

## PHYSIOLOGICAL AND BIOCHEMICAL BIOMARKERS OF MACROPHYTE RESILIENCE TO MILITARY-RELATED TOXIC STRESSORS

Illia Tsyhanenko-Dziubenko<sup>1</sup> , Hanna Kireitseva<sup>1</sup> , Joana Fonseca Araújo<sup>2</sup> <sup>1</sup> Zhytomyr Polytechnic State University,  
103, Chudnivska Str., Zhytomyr, 10005, Ukraine,<sup>2</sup> AmbiTerra Laboratory, Institute of Earth Science (IES), University of Évora,  
Apartado 94, 7006-554, Évora, Portugal  
ke\_miyu@ztu.edu.ua<https://doi.org/10.23939/ep2024.04.227>

Received: 25.09.2024

© Tsyhanenko-Dziubenko I., Kireitseva H., Fonseca Araújo J., 2024

**Abstract.** This study employs state-of-the-art analytical tools to investigate the ecotoxicological impacts of mercury contamination on aquatic macrophytes in post-military zones, focusing on *Typha latifolia* and *Lemna minor* as model organisms. The research methodology integrates multiple analytical techniques: spectrophotometric chlorophyll quantification using the Holm-Wettstein method, atomic absorption spectrometry utilizing a C-115PK Selmis spectrometer (precision  $\pm 0.001$  mg/L), and fluorescence analysis via Fluorat-02-Panorama spectrofluorometer. Through this comprehensive analytical approach, we elucidated the bioaccumulation patterns and physiological responses of these hydrophytes to varying concentrations of mercury (0.35–2.0 mg/L). Results demonstrated differential bioaccumulation capacities between *T. latifolia* and *L. minor*, with the latter exhibiting higher mercury sequestration potential (0.51 mg/kg vs 0.4 mg/kg dry weight). Concentration-dependent phytotoxic effects were observed, manifesting as morphological alterations, chlorophyll degradation, and disruption of photosynthetic processes. Notably, a consistent increase in the chlorophyll b to chlorophyll a ratio was documented, indicative of selective degradation of photosystem II under mercury stress. The study further revealed the inactivation of key Calvin cycle enzymes, leading to attenuated carbon fixation and overall photosynthetic capacity. These findings not only elucidate the mechanistic underpinnings of mercury toxicity in aquatic macrophytes but also underscore their potential as bioremediators in mercury-contaminated aquatic ecosystems. The research provides critical insights for developing targeted phytoremediation strategies and ecosystem restoration protocols in post-military aquatic environments.

**Keywords:** environmental safety, water resources, mercury bioaccumulation, aquatic macrophytes, photosynthetic inhibition, post-military ecosystem restoration.

## 1. Introduction

Contemporary military operations constitute a complex multifactorial environmental stressor that generates profound perturbations in ecosystem homeostasis. The affected ecosystems, characterized by their intricate biogeochemical cycles and trophic interactions, exhibit systemic responses to anthropogenic perturbations. Therefore, a holistic, multimetric approach is imperative for quantifying and ameliorating the environmental sequelae of military activities, necessitating the integration of multiple ecological indicators and biomonitoring methodologies.

The exigent nature of this research is underscored by recent empirical investigations in the Kyiv region of Ukraine, which have documented significant heavy metal contamination in aquatic ecosystems. Maksymenko et al. (2022) conducted comprehensive hydrochemical analyses of water ecosystems in Moschun, revealing elevated concentrations of transition metal ions ( $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ) and amphoteric metal species ( $\text{Al}^{3+}$ ) in both the aqueous phase and benthic sediments. Subsequent investigations by Tsyhanenko-Dziubenko et al. (2023) elucidated the spatio-temporal dynamics of heavy metal distribution in urban hydrotopes, demonstrating differential accumulation patterns between recreational and aquacultural water bodies.

The anthropogenic introduction of heavy metal contaminants into aquatic matrices during military

operations can be attributed to multiple vectors, including infrastructure degradation, munitions deployment, and improper waste management protocols (Kireitseva et al., 2023). These findings emphasize the critical necessity for implementing comprehensive biomonitoring and ecological restoration strategies in conflict-affected regions.

Mercury ( $\text{Hg}^{2+}$ ) represents a particularly problematic xenobiotic due to its exceptional toxicity and bioaccumulation potential. The incorporation of mercury in military ordnance, including explosive devices and artillery components, has been extensively documented. Upon detonation, these materials release mercury species into various environmental compartments, initiating complex biogeochemical cycling processes. Aquatic ecosystems exhibit particular vulnerability to mercury contamination due to enhanced bioaccumulation and biomagnification phenomena within aquatic food webs. Macrophytes, as primary producers and ecosystem engineers, play pivotal roles in aquatic biogeochemistry and community structure. However, the ecotoxicological impacts of mercury on these crucial ecosystem components remain insufficiently characterized.

The environmental fate of mercury involves multiple transport pathways, including atmospheric deposition, surface water transport, and soil leaching processes. In aquatic systems, mercury undergoes complex biotransformation processes, with methylmercury ( $\text{CH}_3\text{Hg}^+$ ) representing the most bioavailable and toxic species. This organometallic compound exhibits enhanced bioaccumulation potential and demonstrates significant biomagnification across trophic levels, posing substantial risks to apex predators and human populations consuming contaminated aquatic organisms (Fliedner et al., 2016).

The present investigation aims to establish a robust scientific framework for understanding mercury-macrophyte interactions in post-military environments, potentially serving as a cornerstone for developing minimally invasive rehabilitation strategies for contaminated hydrotopes. By elucidating the physiological and biochemical responses of aquatic macrophytes to mercury stress, this research provides essential baseline data for designing evidence-based phytoremediation protocols and ecosystem restoration methodologies.

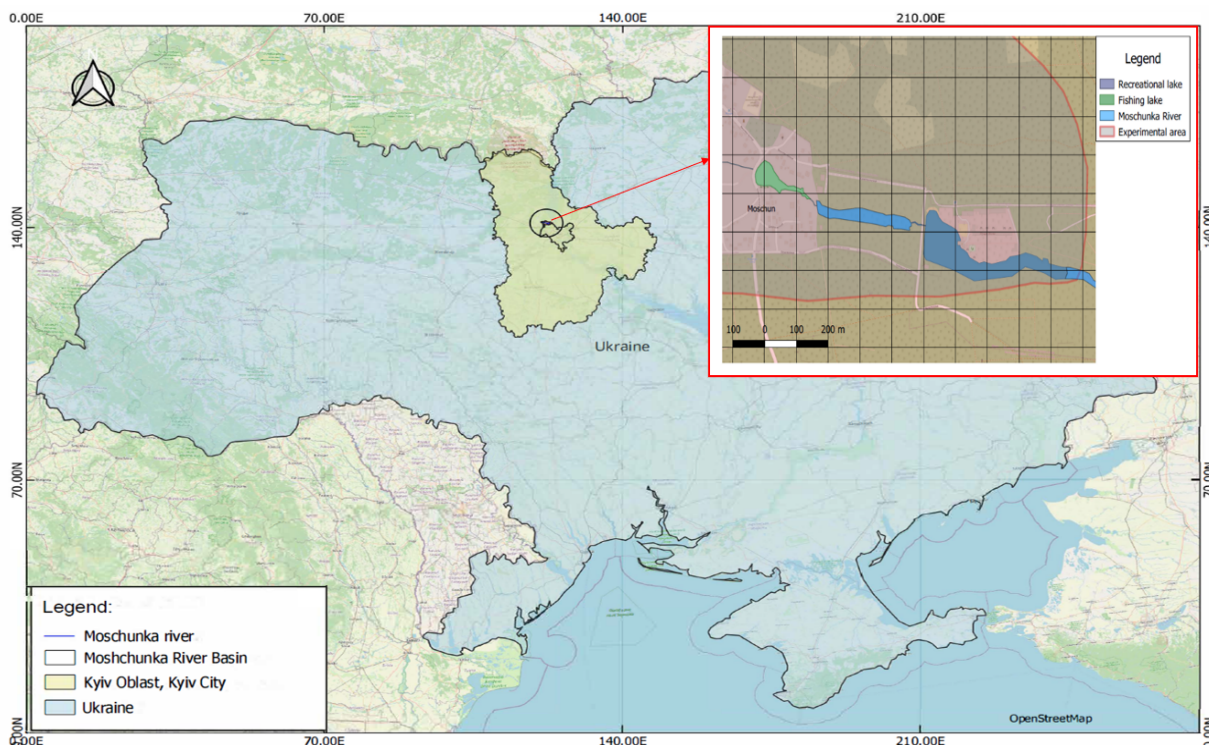
The unprecedented scale of contemporary military operations in Ukraine has created an urgent and critical need for effective methodologies to assess and remediate contaminated aquatic ecosystems. This research addresses this pressing environmental chal-

lenge by providing a comprehensive analytical framework for understanding mercury-macrophyte interactions in post-military environments. The significance of this investigation is particularly heightened by the current lack of standardized protocols for evaluating and remediating military-impacted aquatic ecosystems. Our findings will serve as a foundational scientific basis for developing and implementing highly efficient, minimally invasive recultivation strategies for post-military hydrotopes. This research not only advances our understanding of ecotoxicological processes but also provides practical, nature-based solutions for ecosystem restoration. The methodological approaches and empirical data generated through this study will enable environmental managers and restoration practitioners to design targeted, cost-effective rehabilitation protocols that leverage natural biological processes for the remediation of contaminated aquatic systems. Furthermore, this work establishes a replicable framework for assessing and mitigating heavy metal contamination in conflict-affected regions, potentially serving as a model for future restoration efforts in similar post-military environments worldwide.

This study employs a comprehensive analytical approach integrating field surveys and laboratory experimentation. The methodology encompasses: (1) geobotanical field investigations utilizing route-expedition methods for macrophyte identification and ecological assessment; (2) spectrophotometric analysis of chlorophyll content using the Holm-Wettstein method; (3) atomic absorption spectrometry (C-115PK Selmi) for heavy metal quantification; (4) fluorescence spectroscopy (Flyuorat-02-Panorama) for photosynthetic efficiency evaluation; and (5) statistical analyses employing Student's t-test, Bartlett's test, and Fisher's F-test ( $\alpha = 0.05$ ). This multi-analytical framework enables detailed characterization of mercury-induced physiological and biochemical perturbations in aquatic macrophytes, facilitating the development of targeted phytoremediation strategies for post-military aquatic ecosystem restoration.

## 2. Materials and Methods

To select the most representative plant test object for further toxicological experiments, field studies were conducted in post-military areas. The research materials consist of geobotanical descriptions based on the generally accepted route-expedition method in the water bodies of Moshchun, Kyiv region, in 2022 and 2023 (Fig. 1).



**Fig. 1.** Water bodies within the experimental zone near the village of Moschun, Kyiv region, Ukraine

As a result of the work of the expert group, the most important internal (Table 1) and external factors (Table 2) that affect the use and conservation of water resources in Zhytomyr region were identified and evaluated on a 7-point Miller scale (1 – minimum value, 7 – maximum value).

After field identification of the most optimal macrophyte that could be used in further toxicological experiments (among the identified dominants, subdominants, and associated species of the studied higher aquatic vegetation), a series of toxicological experiments was conducted. The quantitative determination of chlorophylls a and b content in the leaves of aquatic macrophytes was performed using the spectrophotometric method in acetone extract, employing the Holm-Wettstein equations, which consider the contribution of both pigments to the total absorption at wavelengths of 662 and 644 nm. The elemental composition of prepared samples was determined utilizing spectroscopic methods, enabling simultaneous multi-element analysis within a single specimen. This analytical approach is predicated on the examination of atomic absorption and fluorescence spectra in the optical domain, as well as emission and fluorescence spectra in the X-ray region. In all instances, these methodologies are fundamentally based on quantum transitions of valence or inner electrons within atoms between distinct energy states. Quantification of the element of interest is achieved by

measuring the intensity of specific spectral lines. To generate an emission spectrum, additional energy must be imparted to the analyte particles. To this end, during spectral analysis, the sample is introduced into a light source where it undergoes heating and vaporization, with gaseous-phase molecules dissociating into atoms that, upon collision with electrons, transition to excited states. Atoms remain in these excited states for approximately  $10^{-7}$  to  $10^{-8}$  seconds. Upon spontaneous return to their ground or intermediate states, they emit excess energy in the form of light quanta.

For the spectral analysis of Hg ions, plant samples were meticulously rinsed, desiccated to an air-dry state, then to an absolutely dry state, and subsequently incinerated to white ash in a muffle furnace at 450 °C. Heavy metal content in the plant ash was determined using a C-115PK Selmi atomic absorption spectrometer (Ukraine). Metal concentrations were expressed in milligrams per kilogram of dry sample mass.

To assess the status and productivity of plant systems, as well as the resilience of their photosynthetic apparatus to adverse environmental factors, fluorescence-based investigative methods were employed, founded on intensity variations of specific substances. For the determination of chlorophyll a and b concentrations, pigments were extracted from leaf blade samples using anhydrous diethyl ether, and fluorescence spectra were recorded. Luminescence analysis was conducted using a Flyuorat-02-Panorama

spectrofluorometer at three wavelengths: 600 nm (background level), 646 nm (chlorophyll b fluorescence), and 666 nm (chlorophyll a fluorescence).

To investigate chlorophyll content in plants under heavy metal stress, 0.5 g of fresh plant material was utilized. The sample was triturated in a porcelain mortar under cold conditions with the addition of chalk to neutralize acidic environments and a 10-fold acetone solution. Subsequently, the solution was centrifuged at 5000 rpm for 5 minutes. The resultant extract from the centrifuge tube was decanted into a graduated cylinder and brought to a volume of 10 mL (the extract was diluted to achieve an optical density (E) between 0.1 and 0.8 at  $\lambda_{max}$ ).

Concentration calculations were performed using the Holm-Wettstein formula (mg) (1–2):

$$C_{ch.a}=9,784 \times E_{662} - 0,990 \times E_{644}; \quad (1)$$

$$C_{ch.b}=21,426 \times E_{644} - 4,650 \times E_{662}. \quad (2)$$

Subsequently, the pigment content in the investigated material was determined, taking into account the extract volume and mass (3):

$$A = \frac{C \cdot V}{P \cdot 1000}, \quad (3)$$

where  $A$  is the pigment content, expressed in mg/g of fresh mass;  $C$  is the chlorophyll concentration, expressed in mg/L;  $V$  is the volume of extract, measured in mL;  $P$  is the initial mass of plant material, expressed in g of fresh mass.

Experimental results underwent statistical analysis utilizing the integrated statistical functions and the “Data Analysis” add-in of Microsoft Office Excel 2010. Empirical significance criteria (Student’s  $t$ -test, Bartlett’s test, Fisher’s  $F$ -test,  $\chi^2$  test, and others) were juxtaposed with tabulated values at the corresponding degrees of freedom and significance level  $\alpha = 0.05$ .

### 3. Results and Discussion

Based on the results of the research conducted in the post-military area of the Bucha district, Kyiv

region, within the Moshchunka River, 9 phytocenoses were identified, which were later classified as 9 associations united into 4 alliances, 3 orders, and 3 classes of vegetation communities. Among the identified communities, the association *Typhetum angustifoliae* Pignatti 1953 was present, belonging to the alliance *Phragmition* Koch 1926, order *Phragmitetalia* Koch 1926, and class *Phragmiti-Magnocaricetea* Klika et Novak 1941.

Furthermore, the phytosociological analysis revealed the prevalence of *Typha latifolia* L. in the investigated post-military site. The dominance of this helophytic macrophyte indicates the presence of nutrient-rich, hypertrophic conditions in the riparian ecotones of the Moshchunka River. The luxuriant growth of *T.latifolia* L. can be attributed to the anthropogenic eutrophication processes, likely exacerbated by the residual effects of military activities in the region.

The occurrence of *T.latifolia* L. dominated communities in this area underscores the need for further research on the ecological succession patterns and biogeochemical cycling in post-military landscapes. Understanding the synecological dynamics of these phytocenoses can provide valuable insights into the resilience and regenerative potential of war-affected ecosystems.

In conclusion, the post-military site in the Bucha district, Kyiv region, within the Moshchunka River, was characterized by the presence of riparian vegetation dominated by the broadleaf cattail (*T.latifolia* L.), indicative of eutrophic habitat conditions. This finding highlights the importance of studying the ecological implications of military disturbances on aquatic and semi-aquatic ecosystems.

To evaluate the absorptive capacity of *T.latifolia* and *L.minor*, a mercury salt solution ( $Hg(NO_3)_2$ ) with a concentration of 1 mg/L was prepared. The investigation was conducted over a 5-day period under natural illumination conditions at an ambient temperature of +24 – 25 °C.

The temporal variation in mercury ion concentration is presented in Table 1.

Table 1

Temporal Fluctuation of Mercury Ion Concentration in Experimental Containers with *T.latifolia* and *L.minor*

| Macrophyte         | Mercury ion concentration (mg/L) |             |             |             |             |
|--------------------|----------------------------------|-------------|-------------|-------------|-------------|
|                    | Day 1                            | Day 2       | Day 3       | Day 4       | Day 5       |
| <i>L.minor</i>     | 1.00 ± 0.05                      | 0.78 ± 0.04 | 0.65 ± 0.03 | 0.43 ± 0.02 | 0.53 ± 0.03 |
| <i>T.latifolia</i> | 1.00 ± 0.05                      | 0.75 ± 0.04 | 0.70 ± 0.04 | 0.50 ± 0.03 | 0.55 ± 0.03 |

The tabulated data elucidate that mercury nitrate is rapidly sequestered by the phytomass. Residual concentrations on the fifth day exhibit a notable increase in both Typha and Lemna, potentially attributable to the reverse diffusion process of metal-ligand complexes from the plant tissues into the aqueous solution.

The experimental data reveal differential mercury accumulation patterns between two aquatic macrophyte species, *T.latifolia* and *L.minor*. Quantitative analysis of heavy metal content, specifically mercury (Hg<sup>2+</sup>), demonstrates that *L.minor* exhibits a higher bioaccumulation capacity compared to *T.latifolia*. The mercury concentration in *L.minor* tissues reached 0.51 mg/kg dry weight, whereas *T.latifolia* accumulated 0.4 mg/kg dry weight under identical exposure conditions. This disparity in mercury sequestration efficacy between the two species is noteworthy, with *L.minor* demonstrating approximately 27.5 % greater mercury retention than *T.latifolia*. These findings suggest species-specific variations in metal uptake mechanisms, potentially attributable to differences in physiological adaptations, root structure, or cellular detoxification processes. The enhanced bioaccumulation observed in *L.minor* underscores its potential utility in phytoremediation strategies for mercury-contaminated aquatic ecosystems, while also highlighting the importance of species selection in biomonitoring and ecological risk assessment protocols.

During the investigation of metal concentrations in *T.latifolia* and *L.minor*, it was ascertained that *Lemna* demonstrates a higher propensity for heavy metal bioaccumulation compared to *Typha*. Contemporary literature corroborates that the aforementioned species hold significant importance in bioindication processes for heavy metal contamination levels in abiotic ecosystems under anthropogenic stress (Kyrychuk et al., 2024; Pasichna et al., 2023; Tsyhanenko-Dziubenko et al., 2024). The experimental data obtained substantiate that both free-floating and rooted vascular hydrophytes capable of intensive metal accumulation possess bioindication value. Concurrently, there exist discernible disparities in pollutant accumulation processes among plants of diverse taxonomic and ecological groups.

At the maximum concentration (1 mg/L), the plants exhibited severe phytotoxic effects, resulting in complete disintegration of all rosettes, ultimate root loss, and near-total tissue necrosis.

Furthermore, throughout the experimental duration, a reduction in the width and length of *Lemna* minor fronds was observed, indicating that elevated mercury concentrations in the plant negatively impact growth, proliferation, and developmental processes.

The impact of mercury ions (Hg<sup>2+</sup>) on *Lemna* minor is delineated in Table 2, while the effect on *Typha latifolia* is delineated in Table 3.

Table 2

**Alterations in Morphometric Parameters of *Lemna* Under the Influence of Varying Mercury Concentrations**

| Mercury concentration (mg/L) | Time from start of experiment | Rosette division | Root shedding | Necrosis | Chlorosis |
|------------------------------|-------------------------------|------------------|---------------|----------|-----------|
| 0                            | 5d                            | –                | –             | –        | –         |
| 0.35                         | 5d                            | +                | +             | +        | +         |
| 0.51                         | 5d                            | ++               | ++            | ++       | ++        |
| 2                            | 5d                            | +++              | ++            | +++      | +++       |

Table 3

**Alterations in Morphometric Parameters of *Typha* Under the Influence of Varying Mercury Concentrations**

| Mercury concentration (mg/L) | Time from start of experiment | Root system size (cm <sup>2</sup> ) | Stem size (cm <sup>2</sup> ) |
|------------------------------|-------------------------------|-------------------------------------|------------------------------|
| 0                            | 5d                            | 200                                 | 50                           |
| 0.35                         | 5d                            | 150                                 | 45                           |
| 0.51                         | 5d                            | 100                                 | 20                           |
| 2                            | 5d                            | 70                                  | 10                           |

*Lemna* assimilates mercury from the aqueous medium. Partial adverse effects are discernible at a

concentration of 0.35 mg/L. At a concentration of 2 mg/L, the plants experienced severe phytotoxicity,

resulting in complete disintegration of all rosettes, ultimate root loss, and near-total tissue necrosis. Furthermore, a lightening of the *Lemna* fronds is observed, attributed to the degradation of chlorophyll molecules in plant cells, manifesting as depigmentation and chlorosis phenomena.

*Typha* accumulates mercury from sedimentary deposits. With increasing concentrations, inhibition of rhizome growth in *Typha latifolia* is observed across all investigated mercury concentrations. Growth retardation is also characteristic of the stem. Thus, the

degree of mercury's toxic effect on plants is directly proportional to the quantity of  $\text{Hg}^{2+}$  accumulation within the plant.

To investigate the impact of heavy metals on the photosynthetic activity of aquatic macrophytes, aqueous solutions of  $\text{Hg}(\text{CH}_3\text{COO})_2$  at concentrations of 0.03, 0.07, and 0.11 mg/L, prepared by serial dilution, were utilized. The study was conducted over a five-day period under natural illumination conditions at an ambient temperature of +24 – 25 °C. Upon completion, fluorescence intensity was measured. The results are graphically represented in Fig. 2.

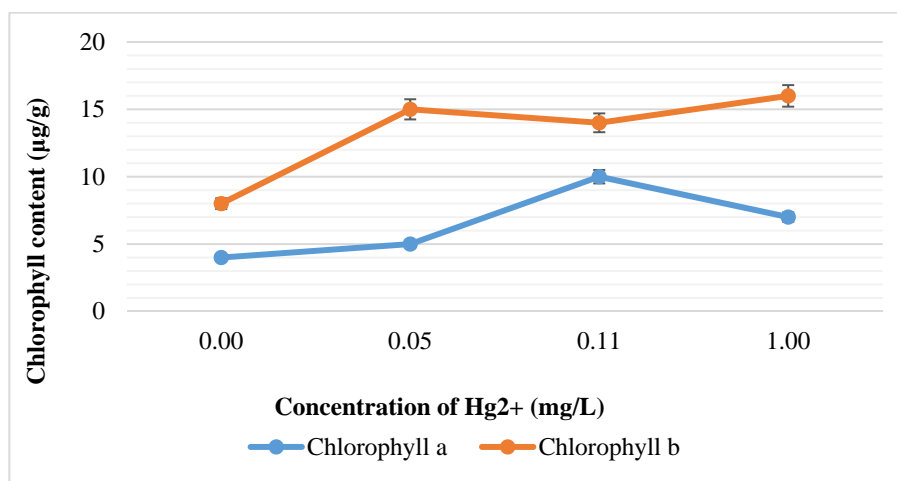


Fig. 2. Correlation between Chlorophyll a and Chlorophyll b Fluorescence Intensity in *L. minor* as a Function of  $\text{Hg}^{2+}$  Acetate Concentration

In the presence of  $\text{Hg}^{2+}$  ions, the quantity of chlorophyll b relative to chlorophyll a exhibits a consistent increase. This phenomenon corroborates the disruption of chlorophyll biosynthesis in the presence of cobalt and mercury salts, wherein these metal ions displace  $\text{Mg}^{2+}$  in the chlorophyll molecules. The reduction in chlorophyll a content is a frequent indicator of heavy metal toxicity.

Consequently, heavy metals exert a deleterious effect on the light reactions of photosynthesis and the structural integrity of photosystems. Data pertaining to

the alteration in the chlorophyll a/b ratio substantiate the preferential degradation of chlorophyll a relative to chlorophyll b under metal stress conditions.

Upon exposure to mercury, the chlorophyll content in *Typha* leaves demonstrates a decline commencing from the third day, as evidenced by the manifestation of yellowing on plant leaves – chlorosis. Thus, Hg impacts the photosynthetic processes of *Typha latifolia*, disrupting both light-dependent and light-independent reactions. The composition of photosynthetic pigments in the plant leaves is delineated in Table 4.

Table 4

**Photosynthetic Pigment Content in *T. latifolia* Leaves under  $\text{Hg}^{2+}$  Ion Contamination, Expressed in mg/g Dry Mass**

| $\text{Hg}^{2+}$ concentration (mg/L) | Chlorophyll a     | Chlorophyll b     | Ratio a/b         |
|---------------------------------------|-------------------|-------------------|-------------------|
| 0 (Control)                           | 1.29600 ± 0.06480 | 0.38515 ± 0.01926 | 3.36258 ± 0.16813 |
| 0.35                                  | 1.21897 ± 0.06095 | 0.37514 ± 0.01876 | 3.27047 ± 0.16352 |
| 0.51                                  | 1.07509 ± 0.05375 | 0.29299 ± 0.01465 | 3.70008 ± 0.18500 |
| 2                                     | 1.06154 ± 0.05308 | 0.31353 ± 0.01568 | 3.78847 ± 0.18942 |



Analysis of the plant pigment complex revealed heightened sensitivity to mercury ions, corroborated by a diminution in the assimilatory capacity of *Typha latifolia* foliar tissues. Furthermore, the data elucidate that the magnitude of mercury-induced contamination effects exhibits a concentration-dependent relationship with the aforementioned ions.

According to various researchers (Pasichna et al., 2023), the observed attenuation in plant assimilation intensity within mercury-contaminated aqueous solutions is attributed to the inactivation of Calvin cycle enzymes integral to carbon reduction processes. This mechanistic explanation posits that mercury ions interfere with the catalytic functionality of key carboxylation and regeneration phase enzymes, thereby impeding the efficacy of the carbon fixation pathway and subsequently diminishing overall photosynthetic capacity.

#### 4. Conclusions

1. The present investigation has elucidated several key findings regarding mercury contamination impacts in post-military aquatic ecosystems. Through systematic analysis of our research variables – mercury concentration (0.35–2.0 mg/L), bioaccumulation patterns, photosynthetic responses, and morphological alterations – we have established comprehensive insights into macrophyte-mercury interactions. The study revealed significant differential mercury sequestration between species, with *L.minor* exhibiting 27.5 % higher bioaccumulation (0.51 mg/kg dry weight) compared to *T.latifolia* (0.4 mg/kg dry weight), demonstrating species-specific uptake efficiencies.

2. Our experimental analyses established clear concentration-dependent toxicity patterns, where exposure to varying mercury concentrations (0.35–2.0 mg/L) produced a gradient of physiological responses in both species. The photosynthetic machinery showed particular sensitivity to mercury stress, evidenced by significant alterations in chlorophyll a:b ratios (3.36258 to 3.78847) with increasing mercury exposure. These biochemical changes were accompanied by quantifiable morphological responses, including dramatic reductions in root system size (200 to 70 cm<sup>2</sup>) and stem dimensions (50 to 10 cm<sup>2</sup>).

3. The impact of this research extends beyond theoretical understanding, providing validated biomonitoring protocols for post-military aquatic ecosystems and establishing quantitative thresholds for mercury toxicity in key macrophyte species. Furthermore, our findings deliver species-specific phytoremediation

efficiency metrics and mechanistic insights into mercury-induced photosynthetic disruption. This comprehensive dataset establishes a robust scientific foundation for implementing evidence-based restoration strategies in mercury-contaminated post-military aquatic environments.

#### Recommendations:

1. Based on our experimental findings, we recommend prioritizing *L.minor* for phytoremediation initiatives due to its superior bioaccumulation capacity. Implementation should follow a sequential harvesting protocol at 4-day intervals to optimize metal removal efficiency, while maintaining mercury concentrations below 0.35 mg/L to ensure sustained remediation effectiveness. These protocols should be integrated into a comprehensive monitoring system that includes regular chlorophyll fluorescence measurements as an early warning mechanism for ecosystem stress.

2. For effective ecosystem management, we propose developing integrated approaches that combine both floating and rooted macrophytes to maximize remediation efficiency. This should be coupled with regular biomass harvesting to prevent mercury rerelease into the aquatic system. The establishment of buffer zones around contaminated areas is crucial to prevent mercury migration and protect adjacent ecosystems from contamination.

3. Future research directions should focus on investigating seasonal variations in mercury uptake efficiency and examining potential synergistic effects with other military-related contaminants. The development of automated monitoring systems for large-scale implementation would significantly enhance the practical application of these findings. These recommendations provide environmental managers and policymakers with a structured framework for implementing effective post-military ecosystem restoration strategies.

4. The integration of these conclusions and recommendations offers a comprehensive approach to addressing mercury contamination in post-military aquatic environments, combining theoretical understanding with practical implementation strategies for ecosystem restoration and management.

5. The findings from this research serve as a pioneering scientific foundation and catalyst for developing highly efficient, minimally invasive recultivation strategies for post-military hydrotopes. Our methodological framework and empirical data provide essential baseline parameters for designing site-specific restoration protocols that maximize remediation efficiency while minimizing ecosystem

disruption. The elucidated species-specific responses and toxicity thresholds enable environmental practitioners to implement precision-based phytoremediation strategies, utilizing natural biological processes for ecosystem recovery. This approach represents a significant advancement in sustainable restoration practices, offering a cost-effective and environmentally sensitive alternative to traditional remediation methods. Furthermore, the established protocols can be adapted and scaled for various post-military aquatic environments, providing a versatile template for future restoration projects. This research thus bridges the gap between theoretical understanding and practical implementation, facilitating the development of evidence-based, minimally invasive recultivation strategies for contaminated military sites worldwide.

## References

- Alpatova, O., Maksymenko, I., Patseva, I., Khomiak, I., & Gandziura, V. (2022). Hydrochemical state of the post-military operations water ecosystems of the Moschun, Kyiv region. *16th International Conference Monitoring of Geological Processes and Ecological Condition of the Environment* (pp. 1–5). doi: <https://doi.org/10.3997/2214-4609.2022580145>
- Gandziura, V. P., Afanasyev, S. O., & Biedunkova, O. O. (2023). The concept of hydroecosystems' health (a review). *Hydrobiological Journal*, *59*(2), 3–17. doi: <https://doi.org/10.1615/hydrobj.v59.i2.10>
- Institute for the Study of War. (2022). *Interactive map: Russia's invasion of Ukraine*. Retrieved from <https://storymaps.arcgis.com/stories/36a7f6a6f5a9448496de641cf64bd375>
- Janowicz, L. M., & Stadniczenko, A. P. (2018). Symptomatic complex of the poisoning of *Planorbarius corneus* (Mollusca, Gastropoda, Pulmonata) with chrom (III)-sulfate of the water environment. *Biology and Ecology*, *4*(2), 100–105. doi: <https://doi.org/10.5281/zenodo.2368735>
- Khilchevskiy, V. K., Grebin, V. V., & Bolbot, H. V. (2022). River basins districts of Ukraine – Comparison with the map of Russia's armed aggression (Summer 2022). *16th International Conference Monitoring of Geological Processes and Ecological Condition of the Environment* (pp. 1–5). doi: <https://doi.org/10.3997/2214-4609.2022580017>
- Kireitseva, H., Šerevičienė, V., Zamula, I., & Khrutba, V. (2024). Internal and external factors of use and conservation of water resources in Zhytomyr region. *Journal Environmental Problems*, *9*(1), 43–50. doi: <https://doi.org/10.23939/ep2024.01.043>
- Kyrychuk, G. Ye., Muzyka, L. V., & Mykula, M. M. (2024). Peculiarities of low concentrations of heavy metal ions' effect on  $\beta$ -carotene content in *Lymnaea stagnalis*. *Hydrobiological Journal*, *60*(4), 72–84. doi: <https://doi.org/10.1615/HydrobJ.v60.i4.50>
- Pasichna, O. O., Gorbatiuk, L. O., Platonov, M. O., Burmistrenko, S. P., & Godlevska, O. O. (2023). Biomonitoring of heavy metals pollution in lakes of Kyiv (Ukraine) using submerged macrophytes and assessment of their phytoremediative potential. *Hydrobiological Journal*, *59*(5), 80–92. doi: <https://doi.org/10.1615/HydrobJ.v59.i5.50>
- Tsyhanenko-Dziubenko, I., Kireitseva, H., & Demchuk, L. (2023). Dynamics of heavy metal compounds allocation in urbohydrotops of Kyiv region in post-military conditions. *17th International Conference Monitoring of Geological Processes and Ecological Condition of the Environment* (pp. 1–5). doi: <https://doi.org/10.3997/2214-4609.2023520066>
- Tsyhanenko-Dziubenko, I., Šerevičienė, V., & Ustylenko, V. (2024). Dissecting biochemical mechanisms that mediate tolerance to military chemical stressors in diverse malacological systems. *Environmental Problems*, *9*(1), 51–58. doi: <https://doi.org/10.23939/ep2024.01.051>