increased from 18 mg kg^{-1} to 34 mg kg^{-1}. Potassium levels also rose considerably, from 145 mg kg^{-1} in control to 230 mg kg^{-1} in T_2 treatments. This increase may be due to high contents of nitrogen and potassium in the sugarcane bagasse biochar (Table 3). Biochar also increased the cation exchange capacity, with values rising from 10.5 cmol kg^{-1} in the control to 18.6 cmol kg^{-1} in T_2 treatments. Similar improvements in cation exchange capacity (CEC) in inland saline soil were reported by Kara *et al.* (2025) as enhanced CEC supports better soil nutrient retention (Domingues *et al.,* 2025). In contrast, the bulk density decreased from 1.42 gcm^{-3} in control to 1.18 gcm^{-3} in T_2 treatment, indicating improved soil structure (Obia *et al.,* 2016). Moisture content increased significantly with biochar application, from 18% in control to 29% in T_2, reflecting enhanced water retention capacity of amended soils.

The growth performance and survival of *Pangasiodon hypophthalmus* reared under different treatments is shown in Table 4. At the end of 90 days experimental period, significant ($p < 0.05$) differences in the growth parameters were observed among the treatments. The improved growth performance may be attributed to enhanced water and sediment quality (Amal *et al.,* 2023; Kara *et al.,* 2025). Weight gain percentage and specific growth rate were significantly higher ($p < 0.05$) in biochar-treated groups. The highest weight gain (573.26%) and specific growth

Table 4: Growth parameters and survival rate of the experimental fish, *P. hypophthalmus*

Parameters	Control	T_1	T_2
Initial weight (g)	15.6±0.16[a]	15.3±0.04[a]	15.15±0.17[a]
Final weight (g)	50.25±0.36[a]	83.22±0.37[b]	102±0.33[c]
Weight gain (%)	222.12 ±15[a]	443.92± 23[b]	573.27± 30[c]
Specific growth rate (% d^{-1})	1.30± 0.19[a]	1.88± 0.52[b]	2.12± 0.54[c]
Feed conversion ratio	1.8±0.06[c]	1.40±0.06[a]	1.41±0.04[b]
Survival rate (%)	90.0 ± 3.5[a]	93.0 ± 4.5[a]	94.0 ± 3.1[a]

Table 5: Haematological and antioxidative enzymes of the experimental fish, *P. hypophthalmus*

Parameters	Control	T_1	T_2
Haematological parameters			
Hemoglobin (g d l^{-1})	5.90±0.15[a]	6.57±.82[b]	7.67±0.33[c]
R.B.C Count (×106m m^{-3})	2.23±0.02[a]	2.55±0.02[b]	5.56±0.01[b]
Total WBC (×103m m^{-3})	11.63±0.28[a]	12.10±0.40[b]	15.60±0.35[c]
Glucose (mgd l^{-1})	52.67± 4.80[a]	55.33±2.20[a]	53.67±2.67[a]
Antioxidative enzymes			
SOD Liver (U mg $protein^{-1}$)	20.08±1.16[b]	19.14±0.44[b]	18.45±0.25[a]
Catalase Liver (µmol H_2O_2 min^{-1} mg $protein^{-1}$)	27.89±1.65[c]	25.12±1.55[b]	24.72±1.30[a]

Values are mean of three replicates ± S.E. Values with various superscripts in the same column differ significantly ($p < 0.05$). C (control pond); T_1 (pond with 1 tons biochar ha^{-1}) and T_2 (pond with 2 tons biochar ha^{-1}).

rate (2.12% d^{-1}) was observed in T_2, followed by T_1 treatments (452.58; 1.88% day^{-1}), while the control group recorded the lowest values (233.63; 1.30% day^{-1}). The lowest feed conversion ratio was found in the treatment group as compared to the control group, lower feed conversion ratio indicate proper feed utilization, which may be also due to improvement in the sediment and water quality in treatment groups as compared to control. No significant difference was observed between T_1 and T_2 treatments. Survival rate did not differ significantly ($p > 0.05$) among the treatments. However, a marginal increase in survival rate was observed in the biochar-treated groups, with T_2 treatment recording the highest survival (94.0 %), followed by T_1 (93.0 %) and control (90.0 %). Haematological parameters are widely accepted indicators of fish health, physiological condition, and environmental stress. In the present study, the hemoglobin levels were significantly higher in T_2 treatment compared to Control and T_1 treatments, respectively, indicating improved oxygen transport capacity and better physiological performance. Enhanced hemoglobin concentration is generally associated with improved metabolic activity and health status in fish reared under optimized culture conditions (Hrubec *et al.,* 2000). In all the treatments, RBC were in normal range ($0.4–5.2×10^6$ cells m m^{-3}), which suggests no adverse effect of biochar on RBC (Esmaeili *et al.,* 2021) (Table 5). The significant increase in WBC count in T_2 indicates activation of immune system (Kara *et al.,* 2025). Elevated leukocyte levels are generally associated with enhanced innate immunity and improved disease resistance in fish (Li *et al.,* 2019).

Blood glucose levels remained statistically non-significant among all treatments, suggesting that none of the biochar applications induced metabolic stress. Stable glucose levels reflect proper energy homeostasis and absence of stress-induced hyperglycemia, which is commonly observed under unfavorable environmental conditions. Antioxidant enzymes such as superoxide dismutase and catalase play a critical role in maintaining cellular redox balance by neutralizing reactive oxygen species. In the present study, SOD activity showed slight variation among treatments, with marginally lower values in T_1 and T_2 compared to Control (Table 5). This suggests a possible reduction in oxidative stress levels in treated systems, resulting in decreased requirement for enzymatic defense activity. A similar trend has been reported in fish exposed to improved environmental conditions, where antioxidant enzyme activity stabilized due to reduced ROS production (Li *et al.,* 2019). Catalase activity also showed a decreasing trend in treated groups compared to control. This indicates reduced accumulation of hydrogen peroxide and improved oxidative balance in fish exposed to biochar-treated ponds. Lower catalase activity under improved rearing conditions has been interpreted as an indicator of reduced oxidative stress burden rather than physiological impairment (Liu *et al.,* 2024).

In conclusion, sugarcane bagasse biochar amendment significantly improved the water quality, sediment properties, growth performance, and physiological health of *Pangasianodon hypophthalmus* juveniles. From a practical perspective, sugarcane bagasse biochar serve as a low-cost and sustainable

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pond sediment amendment to improve pond productivity, fish performance, and environmental quality in freshwater aquaculture. Additionally, the conversion of sugarcane bagasse into biochar provides a value-added use of agricultural waste, while reducing environmental pollution associated with residue burning. Therefore, biochar has considerable potential for application in *Pangasianodon hypophthalmus* farming to enhance fish growth, improve sediment quality, and maintain better water quality.

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Conflict of interest: The authors declare that is there is no conflict of interest.

Data availability: The data that support the findings of this study are available on request from the Corresponding author.

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