

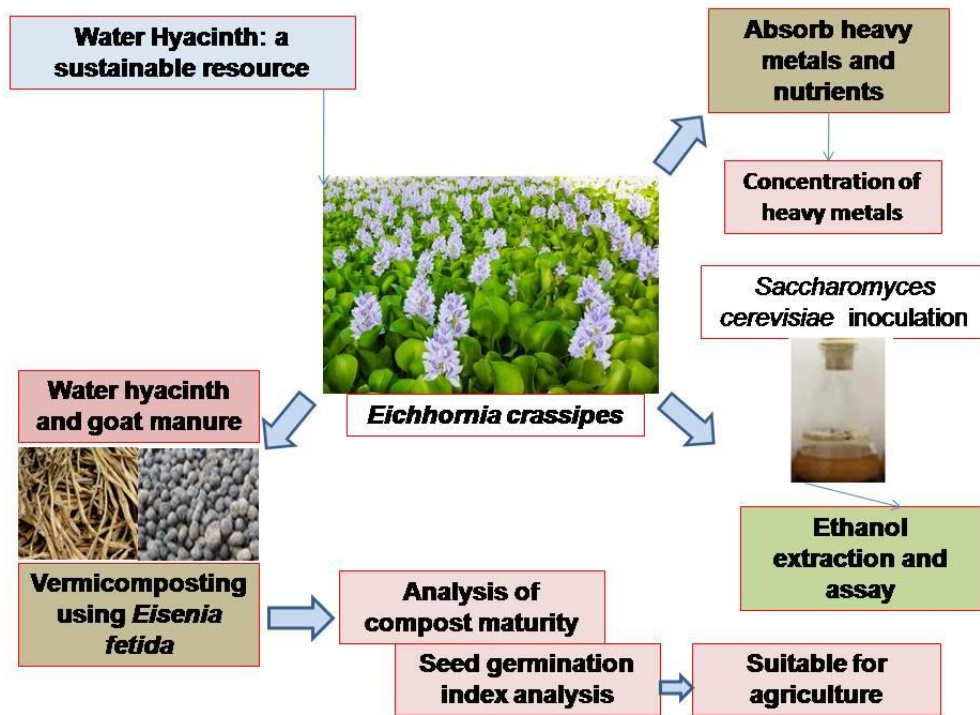
Water Hyacinth: A Sustainable Resource for Water Phytoremediation, Ethanol Production, Soil Nutrient Improvement, and the Dynamics of Microbial C and N in Vermicompost

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GRAPHICAL ABSTRACT



Water Hyacinth: A Sustainable Resource for Water Phytoremediation, Ethanol Production, Soil Nutrient Improvement, and the Dynamics of Microbial C and N in Vermicompost

Amirtha Mani Punitha,^a Chellappa Josephine Priyatharshini,^a Mai Ahmad Alghmdi,^b Dunia A. Al Farraj,^b Mohamed Soliman Elshikh,^b M. Ajmal Ali,^b Selvaraj Arokiyaraj,^c and Stalin Rinna Hamlin^{d,*}

The water hyacinth *Eichhornia crassipes* is a rapidly growing weed that grows in shallow fresh water. It can be used for the removal of nutrients and heavy metals from water, as feedstock for biofuels, and as a bulking agent for the vermicompost process. The present study focused on the use of water hyacinth plants for nutrient and heavy metal removal from water. Water hyacinth removed >95% of ammonium nitrogen, nitrite, and nitrate from the water. The bioaccumulation potentials of water hyacinth for Cu, Cd, Cr, As, Li, and Zn after 30 days of treatment were $80 \pm 2.3\%$, $78.6 \pm 3.2\%$, $73.2 \pm 1.2\%$, $69.6 \pm 2.1\%$, $65.5 \pm 1.9\%$, and $44.2 \pm 2.2\%$, respectively. The heavy metal accumulation in water hyacinth was in the following order: Cu>Cd>Cr>As>Li>Zn. Water hyacinth was pretreated with acid and base. It was further digested with cellulolytic enzymes, and *Saccharomyces cerevisiae* was further inoculated to produce ethanol in a liquid culture. The ethanol yield was 0.09 mL/mL culture. Water hyacinth was cut into small pieces, mixed with goat manure and used for vermicomposting. The microbial C content of the vermicompost ranged between 398 ± 12.8 and 537 ± 11.2 $\mu\text{g/g}$, and the microbial N content ranged from 104.4 ± 2.2 to 254.9 ± 2.2 $\mu\text{g/g}$. The vermicompost had an $84.3 \pm 2.2\%$ germination index after 48 h in the pots treated with 20% vermicompost.

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Keywords: Plant biomass; Weed; Water hyacinth; Phytoremediation; Biofuel; Antibacterial

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is an invasive freshwater weed native to South America, and it has spread throughout almost all countries (Mulugeta *et al.* 2020). This invasive feed initially spread over Africa at the beginning of the 19th century, where it occupied lakes, rivers, and freshwater bodies and caused economic and environmental losses (Merga *et al.* 2020). It is considered one of the worst invasive weeds in the world and is reported as a noxious plant in various countries (Lagoon *et al.* 2019). In addition to Africa, water hyacinth causes substantial economic loss and affects the environment in tropical and subtropical regions (Subash 2016). The pollutants in water bodies provide

nutrients to invasive weeds. In developing and developed countries, water bodies are generally exposed to various unintentional and intentional contaminants that can cause serious public health issues (Lake *et al.* 2020). With industrialization, urbanization, and developmental activities, unintentional pollutant levels have increased in water bodies. These processes are associated with serious health risks. In Asian countries, aquaculture wastewater, industrial effluents, domestic wastes, *etc.*, severely affect water and land ecosystems (Yabanli *et al.* 2016). Water hyacinth is the most productive plant worldwide. The growth of water hyacinth is rapid because of its adaptive strategies and a lack of competitors or natural enemies in the freshwater environment. The productivity of water hyacinth is approximately 270 to 400 tons per hectare of water, and the number of plants is approximately two million per hectare (Kunatsa *et al.* 2013). It is one of the most problematic types of invading freshwater weeds, and its growth is uncontrollable in water bodies such as open ponds and irrigation canals. The growth rate of water hyacinth is very high, and it can grow $>50 \text{ kg/m}^2$ on the water surface and affect the economy (Ganguly *et al.* 2012).

There are several methods used for the removal of pollutants from wastewater, including adsorption, reverse osmosis, leaching, ion exchange, precipitation, oxidation, and solvent extraction (Vedula *et al.* 2013). These methods have several limitations, including poor efficacy and high cost. Hence, an alternative approach has been introduced to remove pollutants from wastewater. The phytoremediation method is highly popular, and this method uses green plants to reduce organic pollutants and inorganic matter from wastewater (Hejna *et al.* 2020). This method has been widely accepted as an inexpensive and eco-friendly method compared with other treatment methods. The aquatic plant-based treatment method is an accepted method for wastewater treatment in emerging countries (Daud *et al.* 2018). *Eichhornia crassipes* grows well in low-oxygen environments, is tolerant to several heavy metals, can potentially absorb heavy metals and nutrients from wastewater, and is considered a popular iconic plant in the field of phytoremediation (Feng *et al.* 2017). Compared with other reported aquatic plants, water hyacinth has potential water purification effects (Mahamadi and Nharingo 2010). In wastewater, *Eichhornia crassipes* has the potential to remove $>99\%$ Cr(VI) within 15 days of culture (Saha *et al.* 2017). The efficacy of lead (Pb) removal from wastewater by *Eichhornia crassipes* was $>99\%$ within 9 days of culture (Suryandari *et al.* 2017). *Pteris ensiformis* accumulated As in the shoots (1091 mg/kg), and *Boehmeria nivea* accumulated a high amount of Cd (490.3 mg/kg) in the shoots (Pan *et al.* 2019). *Eichhornia crassipes* absorbs heavy metals effectively in the water containing a high amount of heavy metals. It absorbed a higher amount of Pb in the root (5.45%), petiole (2.72%), and in and leaf tissues (0.66%) (Malar *et al.* 2016). In summary, water hyacinth is a promising feedstock for removing water contaminants from water bodies.

Technological advancements in water plants for the absorption of contaminants from aquatic environments, the production of animal feed, fertilizer production from slurry or composting, and various ecofriendly beneficial applications support socioeconomic benefits of water hyacinth (Rai 2016). There are several methods to manage and control water hyacinth, including biological, mechanical, and chemical methods. Moreover, water hyacinth also has been collected and used as raw material to produce biofuels (Das *et al.* 2016), vermicompost, and compost.

Composting is the process of conversion of biodegradable organic matter and wastes into stable end products with the aid of beneficial microorganisms, including bacteria, fungi, and actinomycetes. The end products contain “humic-like” substances which

improve soil structure and properties, including, water retention, pH buffering and cation exchange capacity (Yang *et al.* 2021). Vermicomposting is a traditional biological method in which earthworms, such as *Eudrilus eugeniae* and *Eisenia fetida*, are used to prepare organic waste compost. The vermicompost is rich in nutrients, hormones, and enzymes that improve the growth of plants and reduce the bacterial population (Gajalakshmi *et al.* 2002; Pramanik 2010). The maturity of organic compost materials is important because mature compost can be used directly in the soil. The toxicity of compost can be analysed *via* phytotoxicity analysis. Microbial biomass is a key parameter and is an indicator to determine the composting process. Analysis of microbial biomass is useful to determine the compost maturity, and the biomass plays a critical role in the carbon cycle (Khan *et al.* 2014).

The term phytoremediation means that plants are being used to degrade, remove, or immobilize pollutants from wastewater environments (Mustafa and Hayder 2021). These phytoremediators can reduce the toxic effect of several inorganic and organic contaminants by their being converted, reduced, and catabolized by water plants (Sun *et al.* 2015). Water hyacinth is considered a good phytoremediator because of its extensive biomass, broad leaves, and fibrous roots, enabling it to uptake increased concentrations of heavy metals. During metal sorption, water hyacinth releases both cations and protons, and this process has revealed the role of ionic exchange in the heavy metal absorption process (Zheng *et al.* 2009). In water hyacinth, cellulose and lignin are considered as the major cellular components. The presence of amino and carboxyl groups of cellulose allows the adsorption of heavy metals through cation exchange (Elbasiouny *et al.* 2021). The heavily accumulated heavy metals from the water hyacinth can be recycled by extraction *via* physical or chemical methods after harvesting, drying, or burning into ashes (Mahmood *et al.* 2010). Water hyacinth biomass can be converted into compost manure and can replace >50% of the mineral requirement in agriculture (Gezahegn *et al.* 2024). It has been reported that the toxicity of heavy metals in the environment is dependent on bioavailability rather than total metal concentration. The bioavailable fraction of heavy metals may be highly toxic for soil microorganisms and plants. The vermicomposting process of water hyacinth has been shown to reduce the bioavailability and leachability of heavy metals (Singh and Kalamdhad 2013a). Thus, vermicompost could mitigate metal toxicity and improve the nutrient profile in soil for sustainable agriculture. This study aimed to analyse the potential of water hyacinth plants (i) to remove nutrients and heavy metals from wastewater; (ii) to produce ethanol; and (iii) to produce vermicompost with goat manure.

EXPERIMENTAL

Plant Material and Growing Conditions

The aquatic weed *E. crassipes* was obtained from a freshwater pond. Twelve randomly selected plants of equal size and weight were used for phytoremediation experiments in 20 L of water in each tank. The phytoremediation experiment was performed for 30 days, and the efficacy was tested every 5 days. The water samples were collected in BOD bottles, labelled, and stored at 2 to 4 °C. The amounts of ammoniacal nitrogen, nitrite, nitrate, and heavy metals (As, Cd, Cr, Cu, Li, and Zn) were determined. Nutrient and pollutant removal was tested over three different cycles over a 30-day period. Metals were not added to the control container. *E. crassipes* was maintained separately to produce biofuels and vermicompost.

Analysis of Nutrients

The amount of ammonia nitrogen in the water was determined *via* spectrophotometric assay using a Spectronic 200 UV-Vis Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). Absorption spectrometry was used to determine the amount of nitrates in the medium. Briefly, the analytical method is based on the reaction of the diazotization of nitrite ions and 4-amino-benzene sulfonamide at acidic pH values (pH=1.9), which results in the formation of a diazonium salt. This compound was coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride, and the color was measured at 540 nm (Eq.1),

$$\text{Ammonia nitrogen (mg N/L)} = [(A - A_0) \times V_f] / (b \times V_s) \quad (1)$$

where A represents the absorbance(optical density at 540 nm) of the sample; A_0 represents the absorbance(optical density at 540 nm) of the blank; V_f represents the final volume(mL); V_s represents the sample volume(mL); and b represents(Y value) the calibration curve slope.

The amount of nitrates in the water was determined *via* the 2,6-dimethylphenol method. The mixture of sulfuric acid, 2,6-dimethylphenol, and phosphoric acid reacted with nitrate ions and generated 4-nitro-2,6-dimethylphenol. The absorbance of the sample was read at 324 nm, and the result was expressed as mg nitrates/L (Eq. 2),

$$\text{Nitrates (mg N/L)} = (A - A_0) / b \quad (2)$$

where A represents the absorbance(optical density at 324 nm) of the sample, A_0 represents the absorbance(optical density at 324 nm) of the blank, and b represents the calibration curve slope. The average percentages of analyte removed from the wastewater during the experimental period were expressed as phyto remediation yield.

Harvesting

The experimental and control plants subjected to heavy metal stress were harvested from the experimental and control tanks. They were gently harvested, wrapped with wet tissue paper, and aseptically packed in plastic bags.

Analysis of Heavy Metals

Water hyacinth was grown in pond water containing heavy metals (0.5 mg/L As(III), 0.5 mg/L Cd(II), 0.5 mg/L Cr(II), 5 mg/L Cu(II), 2 mg/L Li(II), and 5 mg/L Zn(II)). Every five days, the amount of heavy metals present was determined. The harvested plants were oven dried, and 5 g of dried sample was transferred to crucibles. Then, the mixture was placed in a muffle furnace at 500 °C for 2 h.

The mixture was cooled, and the ash content was dissolved in 10 mL of double distilled water containing 0.01 N nitric acid. The samples were filtered, and the concentrations of the heavy metals (As, Cd, Cr, Cu, Li, and Zn) were analysed *via* an automatic flame photometer (Model 391/392, Dual Channel Flame Photometer, Haryana, India).

The heavy metal content was tested every five days for up to 30 days. The concentrations of the heavy metals selected in this study were based on the permitted threshold levels for the selected heavy metals.

BIOETHANOL PRODUCTION

Water Hyacinth Pretreatment and Enzymatic Hydrolysis

Water hyacinth was pretreated with 0.1 N sulfuric acid and 1% (w/v) sodium hydroxide for 3 h. The mixture was washed with water several times until a neutral pH was attained. The mixture was oven dried at 80 °C until a constant weight was achieved. It was finely powdered and sieved *via* a standard sieve (1.0 to 1.5 mm diameter) for enzymatic hydrolysis. Cellulase (C1184, Sigma Aldrich, St. Louis, MO, USA) and β -glucosidase (Sigma Aldrich, USA) were used for enzymatic hydrolysis. Enzymatic hydrolysis was performed by incubating 50 g of biomass with enzymes (200 units of cellulase and 200 units of β -glucosidase) for 72 h at 32 °C (pH 5.0). The released sugars were determined *via* spectrophotometry.

Fermentation and Ethanol Production

The commercial yeast *Saccharomyces cerevisiae* (MTCC 171) was used to produce ethanol from the solution obtained from the enzyme treatment of biomass. Briefly, the strain was cultured in malt extract yeast extract glucose peptone, which was used as the inoculum. The inoculum was inoculated in water hyacinth (2%) medium in submerged fermentation (SmF). Submerged fermentation was performed for 72 h at 37 °C. After 72 h of treatment, the fermented broth was subjected to distillation between 78 and 79 °C, and ethanol was collected. The amount of ethanol produced was subsequently determined *via* the spectrophotometric method with acidic dichromate solution (Caputi *et al.* 1968).

Vermicomposting of Water Hyacinth and Goat Manure

Water hyacinth was washed with tap water, and the sediment particles were removed from the root system and leaves. The whole water hyacinth was cut into 1.5 to 2.5 cm pieces, and goat manure was collected from the local farm. The important physical and chemical properties of water hyacinth and goat manure are described in Table 1.

Table 1. Physicochemical Properties of the Water Hyacinth and Goat Manure Used for Vermicompost Preparation

Characters	Water Hyacinth	Goat Manure
Ash (%)	87.4 ± 1.1	119.4 ± 2.3
Moisture (%)	87.2 ± 2.4	21.5 ± 2.8
Electrical conductivity (dS/m)	3.2 ± 0.1	4.9 ± 0.5
pH	7.9 ± 0.4	8.8 ± 0.3
Total organic carbon (g/kg)	29.4 ± 0.17	38.2 ± 0.4
Total nitrogen (g/kg)	0.81 ± 0.04	8.3 ± 1.2
Humic acid (g/kg)	18.4 ± 1.5	28.5 ± 1.6

The mixture of water hyacinth and goat manure was placed in a pile, which was covered with a black plastic bag. The mixture was incubated for 20 days, and toxic gases, which are considered toxic to earthworms, were eliminated. In each treatment, beds comprising water hyacinth (5 kg) or goat manure (5 kg) were treated with 250 g of *E. fetida*. The compost was covered, and the moisture content of the compost was maintained at approximately 80% throughout the experiment. Random sampling was performed, and

the survival percentage of the worms was determined. After four months, the earthworms were removed from the beds, and the composite mixture was collected and dried. The compost mixture was ground and stored in an airtight container and used for nutrient analysis.

Physical and Chemical Properties of the Vermicompost

The physical and chemical properties of the collected samples were determined. The compost was mixed with double distilled water and used for the determination of pH. A digital conductivity meter was used for the analysis of electrical conductivity, and the result was expressed as dS/m. The total organic carbon content was determined *via* the method of Nelson and Sommer (1982), and the total Kjeldahl nitrogen content was determined *via* the methods of Bremner and Mulvaney (1982). The moisture content of the compost was determined *via* the method of Alef and Nannipieri (1995), and the organic and inorganic carbon contents were determined (Page 1982; Sánchez-Monedero *et al.* 1996). The amounts of NO_3^- and NH_4^+ were determined *via* the colorimetry method (APHA 1998).

Phytotoxicity Analysis

The germination index of red chili (*Capsicum annuum*) was analysed as described previously by Zucconi *et al.* (1981). Briefly, approximately 60 seeds were sown at a depth of 2 cm in pots containing 2 kg of soil. The pots were maintained for three days. The compost was added at three different percentages to the soil (10, 20, and 30%).

The seed germination index was calculated using the following equation (3),

$$GI = \frac{G_1}{G_2} \times 100 \quad (3)$$

where, GI is the germination index; G_1 is the germination proportion of *C. annuum* seeds cultured in agricultural soil containing 10 to 30% vermicompost; and G_2 is the germination proportion of *C. annuum* seeds cultured in agricultural soil containing any vermicompost.

Determination of Microbial Biomass N and C

The microbial biomass N and C contents of the mature compost were determined as suggested previously by Brookes *et al.* (1985). The amount of microbial biomass C was determined as suggested by Vance *et al.* (1987).

Statistical Analysis

The results were analyzed using analysis of variance (ANOVA), followed by multiple mean comparison using Tukey's HSD test ($p < 0.05$). Different lowercase letters indicate the significant difference among treatments according to Tukey's HSD test.

RESULTS AND DISCUSSION

Phytoremediation of Nutrients

The amounts of ammonium nitrogen, nitrite, and nitrate in the water before and after phytoremediation are depicted in Fig. 1A to 1C. The maximum phytoremediation yield for ammonia nitrogen was identified in the water after 30 days of treatment.

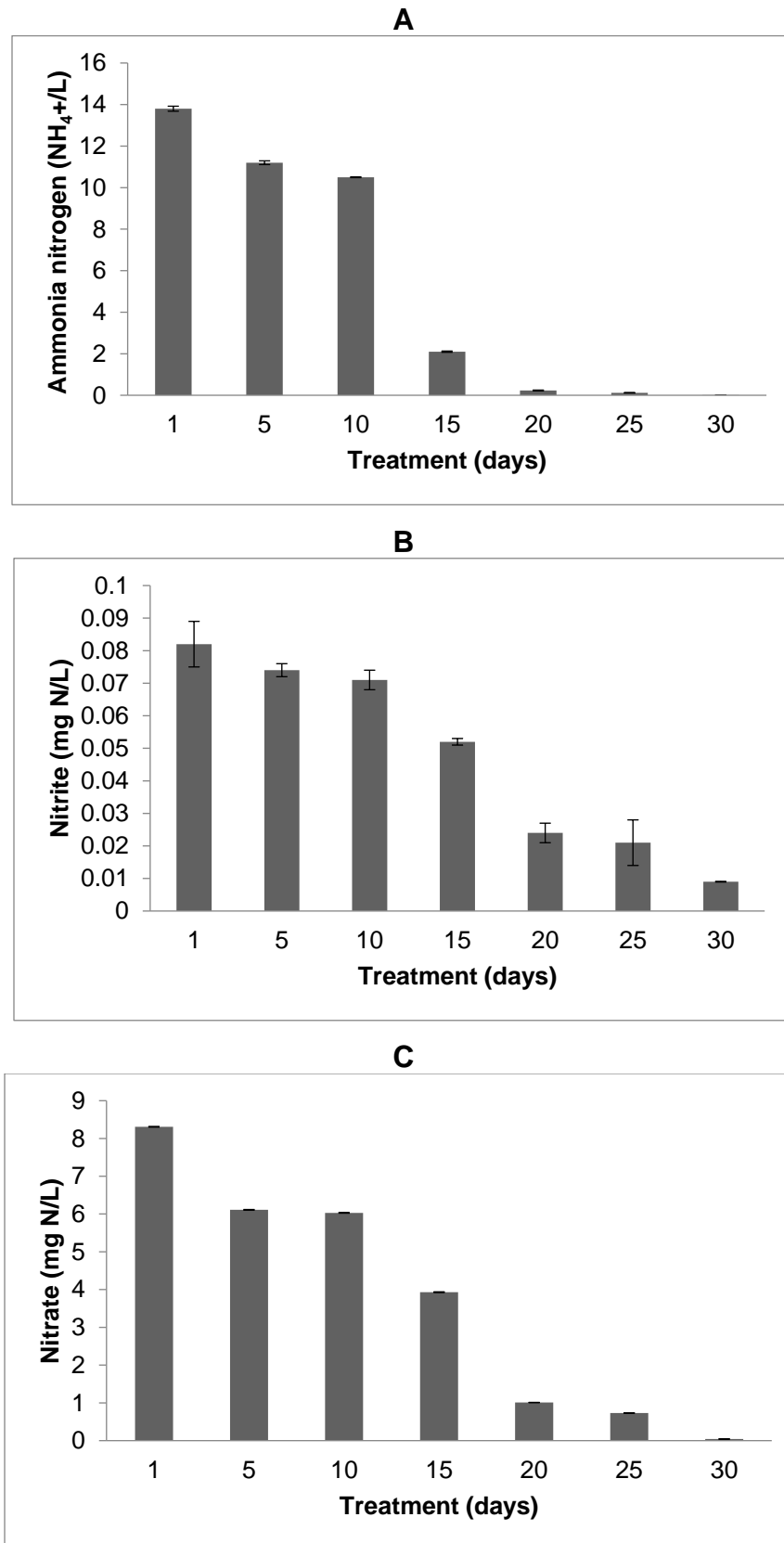
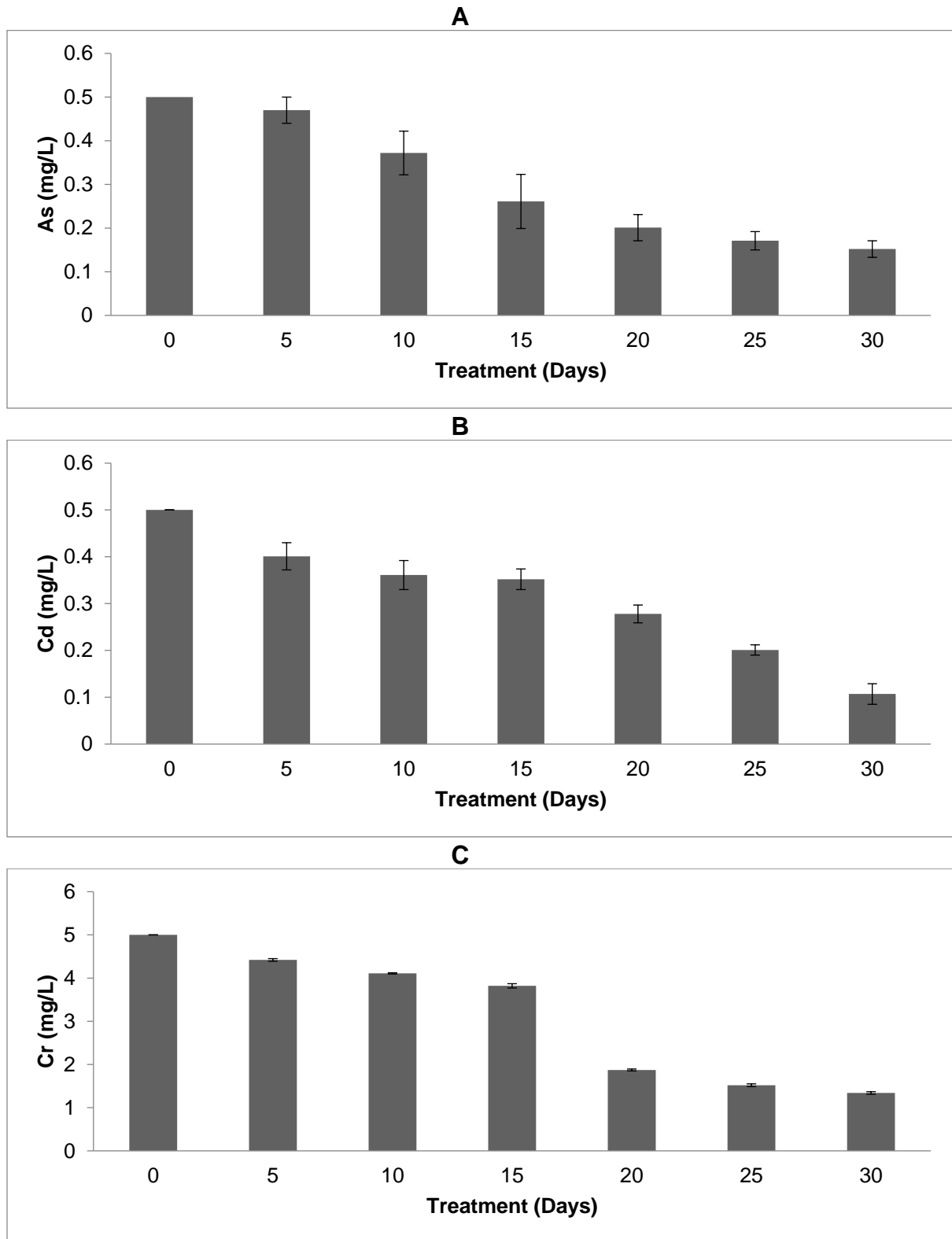


Fig. 1. Determination of ammonia nitrogen (A), nitrite (B), and nitrate (C) in the water after phytoremediation

The initial ammoniacal nitrogen content was 13.8 ± 0.27 mg N/L, which decreased because of water hyacinth growth; >99% ammoniacal nitrogen and 0.08 ± 0.01 mg N/L in the water were utilized after 30 days ($p < 0.05$). Water hyacinth utilizes nitrites and nitrates from wastewater. Initially, the nitrite content was 0.082 ± 0.02 mg N/L, and the plant had a greater capacity to eliminate nitrite from wastewater. After 5 days of treatment, the nitrate content was 0.074 ± 0.01 mg N/L, and the plants eliminated nitrite continuously and reached 0.012 ± 0.03 mg N/L after 30 days ($p < 0.05$). However, the nitrate elimination potential was lower than that of nitrite. Aquatic weeds have been widely utilized for the phytoremediation of wastewater. In wastewater, the available ammonia nitrogen formed from the degradation of organic nitrogen and ammonium ions in water affects water purity (Aziz *et al.* 2020). The *E. crassipes* used in this study showed remarkable accumulation of ammoniacal nitrogen, which is considered to be a nitrogen hyperaccumulator. The bioaccumulation was as high as 50 g/kg (Ting *et al.* 2018). The extent of biodegradation of nutrients varies based on the ecological conditions and concentration of nutrients in wastewater. In a previous study, Fox *et al.* (2008) used a modified Hoagland solution and reported 60 to 85% N removal by hyacinth after 4 weeks. The distribution of ammonium nitrogen in industrial and domestic wastewater has been reported to contribute to eutrophication. Water hyacinth is an important aquatic plant that removes several contaminants and reduces chemical oxygen demand, biological oxygen demand, total solids, total dissolved solids, nutrients, and heavy metals (Rezania *et al.* 2015). The physiological and biochemical mechanism of nutrient removal by water hyacinth was reported previously by Wu *et al.* (2016). Water hyacinth converts nitrogen to ammonia nitrogen, which subsequently increases the levels of glutamic and aspartic acid and decreases sucrose, soluble sugar, and glutamine, which improves the biosynthesis of amino acids and improves plant growth.

Bioaccumulation of Heavy Metals by Water Hyacinth

The water hyacinth utilized in this study accumulated heavy metals from the water, and its efficacy for heavy metal accumulation was determined. The plant material was monitored, and the accumulated heavy metal content was analyzed after 5 to 30 days. The removal efficacy for water hyacinth is shown in Fig. 2. Fifteen days after treatment with water hyacinth, the concentrations of Cu, Cd, Cr, As, Li, and Zn in the water hyacinth were 4.12 ± 0.033 mg, 0.352 ± 0.022 mg, 3.81 ± 0.05 mg, 0.261 ± 0.062 mg, and 3.72 ± 0.022 mg, respectively. The bioaccumulation potentials of water hyacinth for Cu, Cd, Cr, As, Li, and Zn after 30 days of treatment were $80 \pm 2.3\%$, $78.6 \pm 3.2\%$, $73.2 \pm 1.2\%$, $69.6 \pm 2.1\%$, $65.5 \pm 1.9\%$, and $44.2 \pm 2.2\%$, respectively ($p < 0.05$). The heavy metal accumulation in water hyacinth was in the following order: Cu > Cd > Cr > As > Li > Zn. The findings revealed that the heavy metal concentration in the wastewater treated with hyacinth decreased after 30 days of treatment compared with 5 to 10 days. The remaining Cu concentration was only 20%, and the initial Cu concentration was 5 mg/L. In addition, the results presented in Fig. 2 show that with respect to the plant growth conditions of the experimental tanks, the amount of heavy metals (Cu, Cd, Cr, As, Li, and Zn) in the water decreased with increasing treatment time with increasing water hyacinth. Developing and developed countries are dependent mainly on surface water and are polluted daily by several pollutants, including heavy metals (Alarjani *et al.* 2024; Rajaselvam *et al.* 2024). In this study, the selected metal concentration ranged from 0.5 to 5 mg/L, and the selected concentration was based on a previous report (Sajad *et al.* 2019).



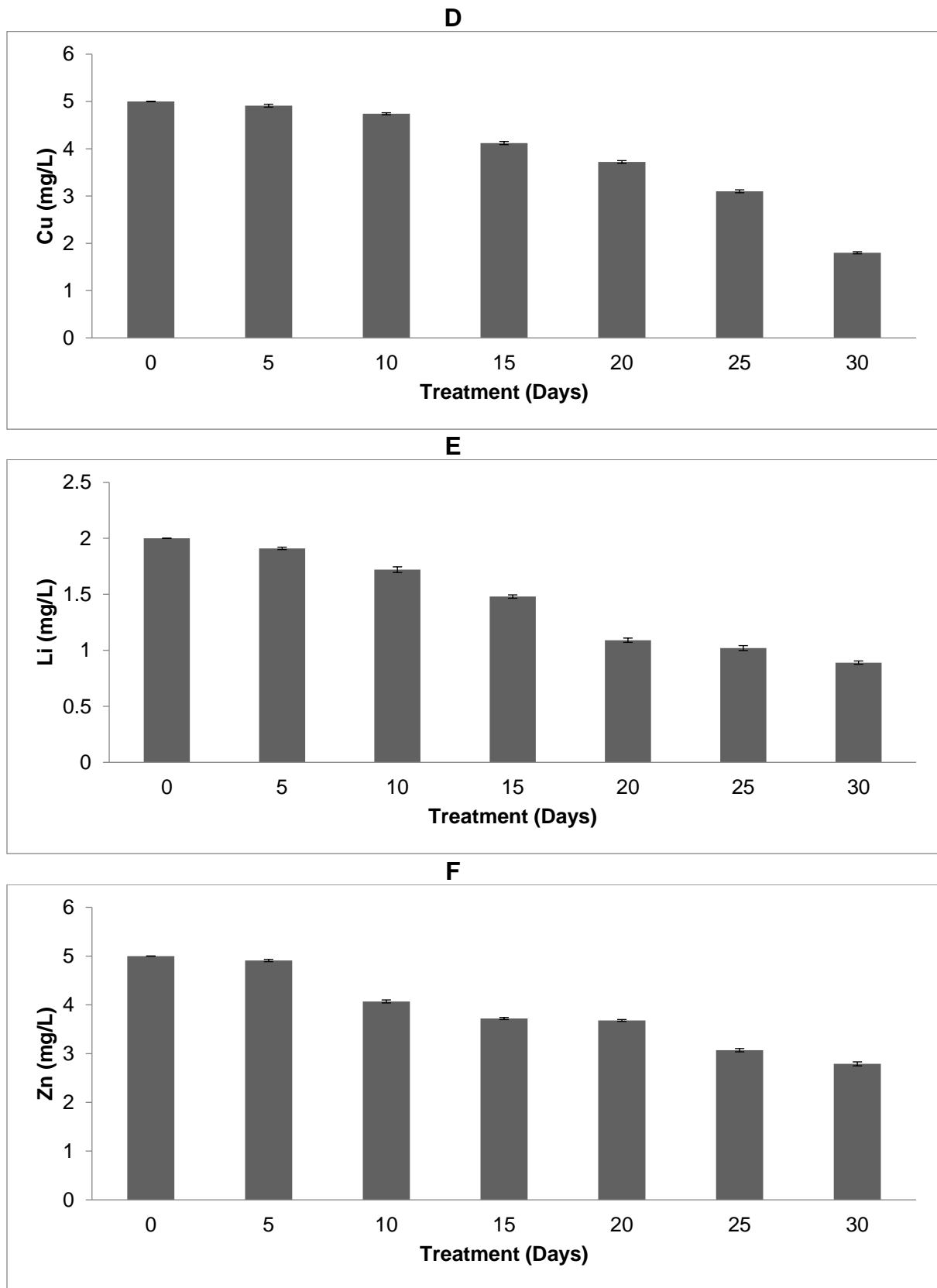


Fig. 2. Bioaccumulation of heavy metals from wastewater by water hyacinth after 30 days of treatment: Accumulation of As (a), Cd (b), Cr (c), Cu (d), Li (e), and Zn (f)

The selected concentrations of the heavy metals used in this study were previously used for *Eichhornia crassipes* (Tabinda *et al.* 2020). The selected Li concentration (2 mg/L) was lower than those of Zn, Cu, and Cr, both of which were 5 mg/L and higher than those of As and Cd (0.5 mg/L), and the concentration selected in this study was appropriate based on a previous report (Shahzad *et al.* 2016). At lower concentrations of these heavy metals, water hyacinth utilizes these metals for growth and has no negative effect at lower concentrations of metals in the water. In the present study, the Li removal efficacy was $65.5 \pm 1.9\%$ after 30 days, and the Li removal efficacy was similar to that in previous reports (Wu *et al.* 2018). The present findings revealed that water hyacinth could remove heavy metals from water. The root system, shoot system, and leaves of *Eichhornia crassipes* are involved in the removal of heavy metals. The root system accounts for significant biosorption of heavy metals, and heavy metals are translocated to the stems and leaves (Hayyat *et al.* 2020).

Enzymatic Hydrolysis and Bioconversion of Ethanol

Commercial cellulolytic enzymes were used for the hydrolysis of water hyacinth after acidic and basic treatment. The hemicellulose and cellulose materials in the pretreated biomass were fermentable sugars. Commercial enzymes have been used to produce sugar monomers from these cellulose materials. The yeast *S. cerevisiae* was used for the bioconversion of ethanol. The ethanol yield was 0.09 mL/mL, and the production was 0.124 mL/L at 2% inoculum. The ethanol yield was similar to that reported in a previous study (Mukhopadhyay and Chatterjee 2010). Agro wastes, including pineapple peel and banana peel were utilized for the production of banana peel to reduce the production cost of ethanol (Kanthavelkumaran *et al.* 2023).

Analysis of Vermicompost

Mature compost was obtained after four months of composting, and the physical and chemical characteristics of the compost are described in Table 2. The composting time was similar to that in previous studies (Bernal *et al.* 2017), and the survival percentage of earthworms was $86.5 \pm 1.1\%$. The present findings revealed that water hyacinth biomass and goat manure promoted the growth of earthworms.

Table 2. Physicochemical Properties of Mature Vermicompost after 120 days of Processing in a Pile

Characters	Results
Electrical conductivity (dS/m)	2.09 ± 0.12
pH	7.83 ± 0.01
Total organic carbon (g/kg)	18.5 ± 0.7
N-NO ₂ -(mg/kg)	7.2 ± 0.01
N-NO ₃ -(mg/kg)	47.1 ± 1.02
N-NH ₄ ⁺ (mg/kg)	1.03 ± 0.4

In the current study, pre-composting was performed to reduce the toxic effect of the compost, which favored the growth of earthworms, similar to previous studies (Mupondiet *et al.* 2011). The pH of the mature compost was 7.83 ± 0.01 , which is suitable for agriculture. The pH of the compost was alkaline, which was mainly due to the presence

of calciferous glands in the water hyacinth, which added extra calcium to the compost. Water hyacinth is considered a natural purifier in freshwater environments and is considered a biofilter of wastewater rich in inorganic and organic nutrients, toxic heavy metals, and several organic compounds (Singh and Kalamdhad 2013b). The compost was stable after 120 days, and the NH_4^+ value obtained in this study reflected the maturity of the compost. These findings reveal that compost can be utilized as fertilizer for agriculture.

Analysis of the Seed Germination Index

The germination index is one of the strategies for analysing compost stability. The vermicompost had a $78.3 \pm 1.1\%$ germination index after 24 h, which increased to $81.2 \pm 2.2\%$ and $84.3 \pm 2.2\%$ after 48 and 72 h, respectively, in the pots treated with 20% vermicompost ($p < 0.05$). The available phytotoxic compounds in the water hyacinth-goat manure vermicompost decreased the germination index (Zirbes *et al.* 2011). Hence, the germination index was lower in the 30% vermicompost-treated soil. The nature of the compost was brownish black and porous. The physical and chemical properties of vermicompost had a potential impact on the germination of tomato seeds. The authors found that tomato seeds treated with vermicompost presented an improved seed germination up to 20% vermicompost. The present findings agreed with previous reports that vermicompost improved the seed germination and productivity of plants (Atiyeh *et al.* 2000). The vermicompost increased the nitrogen level and nutrient accessibility to the plants and thus improved the seed germination (Tripathi *et al.* 2013). Earthworms can increase the nitrogen level in compost by adding their body fluids, gut enzymes, and nitrogenous excretory products, and the present findings are consistent with the previous findings of Suthar (2007) and Suthar and Singh (2008). Vermicompost enhanced the growth of *Capsicum annum* (Linn.) Hepper (Rekha *et al.* 2018) and the germination of maritime pine (*Pinus pinaster* Ait.) (Lazcano *et al.* 2010), seed germination and seedling growth of tomato and lettuce (Arancon *et al.* 2012), and *Linum usitatissimum* L. (Makkar *et al.* 2017).

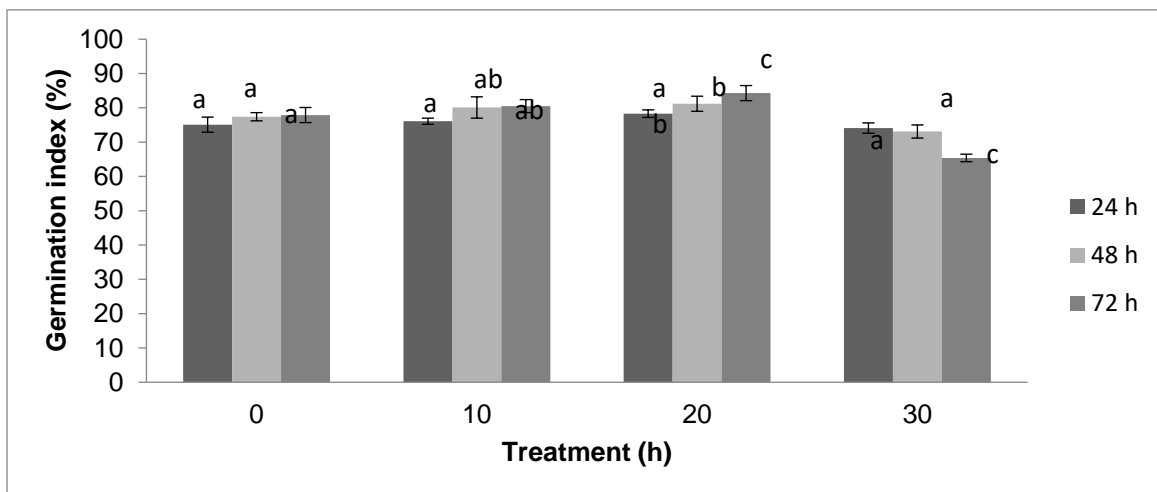


Fig. 3. Seed germination index of tomato plants sown in agricultural soil containing various concentrations of vermicompost (10% to 30%)

Microbial Biomass C and N Levels in the Compost

The microbial C and N contents of the compost were determined in this study, and the microbial biomass C content ranged between 398 ± 12.8 and 537 ± 11.2 $\mu\text{g/g}$. The amount of microbial biomass N ranged from 104.4 ± 2.2 to 254.9 ± 2.2 $\mu\text{g/g}$. The low C/N microbial biomass in the compost indicated the presence of a microbial population in the compost. After 90 days of composting, the increased C/N microbial biomass ratio indicated the dominance of fungal communities. The microbial C and N contents were increased significantly after 90 days of vermicompost ($p < 0.05$). After 30 days of composting, the extent of microbial decomposition increased with increasing microbial enzyme activity. During this thermophilic phase, microbial growth increased because of the high temperature and humidity, which contributed to the compost biomass. At relatively high temperatures, maximum biomass values have been reported (Sarathchandra *et al.* 1984; Singh *et al.* 1989). The N and C values were lower in the cooling phase in the present study, possibly because of the low activity of microbes and the low decomposition extent of organic matter. In one study, microbial C and N dynamics in urban solid waste were reported by Ayed *et al.* (2007). The microbial biomass reached a maximum after five days (4.06 $\mu\text{g/kg}$) and decreased after two months (0.44 $\text{mg } \mu\text{g/kg}$). Wang *et al.* (2022) applied compost to soil, which improved macroaggregative formation, and the supplemented compost improved the soil microbial biomass C (315.2 mg C/kg), which was similar to the results of the present study. In agricultural soil, the application of organic and mineral fertilizers improved microbial biomass.

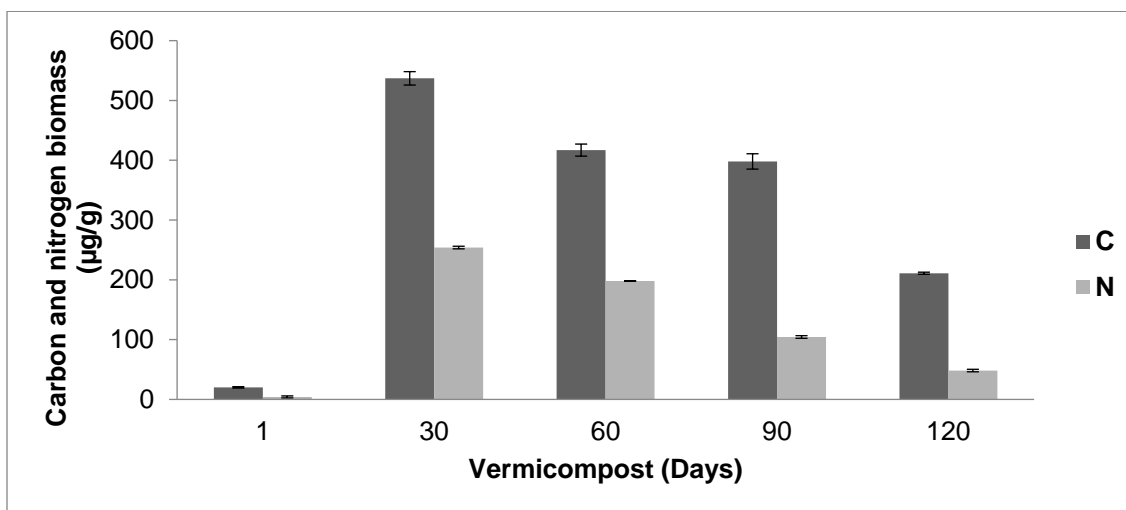


Fig. 4. Microbial biomass C and N levels in the vermicompost for 120 days in a pile; Sampling was performed on the first day and at every 30th day interval, and the microbial C and N contents were determined *via* analytical methods.

CONCLUSIONS

1. Water hyacinth is an aquatic resource for the removal of nutrients and heavy metals. Water hyacinth removed >95% of ammonium nitrogen, nitrite, and nitrate from the water. It accumulated >70% Cu, Cd, and Cr; >60% As and Li; and >40% Zn from the water after 30 days of treatment.

2. Acid- and base-pretreated hyacinth biomass was used as the substrate for ethanol production. The mixture was treated with cellulolytic enzymes, and the converted sugar was utilized by *Saccharomyces cerevisiae* for ethanol production.
3. Carbon-rich water hyacinth and goat manure were used to produce vermicompost. Mature compost was obtained after four months of composting, and the microbial C and N sources were determined for microbial activity. Germination index analysis was found to be suitable for determining the phytotoxicity of vermicompost.

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