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RESEARCH ARTICLE

Dal-lake's dominant submerged aquatic weed valorisation for bioremediation of nitrogenous and metallic stressors from a variety of aquaculture waters

Aalia Bashir[1], K.K. Krishnani*[1 2], A.K. Verma[1], T.I. Chanu[1], Ajay Upadhyay[3], Biplab Sarkar[2], Sanjay K. Gupta[4] and Arpita Sharma[1]

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ABSTRACT

The present study aims to determine ammonia, nitrite, lead (II) and chromium (VI) removal efficiencies of the products developed from aquatic weeds, *Ceratophyllum demersum* and *Hydrilla verticillata,* collected from Dal-lake, for the first time, from a variety of aquaculture waters (pond, aquaponics, and ornamental) under controlled conditions, followed by a wet-lab-based large-scale experiment. Aquatic weeds were found to be effective in the removal of ammonia, nitrite, Pb(II), and Cr(VI) from a variety of aquaculture waters, which can be attributed to adsorption and ion exchange of priority pollutants on functional moieties, alkali metals, and alkaline earth metals present in the aquatic weeds. The experimental results showed that ammonia and nitrite removal was effective at pH 7.5, whereas Cr(VI) removal was observed at pH 2 in treatment with 100 mg/L and 200 mg/L of weed. The average lead biosorption observed was 14 mg/g. The removal efficiencies observed were 60-65% for ammonia, 50% for nitrite, 99% for Cr(IV), and 90% for lead. By integrating bioremediation and livelihood generation, this initiative can not only contribute to environmental restoration but also create sustainable opportunities for local communities, fostering economic growth and social well-being. Utilizing aquatic weeds for bioremediation to generate livelihood opportunities for local communities can be an innovative and sustainable approach. Capacity building of the farmers for plant-assisted bioremediation is also suggested, enabling them to effectively integrate these techniques into their aquaculture operations.

Keywords: Dal-lake, Aquatic weeds, Aquaculture, Toxicants, Bioremediation, Circular bioresource utilisation, Environmental sustainability

INTRODUCTION

Globally, aquatic foods provide about 17 per cent of animal protein. Total fisheries and aquaculture production reached a record 214 million tonnes in 2020, comprising 178 million tonnes of aquatic animals and 36 million tonnes of algae, largely due to the growth of aquaculture, which refers to the rearing of aquatic organisms such as fish, molluscs, crabs, and plants (FAO 2022). The evolution of aquaculture operations from traditional to semi-intensive and intensive culture operations results in increased aquaculture production levels. However, intensive aquaculture operations lead to the deterioration of pond water quality due to the generation of high nitrogen and phosphorus metabolic waste, which can induce eutrophication (Abisha *et al.* 2022). The most prevalent toxicants in pond water are ammonia and nitrite through the decomposition of unconsumed feed and excretion by aquatic organisms in protein catabolism. The other class of pollutants is heavy metals. By accumulating in sediments, these contaminants can enter the food chain through plants and aquatic animals and can cause acute or chronic toxicity, leading to mortality in extreme cases. Lead can accumulate in the body and disrupt the health of humans, animals, and phytoplankton. Lead primarily binds to sulhydryl and oxo-groups in enzymes, affecting nearly every stage of haemoglobin synthesis and metabolism (Jomova *et al.* 2024). Chromium contamination of water bodies occurs as a result of both natural and anthropogenic sources. The hexavalent form Cr (VI) is of special concern due to its high toxicity, because of intense oxidation capabilities, and its effects on humans and animals are carcinogenic, mutagenic, and teratogenic (McCarroll *et al.* 2010), and many of its derivatives are highly water-soluble, making it readily bioavailable (Kotas 2000). Thus, it becomes necessary to remove these toxicants to maintain a healthy aquaculture operation.

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[1] ICAR-Central Institute of Fisheries Education, Andheri (W), Mumbai, Maharashtra 400061, India

[2] ICAR-Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand 834010, India

[3] ICAR-National Research Centre for Grapes, Pune, Maharashtra 412307, India

[4] ICAR-Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu 600028, India

* Corresponding author email: kishore18111966@gmail.com

There is a big challenge to mitigate inorganic and organic contaminants, which together have been coined as contaminants of environmental and emerging concern (CEECs) (Krishnani *et al.* 2023). The most common methods for ammonia and heavy metals removal are ion exchange using zeolites, adsorption, filtration, chemical precipitation, and water exchange (Arunkumar *et al.* 2023, Chakraborty *et al.* 2024). These technologies, on the other hand, have some disadvantages, such as low efficiency and high operating and maintenance costs. Hence, to maintain a healthy aquatic environment and prevent and control disease infections, it becomes essential to develop a sustainable and eco-friendly aquaculture technology. Plants are considered to possess a natural ability to remove inorganic pollutants from water in an eco-friendly and low-cost process called plant-assisted bioremediation. Non-living plant biomass can be used profitably for the uptake and recuperation of contaminants, which is one way to put this principle into practice, and has successfully been demonstrated by Krishnani *et al.* (2021, 2023) and Singh *et al.* (2025a, 2025b). Successive use of dried and dead plant biomass to remove nitrogenous and metallic toxicants from industrial effluents, municipal waste water, and sewage water has gained popularity in the past few years because it is easily handled and is an affordable natural product (Parnian *et al.* 2022, Eliasova *et al.* 2021).Phycoremediation/Algae-assisted bioremediation in brackish water/coastal aquaculture has gained popularity (Kashem *et al.* 2023). Aquatic weed-assisted bioremediation may be considered a new, eco-friendly, and low-cost practice of removing contaminants from aquaculture water. However, reports on aquatic weed-assisted bioremediation for freshwater aquaculture are scanty.

Aquaponics combines hydroponics and aquaculture - a recirculatory system of the farming of aquatic organisms into a single system, where the nutrient-rich water from aquaculture is used as fertiliser for the cultivated plant, and the plants clean the water, creating a suitable environment for the fish to grow (John *et al.* 2022, Meena *et al.* 2023) and microorganisms, act as the third vital component because of their crucial role in the transformation of nutrients (Eck *et al.* 2019).

Freshwater habitats are under serious threat due to the diverse pressures of development, and the restoration of these ecosystems is an important challenge in the present era. Dal-lake (Union Territory of Jammu and Kashmir) is an urban lake that, being the state's most picturesque lake, is vital for tourism and enjoyment. The Dal-lake annually produces about 70,000 tonnes of lake waste, according to data from the Jammu and Kashmir Lake Conservation and Management Authority (LCMA). The floating/ submerged aquatic ecology of Dal-lake is a living repository of aquatic weed plants. The lake, which once covered an area of 75 square kilometres, has shrunk to 12 square kilometres in the last two decades. The lake's depth has also come down by nearly 12 metres, and it is a grave sign of the dangers the lake faces. The manual de-weeding of iconic Dal-lake might be an uphill task in restoring the pristine glory of the water body. The project is being implemented to convert lake weeds into usable organic manure and allied products, with the major objective of significantly promoting the cleaning of Dal-lake as it will not only eradicate the organic waste from the lake but also aid scientific treatment of all organic waste and create a useful product.

Enormous quantities of organic wastes such as sewage sludge (SS) and aquatic weed compost (AWC) are produced in large quantities on the banks of Dal-lake. It is a challenging task for authorities to manage them properly (Dar *et al.* 2023). From time to time, many scholars have focused their efforts on various ecological features of Dal-lake. Qadri *et al.* (2022) presented a case study of *Certophyllum demersum* as an efficient tool for heavy metal remediation in the order of $Co^{2+} > Cd^{2+} > Mn^{2+}$, followed by other metals in Dal-lake, a natural freshwater system in the Union territory of Jammu and Kashmir, India. However, the use of non-living biomass of aquatic weeds for biosorption of heavy metals and removal of ammonia and nitrite in aquaculture waters has been scantly reported. The present study evaluates the phytoremediation efficiency of aquatic weeds to determine nitrogenous and metallic toxicants for the first time from a variety of aquaculture waters. In this regard, aquatic weeds were collected from Dal-lake for the development of the product. The aquatic weeds were identified as *Ceratophyllum demersum and Hydrilla verticillata* and used in powder form to study ammonia and nitrite removal, biosorption of Pb (II), and detoxification of chromium Cr (VI).

MATERIALS AND METHODS

Collection of the aquatic weed for the preparation of the product

The aquatic weeds used in this study were collected from the freshwater habitat of Dal-lake (Union Territory of Jammu and Kashmir, India) in clean plastic bags. Plants were carefully washed using tap water to remove visible debris. After drying under sunlight, the plants were crushed and powdered in an electric mixer and passed through a 60-mesh sieve. Collections and product development were done from October 2020 to December 2020.

FTIR of aquatic weed-based product

Fourier Transform Infrared spectroscopy (FTIR) was used to characterize the functional moieties of an aquatic weeds. FT-IR spectra were recorded using an FTIR-Imaging system (Thermo Fisher Scientific) using the potassium bromide (KBr) pellet technique. The sample was scanned from 4,000 to 400 $cm^{"1}$ wave number.

Removal of ammonia and nitrite from aquaculture water

Total ammonia-N (TAN) and nitrite-N concentrations in different water samples (fish pond, aquaponics, and ornamental) were estimated in separate experiments using a UV-VS Spectrophotometer (2080 UV/Vis Spectrophotometer) at 640 nm and 540nm of wavelength (APHA, 2005) and compared with the standard graph. The concentration was expressed as mg/L. For experimenting on aquatic weed-assisted bioremediation of ammonia and nitrite, spiking of three different water samples was done with 1000 mg/L stock solution of $(NH_4)_2SO_4$ to obtain a 1.5 mg/L concentration in addition to traces of TAN already present in collected water samples from aquaponics and ornamental. Spiking of three different water samples was done with a stock solution of $NaNO_2$ to obtain 0.5mg/L for estimation of Nitrite-N. The experiments were assigned different concentrations of the aquatic plant in different sources of water, with a Control (0 mg/L), and all were arranged with three replicates in 50 mL flasks. The test durations were kept at 3, 6, 12, 24, 48, and 72 hours, respectively. The solutions were agitated using a rotary shaker. In all the experiments, the plant biomass-contained solutions were filtered using a no. 41 Whatman filter papers. After getting successful results for ammonia removal activity, a large-scale experiment was carried out in eighteen numbers of 140 L tanks containing 100 L of water from an Aquaponics facility spiked with a stock solution of TAN for obtaining 1.5mg/L ammonia. A completely randomized design was followed for the experiment, using three replicates for each treatment. The experiment was assigned with treatments as 100 mg/L, 200 mg/L, and 0 mg/L (Control) of *Ceratophyllum*, and *Hydrilla verticillata* and all treatments were arranged with 3 replicates in nine 140L capacity tanks containing 100 L of water. The stocking density was kept at 6 per 100L. The experiment was conducted for 72 hours for TAN removal by keeping fish without feed, and then was extended up to 30 days by feeding fish twice daily and siphoning after a 4-day interval for measuring growth parameters. *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings of length (cm) and weight (g) were stocked in the experiment. During the experimental period, the following parameters were maintained: pH 7.5 ± 0.5, water temperature 27 ± 2.0°C. The percentage removal efficiency was calculated according to the following formula.

$$\%\, efficiency = \frac{co - c1}{co} \times 100$$

where C0 and C1 are initial and final concentrations of ammonia in a medium (mg/L).

Cr (VI) detoxification

Hexavalent Cr in the pond water sample was determined spectrophotometrically (Standard Methods, 1989) by measurement of the intense red-violet complex formed by the reaction of Cr (VI) with 1,5-diphenyl carbazide at different pH (2, 3, 4, 5, 6, 7) and compared with a standard graph. The concentration was expressed as mg/L. Spiking of different water samples was done with 100mg/L stock solution of $K_2Cr_2O_7$ to obtain a 2 mg/L concentration of Cr (VI) using the equation($N_1V_1=N_2V_2$). Nitric acid was used to adjust the pH of the pond water in which the chromium samples (Cr-VI) were prepared. The experiment was assigned three different concentrations of the aquatic plant, with 0 mg/L as the Control, and all concentrations were arranged with three replicates in 50 mL flasks. The test durations were kept at 3, 6, 12, and 24 hours, respectively. The solutions were agitated using a rotary shaker. In all the experiments, the plant biomass-containing solutions were filtered using a no. 41 Whatman filter papers.

Lead biosorption

Water samples were collected from the pond, and spiking of the water samples was done with a stock solution of $PbNO_3$ to obtain a 10 mg/L Pb concentration. The test duration was kept at 24 hours. The solutions were agitated using a rotary shaker. In all the experiments, the plant biomass-containing solutions were filtered using No. 41 Whatman filter papers. The samples were analysed by Inductively Coupled Plasma Mass spectrometry (ICP-MS-Agilent Technologies Model 7800).

All reagents were analytical grade, and instruments were pre-calibrated appropriately prior to measurement. Replicate analyses were carried out for each determination to ascertain reproducibility and quality assurance. The treated samples were also analyzed by Flame photometry (Labard LIM-204) to determine the effective ion-exchange mechanism.

All the above experiments and analyses were carried out from February 2021 to July 2021.

RESULTS AND DISCUSSION

Identification of aquatic weeds

To determine the nitrogenous and metallic toxicants removal activity of an aquatic weed-based product, the aquatic weeds were collected from Dal-lake, India (**Figure-1 A, B**) and identified as *Ceratophyllum demersum* (**Figure-1C**) and *Hydrilla verticillata* (**Figure-1D**). *Ceratophyllum demersum* known as coon tail, is a submerged macrophyte commonly seen in freshwater ponds and is well-adjusted to aquarium conditions in temperate climates. *Ceratophyllum demersum* species is rootless; a completely submerged dicotyledon's seed belonging to the family Ceratophyllaceae grows well in subtropical and tropical weather regimes and is commonly seen in ponds, lakes, ditches, and quiet streams. It does not produce roots; instead, it absorbs all the nutrients it requires from the surrounding water.

Determination of functional moieties of aquatic weed using Fourier Transform Infrared spectroscopy (FTIR)

The intense peak at 3433.15 cm^{-1} was observed, which indicated that the hydroxyl functional group is present in an aquatic weed. Also, OH stretching of the carboxylic acid functional group was present in 2928.10 cm^{-1} and 2360.14 cm^{-1}, and carbonyl (C=O) group stretching occurred at 1646.14cm^{-1}, and C-O stretching appeared in 1026.81 cm^{-1}. FTIR spectra revealed the presence of various functional moieties in the aquatic weed. The other absorption peaks appeared at 875.31, 712.7, 666.68, 572.73, and 506.98 cm^{-1} (**Figure 2**).

Determination of TAN removal activity in different water samples under lab conditions

The effect of four different concentrations of *Ceratophyllum* (40, 100, and 200 mg/L) on the removal of the initial TAN concentration of 1.37 mg/L from pond water, aquaponics water and ornamental water at different time intervals is presented in **Figure 3** (A, B, C). This shows that TAN levels decreased up to 31%, 45%, and 67% in treatments with 100 mg/L of *Ceratophyllum* and decreased up to 45%, 63%, and 67% in treatment with 200 mg/L of *Ceratophyllum* in 24, 48, and 72 hours respectively

(A). Dal-lake

(B). Collection of aquatic weeds

(C). *Ceratophyllum demersum* (Dried)

(D). *Hydrilla verticillata* (Dried)

Figure 1. Dal-lake and the Site of sample collection and aquatic weeds

(**Figure 3A**). Similarly, from aquaponics water, TAN levels decreased 10%, 27%, and 36% with 100 mg/L of *Ceratophyllum* and decreased up to 14%, 43%, 61% with 200 mg/L of *Ceratophyllum* in 24, 48, and 72 hours respectively (**Figure 3B**). In ornamental water, aquatic weed was able to reduce TAN 18%, 34%, and 40% with 100 mg/L of *Ceratophyllum* and decreased up to 20%, 48%, and 51% with 200 mg/L of *Ceratophyllum* in 24, 48 and 72 hours respectively (**Figure 3C**).

The effect of four different concentrations of *Hydrilla verticillata* (40, 100, and 200 mg/L) on the removal of the initial TAN concentration of 1.37 mg/L from pond water, aquaponics water and ornamental water at different time intervals is presented in **Figure 4** (A, B, C). This shows that TAN levels decreased up to 29% and 53% in treatments with 100 mg/L of *Hydrilla verticillata* and decreased up to 55%, and 60% in the treatment with 200 mg/L of *Hydrilla verticillata* in 48 and 72 hours, respectively. Similarly, from aquaponics water, TAN levels decreased 26% and 33% with 100 mg/L of *Hydrilla verticillata* and decreased up to 40% and 53% with 200 mg/L of *Ceratophyllum* in 48 and 72 hours, respectively. In ornamental water, aquatic weed was able to reduce TAN by 33% and 39% with 100 mg/L of *Hydrilla verticillata* and decreased up to 47% and 49% with 200 mg/L of *Hydrilla verticillata* in 48 and 72 hours, respectively.

Determination of Nitrite-N removal activity in different water samples under lab conditions

The effect of four different concentrations of *Ceratophyllum* (40, 100, and 200 mg/L) on the removal of the initial Nitrite-N concentration of 0.5 mg/L at different time intervals in pond water, aquaponics water and ornamental water is presented in **Figure 5** (A, B, C). The nitrite-N level decreased from 0.46 to 0.38, 0.45 to 0.28 (37%) in 48 and 72 hours, respectively with 100 mg/L of *Ceratophyllum*. Nitrite-N levels showed a further decrease from 0.46 to 0.30 (34%), and 0.45 to 0.22 (51%) in 48 and 72 hours, respectively, with 200 mg/L of *Ceratophyllum* (**Figure 5A**). Similarly, in Aquaponics water, the effect of various concentrations of *Ceratophyllum* (0,

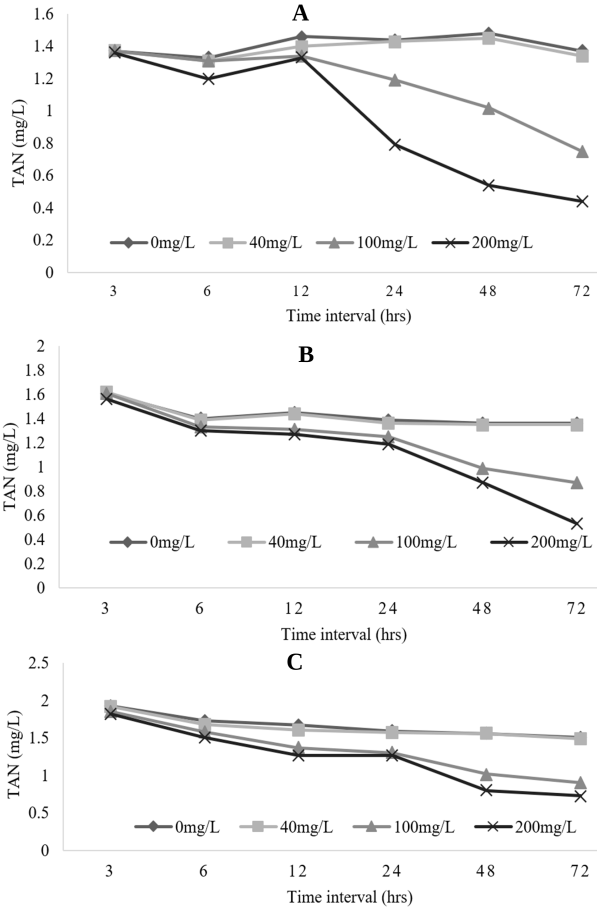


Figure 3. TAN removal from (A). Pond water (B). Aquaponics water (C). Ornamental water using *Ceratophyllum* at different time intervals under lab conditions

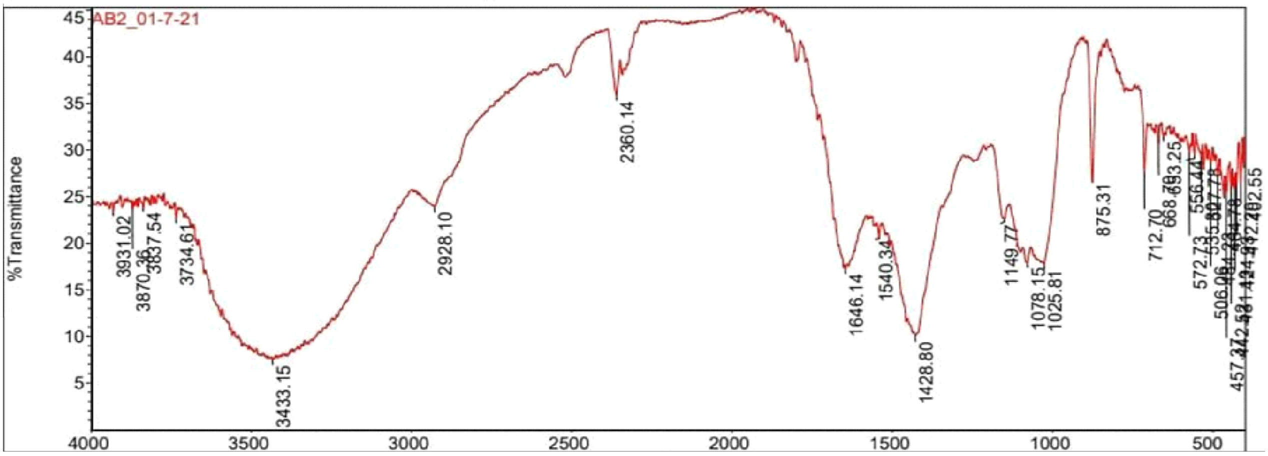


Figure 2. FT-IR of *Ceratophyllum* product

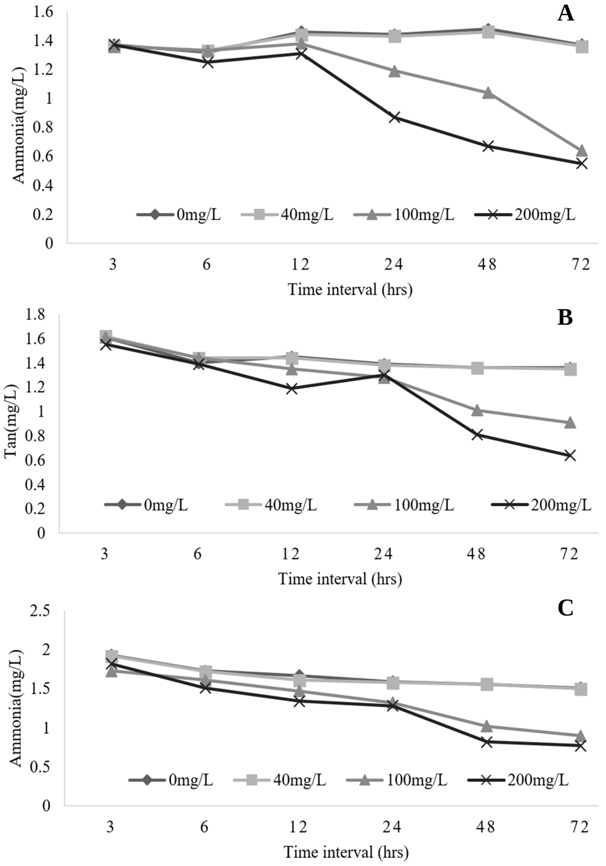


Figure 4. TAN removal from (A). Pond water (B). Aquaponics water (C). Ornamental water using *Hydrilla verticillata* at different time intervals under lab conditions

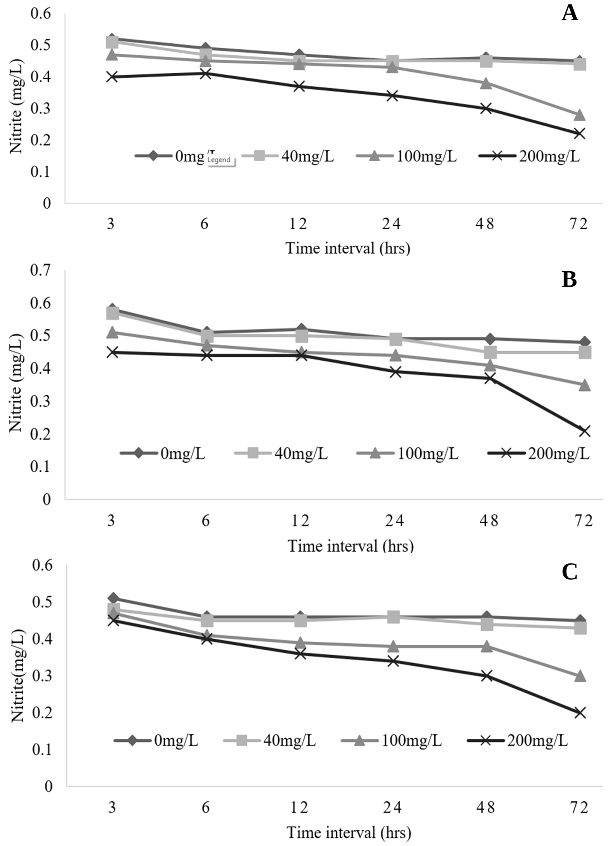


Figure 5. Nitrite-N removal from (A). Pond water (B). Aquaponics water, (C). Ornamental water using *Ceratophyllum* at different time intervals under lab conditions

40, 100, and 200 mg/L) on the removal of the initial concentration of 0.58 mg/L Nitrite-N in 72 h is shown in **Figure 5B**. The nitrite-N level decreased from 0.49 to 0.42 (14%), and from 0.48 to 0.35 (27%) in 48 and 72 hours, respectively, with 100 mg/L of *Ceratophyllum.* A nitrite-N level further showed a decrease from 0.49 to 0.38 (22%), and 0.48 to 0.21 (56%) in 48 and 72 hours, respectively, with 200 mg/L of *Ceratophyllum.* The effect of various concentrations of *Ceratophyllum* in ornamental water ranging from 0 to 200 mg/L on the removal of Nitrite-N in 72 hours is shown in **Figure 5C**. The nitrite-N level decreased from 0.46 to 0.38(17%), and from 0.45 to 0.30 (33%) in 48 and 72 hours, respectively, with 100 mg/L of *Ceratophyllum*. Nitrite-N level decreased from 0.46 to 0.30(34%), 0.45 to 0.20(55%) in 48 and 72 hours respectively with 200 mg/L of *Ceratophyllum.* The effect of four different concentrations of *Hydrilla verticillata* (40, 100, and 200 mg/L) on the removal of the initial Nitrite-N concentration of 0.5 mg/L at different time intervals in pond water, aquaponics water and ornamental water is presented in **Figure 6** (A, B, C). Nitrite-N decreased from 0.46 to 0.42(9%),0.45 to 0.32(28%) with 100 mg/L of *Hydrilla verticillata* and it decreased from 0.46 to 0.37(19%),0.45 to 0.28(37%) in 48 and 72 hours respectively with 200 mg/L of *Hydrilla verticillata.* In aquaponics, nitrite-N decreased from 0.49 to 0.42(14%),0.48 to 0.36(25%) with 100 mg/L of *Hydrilla verticillata,* and it decreased from 0.49 to 0.37(24%),0.48 to 0.26 (45%) in 48 and 72 hours, respectively, with 200 mg/L of *Hydrilla verticillata.* Similarly, in ornamental water, Nitrite-N decreased from 0.46 to 0.38(17%), 0.45 to 0.30(33%) with 100 mg/L of *Hydrilla verticillata*, and it decreased from 0.46 to 0.30(34%), 0.45 to 0.23 (49%) in 48 and 72 hours, respectively, with 200 mg/L of *Hydrilla verticillata.*

Determination of TAN removal activity of *Ceratophyllum* and *Hydrilla verticillata* under wet lab conditions

Under wet lab conditions, TAN levels decreased from 1.64 to 1.52(7%), 1.41(10%), 1.38(10%), 1.34 (15%), 1.19 (20%), and 0.92(33%) with 100 mg/L of *Ceratophyllum* and TAN decreased from 1.64 to 1.48(10%), 1.31(16%), 1.35(12%), 1.26(20%), 0.87(41%), and 0.53(61%) with 200 mg/L of

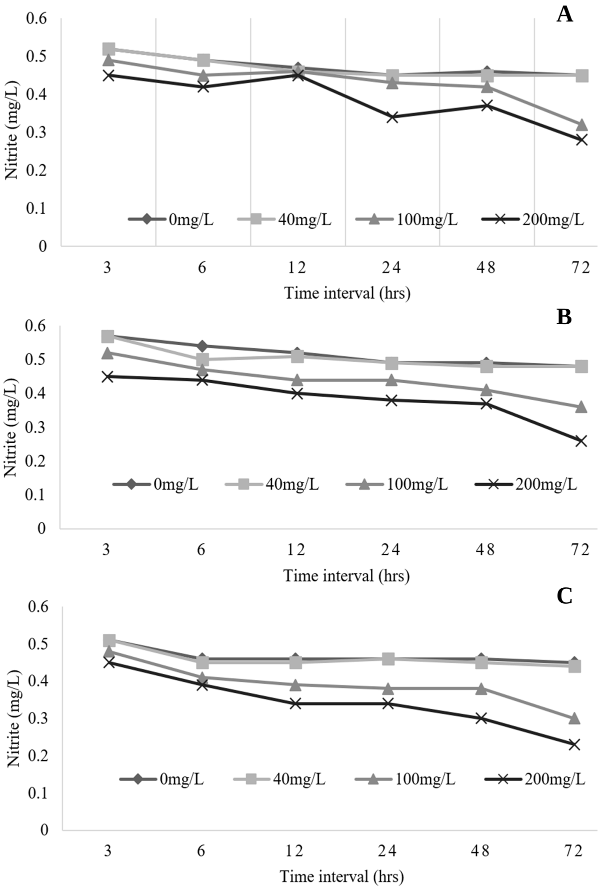


Figure 6. Nitrite-N removal from (A). Pond water, (B). Aquaponics water (C). Ornamental water using *Hydrilla verticillata* at different time intervals under lab conditions

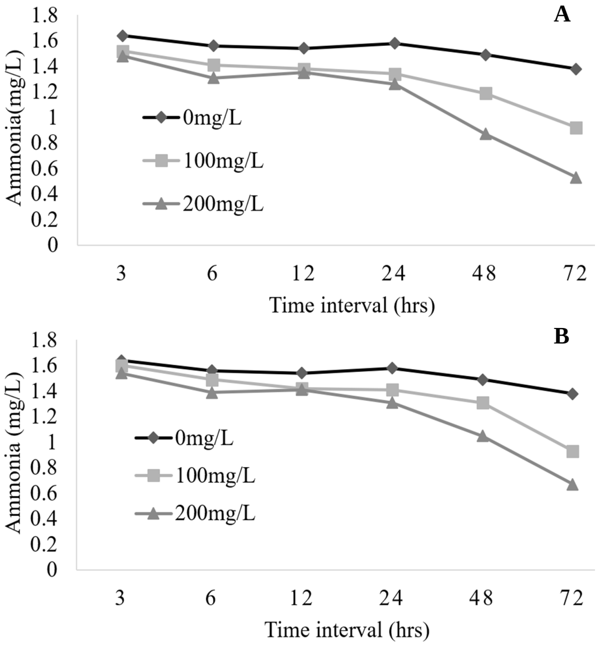
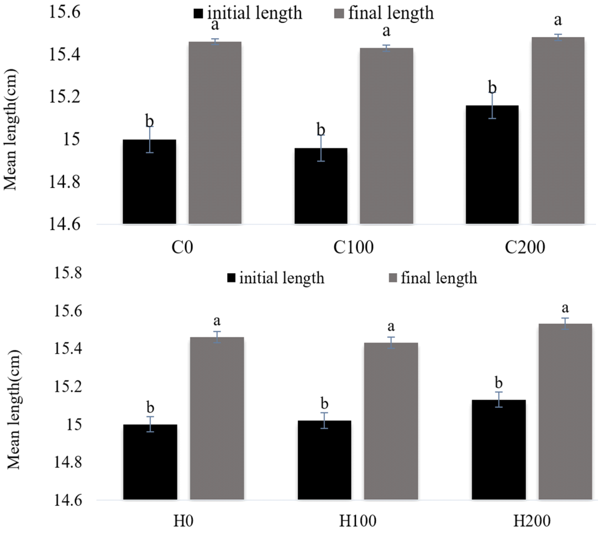


Figure 7. Effect on TAN removal activity using (A). *Ceratophyllum* and (B). *Hydrilla* (under wet lab conditions)



Figures 8. Mean Length of fish in treatment with *Ceratophyllum* and *Hydrilla*

Ceratophyllum in 3, 6, 12, 24, 48, and 72 hours respectively (**Figure 7A**). TAN level decreased from 1.49 to 1.31 (12%) and from 1.38 to 0.93(32%) in 48 and 72 hours, respectively, with 100mg of *Hydrilla verticillata*. TAN level showed further decrease from 1.49 to 1.05(29%) and1.38 to 0.67(51%) in 48 and 72 hours respectively with 200 mg/L of *Hydrilla verticillata* (**Figure 7B**).

The present analysis has revealed the excellent performance role of the aquatic weed-based product developed from *Ceratophyllum and Hydrilla verticillata* in phytoremediation technology for TAN and Nitrite-N removal from a variety of aquaculture waters, including the pond, aquaponics, and ornamental systems in a 72-hour experimentation period. The present findings gain support from the work of Krishnani *et al.* (2002), who have found a similar trend of reduction in TAN removal activity using neem oil, neemazal, and neemgold at 90 mg/L in decreasing the total TAN nitrogen (TAN) level of 0.40–0.45 mg/L in 96 hours. The removal efficiency after a 72-hour time duration was observed to be higher in pond water and aquaponics water than in ornamental water, which may be due to the presence of beneficial bacteria and enhanced periphytic bacterial growth due to the addition of the plant material that causes the removal of TAN from the water. These studies are in line with the findings of Krishnani *et al.* (2006), where bagasse was used for nitrate removal.

The present findings gain support from the work of Silva *et al.* (2006), who found the removal of NH_3-N by *E. crassipes* and *P. stratiotes* in catfish aquaculture wastewater that reached up to 74% and 78%, respectively, and 69% and 65% respectively, in tilapia fish aquaculture wastewater. Katsuya (2002) reported removing nitrate, nitrite-N, ammonium, and phosphate ions from water by the aerial microalga *Trentepohlia aurea*. Shahar *et al.* (2021) have reported *the* removal of nitrogen, particularly nitrate, from fishpond effluent using *Ulva fasciata* and periphyton. When the experiment was conducted on a large scale with fish, *Pangsianodon hypothalamus*, with 100 and 200 mg/L concentrations of *Ceratophyllum* and *Hydrilla* in 100L of water, the same results were obtained. The water quality parameters like DO, Temperature, alkalinity, hardness, pH, phosphate, and nitrite-N were maintained within normal ranges in both experiments during the 30 days. The growth parameters were also studied during the experiment, and it was found that the maximum weight gain observed was 2.9%, and the mean length was 15.48±0.05cm at 200 mg/L of *Ceratophyllum*. Hence, it was observed that in the experiment with different treatments, no significant difference was observed in weight gain, SGR, and mean length, respectively. Thus, it was concluded that among 40, 100, and 200 mg/L of plant product used, 100 and 200 mg/L doses showed a significant reduction in TAN and nitrite-N levels from the pond, aquaponics, and ornamental water, respectively.

Determination of the heavy metal removal capacity of the aquatic weed-based products

Cr (VI) Detoxification using *Ceratophyllum*

It was observed that chromium removal activity was significantly higher at pH 2 in treatment with 100 mg/L and 200 mg/L of *Ceratophyllum* in 24 hr, which indicates that the *Ceratophyllum* was able to reduce Chromium from 2.28 to 0.07(96%) and 0.01(99%) at 100 and 200 mg/L, respectively as shown in **Table 1**. The effect of pH 2 on chromium removal showed a significant difference. Similarly, the effect of the concentration of the plant at 100 and 200 mg/L showed a significant difference for the removal of chromium.

Cr Removal using *Hydrilla*

It was observed that chromium removal activity was significantly higher at pH = 2 at 200 mg/L of *Hydrilla* in a 24 h time interval, which indicates that the plant was able to remove Chromium from 1.66 to 1.38(17%) as shown in **Table 2**. The effect of pH=2 on Chromium Removal showed a significant difference. Similarly, the effect of the concentration of the plant at 200 mg/L showed a significant difference for the removal of Chromium.

Table 1. Concentration of total Cr (VI) at different pH levels in treatment with different doses of *Ceratophyllum demersum*

Weed dose (mg/L)	pH	3hr	6hr	12hr	24hr
0	2	1.81	2.04	2.13	2.28
0	3	1.74	1.68	1.71	1.78
0	4	1.81	1.81	1.84	1.86
0	5	1.79	1.64	1.76	1.87
0	6	1.71	1.75	1.82	1.94
0	7	1.86	2.41	2.45	2.67
40	2	1.62	1.75	1.16	0.58
40	3	1.72	1.50	1.63	1.77
40	4	1.75	1.70	1.83	1.86
40	5	1.74	1.56	1.69	1.86
40	6	1.72	1.73	1.76	1.91
40	7	1.79	2.12	2.24	2.17
100	2	1.5	1.55	0.40*	0.07*
100	3	1.63	1.40	1.38	1.55
100	4	1.6	1.49	1.57	1.83
100	5	1.71	1.55	1.62	1.80
100	6	1.67	1.61	1.61	1.79
100	7	1.8	2.12	2.03	2.12
200	2	0.74	0.62	0.15*	0.01*
200	3	1.52	1.31	1.36	1.45
200	4	1.46	1.40	1.53	1.83
200	5	1.64	1.44	1.51	1.72
200	6	1.63	1.45	1.40	1.61
200	7	1.73	2.05	1.94	2.11
	SEM	0.005	0.04	0.01	0.01

*Indicates significant difference ($p<0.05$); Individual effect on Chromium removal activity using *Ceratophyllum* product

Ceratophyllum conc(mg/L)	3hr	6hr	12hr	24hr
0	1.79	1.88	1.94	2.06
40	1.72	1.72	1.71	1.68
100	1.65	1.62	1.43	1.52*
200	1.45	1.37	1.31	1.45*
SEM	0.002	0.01	0.01	0.01
pH				
2	1.42	1.49	0.96	0.74*
3	1.65	1.47	1.52	1.64
4	1.66	1.60	1.69	1.84
5	1.72	1.55	1.64	1.81
6	1.68	1.63	1.64	1.81
7	1.79	2.18	2.16	2.26
SEM	0.003	0.02	0.01	0.01

*Indicates significant difference ($p<0.05$)

In the present study, the best removal of chromium was observed at pH 2 at 100 and 200 mg/L *Ceratophyllum* during the 24-hour experimentation period. Since Chromium is negatively charged in aqueous solutions, it offers repulsion to functional moieties present in the plant material; hence, by decreasing pH, H+ ions cause adsorption of Cr (VI) on plant material. Also, the presence of lignin in plant material acts as an electron donor, reducing toxic Cr (VI) to less toxic Cr (III). A similar study was conducted to ascertain the removal efficiency of chromium (VI), and it was found that the adsorption process was optimal at pH 2 for Cr (VI) (Alemu *et al.* 2018). The result was consistent with the previous observations conducted for the removal of Cr (VI)

Table 2. Concentration of total Cr(VI) at different pH in treatment with different doses of *Hydrilla verticillate* at different pH

Treatment	3hr	6hr	12hr	24hr
Effect of Hydrilla pH Chromium Concentration (mg/L)				
H0P2	1.84	1.92	1.88	1.66
H0P3	2.20	2.43	2.21	1.98
H0P4	1.86	1.87	1.87	1.51
H0P5	1.71	1.77	1.69	1.76
H0P6	1.78	2.00	1.76	1.76
H0P7	1.79	1.97	1.87	1.85
H40P2	1.61	1.92	1.62	1.66
H40P3	1.92	2.19	2.15	1.78
H40P4	1.76	1.75	1.76	1.52
H40P5	1.68	1.73	1.69	1.73
H40P6	1.69	1.94	1.76	1.69
H40P7	1.78	1.86	1.84	1.79
H100P2	1.60	1.51	1.59	1.53
H100P3	1.84	2.19	2.03	1.70
H100P4	1.61	1.69	1.76	1.49
H100P5	1.66	1.68	1.67	1.72
H100P6	1.66	1.66	1.74	1.69
H100P7	1.73	1.81	1.82	1.75
H200P2	1.52	1.51	1.51	1.38
H200P3	1.84	2.15	1.86	1.57
H200P4	1.53	1.64	1.62	1.47
H200P5	1.65	1.66	1.66	1.70
H200P6	1.64	1.84	1.72	1.67
H200P7	1.69	1.74	1.81	1.67
SEM	0.01	0.01	0.01	0.01
P Value	<0.001	<0.001	<0.001	<0.001
Individual effect on Chromium removal activity				
Effect of Hydrilla Concentration				
H0	1.86^{a}	1.99^{a}	1.87^{a}	1.75^{a}
H40	1.64^{d}	1.76^{c}	1.69^{d}	1.59^{d}
H100	1.73^{b}	1.90^{b}	1.80^{b}	1.69^{b}
H200	1.68^{c}	1.76^{c}	1.76^{c}	1.64^{c}
SEM	0.01	0.002	0.003	0.002
P Value	<0.001	<0.001	<0.001	<0.001
Effect of pH				
P2	1.64^{d}	1.72^{e}	1.65^{e}	1.56^{e}
p3	1.94^{a}	2.24^{a}	2.06^{a}	1.76^{b}
p4	1.69^{c}	1.74^{d}	1.75^{c}	1.49^{f}
P5	1.68^{c}	1.71^{f}	1.68^{d}	1.73^{c}
P6	1.69c	1.85^{b}	1.75^{c}	1.71^{d}
P7	1.75^{b}	1.84^{c}	1.84^{b}	1.77^{a}
SEM	0.007	0.003	0.003	0.002
P Value	<0.001	<0.001	<0.001	<0.001

Values with the same superscript in a column did not show any significant difference($p>0.05$); H = *Hydrilla;* 0, 40,100, 200 = Concentrations of plant in mg/l; P=pH

when the material was charred with sulphuric acid, with five different products prepared from bagasse for evaluating the detoxification of Cr (VI) from high saline coastal water (Krishnani *et al.* 2004). Other aquatic plant species such as *Eichhornia crassipes*, *Potamogeton lucens*, and *Salvinia herzegoi* have been reported as excellent biosorbent materials used successfully in several studies for removing Cr, Ni, Cd, Zn, Cu, and Lead (Wang *et al.*1998, 2019).

Table 3. Biosorption capacities of *Ceratophyllum demersum* and *Hydrilla verticillate* on lead (data shown are mean values)

Treatment Conc	Ceratophyllum		Hydrilla	
	mmol	mg/g	mmol	mg/g
40	0.08	16.93	0.12	25.82
100	0.07	14.99	0.06	12.93
200	0.05	10.07	0.05	9.63
Av.	0.07	14.00	0.08	16.13

Lead removal using *Ceratophyllum*

It was also observed that lead concentration decreased from 10.44 mg/L to 7.17 mg/L, 3.21 mg/L, and 0.72 mg/L in treatment with 40, 100, and 200 mg/L of *Ceratophyllum,* respectively, after a 24-hour time duration and there was a significant difference in Lead removal at different concentrations of the plant used and maximum removal was observed at 200 mg/L. Biosorption capacities of *Ceratophyllum* for Lead were varying from 0.05 to 0.08 mmol/g as shown in **Table 3**.

Lead removal using *Hydrilla*

It was observed that Lead concentration decreased from 10.44 mg/L to 5.45 mg/L, 4.2 mg/L, 1.14 mg/L in treatment with 40, 100, 200 mg/L of *Hydrilla* respectively after 24-hour time duration and there was a significant difference in Lead removal at different concentrations of the plant used and maximum removal was observed at 200 mg/L. Biosorption capacities of *Hydrilla* for Lead were varying from 0.05 to 0.08 mmol/g as shown in **Table 3**.

Characterisation of the *Ceratophyllum* using FT-IR spectroscopic technique indicated the presence of functional moieties -COOH, -OH, -CO that is negatively charged attract positively charged ammonium and metal ions, due to which efficient ion exchange occurs (Krishnani and Ayyappan, 2006). This result was consistent with the previous observations showing that pollutants and heavy metals are naturally absorbed by aquatic plants (Pratas *et al.* 2014). The most efficient and profitable method of removing various heavy metals and other contaminants is aquatic plants (Ali *et al.* 2013, Guittonny-Philippe *et al.* 2015).

The use of biological materials for heavy metal removal and recovery technologies (Biosorption) has gained significant recognition in recent years due to its good performance and inexpensive cost. In the present study, the aquatic weed-based product was tested for the removal of Lead from aqueous solutions having an initial lead concentration of 10 mg/L. The present investigation has revealed the role of the aquatic weed-based product developed from *Ceratophyllum* for *the* biosorption of lead. The *Ceratophyllum* was able to reduce lead from 10.44 to 7.17 mg/L, 3.21 mg/L, and 0.72 mg/L at 40, 100, and 200 mg/L, respectively, during a 24-hour time duration. The average value of biosorption capacity found was 14 mg/g. A similar study was conducted where water hyacinths (*Eichornia crassipes*) dried roots showed the potential to remove cadmium and lead effectively from wastewater (Wang *et al*, 1998). The flame photometry analysis indicated that there is a significant ion-exchange mechanism taking place between lead with calcium and potassium, which suggests that the plant materials possess good ion-exchange properties.

Metal ions have been removed by both living and dead (metabolically inactive) biological materials. It was discovered that certain functional groups present on their cell wall produce certain forces of attraction for metal ions, resulting in high removal efficiency. This explains the ion exchange mechanism for heavy metal removal. The results of the present study coincide with the work carried out by Krishnani *et al.* (2008), where paddy straw was used for heavy metal biosorption. In the present study, the different concentrations of plant material used (40, 100, and 200 mg/L) resulted in significant reduction and biosorption of Lead, and the high removal of Lead was observed with 200 mg/L in a 24-hour time duration.

Metal removal mechanism using flame photometry

The flame photometry analysis indicated that there is a significant ion-exchange mechanism taking place between lead and calcium and potassium, while sodium showed no significant difference in ion-exchange mechanism with lead, which indicated that sodium has no role to play in ion-exchange with lead as presented in **Table 4**. Silver has also been reported in *Hydrilla*, which may be attributed to the natural occurrence of silver in aquatic plants in Dal-lake. During the last decade, there has been considerable work done in the field of nanobiotechnology for the synthesis of nanoparticles by using plants, animals, fish and microorganisms (Krishnani *et al.* 2022). Terrestrial and aquatic plants can synthesise silver nanoparticles under natural conditions. Sable *et al.* (2012) reported the extracellular phytosynthesis of silver nanoparticles (65 nm) using aquatic plants *Hydrilla verticilata* and re4commended that the phytofabricated Ag-NPs can be used in the field of medicine and agriculture, due to their antimicrobial potential. Jha *et al.* (2009) synthesized AgNPs (2–5 nm) using plant extracts of *Bryophyllum* sp., *Cyperus* sp. and *Hydrilla* sp.

Fish growth parameters

It was observed that there was no significant difference in the mean length, weight gain, and SGR of fish when treated with 100 and 200 mg/L of plant *Ceratophyllum* and *Hydrilla,* probably due to the short duration of the experiment under the wet lab conditions (**Figure 8A, B**).

Submerged macrophytes distributed in freshwater ecosystems are highly important for maintaining water quality as well as the ecological functions of such ecosystems. Eliasova *et al.* (2021) found that *Ceratophyllum demersum* is a good source of flavonoids/glycosides (Tricetin, Luteolin, Selgin, Apigenin, Tricin, and Chrysoeriol) with good antioxidant properties, which were induced by ammonium. Nitrogenous toxicants such as ammonia and nitrite removal from discharge water has been of concern. Gopal (2014) studied the removal efficiency of organic load and nutrients from sewage water and observed that the physicochemical properties such as turbidity, ammonia, phosphate, Chemical Oxygen Demand, and Biological Oxygen Demand showed a significant decrease in values due to bio-digestion of organic nutrients during phytoremediation. Foroughi *et al.* (2013) have demonstrated that the *Ceratophyllum demersum* reduced ammonium and nitrate by more than 62% and 41.66% from wastewater. Parnian *et al.* (2022) have successfully demonstrated that hydrophyte (*Ceratophyllum demersum* L.) has good potential for the phytoremediation of cadmium and nickel from a saline aquatic environment under controlled conditions. The potential of soft submerged aquatic macrophytes, including *Ceratophyllum demersum* as

Table 4. Concentration of K, Ca and Na in water samples treated with *Ceratophyllum demersum* and *Hydrilla verticillata*

Treatment	*Ceratophyllum demersum*			*Hydrilla verticillata*				
	Potassium (K^+)	Calcium (Ca^{2+})	Sodium (Na^+)	Potassium (K^+)	Calcium (Ca^{2+})	Sodium (Na^+)	Ag	Cu
0	10.20 ± 0.15^{c}	26.4 ± 1.4^{c}	45 ± 0.90^{a}	10.2 ± 0.15^{c}	26.4 ± 1.44^{b}	45 ± 0.90^{a}	--	--
40	11.27 ± 0.16^{c}	30.5 ± 1.0^{b}	44.8 ± 0.15^{a}	10.7 ± 0.06^{c}	27 ± 0.85^{b}	45.5 ± 0.24^{a}	0	0.18
100	12.67 ± 0.20^{b}	29.6 ± 0.4^{b}	45.8 ± 0.52^{a}	11.3 ± 0.25^{b}	30.9 ± 0.54^{a}	44.3 ± 0.57^{a}	0.01	0.73
200	14.60 ± 0.70^{a}	34.9 ± 0.55^{a}	46.2 ± 0.16^{a}	11.9 ± 0.13^{a}	32.6 ± 1.50^{a}	44.9 ± 0.51^{a}	0.03	1.43
P Value	<0.001	<0.001	0.259	<0.001	<0.001	0.58		

a feed ingredient for herbivorous/omnivorous fish, such as tilapia, has also been demonstrated (Balkhashera *et al.* 2021).

Beheary *et al.* (2019) recommended the application of *C. demersum* in tilapia farms for the phytoremediation of contaminants from aquaculture wastewater. Aquaculture wastes and sub-products need to be reutilized to achieve a more efficient production system based on the application of circular bio-economy and the One-Health concept, protecting human, animal health and the environment (Fraga-corral *et al.* 2022; Krishnani *et al.* 2021; 2022; 2023). Climate change-induced abiotic stresses are an inevitable event that obstructs the output of aquaculture farms (Patel *et al.* 2022) and culture-based fisheries in open waters (Abisha *et al.* 2022). Mitigating abiotic stresses using natural and modified stilbites, synergizing with changes in oxidative stress markers in aquaculture, has been successfully demonstrated by Arunkumar *et al.* (2023).

The study proposes a solution through plant-assisted bioremediation using aquatic weeds (*Ceratophyllum demersum* and *Hydrilla*). Local communities can be involved in harvesting and collecting aquatic weeds, creating direct livelihood opportunities. Thereafter, collection centres can be made where harvested weeds can be brought and processed. For value addition, the local communities can be trained in processing techniques, such as drying, grinding and pelletizing to develop harvested aquatic weeds into usable products. Alternatively, local entrepreneurs can develop bioremediation marketable products from the processed aquatic weeds, and the Government can assist local entrepreneurs in establishing marketing channels for their products. Later, the formation of community-based organizations/cooperatives/self-help groups focusing on aquatic weed bioremediation can be encouraged so that these organizations can collectively manage harvesting, processing, and marketing efforts, ensuring equitable distribution of benefits. By integrating these strategies, Dal-lake's aquatic weed bioremediation initiative can not only contribute to environmental restoration but also create sustainable livelihood opportunities for local communities, fostering economic growth and social well-being.

The DWR developed and demonstrated a sequential, multi-weed phytoremediation system using combinations of semi-aquatic, free-floating, and submerged weeds (e.g., *Arundo donax*, *Eichhornia crassipes* and *Hydrilla verticillata*) to treat wastewater effectively (Khankhane and Varshney 2011; Khankhane *et al.* 2014). The studies primarily focused on the removal of heavy metals, including iron (Fe), cadmium (Cd), manganese (Mn), nickel (Ni), and copper (Cu), from sewage and polluted pond water.

Conclusions

Present study is the first report of using non-living biomasses of the aquatic weeds *Ceratophyllum demersum* and *Hydrilla verticillata* of Dal-lake for uptake and removal of nitrogenous stressors (ammonia, nitrite) and metallic stressors - lead (II), and chromium (VI) : a solution through plant-assisted bioremediation from different types of aquaculture waters, including ponds, aquaponics and ornamental setups, which can be attributed to functional moieties for adsorption and cations for ion exchange present in the aquatic weeds. This has future potential applications in circular bioresource utilization of aquatic weeds for aquaculture applications, which will help treat wastewater generated from aquaculture and industrial discharges to protect aquatic life from adverse impacts. These aquatic plants play a major role in the environmental conditions of stagnant and flowing waters, and could adsorb elements and decrease pollution.

Research and development in the field of aquaculture bioremediation for exploring the potential of various aquatic plant species in removing pollutants from aquaculture waters is necessary. As a policy suggestion, it is recommended to establish a comprehensive water quality monitoring programme for aquaculture systems. Regular monitoring of parameters such as ammonia, nitrite and heavy metals is suggested as it will help in early detection of pollution issues and ensure timely corrective actions. Based on the positive results of this study, some of the policy recommendations suggested are incorporating plant-assisted bioremediation techniques using aquatic weeds. Government agencies and regulatory bodies can provide incentives and guidelines for implementing such practices to reduce the impact of toxicants on aquatic ecosystems. Capacity building of the aquaculture farmers and industry professionals on the benefits of plant-assisted bioremediation is required so that they can effectively integrate these techniques into their aquaculture operations. These are some policy recommendations that can enhance the aquaculture sector's sustainability, reduce pollution, and contribute to the overall health of aquatic ecosystems.

Dal-lake, it is known that the lake is besieged by obnoxious weeds. By encouraging public-private partnerships and collaborations between government agencies, research institutions and private aquaculture enterprises, the implementation of bioremediation technologies on a larger scale can be undertaken, which will help in the adoption of sustainable practices in the fisheries and aquaculture sector. For this, awareness campaigns within local communities to highlight the positive impact of using aquatic weeds for bioremediation and livelihood generation, along with advocacy for policy support and recognition of this innovative approach at the regional and national levels, are suggested.

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