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RESEARCH ARTICLE
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The Effect of Biotechnological Treatment using Vascular Aquatic Plants Azolla (*Azolla caroliniana*) and Duckweed (*Lemna minor*) on Changes in Polluted Water Parameters

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ABSTRACT

This article examines the potential of biotechnological purification of polluted water using higher aquatic plants such as Azolla (*Azolla caroliniana* Willd.) and duckweed (*Lemna minor L.*). During the study, the main physicochemical parameters, including pH, dissolved oxygen (DO), total nitrogen, ammonium ions (NH4+), nitrates (NH3+), phosphates (PO4+) and heavy metal ions (e.g., Pb2+, Cd2+), were determined in water samples polluted by industrial waste, agricultural wastewater, and other anthropogenic sources. The dynamics of their changes under the influence of *A. caroliniana* Willd*.,* and *L. minor* L. were analyzed. Due to their rapid growth rate, biosorption capacity, and environmental adaptability, plants significantly reduce water pollutants within a short period. The results indicate that these higher aquatic plants can serve as effective agents in biological remediation processes. Specifically, when *A. caroliniana* Willd. When *L. minor* L. and *Limnaea stagnalis* were combined, their purification efficiency increased further. This study proposes relevant solutions using biotechnological approaches to ensure environmental safety, restore the natural state of water bodies, and achieve sustainable development. The results of the article provide a scientific and practical basis for the rehabilitation of natural water bodies, the preparation of water resources for secondary use, and the improvement of environmental monitoring systems.

Keywords: Azolla, Duckweed, Biotechnological remediation, Phytoremediation.

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INTRODUCTION

Lemna minor L. has been widely recognized for its high phytoremediation potential, particularly its ability to absorb nitrogen, phosphorus, and heavy metals, thereby, significantly improving water quality (Zhou et al., 2023). However, the effects of *Azolla caroliniana* Willd. on key polluted water parameters, including physicochemical and heavy metal dynamics, remain insufficiently investigated. Previous studies have demonstrated that *Azolla* and water hyacinth can achieve up to 90% phosphorus removal from wastewater. Nevertheless, the influence of biotechnological treatment on other critical water quality parameters, as well as the specific role of *L. minor* L. within such treatment systems, has not been comprehensively addressed (Rezania et al., 2021). Several investigations into phytoremediation using higher aquatic plants have primarily focused on species such as *Lythraceae, Nymphaea, Myriophyllum, Hydrilla verticillata*, and *Vallisneria,* while the biotechnological treatment efficiency

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of *A. caroliniana* Willd. and *L. minor* L. has received limited attention (Jia et al., 2023). In addition, recent wastewater treatment research has increasingly emphasized microalgal–bacterial consortia, while the potential of higher aquatic plants, particularly *A. caroliniana* Willd., has been largely overlooked. and *L. minor* L., to improve polluted water parameters comparatively underexplored (Vargas et al., 2024). Although *A. caroliniana* has been shown to effectively remove ammonia from aquaculture wastewater, studies evaluating the combined or comparative effects of *L. minor* L. and other water quality parameters within a biotechnological treatment framework remain scarce (Carlozzi & Padovani, 2016).

Recent studies on wastewater treatment have emphasized artificial macrophytes and biofilm-based systems due to their high purification efficiency; however, the role of higher aquatic plants, such as *A. caroliniana* Willd. and *L. minor* L. in influencing water quality parameters have not been sufficiently examined, particularly with respect to ecological and biotechnological sustainability issues, including the spread of antibiotic resistance genes (Ge et al., 2023). Although the phytoremediation potential of various aquatic plants for heavy metal removal has been widely reported, the biotechnological application of *A. caroliniana* Willd. and *L. minor* L. and their effects on comprehensive changes in polluted water parameters remain inadequately explored (Ali et al., 2020).

Several investigations have demonstrated that *L. minor* L. can significantly reduce total ammonia nitrogen and total phosphorus concentrations in aquaculture systems. Nevertheless, comparative or integrated assessments involving *A. caroliniana* Willd. or alternative plant-based biotechnological treatments and their broader impacts on water-quality parameters remain limited (Sarkheil & Safari, 2020). The efficiency of aquatic plants, including *L. minor* L., in removing heavy metals and improving water quality indicators such as total dissolved solids, biological oxygen demand, and chemical oxygen demand has been documented; however, the contribution of *A. caroliniana* Willd. to these processes has received comparatively little attention (Pang et al., 2023). Studies involving *A. filiculoides* have demonstrated significant reductions in NH_4–N, NO_3–N, PO_4–P, and selenium concentrations in synthetic wastewater, resulting in improved water quality and reduced toxicity to aquatic organisms. However, these findings cannot be directly extrapolated to *A. caroliniana* Willd., and the phytoremediation performance of duckweed under comparable conditions has not been explicitly evaluated (Miranda et al., 2016). Investigations of algoflora diversity in the water reservoirs of the Western Zarafshan Ridge identified more than 120 algal species, with community structure strongly influenced by environmental factors such as water mineralization and temperature. These results underscore the importance of physicochemical conditions in shaping aquatic biota, but do not address the role of higher aquatic plants in regulating water quality parameters (Dustov et al., 2024).

Azolla species have been broadly recognized as effective bioremediators in polluted aquatic environments, with reported potential to modify nutrient concentrations and contaminant levels. Nevertheless, species-specific effects of *A. caroliniana* Willd. and *L. minor* L. on key water quality parameters remain insufficiently characterized (Akhtar et al., 2021). Recent assessments of PFAS uptake by *A. filiculoides* revealed no detectable phytotoxic effects and limited bioconcentration, suggesting that *Azolla* spp. alone may have a restricted capacity to reduce PFAS contamination in aquatic systems. These findings further highlight the need for species-specific and multi-parameter evaluations involving alternative macrophytes, including *A. caroliniana* Willd. and duckweed (Lintern et al., 2024). Although aquatic plants such as Azolla and duckweed are widely applied in wastewater purification due to their ability to accumulate pollutants and enhance treatment efficiency, the effectiveness of these processes is strongly influenced by environmental factors, including temperature, pH, and microbial interactions. Despite this, comparative analyses of *A. caroliniana* Willd. and *L. minor* L. under controlled biotechnological treatment conditions remain limited (Qu et al., 2023). Recent aquaculture studies have explored the use of Azolla as a substitute for fish meal, demonstrating positive effects on the growth performance of red tilapia fingerlings under varying salinity conditions. However, such investigations do not evaluate the role of Azolla–duckweed–based biotechnological treatments in modifying polluted water parameters (Sallam et al., 2024). The bioremediation potential of Azolla has been widely reported, particularly its capacity to remove trace metals and organic pollutants from wastewater. Nevertheless, the contribution of *L. minor* L. to the regulation of water quality parameters in comparable treatment systems remains insufficiently examined (Kollah et al., 2016). Comprehensive reviews addressing water pollutants, their ecological impacts, and conventional treatment approaches have emphasized the urgency of sustainable remediation strategies.

Despite this, species-specific assessments of *A. caroliniana* Willd. and *L. minor* L. within biotechnological treatment frameworks are still lacking (Kumar et al., 2020). Field-based phytoremediation studies have demonstrated the effectiveness of macrophytes such as *Phragmites australis* and *Sparganium erectum* in improving water quality in river systems. However, the applicability of *A. caroliniana* Willd. and *L. minor* L. for regulating polluted water parameters under similar conditions has not been systematically evaluated (Karam et al., 2022). Duckweed species such as *Landoltia punctata* have been shown to suppress cyanobacterial blooms, achieving notable reductions in microcystin concentrations. In contrast, the potential roles of *A. caroliniana* Willd. and *L. minor* L. in modifying broader polluted water parameters remain poorly documented (Li et al., 2020). The utilization of dead biomass from aquatic plants, including Azolla and duckweed, has been proposed as a cost-effective approach for heavy metal adsorption in wastewater treatment. While environmentally advantageous, this approach does not address the dynamic effects of living plants on physicochemical water parameters within biotechnological remediation systems (Singh & Kumar, 2023). Constructed wetland studies have primarily focused on macrophytes such as water hyacinth, demonstrating their effectiveness in wastewater treatment applications. Nonetheless, the performance of *A. caroliniana* Willd. and *L. minor* L. remain underrepresented in comparable biotechnological contexts (Thakur et al., 2023). Although a wide range of phytoremediation techniques and plant species have been reported for contaminant removal, integrated evaluations of *A. caroliniana* Willd. and *L. minor* L. targeting comprehensive parameters of polluted water are still limited (Jeevanantham et al., 2019). Recent investigations into aquaculture wastewater treatment have primarily emphasized the pollutant-removal capacity of stable bioreactor systems and the associated microbial community dynamics. While effective, these approaches provide limited insight into the role of higher aquatic plants, such as Azolla and duckweed, in regulating water quality parameters through biotechnological treatment strategies (Su et al., 2025). Duckweed species (*Lemnaceae*) have demonstrated moderate nutrient removal efficiency in aquaculture wastewater, with reported reductions of approximately 31% for nitrogen and 29% for phosphorus. However, the phytoremediation potential of *A. caroliniana* Willd. and its comparative influence on key polluted water parameters remains insufficiently explored (Paolacci et al., 2022).

A growing body of research has focused on the application of aquatic plants as feed additives in aquaculture systems. The inclusion of ecological plant-based supplements has been shown to enhance the growth performance of herbivorous fish species by 12–18% and to stabilize pond water quality (Skokov et al., 2023). Similarly, the use of fermented *Lemna* and *Spirodela* biomass increased the growth rate of Piaractus brachypomus by 17.3±1.2% and reduced the feed conversion ratio (FCR) from 1.89 to 1.54 (Velásquez, 2014). Comparable results have been reported for freshwater species such as *Osteochilus vittatus* and *Barbonymus gonionotus*, where diets incorporating *Lemna* in fresh, dried, or fermented forms enhanced growth by 15.6±1.4% and reduced feed costs by 18–22% (Iskandar et al., 2020). Under intensive cultivation conditions, *L. minor* and *Eichhornia crassipes* achieved biomass yields of 2.8–3.5kg/m^2, while fermentation of these plants further improved fish growth by 19.2±1.1% and reduced FCR from 1.71 to 1.43 (Pratiwy et al., 2024). In recent years, fast-growing aquatic macrophytes such as *Azolla* and *Ipomoea aquatica* have attracted increasing attention as sustainable feed resources due to their high protein content, balanced amino acid composition, and low levels of anti-nutritional factors. Diets based on these plants have been shown to significantly improve fish growth performance, achieving average daily weight gains of 0.85g/day and FCR values as low as 1.3, indicating their competitiveness with conventional feeds (Omweno et al., 2024).

Despite these promising results, the quantity and quality of aquatic plant biomass are strongly influenced by environmental conditions, particularly light availability. Studies on *Chelidonium majus* L. cultivated under varying light regimes demonstrated that optimized lighting increased biomass production by 20–25%, underscoring the importance of agrotechnical optimization when cultivating aquatic and semi-aquatic plants for biotechnological applications (Hamrayeva et al., 2025). The development of a stable local supply of raw materials for pharmaceutical production and the cultivation of medicinal plants remain among the most pressing challenges in many regions (Bobokandov et al., 2024). Under the medium-saline soil conditions of the Bukhara region, the cultivation methodology, phenological development, and maturation dynamics of the *Cynara scolymus* L. cultivars *Imperial Star* and *Violetto* were investigated, thereby identifying key adaptability traits in Uzbekistan's slightly salinized soils (Isomov et al., 2024). In addition, the anatomical structure of the vegetative organs of the artichoke cultivar Green Gold, cultivated in medium-salinity soils of the Bukhara region, was examined for the first time, providing new insights into its structural adaptation mechanisms (Isomov et al., 2025). Global climate change, particularly rising temperatures and prolonged droughts, has accelerated habitat degradation across Central Asia, resulting in an increase in endangered plant species and a severe decline in vegetation cover. Intensive anthropogenic pressure combined with climate-driven water scarcity has further exacerbated ecosystem instability in the region (Akhmedov et al., 2025). In parallel, the use of plant-based resources in animal nutrition and aquaculture is constrained by the presence of anti-nutritional compounds. Fermentation and thermal–mechanical processing have been identified as effective strategies to mitigate these limitations. Fermentation has been shown to reduce tannin and phytate contents by 25–30%, thereby improving nutrient digestibility (Velásquez, 2014). Moreover, processing techniques such as autoclaving, pH adjustment, boiling, and microwave treatment have been reported to significantly alter the protein, ash, and fiber composition of *Azolla pinnata* biomass, enhancing its nutritional value (Kaur et al., 2024). Overall, the available literature suggests that aquatic plants, particularly *Azolla* species, represent a highly efficient and environmentally sustainable feed resource when combined with appropriate fermentation and processing technologies. However, most studies have not sufficiently addressed the influence of site-specific ecological conditions, local climatic factors, or long-term biomass productivity. Consequently, further research under integrated and region-specific conditions remains essential. Evidence from animal nutrition studies also highlights the importance of processing strategies: while duckweed inclusion reduced protein digestibility in dogs, supplementation with phytase enzymes effectively mitigated this limitation, demonstrating that fermentation or enzymatic treatments can significantly enhance nutrient bioavailability (Brown et al., 2013). Aquatic macrophytes and microalgae are rich sources of protein and bioactive compounds. Protein contents ranging from 45–65%, polyphenol concentrations of 3.5–6.8mg/g, and high carotenoid levels have been reported in *Chlorella, Arthrospira*, and *L. minor* (Song et al., 2025). Cultivation of L. minor in human urine produced biomass containing 34.1±1.2% protein and 23.7±0.9% starch, confirming its strong potential as a sustainable bioprotein source (Iatrou et al., 2015). In addition, processing techniques such as autoclaving, pH adjustment, boiling, and microwave treatment have been shown to significantly modify the protein, ash, and fiber composition of *Azolla pinnata* biomass, thereby improving its nutritional profile (Kaur et al., 2024). Beyond their nutritional value, aquatic plants play a critical role in phytoremediation by efficiently absorbing nutrients and contaminants from polluted water.

Nitrogen and phosphorus removal efficiencies of 50–75% have been reported for various aquatic macrophytes (Yungjie et al., 2025). Comparative studies have shown that L. minor reduced biological oxygen demand by 12.4% more than *Azolla* under similar conditions (Amare et al., 2018). Moreover, *L. minor* demonstrated substantial heavy metal removal capacity, eliminating 63.5±3.2% of Cd^{2+} and 71.8±2.7% of Pb^{2+} from contaminated water (Neagu et al., 2014). Aquatic macrophytes also exhibit species-specific biotransformation capacities for organophosphorus pesticides, with *Myriophyllum aquaticum* showing particularly high degradation efficiency (Gao et al., 2000). In intensive aquaculture systems, natural feeds typically contribute less than 10% of total feed inputs, whereas commercial feeds account for more than 90% and represent approximately 60–70% of total production costs (Li et al., 2022). Consequently, incorporating locally available, low-cost aquatic plants as feed alternatives offers clear economic advantages. Feed cost reductions of 11–13% have been reported when aquatic plants are included in formulated diets (Iskandar et al., 2020), and integrated aquatic plant cultivation systems have the potential to reduce reliance on commercial feeds by 20–25% (Pratiwy et al., 2024). At the ecosystem scale, the phytoremediation performance of mixed aquatic plant assemblages has been evaluated in pilot-scale constructed wetlands, alongside analyses of their rhizosphere microbial communities. These studies demonstrated that multi-species aquatic plantings can effectively remove pollutants while sustaining diverse and metabolically active microbial communities, highlighting both their environmental remediation potential and the importance of monitoring pollutant bioaccumulation risks (Zhang et al., 2024). Previous research demonstrates that the uptake of gold nanoparticles by plants depends on particle size and levels of dissolved organic carbon, indicating that contaminant properties and water chemistry critically influence pollutant uptake by aquatic vegetation (Glenn & Klaine, 2013). Investigations into the use of *Azolla* in combination with polymer carriers for the removal of pesticides and pharmaceuticals revealed degrees of phytotoxicity under certain conditions, suggesting that treatment efficiency may vary with pollutant type and plant interactions (Forini et al., 2020). Integrated systems combining aquaculture, wastewater treatment, and bioenergy production have gained attention, highlighting the multifunctional potential of aquatic macrophytes not only for remediation but also for resource recovery. In particular, duckweed (*L. minor* L.) has shown promise as a bioenergy feedstock in biogas and ethanol production due to its rapid growth and high biomass yield, supporting its role within a sustainable circular bioeconomy (e.g., biogas/ethanol potential). Comparable research identifies *Azolla pinnata* as a viable feedstock for biodiesel production, reinforcing the multifunctional utility of floating plants in environmental biotechnology (Prabakaran et al., 2021). Experimental evidence further suggests that *Azolla* species can improve general water quality parameters by removing heavy metals and a range of inorganic and organic pollutants, demonstrating their applicability in phytoremediation efforts beyond single-contaminant systems. Moreover, floating aquatic plants have been observed to effectively remove arsenic from contaminated water, confirming their ecological safety and cost-effectiveness as a remediation approach (Rahman & Hasegawa, 2011). Although interactions between pollutants and aquatic plants can induce oxidative stress and physiological changes in aquatic organisms—highlighting potential ecological risks—these findings also underscore the need to better understand plant responses under mixed pollution scenarios. Floating aquatic plants have been recognized for their ability to significantly improve water quality by removing organic and inorganic pollutants in various environmental contexts. For instance, the combined use of *L. minor* and *A. caroliniana* has demonstrated an effective reduction in pollutants and improved physicochemical water parameters under field conditions, supporting their application in sustainable wastewater treatment systems. However, analysis of available sources indicates that while both *A. caroliniana* Willd. and *L. minor* L. have been studied individually for water treatment potential, there is limited data on their comparative performance and effectiveness under the specific environmental conditions of the central regions of Uzbekistan, despite observed rapid improvements in polluted water parameters during preliminary trials. The objectives of the present study are as follows: To assess and compare the growth performance and biomass accumulation of *A. caroliniana* Willd. and *L. minor* L. in polluted water environments. To determine the efficiency of pollutant removal (e.g., chemical oxygen demand, nutrients, and general water quality parameters) by each species over the experimental period. To evaluate species-specific differences in phytoremediation potential under environmental conditions typical of the central regions of Uzbekistan, thereby addressing gaps in regional empirical data.

MATERIALS & METHODS

In recent years, due to the rapid development of industry, increasing population density, and expanding agricultural activities, pollution of natural water resources has become a global environmental problem. In particular, nitrogen and phosphorus compounds, heavy metal ions, and organic substances found in wastewater not only disrupt the biological balance of water bodies but also pose a serious threat to human health and ecosystem stability. Traditional cleaning methods, namely physical-chemical methods, often require expensive, complex technologies and produce environmentally harmful by-products. In this regard, there is a growing need for sustainable, affordable, environmentally friendly, and effective cleaning methods. Phytoremediation based on biotechnological approaches - that is, the cleaning of water and soil environments using plants - is recognized as a promising direction in this field. The effectiveness of phytoremediation, especially when carried out by higher aquatic plants, depends on their ability to biosorb and bioaccumulate pollutants. Higher aquatic plants such as *A. carliniana* and *L. minor* are characterized by their high growth rate, biological activity, and environmental adaptability. They accumulate nutrients and heavy metals in their bodies from polluted aquatic environments, thereby improving water quality and ecological status. In this study, the effects of these plants on the leading indicators of polluted waters from various sources were examined.

Azolla is a small, free-floating aquatic fern belonging to the family Salviniaceae, division Pteridophyta, and kingdom Plantae. It is characterized by its delicate, overlapping leaves forming a dense mat on the water surface (Fig. 1). The plant's leaves are bilobed, with the upper lobe containing chlorophyll and the lower one submerged in water. Its roots are delicate and fibrous, hanging freely in the water. Azolla maintains a symbiotic relationship with the cyanobacterium *Anabaena azollae*, which fixes atmospheric nitrogen, making the plant an excellent natural biofertilizer. Ecological requirements: Azolla thrives in neutral to slightly acidic water (pH 5–7), with an optimal temperature for growth of 18–28°C. It prefers shallow, still or slow-moving water bodies such as ponds, rice paddies, and irrigation canals. Under optimal conditions, Azolla doubles its biomass every 3–5 days. Direct strong sunlight may inhibit its growth; therefore, 25–50% partial shading is recommended. The plant grows best when supplied with organic manure (especially cow dung extract) and phosphorus-based fertilizers such as superphosphate. Geographical distribution: The genus Azolla includes seven known species, widely distributed across tropical and subtropical regions worldwide. *Azolla pinnata* - Asia, Africa, Australia (most common in rice fields). *A. filiculoides* - Europe, North and South America. *A. caroliniana* - North America. *A. mexicana* - Mexico and southern USA. *A. microphylla* - Central and South America, tropical Asia. *A. nilotica* - Africa (Nile basin). *A. rubra* - Australia and New Zealand.

Lemna belongs to the family Lemnaceae and includes small, free-floating aquatic plants. The plant body is simple, consisting of a leaf-like frond and a single thin hanging root. Flowers are tiny, unisexual, and reproduction mainly occurs vegetatively. The fruit is a small, one-seeded nutlet that usually sinks in water. Species of *Lemna* are cosmopolitan and found almost worldwide except in polar regions. They are widespread in Europe, Asia, Africa, North and South America. The most common species, *L. minor* and *Lemna gibba*, grow in stagnant or slow-moving waters such as ponds, canals, and irrigation ditches. In Uzbekistan, *Lemna* species occur naturally in river valleys, reservoirs, and artificial water bodies. *Lemna* prefers calm, nutrient-rich freshwater environments. The optimal temperature range is 18–30°C, with a pH between 5.5 and 7.5. The plant often forms dense mats on the water surface, reducing light penetration and limiting algal growth. Additionally, *Lemna* absorbs excess nitrogen and phosphorus, playing an important role in water purification through phytoremediation.

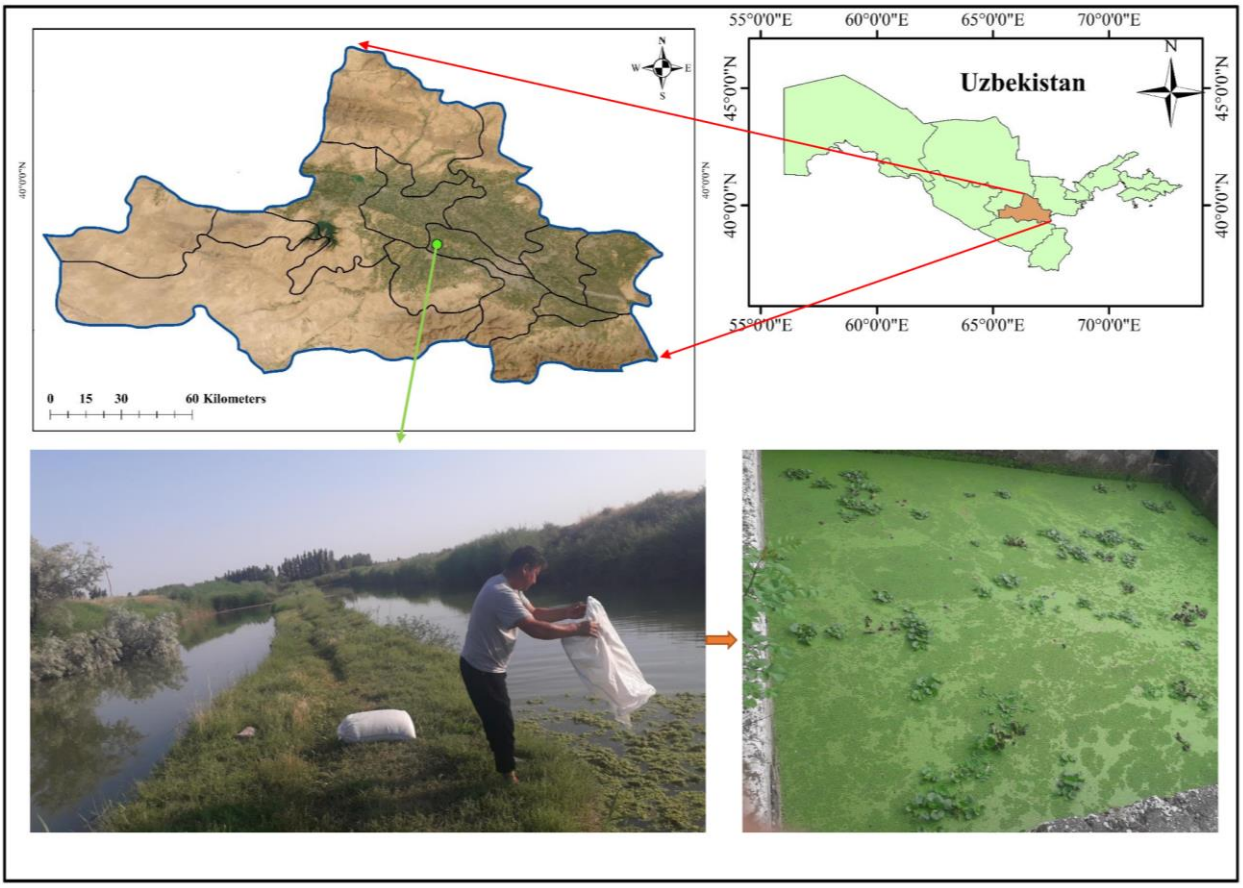

Fig. 1: Geographic location of the study area. Place where the experiments were conducted. Experimental biohydrocomplex for intensive cultivation of *Azolla* and *Limna* plant species (Photo by V. Rakhmonov).

The results of the study demonstrate the importance of biological treatment using *A. carliniana* and *L. minor* in ensuring ecological safety and substantiate the possibilities of their practical application as biofilters. This creates a scientific basis for the development of advanced technologies aimed at conserving, restoring water resources, and ensuring environmental protection. The main goal of the research is to study the process of purifying polluted water using higher aquatic plants such as *A. carliniana* and *L. minor*, and to scientifically evaluate the effect of these plants on the physical and chemical parameters of water. To select samples of polluted water, determine their initial physical and chemical composition (pH, DO, NH_4^+, NO_3^-, PO_4^{3-}, heavy metals). To organize the purification process by growing *A. carliniana* and *L. minor* plants in experimental conditions and placing them in polluted water. To monitor the changes in water composition under the influence of the plants over a specific period of time. To compare the purification efficiency of *A. carliniana* and *L. minor* plants separately and in combination. Based on the results obtained, phytoremediation technologies are recommended as an environmentally safe and cost-effective alternative. Contaminated water samples from industrial zones and agricultural areas of the Samarkand region were selected as research material. For phytoremediation, *A. carliniana* and *L. minor* plants were grown under special conditions and prepared for the experiment. The experimental study was conducted using the following methods: Laboratory experiments: Plants were placed in contaminated water in 5-liter transparent containers.

Each experimental variant was replicated 3 times:
Variant 1: Azolla only
Variant 2: Dckweed only
Variant 3: Azolla and duckweed (combined).

Monitoring and Measurements

During the experiment, the following parameters were measured at 7-day intervals: pH, dissolved oxygen content (DO), ammonium ions (NH4), nitrates (NO3-), phosphates (PO43-), and heavy metals (Pb^{2+} and Cd^{2+}) – by atomic absorption spectrophotometry.

Biomass growth rate: The daily increase in plant biomass was monitored in each option. The results were statistically analyzed using Microsoft Excel and SPSS software. Differences between groups were evaluated using analysis of variance (ANOVA), and statistical significance was determined at a confidence level of $P<0.05$. The methods used in the study were adapted to international environmental monitoring standards. Experiments conducted during the study, involving azolla and duckweed plants, showed significant positive changes in the physical and chemical parameters of polluted water. The results obtained based on 10 days of observation for all three experimental options are presented below.

RESULTS AND DISCUSSION

The pH level was measured electrometrically using a digital pH meter (HANNA, Mettler Toledo). The instrument was calibrated with standard buffer solutions (pH 4.0, 7.0, 10.0) before each measurement, and the results were returned with an accuracy of ±0.05. In polluted water with an initial pH of 7.8-8.1, the pH decreased to 7.0-7.4 with the presence of azolla and duckweed. This indicates neutralization of the water, especially in the combined variant, this result was more stable.

This table clearly shows that azolla and duckweed are effective when used separately, but they yield the greatest results when used together.

The photosynthetic and metabolic activities of plants reduced the alkalinity of the water, bringing the pH closer to neutral (Table 1). Dissolved oxygen (DO) was determined electrochemically (membrane or optical sensor) using a digital DO meter (YSI Pro20 or Hach HQ40d). The sensor was placed directly into the sample and measured in real time. If the results were inmg/L, then over a 10-day period this indicator would be:

Table 1: Water pH level (by days)

Day	Azolla (pH)	Duckweed (pH)	Azolla + Duckweed (pH)
1	$7.90±0.32^a$	$7.85±0.30^a$	$8.00±0.31^a$
3	$7.55±0.29^b$	$7.50±0.26^b$	$7.45±0.30^b$
5	$7.25±0.27^c$	$7.20±0.27^c$	$7.15±0.28^c$
7	$7.10±0.26^d$	$7.00±0.23^d$	$6.95±0.22^d$

Water pH was measured electrometrically using a digital pH meter (HANNA, Mettler Toledo) calibrated with standard buffer solutions (pH 4.0, 7.0, 10.0). Values (n = 3) are presented as mean±SD. Different superscript letters within a row indicate significant differences at P<0.05 (one-way ANOVA followed by Tukey's post hoc test). The initial pH of polluted water (7.8–8.1) decreased to 7.0–7.4 in the presence of Azolla and Duckweed, with the combined treatment showing a more stable neutralization effect.

1. With Azolla: 4.6±0.1mg/L
2. With Duckweed: 4.2±0.09mg/L
3. With Azolla + duckweed: increased to 5.1±0.1mg/L.

This indicates that the amount of oxygen in the water, which is essential for life, has increased as a result of the plants releasing oxygen during photosynthesis.

Plants produce oxygen through photosynthesis, which increases the amount of dissolved oxygen in the water (Fig. 2).

Ammonium ions (NH_4^+) were determined by the colorimetric (phenol-hypochlorite) method. The analysis was carried out using a spectrophotometer, and the color intensity formed after reacting with the reagents was compared with standard solutions and evaluated.

Plants use nitrogenous substances as nutrients, which has led to their decline (Fig. 3). Nitrates (NO_3^-) were determined by UV spectrophotometry (220/275nm wavelength) or calorimetrically. The nitrate concentration in the water was calculated inmg/L by UV irrigation.

Changes in nitrate (NO3⁻) concentrations (mg/L) in water over 7 days for different treatments: Azolla, Duckweed, and their combination (Azolla + Duckweed). Data represent the mean values (n = 10) with significant differences indicated at P<0.05. The co-cultivation of Azolla and Duckweed resulted in the most pronounced nitrate reduction compared to single-species treatments, highlighting the enhanced nutrient-removal efficiency of the combined treatment (Fig. 4). Phosphates (PO_4^{3-}) were determined using the molybdenum blue colorimetric method. After the sample was reacted with the appropriate reagents, the optical density of the resulting blue color was measured using a photometer.

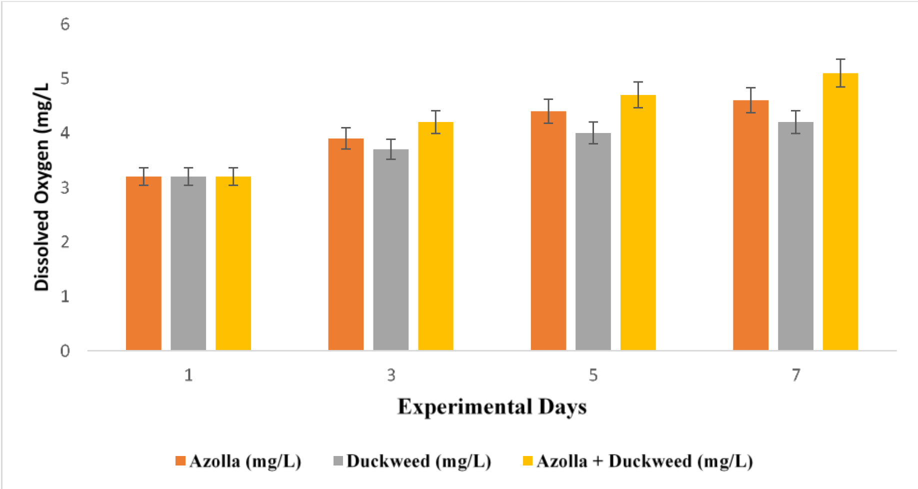

Fig. 2: Dissolved oxygen (mg/L). (n=10, P<0.05).

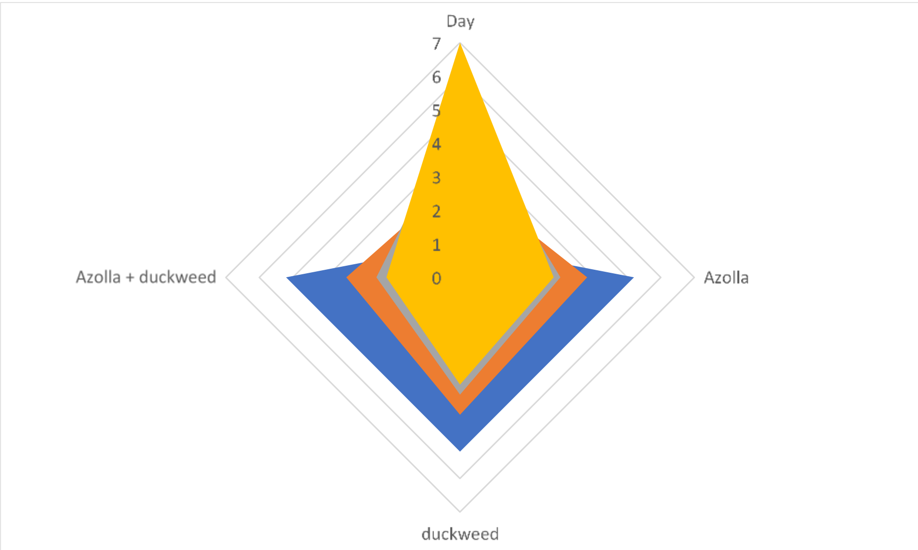

Fig. 3: Ammonium ion (NH_4^+) change (mg/L).

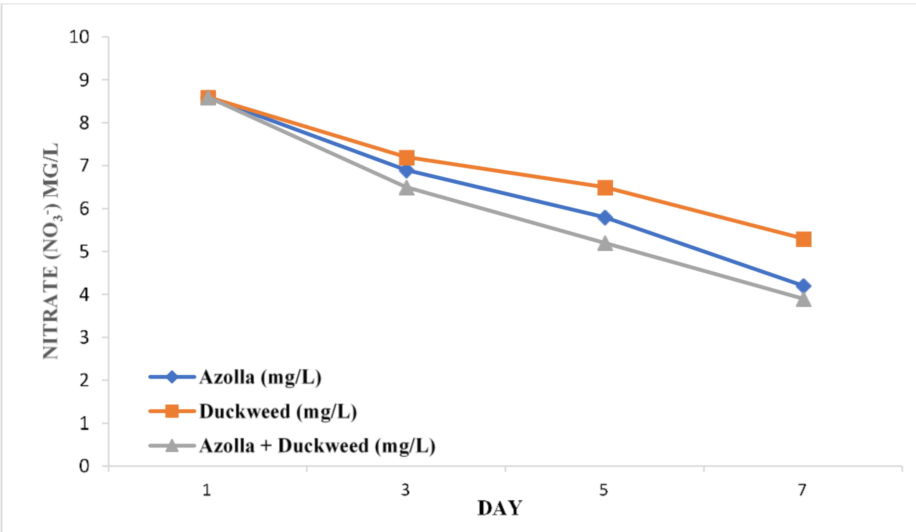

Fig. 4: Nitrate (NO_3^-) level change (mg/L). (n=10, P<0.05).

Phosphates are essential for plant growth, and their reduction indicates assimilation (Fig. 5). The amount of nitrogen and phosphorus pollutants has decreased as follows:

The reduction efficiencies of NH_4^+, NO_3^-, and PO_4^{3-} by Azolla, Duckweed, and their combination are presented in Table 2. On average, Azolla removed 47, 51, and 49% of NH_4^+, NO_3^-, and PO_4^{3-}, respectively, while Duckweed removed 39, 45, and 42% of these nutrients. The combined treatment of Azolla and Duckweed showed the highest removal efficiency, achieving 52, 55, and 54% for NH_4^+, NO_3^-, and PO_4^{3-}, respectively. Statistical analysis indicated that values with different letters in the same column were significantly different ($P<0.05$), demonstrating that co-cultivation of Azolla and Duckweed enhances the removal of these nutrients from water compared to single-species treatments (Table 2).

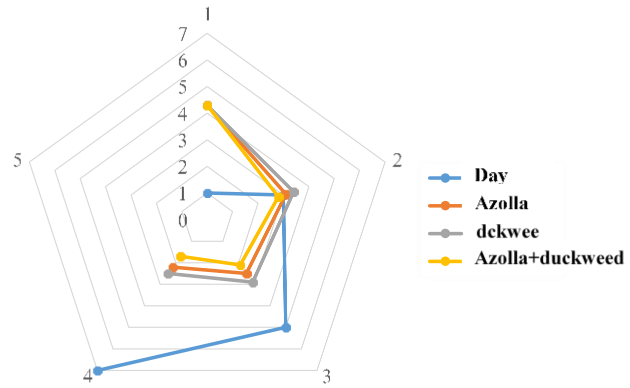

Fig. 5: Phosphate (PO_4^{3-}) change (mg/L).

Table 2: Reduction efficiency of NH_4^+, NO_3^-, and PO_4^{3-}, in water by *Azolla, Lemna* and their combination

Substance	Initial (mg/L)	Azolla (%)	Duckweed (%)
$NH4^+$	5.2±0.16	47±1.2^a	39±1.1^b
$NO3^-$	8.6±0.34	51±1.5^a	45±1.4^b
$PO4^{3-}$	4.3±0.11	49±1.0^a	42±0.9^b

Values (n = 3) are presented as mean±SD. Different superscript letters within a row indicate significant differences at P<0.05 (one-way ANOVA followed by Tukey's post hoc test).

Heavy metals (Pb^{2+} and Cd^{2+}) concentrations were analyzed by atomic absorption spectrophotometry (AAS) using a modern device (Perkin Elmer AA Analyst or Shimadzu AA-7000). Water samples were acidified with nitric acid (HNO_3) and concentrated if necessary. Appropriate wavelengths were used for each element: 283.3 nm for Pb^{2+} and 228.8 nm for Cd^{2+}. On day 1, Pb^{2+} and Cd^{2+} concentrations were similar across all groups: 1.7±0.03–1.7±0.031 and 0.9±0.018–0.9±0.020mg/L, respectively. By day 7, Pb^{2+} decreased to 0.76±0.016mg/L in Azolla, 0.88±0.017mg/L in Duckweed, and 0.60±0.014mg/L in the combined treatment. Similarly, Cd^{2+} decreased to 0.35±0.010, 0.42±0.010mg/L, and 0.30±0.011mg/L in Azolla, Duckweed, and the combination, respectively. These results indicate that the co-cultivation of Azolla and Duckweed was the most effective in reducing Pb^{2+} and Cd^{2+} concentrations in water.

Azolla and duckweed can absorb heavy metals into their parenchymatous tissues, facilitating their removal from water. Therefore, Azolla and duckweed significantly reduced the concentration of heavy metal ions by absorbing them into their bodies (Table 3). Rakhmonov et al. (2025) studied the intensive cultivation of aquatic plants in the central regions of Uzbekistan and their use as feed for herbivorous fish. According to the results, *Lemna, Azolla, Elodea,* and *Ceratophyllum* species showed optimal growth at temperatures of 22–28°C and pH levels of 6.5–7.5. The biomass yield reached 10–15kg/m^2, and the prepared feed increased the growth rate of herbivorous fish by 18–22%. The authors recommended aquatic plants as a high-protein, environmentally friendly, and economically efficient feed source for sustainable aquaculture. Biomass gain is a measure of plant growth, pollutant utilization, and total phytomass production. This parameter plays a vital role in determining the environmental and economic efficiency of biotechnological treatment processes. Biomass gain is usually expressed in grams/m^2 or g/L and is measured in two primary forms:

- Wet (fresh) biomass – the mass of plants directly collected, filtered and immediately weighed.
- Dry biomass – plants are dried in a thermostat or drying cabinet at 60-70°C until they reach constant weight, and then weighed.

Table 3: Reduction Pb^{2+} and Cd^{2+} by Azolla, duckweed, and their combination (mg/L)

Day	Substance	Azolla (mg/L)	Duckweed (mg/L)	Azolla + duckweed (mg/L)
1	Pb^{2+}	1.7±0.03^a	1.7±0.029^a	1.7±0.031^a
7	Pb^{2+}	0.76±0.016^b	0.88±0.017^c	0.60±0.014^a
1	Cd^{2+}	0.9±0.018^a	0.9±0.019^a	0.9±0.020^a
7	Cd^{2+}	0.35±0.010^b	0.42±0.010^c	0.30±0.011^a

Values (n = 3) are presented as mean±SD. Different superscript letters within a row indicate significant differences at P<0.05 (one-way ANOVA followed by Tukey's post hoc test).

At the beginning of the experiment (day 1), a specified amount (e.g., 100g/m^2) of azolla and/or duckweed, or a mixture of them, is spread. This is recorded as the initial biomass. At the end of the experiment (day 7), the plants were removed from the water, excess water was shaken off, and they were weighed wet. The samples are then dried at 60-70°C for 24-48 hours (until constant weight is reached). The dry biomass is weighed on an analytical balance (Table 4).

Biomass gain is calculated using the following formulas:

Wet biomass gain:

$B_w=W_7-W_1$

- B_w – wet biomass gain (g/m^2)
- W_7 – Biomass measured at the end of day 7
- W_1 – initial biomass

Dry biomass increase (%):

$$Bq\ (\%) = \left(\frac{DW^7}{DW^7 - DW^1}\right) \bullet 100$$

B_d – dry biomass increase percentage

DW_7, DW_1 – biomass (g/m^2) on day 7 and day 1, respectively.

Table 4: Process for determining biomass growth of Azolla and duckweed plants (with steps and explanations)

Stage	Practical activity	Unit of measure	Note
1	Initial biomass is measured (day 1)	g/m^2	The total weight of azolla and duckweed spread on the water surface is determined (e.g. 100g/m^2).
2	Harvested at the end of the experiment (day 7)	g/m^2 (wet)	The plants are removed from the water, excess water is shaken off and weighed while wet.
3	Drying process	-	Dry at 60-70°C for 24-48 hours.
4	Dry biomass is weighed	g/m^2	Dry biomass is weighed on an analytical balance. This is considered the actual biomass.
5	Biomass gain is calculated	g/m^2 and %	Wet biomass increase: $B_h=W_7-W_1$. Dry (%): $Bq\ (\%) = \left(\frac{DW^7}{DW^7 - DW^1}\right) \bullet 100$
6	The results are displayed in tables and graphs.	-	The growth rate for each option is compared.

Measuring instruments:

- Analytical balance (accuracy 0.001g)
- Drying cabinet or thermostat (60-70°C)
- Filter paper or gauze
- Metal grid trays (for collecting plant samples)

A correlation analysis is conducted between the increase in biomass and the reduction in water pollution. This allows us to numerically demonstrate the bioremediation potential of plants. The increase in biomass not only increases the cleaning efficiency but also provides additional economic benefits. Plants quickly occupy the water surface within 10 days. Biomass growth increased by 70% for azolla, 62% for duckweed, and 85% for azolla + duckweed. This indicates their rapid reproduction and the ability to accelerate the cleaning process (Table 5).

- Percentage decrease (-%) indicates a decrease in the amount of pollutants in the water.
- Increase (+%) in dissolved oxygen (DO) is indicated
- Increase in biomass indicates the activity of plants in purification.

Table 5: Comparison of the purification efficiency of Azolla and duckweed plants

Indicators	Initial amount (mg/L)	Azolla (%)	Duckweed (%)	Azolla and duckweed (%)
NH_4+	5.2±0.1	47±2.1^b	39±1.8^c	58±2.4^a
NO_3−	8.6±0.26	51±2.6^b	45±2.0^c	63±2.8^a
PO_4^{3-}	4.3±0.09	49±2.3^b	42±1.9^c	60±2.5^a
Pb^2+	1.7±0.03	55±2.8^b	48±2.1^c	65±3.0^a
Cd^2+	0.9±0.02	61±3.1^b	53±2.4^c	67±3.3^a
Dissolved O_2 (DO)	3.2±0.06	44±1.9^b	31±1.5^c	59±2.2^a
Biomass increase	–	70±3.4^b	62±2.9^c	85±3.8^a

Values (n = 3) are presented as mean±SD. Different superscript letters within a row indicate significant differences at P<0.05 (one-way ANOVA followed by Tukey's post hoc test).

During the experiment, the efficiency of *A. caroliniana* Willd. or *L. minor* L. plants, both individually and in combination, to absorb pollutants (ammonium, nitrate, phosphate, heavy metals) from the aquatic environment was evaluated. Shernazarov et al. (2024) studied the role of Azolla aquatic plants in creating a natural and sustainable nutrient environment in fisheries, showing that they enhance nitrogen fixation and biological nutrient cycling in marine ecosystems. The purification efficiency was determined as a percentage based on 7-day monitoring for all three options. According to the results, although both plants have phytoremediation potential individually, their combined use further increased efficiency. The differences in resource utilization and symbiotic effects between the plants explain this.

Azolla absorbs nitrogen compounds (NH_4^+ and NO_3^-) from water at a high level. Its rapid growth, complete surface coverage, and enhanced photosynthesis significantly increase the amount of oxygen produced. The absorption rate of heavy metals (Pb_2^+, Cd_2^+) is higher than that of duckweed.

Duckweed plant efficiency: mainly absorbs phosphorus compounds (PO_4^{3-}). Its rapid growth on the water surface allows it to effectively clean surface pollution. Although its ability to absorb heavy metals is slightly lower than that of Azolla, it plays an important role in maintaining the nitrogen-phosphorus balance. The combined system (Azolla + duckweed): showed the highest cleaning efficiency. The specific ecological and physiological properties of both plants had a complementary effect. A synergistic effect was observed, especially in the level of heavy metal absorption.

The orange line represents annual observations, while the black line indicates the smoothed (glatting) trend. The mean temperature (13–16°C) is below the optimal growth range for *A. caroliniana* and *L. minor* (20–30°C), suggesting that these aquatic species grow most intensively during the warm season (Fig. 6).

Climatic Trend Analysis of the Study Area (1901–2023)

Fig. 6 illustrates the temporal dynamics of the annual mean air temperature in the study area during 1901–2023, based on the Climatic Research Unit Time Series (CRU TS3.10) dataset. The long-term trend reveals a clear and statistically consistent warming tendency over the past century.

1) Early 20th century (1901–1950): During this period, the mean annual temperature remained relatively stable, fluctuating between 13.2 and 14.1°C. Short-term cooling episodes were observed in the 1930s and 1940s, likely linked to global climatic oscillations.

2) Mid-20th century (1950–1980): A slight cooling phase occurred, with temperatures occasionally dropping to around 13°C in the 1960s. This period corresponds to the mid-century global cooling observed in many continental regions.

3) Recent decades (1980–2023): From the early 1980s onwards, the region entered a persistent warming phase. Since the mid-1990s, annual mean temperatures have exceeded 15°C, reaching 15.5–16.2°C in the most recent years (2016–2023). This trend is consistent with global climate warming patterns and regional temperature increases observed across Central Asia (Fig. 6).

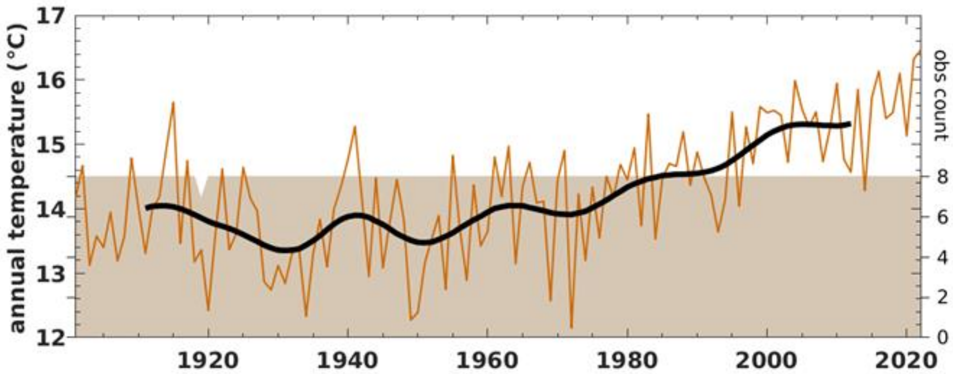

Fig. 6: Long-term dynamics of the mean annual air temperature in the study area (1901–2023), based on CRU TS3.10 data.

Overall Trend (1901–2023)

The analysis indicates that the mean annual temperature in the study area has increased by approximately +1.8–2.0°C over the last 120 years. Such a sustained warming trajectory implies significant shifts in the regional thermal regime and associated ecological processes.

The thermal regime of the study area directly influences the growth and productivity of floating aquatic plants such as *A. caroliniana* and *L. minor*. According to published physiological studies, these species exhibit the following temperature preferences (Table 6).

Given that the mean annual air temperature of the study area is 13–16°C, it remains below the optimal range for these species' growth. Thus, active vegetative development of *Azolla* and *Lemna* occurs primarily during the warm season (May–August), when both air and water temperatures approach their optimal physiological levels. However, the gradual warming observed after 2000 has likely extended the vegetative growth period by 3–4 weeks, enhancing biomass accumulation and expansion of surface cover.

Long-term precipitation trends in the study area from 1901 to 2023 were analyzed using the CRU TS3.10 climate dataset (Climatic Research Unit). This analysis provides insights into both interannual and decadal variability of rainfall and its ecological effects.

1. Long-term Precipitation Trends

• 1901–1950: Annual precipitation ranged between 90 and 130mm, reflecting relatively dry and semi-arid conditions. The long-term average during this period was approximately 110mm.

• 1950–1980: A slight increase in precipitation was observed, with occasional peaks in some years.

• 1980–2023: From the 1980s onward, precipitation fluctuated moderately between 120 and 140mm, occasionally reaching 130–140mm.

Overall, over the 120-year period, temperatures have increased significantly, while the precipitation regime has remained relatively stable.

2. Hydro-ecological Impacts on *A. caroliniana* and *L. minor*

Stable precipitation is essential for maintaining water level consistency, nutrient inflow, and habitat stability in aquatic ecosystems. Both *A. caroliniana* and *L. minor* benefit from these conditions:

• Annual precipitation averaging 130–140mm provides favorable moisture conditions in wetlands and irrigation basins.

• Consistent rainfall prevents extreme fluctuations in water levels, which could disrupt macrophyte growth.

• During drier years, *Azolla* biomass accumulation may decline due to reduced nitrogen availability, while. *L. minor* is similarly affected by lower water levels.

Thus, CRU TS3.10-based precipitation analysis confirms that the region's hydrological regime is sufficiently stable to support *Azolla* and *Lemna* populations. Combined with recent warming trends, these conditions promote an extended growing season, increased biomass productivity, and enhanced ecological and bioresource potential of these floating macrophytes in regional aquatic ecosystems.

Long-term annual precipitation (1901–2023) and decadal averages in the study area based on CRU TS3.10 data. Shaded areas indicate periods of lower and higher precipitation, highlighting stable moisture conditions favorable for aquatic macrophytes (Fig. 7).

This indicates that current climatic conditions are increasingly favorable for the cultivation or natural proliferation of these hydrophytes, especially in shallow and micro-warm aquatic habitats.

The paper focuses on algal consortia for wastewater treatment, not on higher aquatic plants such as Azolla or duckweed. It discusses phycoremediation's effectiveness in removing pollutants but does not provide details on these particular plants' effects (Walters et al., 2025). The paper does not investigate *A. caroliniana* Willd. or *L. minor* L. It focuses on *Pistia stratiotes, Spirodela polyrhiza*, and *Eichhornia crassipes* for heavy metal removal, achieving over 79% reduction in wastewater pollutants over 15 days (Rai, 2019). The paper does not investigate *A. caroliniana* Willd. or *L. minor* L. but focuses on *Eichhornia crassipes* and *Pistia stratiotes*. It highlights their role in promoting

Table 6: Implications for the Growth Ecology of Aquatic Macrophytes

Species	Optimal growth range (°C)	Lower growth limit (°C)	Upper tolerance (°C)	Reference
Azolla caroliniana	20–28	10–12	35	Wagner (1997); Carrapiço (2010)
Lemna minor	20–30	8–10	33–35	Landolt (1986); Appenroth et al. (2015)

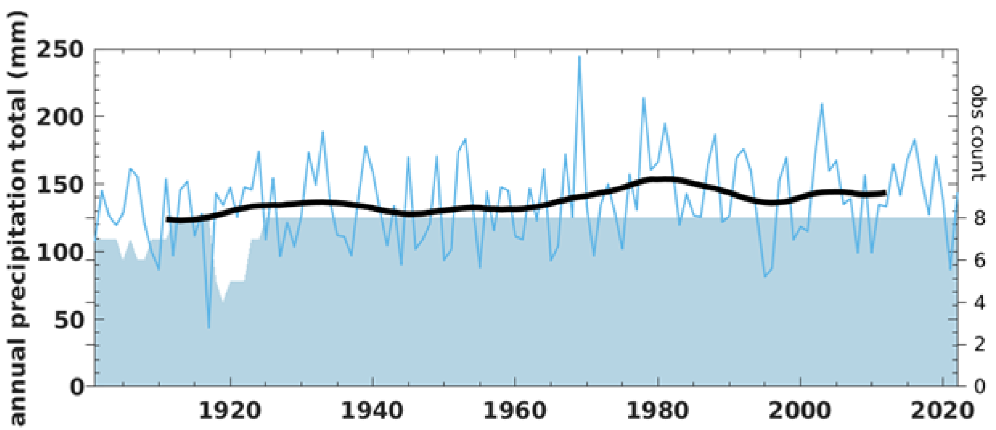

Fig. 7: The study area is integrated with the CRU TS3.10 research section of the climate dataset. Annual Precipitation Dynamics and Its Ecological Implications.

rhizospheric bacteria and reducing water/oil interfacial tension in oil-contaminated environments (Zhilkina et al., 2024). The paper does not specifically address the effects of *A. caroliniana* Willd. and *L. minor* L. on polluted water parameters. It focuses on the performance of five different aquatic plants in constructed wetlands for nutrient removal and microbial diversity (Zhang et al., 2024). The paper does not address the effects of biotechnological treatment using *A. caroliniana* Willd. and *L. minor* L. on polluted water parameters. It focuses on the purification efficiency of a mosaic system of submerged–emerged plants (Chang et al., 2024). The paper focuses on *Azolla filiculoides*, not *A. caroliniana* Willd. or duckweed. It discusses Azolla's phosphorus accumulation and growth response to nutrient availability, but does not explicitly address biotechnological treatments or changes in polluted water parameters (Temmink et al., 2018). The study demonstrated that using aquatic plants with plant growth-promoting Rhizobacteria achieved significant removal rates of pollutants: COD (89.18%), NH4+-N (59.65%), NO3--N (69.50%), and TP (75.61%), effectively improving water quality in polluted environments (Xu et al., 2023). The paper does not explicitly address the effects of biotechnological treatment using *A. caroliniana* Willd. and *L. minor* L. on polluted water parameters. It focuses on the phytoremediation efficiency of various aquatic macrophytes under different pollutant concentrations (Xiao et al., 2021). The present study demonstrated that the presence of Azolla significantly reduced pollutant concentrations in the aquatic environment. A clear correlation was observed between biomass accumulation and the decrease in heavy metal concentrations, indicating that Azolla effectively removes contaminants through bioaccumulation and biosorption mechanisms. These findings are consistent with previous reports highlighting the phytoremediation potential of Azolla in heavy-metal-contaminated waters (Sood et al., 2012). The reduction of toxic elements can be attributed to the physiological and morphological characteristics of floating aquatic macrophytes, which facilitate the absorption and immobilization of pollutants. This observation is consistent with the comprehensive review by Dhir et al. (2009), who described the mechanisms by which aquatic plants sequester inorganic and organic contaminants. Furthermore, the current results corroborate classical findings by Zayed et al. (1998), confirming the capacity of wetland plants to phytoaccumulate trace elements from contaminated water bodies. Improvement in general water quality parameters during the experiment suggests that Azolla can remove not only heavy metals but also other inorganic and organic pollutants. These results align with the conclusions of Ali et al. (2013), who emphasized the ecological safety, cost-effectiveness, and sustainability of plant-based remediation approaches. Overall, the findings indicate that Azolla is a promising biological resource for sustainable remediation of polluted aquatic ecosystems. Its integration into environmentally friendly water treatment systems may offer an effective solution for improving water quality and mitigating ecological risks.

Conclusion

The results of the research have shown that higher aquatic plants such as *Azolla caroliniana* Willd. or *Lemna minor* L. can effectively clean polluted water environments naturally, that is, through phytoremediation. During the 7-day monitoring period, the presence of these plants significantly reduced the levels of the primary pollutants in the water. Both Azolla and duckweed individually had a positive effect on the following indicators: the pH was neutralized from an initial 8.0 to 6.9, indicating that the aquatic environment had returned to a stable state. Increased dissolved oxygen (DO): Plants increased the amount of oxygen in the water from 3.2 to 5.1mg/L through photosynthesis. Reduction of nitrogen and phosphorus compounds: NH_4^+, NO_3^-, PO_4^{3-} A 40-65% reduction in nutrients such as Heavy metals Pb^{2+}, Cd^{2+} absorption decreased to 65 and 67%, This indicates that they accumulate in plant cells through the mechanism of biosorption. It is especially noteworthy that the combined use of azolla and duckweed significantly increased the cleaning efficiency. This is explained by the high potential for reducing pollution through a synergistic effect. The rapid increase in plant biomass also indicates that they can act as active filters in the ecological environment. Their simple maintenance, adaptability to local conditions, and economic efficiency create vast opportunities for the practical application of this method. In general, higher aquatic plants such as *A. caroliniana* Willd. or *L. minor* can be used as a biotechnologically effective tool for the natural purification of polluted waters. In particular, they are an environmentally safe and economically viable solution for the purification of industrial waste, agricultural drainage water, and urban wastewater. The results of the study expand the possibilities for using these plants as biofilters and demonstrate their importance in protecting water resources.

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Author's Contribution: This study was a collaborative effort among all authors. Vakhob Rakhmonov, Khurmatoy Turdalieva, Nodirjon Bobokandov, Barno Kobulova, Sobir

Mustanov, Pakhlavon Nurimov, Makhsuda Bekmuradova, Mashrab Yusupov, Gulchekhra Tastanova, Dilafruz Ishankulova, Mahliyo Narzullayeva, Chinara Sadikova, Zebo Egamberdieva and Yigitali Tashpulatov contributed to the study design and data analysis. Vakhob Rakhmonov, Yigitali Tashpulatov, Nodirjon Bobokandov, conducted the laboratory experiments, analyzed the data, interpreted the results, and drafted the manuscript. All authors reviewed and approved the final manuscript.

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