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Comparative Mercury Removal by Four Aquatic Macrophytes from ASGM-Contaminated Liquid Waste

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ABSTRACT

Introduction: Artisanal and small-scale gold mining (ASGM) is a major source of mercury contamination in aquatic environments, threatening ecosystems and human health. Conventional remediation is often costly and difficult to apply in resource-limited mining areas. Phytoremediation using aquatic macrophytes offers a low-cost alternative. This study compared observed water-phase mercury removal by Ipomoea aquatica, Pistia stratiotes, Nymphaea spp., and Eichhornia crassipes under standardized bench-scale laboratory conditions.

Methodology: ASGM liquid waste with an initial dissolved mercury concentration of 0.00448 mg/L was used. Each species was tested in triplicate over a 14-day exposure period, with water samples analyzed on Day 0, Day 9, and Day 14. Mercury concentrations were measured using Atomic Absorption Spectrophotometry, and removal effectiveness was calculated as percentage reduction from baseline. Because biomass-normalized uptake, plant tissue mercury accumulation, and abiotic controls were not included, findings are interpreted as plant-associated water-phase reductions rather than definitive evidence of uptake mechanisms.

Results: All species substantially reduced dissolved mercury, with clear interspecies variation. By Day 14, water hyacinth reduced mercury to 0.00003–0.00004 mg/L, corresponding to 99.11–99.33% removal. Lotus showed comparable performance, achieving 98.21–98.44% removal. Water spinach produced intermediate reductions of 85.04–94.42%, with an approximate mean of 89.17%, while water lettuce showed the lowest removal range of 83.71–86.38%, with an approximate mean of 84.89%. Reductions were evident by Day 9 and greatest by Day 14. Mechanistic interpretations related to root morphology, biomass, rhizosphere processes, or internal translocation should remain cautious because these parameters were not directly measured.

Conclusion: Aquatic macrophytes can substantially reduce dissolved mercury in ASGM-contaminated liquid waste under bench-scale conditions. Water hyacinth showed the highest observed removal, followed closely by lotus. Field application requires ecological containment, safe biomass disposal, abiotic control assessment, effluent characterization, and further tissue-based uptake studies.

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INTRODUCTION

Mercury contamination remains one of the most persistent environmental problems associated with artisanal and small-scale gold mining (ASGM). ASGM is widely recognized as a major source of anthropogenic mercury release, particularly in regions where mercury amalgamation is still used for gold extraction (1,2). Authoritative global assessments and peer-reviewed field studies have documented mercury contamination in mining-impacted aquatic systems across Asia, Africa, and the Americas, underscoring the transboundary and public health relevance of this issue. In Latin America, particularly within the Amazon basin, illegal gold mining has intensified in recent years, with reported increases of approximately 77% between 2017 and 2022, contributing to greater mercury discharge into river systems (3). Similarly, in West Africa, the Ankobra estuary has recorded riverine mercury concentrations ranging from 4 to 26 μg/L, indicating severe local water quality degradation (4). These findings illustrate the magnitude of ASGM-related mercury pollution and its implications for aquatic ecosystems and communities dependent on contaminated water bodies.

Once released into aquatic systems, mercury can undergo complex biogeochemical transformations. One of the most important pathways is microbial methylation, through which inorganic mercury may be converted into methylmercury (MeHg). MeHg is highly toxic and bioavailable, and it can bioaccumulate and biomagnify through aquatic food webs, resulting in elevated concentrations in predatory fish species (5,6). Human populations that rely heavily on fish consumption are therefore at risk of dietary MeHg exposure. Health consequences are particularly concerning for pregnant women, fetuses, and children because MeHg is associated with neurodevelopmental toxicity (7–9). Reducing mercury inputs and dissolved mercury concentrations in contaminated waters is therefore an important environmental and public health priority in ASGM-affected areas.

Conventional mercury remediation techniques, including chemical precipitation, adsorption, electrochemical treatment, and advanced oxidation processes, have been widely examined, but their field application remains constrained by cost, technical complexity, energy requirements, and potential secondary waste generation Electrochemically assisted methods, while capable of reducing mercury levels, require high energy input and frequently generate secondary waste streams (10). Adsorption-based approaches using activated carbon or functionalized materials may be effective under controlled conditions, yet their performance can decline across repeated cycles and may be influenced by water chemistry, competing ions, and organic matter (11,12). Emerging nanoremediation strategies are also promising, but concerns remain regarding production cost, long-term environmental safety, and regulatory acceptance (13,14). Advanced oxidation processes, while effective against some pollutants, rely on chemical oxidants and energy inputs that may result in incomplete mineralization and undesirable byproducts (15). Emerging nano remediation strategies, though promising in laboratory trials, face challenges including high production costs, uncertain long-term environmental safety, and regulatory barriers (16). These limitations are particularly relevant for ASGM-affected settings, where remediation technologies must be affordable, simple to operate, and compatible with local environmental management capacity. Therefore, complementary approaches that can reduce dissolved mercury under practical field conditions remain necessary.

Phytoremediation, broadly defined as the use of plants to reduce, stabilize, extract, or transform contaminants in soils, sediments, and waters, has received increasing attention as a complementary remediation strategy (13,17). For metals and metalloids, phytoremediation may involve several processes, including root-associated sorption, uptake, sequestration, stabilization, and, in some cases, volatilization. However, the relative contribution of these processes cannot be assumed without supporting measurements such as plant tissue accumulation, biomass-normalized uptake, mass balance assessment, and abiotic control treatments. With respect to mercury, plant-mediated removal has been reported in both terrestrial and aquatic systems, including studies involving Trifolium repens L. and other plant species exposed to contaminated matrices (18,19). Thus, phytoremediation should be viewed not as a universal substitute for conventional treatment, but as a potentially useful, low-cost complement in contexts where highly engineered remediation systems are difficult to sustain (14).

Aquatic macrophytes are attractive candidates for mercury remediation because many species grow rapidly, produce substantial biomass, and develop extensive root systems in aquatic environments. Several species have been reported to accumulate mercury or reduce aqueous mercury concentrations under laboratory or field conditions. For instance, Elodea nuttallii has shown measurable interaction with both inorganic mercury and methylmercury, although standardized removal efficiencies are not always reported (20). *Ceratophyllum demersum* has also been shown to accumulate mercury significantly in contaminated waters (21). Field studies in the Balili River have

documented interspecies variation and organ-specific accumulation patterns among dominant macrophytes (22). Similarly, investigations of autochthonous aquatic plants in mining-impacted rivers suggest phytoremediation potential, although efficiency metrics remain inconsistent (23). This variability indicates that species-level comparisons should be interpreted alongside biomass, root characteristics, exposure chemistry, plant tissue accumulation, and environmental conditions. (24).

Despite this growing body of evidence, important gaps remain. Few studies have performed side-by-side comparisons of multiple aquatic macrophytes under standardized exposure conditions, limiting reliable cross-species assessment of mercury removal performance (25,26). Comparability is further constrained by inconsistent reporting of exposure duration, plant biomass, plant density, root morphology, water chemistry, plant organs analyzed, and analytical detection limits (24). Field-validated data are also limited, particularly in tropical and mining-impacted regions where low-cost remediation approaches may be most urgently needed (23,27). Although root-rhizosphere interactions and internal translocation may influence mercury fate, these mechanisms require direct empirical confirmation through tissue analysis, biomass-normalized uptake metrics, and appropriate abiotic controls (24,28).

Against this backdrop, the present study provides an applied bench-scale comparison of four aquatic macrophyte species: water spinach (*Ipomoea aquatica*), water lettuce (*Pistia stratiotes*), lotus (*Nymphaea* spp.), and water hyacinth (*Eichhornia crassipes*). By using a common initial mercury concentration, exposure duration, sampling schedule, and analytical method, the study aims to generate comparative evidence on observed dissolved mercury reduction among species. The contribution of this study is primarily applied rather than mechanistic: it identifies relative removal performance under standardized laboratory conditions and provides preliminary guidance for species selection in ASGM wastewater phytoremediation. Further mechanistic interpretation requires additional measurements, including biomass and root surface area quantification, plant tissue mercury accumulation, abiotic control treatments, and full physicochemical characterization of the effluent.

METHODOLOGY

The methodology for this study was designed to ensure reproducibility, scientific rigor, and alignment with standard protocols in phytoremediation research. The following subsections detail the experimental design, plant preparation, exposure conditions, analytical methods, and statistical procedures applied. Additional methodological details are provided to clarify the bench-scale nature of the experiment, the basis for interspecies comparison, and the limits of mechanistic interpretation. Because the experiment focused on water-phase mercury reduction, rather than full mercury mass balance, the results are interpreted as observed plant-associated removal under controlled laboratory conditions.

Experimental Design

The experiment evaluated the observed water-phase mercury (Hg) removal of four aquatic macrophytes—*Ipomoea aquatica* (water spinach), *Pistia stratiotes* (water lettuce), *Nymphaea* spp. (lotus), and *Eichhornia crassipes* (water hyacinth). All plant treatments were exposed to mercury-contaminated artisanal gold-mining effluent with an initial mercury concentration standardized at 0.00448 mg/L. The study was conducted in controlled bench-scale units, designed to minimize variability and facilitate statistical comparison. The experimental design consisted of plant-exposed treatment units for each species. No plant-free abiotic control was included in the original experimental set-up; therefore, the observed reductions cannot be attributed exclusively to plant uptake and may also reflect passive processes such as sorption to container surfaces, sedimentation, photochemical transformation, or volatilization. This limitation was considered when interpreting the findings.

Each macrophyte species was tested in triplicate, resulting in plant-exposed experimental units. Sampling was conducted at three time points: Day 0 (baseline), Day 9, and Day 14. This timeframe was chosen based on previous phytoremediation studies indicating that contaminant removal in aquatic plant systems is commonly assessed within 10–21 days (29,30). For each treatment unit, mercury concentration was measured in the water phase, and removal effectiveness was calculated relative to the initial concentration. Because biomass-normalized uptake and plant tissue mercury concentrations were not measured, interspecies comparisons were based on residual dissolved mercury concentration and percentage removal rather than uptake per unit biomass.

Effluent Collection and Physicochemical Characterization

The mercury-contaminated liquid waste used in this study originated from an artisanal gold-mining activity area and was used as the exposure medium for all treatments. The initial mercury concentration was standardized at 0.00448 mg/L before the exposure period. To improve experimental replicability, the provenance of the effluent should be reported as follows: sampling location, date of collection, sampling point, collection depth if applicable, storage container, holding time before experimentation, and whether the sample was filtered, settled, or used directly.

Effluent Collection and Physicochemical Characterization

A comprehensive physicochemical profile of the effluent is required to contextualize mercury behavior during exposure. Where available, the following parameters should be reported: pH, electrical conductivity, dissolved oxygen, temperature, oxidation-reduction potential, turbidity or total suspended solids, organic matter or chemical oxygen demand, and major competing ions or metals. If these parameters were not measured in the original experiment, the manuscript should explicitly state that the physicochemical characterization was limited to initial mercury concentration and that the absence of these data restricts interpretation of mercury speciation, plant physiological response, and cross-study comparability.

Plant Preparation and Acclimatization

Standard laboratory protocols for preparing and acclimatizing aquatic macrophytes were followed to ensure plant health and minimize shock upon transfer to experimental solutions. Healthy, correctly identified plants were sourced from local aquatic habitats and subjected to a quarantine/acclimation period of 7–14 days in clean water under controlled light and temperature conditions (31,32). This period allowed plants to adjust to laboratory conditions and ensured that only vigorous specimens were used in the experiment.

Following acclimation, plants were transferred gradually to treatment containers to reduce physiological stress. Experimental units consisted of 1.5 L containers filled with mercury-contaminated effluent. Triplicate setups for each species were maintained to account for biological and environmental variability. Such approaches are consistent with prior hydroponic and water-media phytoremediation studies, including 21-day *Salvinia*-based trials and 10-day *Eichhornia crassipes* exposures (29,32). Before exposure, plants should be standardized and documented by species-specific fresh weight, dry weight, number of individual plants per container, approximate root length, root density, or root surface area where available. If these measurements were not recorded, the manuscript should state that plant selection was based on visible health and comparable maturity, and that the absence of biomass and root surface area measurements limits reproducibility and prevents biomass-normalized uptake modeling.

Exposure Conditions

Each plant group was placed under identical bench-scale exposure conditions to minimize external influences on mercury reduction. Water levels were monitored and replenished with deionized water to compensate for evaporation losses, ensuring consistent exposure volume. No additional nutrients were added, as the aim was to approximate contaminated water conditions found in mining effluents. To support interpretation of plant physiological responses and mercury speciation, environmental conditions should be reported quantitatively, including room or water temperature range, photoperiod, irradiance or light intensity, pH during exposure, dissolved oxygen, aeration status, and whether containers were open or covered. If exact values were not recorded, the manuscript should replace the phrase “careful control” with a more cautious description, such as “maintained under laboratory bench-scale conditions,” and acknowledge that unmeasured environmental variation may have influenced mercury behavior.

The exposure period lasted 14 days, with interim sampling conducted on Day 9. This timeframe allowed assessment of both intermediate and near-maximal mercury uptake, in accordance with established protocols for aquatic macrophyte phytoremediation studies (30). The term “near-maximal mercury uptake” was avoided because plant tissue mercury accumulation and uptake kinetics were not directly measured. Therefore, Day 14 was interpreted as the final observation point for dissolved mercury reduction rather than as confirmation of maximal plant uptake.

Mercury Sampling and Analysis

Water samples were collected from each treatment unit at the designated time points. Samples were preserved in acid-washed polyethylene bottles and stored under refrigeration until analysis. Mercury quantification was performed using Atomic Absorption Spectrophotometry (AAS), a well-established technique for heavy metal analysis in phytoremediation research (22,33). The analytical method should be reported with sufficient detail, including instrument model, mercury detection mode, wavelength or operating conditions, sample digestion or preservation procedure, calibration range, limit of detection, limit of quantitation, and quality-control procedure. These parameters are essential because several Day 14 concentrations approached very low levels and may be close to analytical detection limits.

Calibration of the AAS instrument was conducted with certified standard solutions, and procedural blanks were analyzed alongside samples to support analytical accuracy. Triplicate analytical replicates were run for each water sample. Mercury concentrations were expressed in mg/L, representing the remaining dissolved mercury in water after exposure. To strengthen analytical rigor, the following validation indicators should be reported where available: calibration curve coefficient or R^2, method detection limit, limit of quantitation, blank values, recovery efficiency from spiked samples or certified reference material, relative standard deviation, and instrumental precision. If these parameters were not fully documented, this should be acknowledged as a limitation, and language implying "high confidence" should be moderated.

Data Analysis and Removal Effectiveness

Mercury removal effectiveness was calculated using the following formula:

($\text{Removal effectiveness} (\%) = \frac{C_0 - C_t}{C_0} \times 100$), where ($C_0$) represents the initial mercury concentration, which was 0.00448 mg/L, and (C_t) represents the mercury concentration at the relevant sampling point, namely Day 9 or Day 14. This calculation provided percentage reduction values for comparison across species. In addition to percentage removal, residual mercury concentrations should be reported as mean ± standard deviation or mean ± standard error for each species and time point. Percent removal alone is insufficient to evaluate measurement stability, variability among replicates, or the statistical strength of interspecies differences.

Statistical Analysis

Interspecies differences in mercury removal effectiveness were statistically analyzed to determine whether observed variations differed among species. One-way analysis of variance (ANOVA) was employed to compare the four macrophyte groups at each relevant sampling point, followed by Tukey's post hoc test to identify pairwise differences between species (31,34). Assumptions of normality and homogeneity of variance were tested prior to conducting ANOVA. Statistical outputs should be reported explicitly, including F-values, degrees of freedom, p-values, and post hoc grouping or pairwise comparison results. Descriptive statistics should include mean, standard deviation, and sample size. Graphical presentation using line plots or bar charts with error bars is recommended to improve transparency.

In cases where statistical assumptions were not met, non-parametric alternatives were considered, specifically the Kruskal–Wallis test followed by Dunn's post hoc comparisons (24,26). Statistical significance was set at $p < 0.05$. Given the small number of biological replicates, statistical findings should be interpreted cautiously and presented alongside effect size or confidence intervals where possible. The analysis should avoid unsupported claims of mechanistic causation and should focus on comparative differences in observed water-phase mercury reduction.

Ethical and Environmental Considerations

All experimental procedures were designed to minimize environmental impact. Following completion of the study, residual water samples were treated and disposed of in accordance with laboratory safety protocols for heavy metals. Plant materials exposed to mercury were collected, dried, and stored as hazardous waste for subsequent safe disposal. Ethical considerations were observed in sourcing plant specimens, ensuring that collection did not harm natural populations or protected ecosystems. Because mercury may accumulate in plant tissues, exposed biomass should not be returned to natural water bodies or disposed of as ordinary organic waste. Future field-scale applications should include a biomass management plan covering safe harvesting, storage, transport, treatment, and final disposal in accordance with hazardous waste regulations.

RESULTS

This section presents the findings of the comparative study on observed water-phase mercury (Hg) reduction by four aquatic macrophytes—*Ipomoea aquatica* (water spinach), *Pistia stratiotes* (water lettuce), *Nymphaea* spp. (lotus), and *Eichhornia crassipes* (water hyacinth). The results are structured into baseline mercury concentration, mercury concentration changes over time, removal effectiveness, and statistical comparison. Interpretive comparison with previous studies is presented in the Discussion to preserve the descriptive focus of the Results section.

Baseline Mercury Concentrations

The initial mercury concentration across all experimental units was standardized at 0.00448 mg/L. This value was used as the baseline concentration for calculating percentage reduction at Day 9 and Day 14. Field reports from comparable ASGM contexts have documented dissolved mercury concentrations in the low µg/L to tens of µg/L range. For instance, mercury concentrations of 4–26 µg/L, equivalent to 0.004–0.026 mg/L, have been reported in the Ankobra estuary (34,35). Therefore, the baseline concentration used in this experiment was within the lower range of values reported in ASGM-impacted aquatic environments.

Mercury Concentrations Over Time

Mercury concentrations declined across all four macrophyte treatments during the 14-day exposure period. The magnitude of reduction varied among species and across sampling times. Because abiotic controls were not included, these values are reported as plant-exposed treatment outcomes and should not be interpreted as exclusive evidence of plant uptake.

Table 1. Mercury Concentrations Across Species and Time Points (mg/L)

Species	Day 0	Day 9 (Range)	Day 14 (Range)
Water spinach (*I. aquatica*)	0.00448	0.00068 – 0.00113	0.00025 – 0.00067
Water lettuce (*P. stratiotes*)	0.00448	0.00110 – 0.00123	0.00061 – 0.00073
Lotus (*Nymphaea* spp.)	0.00448	0.00043 – 0.00044	0.00007 – 0.00008
Water hyacinth (*E. crassipes*)	0.00448	0.00049 – 0.00050	0.00003 – 0.00004

By Day 9, all species demonstrated measurable reductions in water-phase mercury concentration. Lotus and water hyacinth had lower residual mercury concentrations than water spinach and water lettuce. By Day 14, lotus and water hyacinth showed the lowest residual concentrations, ranging from 0.00007–0.00008 mg/L and 0.00003–0.00004 mg/L, respectively. Water spinach and water lettuce also showed reductions, although their residual concentrations remained higher than those observed in lotus and water hyacinth treatments. The term "significant" is not used here unless supported by complete inferential statistics, including variance measures, ANOVA outputs, and post hoc comparisons.

Removal Effectiveness

Removal effectiveness, expressed as a percentage reduction from the baseline concentration, varied by species and sampling time. Because percentage values alone do not show replicate variability, removal effectiveness should be reported together with dispersion metrics such as standard deviation, standard error, or confidence intervals.

Table 2. Mercury Removal Effectiveness (%) at Day 14

Species	Removal Effectiveness (%)
Water hyacinth (*E. crassipes*)	99.11 – 99.33
Lotus (*Nymphaea* spp.)	98.21 – 98.44
Water spinach (*I. aquatica*)	85.04 – 94.42 (mean ≈ 89.17)
Water lettuce (*P. stratiotes*)	83.71 – 86.38 (mean ≈ 84.89)

Water hyacinth showed the highest Day 14 removal range, exceeding 99%, followed by lotus with removal values above 98%. Water spinach and water lettuce showed lower removal ranges, although both exceeded 80% by Day 14. These findings indicate a clear descriptive ranking of observed water-phase mercury reduction: water hyacinth > lotus > water spinach > water lettuce. Inferential conclusions regarding statistical differences should be supported by ANOVA or non-parametric test outputs.

DISCUSSION

The comparative analysis showed substantial interspecific variation in observed water-phase mercury reduction among the four aquatic macrophytes. Eichhornia crassipes and Nymphaea spp. produced the lowest residual mercury concentrations by Day 14, followed by Ipomoea aquatica and Pistia stratiotes. The contribution of this study is primarily applied rather than mechanistic: it provides comparative evidence on species-level performance under standardized bench-scale exposure conditions. Because abiotic controls, plant tissue mercury analysis, biomass-normalized uptake, and full mass balance were not included, mechanistic explanations should be treated as plausible interpretations rather than confirmed causal pathways.

Integration with Previous Studies and Contextual Benchmarks

The high observed removal by water hyacinth in this study, ranging from 99.11% to 99.33% by Day 14, is consistent with previous evidence identifying E. crassipes as one of the most effective floating macrophytes for heavy metal remediation (37). Controlled trials in artisanal and small-scale gold mining (ASGM)-related contexts have reported substantial reductions in aqueous mercury after short exposure periods, while reviews of floating macrophytes identify Eichhornia as a frequently studied taxon due to its high biomass and extensive root system (38).

The present findings also align with published ranges of mercury reduction achieved by aquatic macrophytes over comparable short-term exposure periods, particularly within 7–14 days. Similar hydroponic and controlled exposure trials lasting approximately 10–14 days have demonstrated measurable mercury uptake across diverse macrophyte species (22,31,32). However, previous studies often emphasize mercury accumulation in plant tissues rather than explicit reductions in water-phase mercury concentration. This distinction is important because tissue accumulation does not always directly correspond to quantified removal from the water column. Nevertheless, reported root accumulation factors ranging from approximately 100 to 270 times the initial water concentration demonstrate the strong capacity of aquatic macrophytes to accumulate mercury from contaminated aquatic environments (23). In this context, the reductions observed in the present study are consistent with the broader phytoremediation literature, but they should not be interpreted as direct confirmation of plant uptake alone because passive mercury losses were not quantified through abiotic controls. The results are best interpreted as strong plant-associated reductions in dissolved mercury under bench-scale conditions. Mercury removal or accumulation has also been reported in other aquatic macrophytes, including Ceratophyllum demersum and Elodea nuttallii (20,21). These findings support the need for species-specific comparison rather than assuming uniform remediation performance across aquatic plants. Species- and site-specific variability has been documented in previous macrophyte studies, indicating that remediation outcomes may depend on plant morphology, exposure duration, mercury speciation, water chemistry, and environmental conditions (20–22). In the present study, lotus achieved 98.21–98.44% observed water-phase mercury reduction by Day 14, placing it close to water hyacinth under the tested conditions. Although explicit data on lotus-related mercury uptake, particularly for Nymphaea spp. or Nelumbo nucifera, remain comparatively limited, its performance in this study suggests a strong but less well-documented capacity for mercury reduction. Therefore, lotus should be described as a promising candidate for further testing rather than as a confirmed high-uptake species.

Field-reported mercury concentrations, such as those documented in the Ankobra estuary, provide contextual validation for the environmental relevance of the reductions observed in this study (4). In addition, prior documentation of organ-specific uptake patterns in Balili River macrophytes emphasizes the importance of considering intra-plant variation when interpreting phytoremediation efficiency (22). Such evidence indicates that mercury distribution within plant organs, particularly between roots and shoots, should be considered in future studies to clarify whether observed water-phase reductions are mainly attributable to root accumulation, translocation, surface adsorption, or other processes.

Possible Explanations for Species-Level Differences

The descriptive ranking observed in this study may be partly related to interspecies differences in root architecture, plant surface area, biomass, and root-water contact. Water hyacinth and lotus showed lower residual mercury concentrations than water spinach and water lettuce, which is consistent with literature suggesting that root-associated processes can influence heavy metal removal by aquatic macrophytes. Mercury has been shown to accumulate predominantly in roots, and root architecture, total biomass, and root surface area may strongly influence uptake capacity (22,24,27). Rhizosphere interactions may also affect mercury transformation, including processes related to methylmercury formation and uptake, thereby influencing root-to-shoot partitioning (22,24,27).

These mechanisms, however, were not directly measured in the present experiment. Therefore, references to root morphology, rhizosphere activity, methylmercury formation, or translocation should be framed as possible explanations requiring confirmation through plant tissue analysis, root surface area quantification, biomass-normalized uptake, and mercury mass balance studies. The stronger performance of water hyacinth and lotus may plausibly reflect more extensive root-water contact and greater biomass, but this interpretation remains inferential until supported by direct morphological and tissue-based measurements.

Exposure Time and Removal Efficiency

Exposure time emerged as a key driver of observed mercury reduction. Measurable reductions occurred within 9 days, but maximal removal was observed by Day 14. This pattern is consistent with prior short-term phytoremediation trials showing time-dependent increases in mercury uptake or removal over 7–21 days (30–32,36). The progressive decline in water-phase mercury concentration suggests that longer contact between contaminated water and plant root systems may enhance removal efficiency, particularly where root-associated accumulation and adsorption processes are active.

Nevertheless, the interpretation of removal efficiencies exceeding 95% requires caution. While such values strongly suggest plant-associated mercury reduction, mass balance assessments are needed to confirm the dominant mechanisms and account for potential sorption, sedimentation, volatilization, container-wall adsorption, or changes in mercury speciation. Therefore, future studies should combine repeated water-phase measurements with plant tissue analysis, sediment analysis, and abiotic controls to determine whether mercury is primarily accumulated in plant tissues or redistributed among other compartments.

Ecological and Management Constraints of Water Hyacinth

Although water hyacinth showed the highest observed removal in this study, its field deployment requires careful ecological evaluation. E. crassipes is widely recognized as an invasive aquatic plant that can rapidly colonize waterways, alter hydrological conditions, reduce dissolved oxygen, and displace native species. These risks are especially relevant if the species is introduced into open water systems. Therefore, any applied use of water hyacinth for mercury remediation should be restricted to controlled systems such as lined ponds, enclosed treatment units, or engineered wetlands with strict containment and harvesting protocols (39).

Biomass management is also a critical consideration. Mercury-laden plant material, if left to decompose or disposed of improperly, may reintroduce mercury into aquatic systems. Regulatory requirements may also apply because contaminated biomass can constitute hazardous waste. Accordingly, high removal performance in the water phase should be evaluated together with the feasibility of harvesting, storing, transporting, treating, and safely disposing of contaminated biomass. Laboratory-scale removal percentages should not be directly generalized to field-scale systems without considering ecological containment, biomass handling, and long-term monitoring (39).

Lotus as a Viable Alternative

The close performance of lotus relative to water hyacinth suggests that lotus may offer a promising alternative, particularly in contexts where the invasiveness of E. crassipes limits its acceptability. In this study, lotus achieved 98.21–98.44% observed mercury reduction by Day 14, compared with 99.11–99.33% for water hyacinth. Beyond its treatment performance, lotus may have cultural and ecological advantages in several Asian settings, where it has symbolic, ornamental, and utilitarian value (40,41). These characteristics may improve community acceptance if lotus-based remediation is developed in participatory and locally governed treatment systems.

Although literature specifically addressing lotus and mercury uptake remains limited, evidence from broader phytoremediation studies suggests that lotus may contribute to contaminant reduction under suitable conditions (38). The present study supports further evaluation of lotus as an applied candidate for ASGM wastewater treatment, but it does not establish the mechanism of mercury removal. Future studies should assess lotus biomass, root surface area, plant tissue mercury accumulation, root-to-shoot translocation, and removal performance under varying effluent chemistry. As with any field application, containment infrastructure and ecological risk assessment remain necessary.

Field-Scale Implications for ASGM Remediation

The results have applied implications for designing pilot-scale remediation interventions in ASGM-impacted regions. Engineered wetlands, lined ponds, or enclosed treatment units may provide practical systems for containing macrophytes, limiting ecological spread, and allowing scheduled biomass harvesting. However, the present bench-scale findings should be translated cautiously because field systems are influenced by hydraulic retention time, sediment load, pH, dissolved organic matter, competing ions, fluctuating mercury speciation, and seasonal environmental variation (37).

Biomass management must be prioritized in any field-scale application. Options such as controlled drying, stabilization, thermal treatment, or conversion to biochar have been discussed as possible pathways for managing contaminated biomass (42). However, any valorization approach must be evaluated carefully because mercury-contaminated biomass may pose secondary exposure risks. Composting or reuse as soil amendment should not be recommended unless mercury stabilization, leaching risk, and regulatory compliance have been demonstrated. The accumulation of mercury in plant tissues represents both a remediation success and a management challenge, making safe post-harvest handling essential for preventing secondary contamination (14,24,26).

Rigorous monitoring is indispensable for field translation. Atomic Absorption Spectrophotometry provides a widely used analytical platform for quantifying mercury concentrations in water and plant tissues (22). Monitoring should include not only dissolved mercury in water, but also mercury in plant roots, shoots, sediments, and residual biomass to establish a more complete mass balance. Analytical reporting should include detection limits, quantitation limits, recovery efficiency, calibration statistics, and precision estimates.

The sustainability of phytoremediation interventions also depends on governance and stakeholder engagement. Participatory approaches involving local communities, mining groups, environmental authorities, and public health institutions may enhance legitimacy and ensure that remediation strategies are adapted to local socio-ecological conditions (41). For ASGM settings, phytoremediation should be integrated with broader mercury reduction policies, safer mining practices, exposure monitoring, and community health protection measures rather than treated as a stand-alone solution.

Limitations and Future Research

Several limitations should be considered when interpreting this study. First, the absence of plant-free abiotic controls prevents clear separation of plant-mediated removal from passive processes such as sorption, sedimentation, volatilization, or container-wall adsorption. Second, plant biomass, dry weight, plant density, root length, and root surface area were not explicitly quantified, limiting reproducibility and preventing biomass-normalized uptake modeling. Third, the physicochemical profile of the effluent, including pH, conductivity, dissolved oxygen, organic matter, and competing ions, was not comprehensively reported, restricting interpretation of mercury speciation and cross-study comparability. Fourth, plant tissue mercury concentrations were not measured, so internal uptake, root accumulation, and translocation cannot be confirmed. Fifth, analytical validation parameters, including detection limit, quantitation limit, recovery, calibration statistics, and instrumental precision, should be fully reported to support confidence in very low residual mercury values.

Future studies should include abiotic controls, plant-free controls, and, where relevant, dead-biomass controls to distinguish biological uptake from passive removal. Experiments should also quantify fresh and dry biomass, root surface area, and mercury accumulation in roots and shoots. Full effluent characterization should be performed before and during exposure, including pH, conductivity, dissolved oxygen, temperature, organic matter, suspended solids, and competing ions. Future analyses should report mean ± standard deviation, confidence intervals, effect sizes, ANOVA or non-parametric test outputs, and visualizations with error bars. Field-scale studies are also needed to

validate whether the high bench-scale removal observed here can be sustained under realistic ASGM wastewater conditions.

CONCLUSION

This study compared four aquatic macrophytes—*Ipomoea aquatica*, *Pistia stratiotes*, *Nymphaea* spp., and *Eichhornia crassipes*—for their observed water-phase mercury reduction in artisanal and small-scale gold mining (ASGM) liquid waste. Over the 14-day exposure period, all plant-exposed treatments showed marked reductions in dissolved mercury concentration. Water hyacinth demonstrated the highest observed removal, approximately 99.11–99.33%, followed closely by lotus at approximately 98.21–98.44%. Water spinach and water lettuce showed lower but still notable reductions, exceeding 80% by Day 14. Because complete inferential statistics and abiotic controls were not included in the present reporting, these findings should be interpreted as descriptive evidence of comparative treatment performance rather than definitive proof of statistically significant plant uptake.

The findings indicate that mercury reduction increased over time, with lower residual concentrations observed at Day 14 than at Day 9. However, the mechanism of removal cannot be attributed solely to plant uptake because plant tissue mercury accumulation, root surface area, biomass-normalized uptake, and plant-free abiotic controls were not assessed. Therefore, factors such as root morphology, biomass, rhizosphere interactions, sorption, sedimentation, and volatilization should be considered possible explanatory factors rather than confirmed mechanisms. The study contributes an applied side-by-side comparison of four aquatic macrophytes under standardized bench-scale conditions and provides preliminary benchmarks for selecting candidate species in ASGM wastewater phytoremediation research.

Beyond environmental remediation, these findings have potential public health relevance because reducing mercury contamination in aquatic systems may help limit downstream exposure pathways associated with methylmercury formation and fish consumption. Nevertheless, direct health risk reduction was not measured in this study and should not be inferred without additional exposure, food-chain, and biomonitoring data. Future research should validate these results under field conditions, include plant-free and abiotic controls, quantify fresh and dry biomass, assess mercury accumulation in plant tissues, characterize effluent physicochemistry, report analytical validation parameters, and develop safe biomass management strategies. Such work is needed to support durable, ecologically contained, and community-centered remediation approaches for ASGM-impacted waters.

AUTHOR'S CONTRIBUTION STATEMENT

Nur Rismawati contributed to the conceptualization of the study, research design, field data collection, laboratory observation coordination, data analysis, interpretation of findings, and preparation of the original manuscript draft. She also served as the corresponding author and was responsible for manuscript organization, revision, and final submission.

Hamidah contributed to the development of the research methodology, supervision of the phytoremediation experiment, validation of research procedures, interpretation of environmental health implications, and critical review of the manuscript. She also provided intellectual input in refining the discussion and ensuring the scientific coherence of the article.

Abdullah Rahman Zain contributed to the selection and characterization of aquatic macrophytes, technical guidance on plant-based remediation processes, interpretation of phytoremediation performance, and review of the manuscript from the perspective of agricultural and environmental sciences.

All authors contributed substantially to the research process and manuscript development. All authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest related to this study. There are no financial, personal, institutional, or professional relationships that could have influenced the design, implementation, analysis, interpretation, or reporting of the research findings.

The authors further confirm that the study was conducted independently and objectively, without any undue influence from external parties, organizations, or funding bodies that may affect the integrity and impartiality of the research. All authors are responsible for ensuring the accuracy, transparency, and credibility of the manuscript.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used generative AI and AI-assisted technologies namely ChatGPT to support language refinement, improve clarity, enhance readability, and assist in the organization of manuscript structure. These tools were used solely to improve the quality of academic writing and did not contribute to the generation of research data, data analysis, interpretation of results, or formulation of scientific conclusions.

All scientific content, research design, methodology, findings, interpretation, and conclusions presented in this manuscript remain the full responsibility of the authors. The authors carefully reviewed, edited, and verified all AI-assisted outputs to ensure accuracy, originality, academic integrity, and compliance with ethical publication standards.

The authors affirm that the use of AI-assisted technologies did not replace human authorship, critical judgment, or scholarly responsibility in the development of this manuscript.

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