Lemna minor in studying the combined effects of Mn^{7+} and pyrocatechol

Zura Yessimsiitova[1,2], *Victoria* Sukhovnina[3], *Aleksandr* Kupchinsky[3], *Aizhan* Konysbaeva[1], *Mihail* Tolstoy[4], and *Devard* Stom[3,4,5*]

[1] Al-Farabi Kazakh National University, 050040 71 al-Farabi Ave., Almaty, Republic of Kazakhstan
[2] Scientific Production - Technical Center «Jalyn», Almaty, Republic of Kazakhstan
[3] Baikal Museum of the SB RAS, 664520 Listvyanka, 1A, Akademicheskaya Street, Russia
[4] Irkutsk National Research Technical University, 664074 Irkutsk, 83, Lermontov Street, Russia
[5] Irkutsk State University, 664003, Irkutsk, 1, Karl Marx Street, Russia

Abstract. The work investigated the combined effect of pyrocatechol (0.001n and 0.1n) with $KMnO_4$ (0.001n and 0.01n) on the *Lemna minor*. The range of ratios of equivalent concentrations of metal ion and pyrocatechol was from 1:100 to 10:1. It was shown that individual solutions of pyrocatechol exhibited a pronounced damaging effect towards *L. minor* starting from a concentration of 0.1n, and $KMnO_4$ – from 0.01n. It was revealed that the toxic effect of a 0.1n solution of pyrocatechol decreased with the addition of 0.01n and, to a greater extent, 0.001n solutions of $KMnO_4$. A significant reduction in the damaging effect of 0.01n $KMnO_4$ solution was established when adding 0.001n pyrocatechol to a 10-fold excess of the equivalent concentration of potassium permanganate (2.0-2.4 times). On the contrary, the yield of electrolytes increased in equinormal 0.001 n mixtures of solutions of pyrocatechol and a metal compared to individual solutions. Based on the toxicological data, analysis of UV-VIS spectra, an attempt was made to explain the mechanisms of interaction between pyrocatechol and $KMnO_4$ during their action on plant. The data can be useful for understanding the processes of self-purification of water bodies, be of interest when using plants to clean water bodies, predicting environmental risks.

1 Introduction

Heavy metals are ubiquitous pollutants [1-5]. Their danger lies in particular in their very high resistance. Of the organic pollutants in wastewater, phenolic compounds are usually present [6-9]. Very often these two groups of pollutants occur simultaneously in polluted waters. Although there are many publications devoted to the effects of these substances on various organisms, there is very little literature on their combined effects [10-12]. At the same time, both polyphenols and many heavy metals are very, very highly reactive compounds, especially easily involved in redox reactions. Naturally, this should significantly change their biological effects, in particular toxicity. In this regard, to understand the processes of self-

* Corresponding author: stomd@mail.ru

purification of water bodies, the use of aquatic plants to clean water bodies, and predict environmental risks, it is important to study their complex actions.

The purpose of this work was to study the combined toxic effect of manganese (VII) salts and pyrocatechol in relation to the aquatic plant *Lemna minor*.

2 Materials and methods

2.1 Test-object

Lemna minor L. (duckweed) is an aquatic plant. Systematic position of the test object under study: order *Alismatáles*, class *Liliopsida*, family *Lemnaceae*, subfamily *Lemnoideae*. This aquatic plant is very unpretentious and has a high growth rate. It grows abundantly on the surface of reservoirs with slow-flowing or standing water. *L. minor* is widely used to remove nutrients (nitrogen salts, phosphorus) from water [13, 14], which prevents the "blooming" of water bodies, and its biomass serves as food, for example, in poultry farming [15]. In addition, the effectiveness of using duckweed for phytoremediation of water bodies contaminated with heavy metals has been shown [16-19].

L. minor was selected for research in small reservoirs with slow-flowing water. In laboratory conditions, duckweed was cultivated in glass vessels at a temperature of 20-22 °C and artificial lighting of 650-850 Lux on Steinberg's nutrient medium (composition in mg/l: KNO_3 – 350.0; $Ca(NO_3)_2 \cdot 4H_2O$ – 295.0; KH_2PO_4 – 90.0; K_2HPO_4 – 12.6; $MgSO_4 \cdot 7H_2O$ – 100.0; H_3BO_3 – 0.12; $ZnSO_4 \cdot 7H_2O$ – 0.18; $Na_2MoO_4 \cdot 2H_2O$ – 0.044; $MnCl_2 \cdot 4H_2O$ – 0.18; $FeCl_3 \cdot 6H_2O$ – 0.76; Titriplex III (EDTA) – 1.5) [20]. The light period during cultivation was 8 hours.

2.2 Toxicants

Phenolic compounds of various natures, as a rule, contain 3 main nuclei: ortho-, para- and meta-isomers. But polyphenolic compounds of natural origin are most often represented by ortho-isomers of dihydroxybenzene, which have a pyrocatechol core. In this regard, the work investigated the diatomic phenol ortho-dihydroxybenzene (pyrocatechol, or 1,2-dihydroxybenzene) – one of three possible isomers of dihydroxybenzene with the molecular formula C_6H_{42}. In Fig. 1. The structural formula of this diatomic phenol is given. Before the experiments, pyrocatechin was preliminarily purified by the reagent method of sublimation under vacuum.

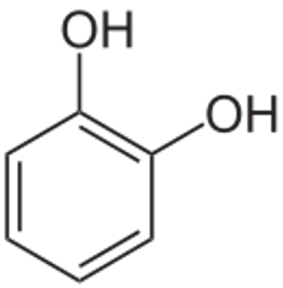


Fig. 1. Structural formula of the diatomic phenol used in this work - pyrocatechol (ortho-dihydroxybenzene, 1,2-dihydroxybenzene)

Among metals, manganese compounds have a particularly high oxidizing ability. In this regard, Mn(VII) was taken as pollutants in the work. The source of MnO_4^- ions was potassium permanganate $KMnO_4$ (chemically pure, JSC Vekton, Russia).

The concentrations of $KMnO_4$ in the experiments were 0.001 n and 0.01 n, pyrocatechin – 0.001 n and 0.1 n. In this case, the equivalent contents of $KMnO_4$ and pyrocatechol were correlated as 1:10, 1:100, 10:1 and 1:1.

2.3 Method for studying the combined toxicity of substances

As a test reaction, the work used a change in the electrical conductivity of the aquatic environment after keeping *L. minor* shoots in it, pre-treated with a toxicant. This test is based on the violation of the barrier properties of plant cell membranes and the release of electrolytes from them. The experiments were carried out as follows.

Solutions of the studied pollutants and their mixtures were prepared (concentrations are indicated in paragraph 2.2).

L. minor biomass (shoots with three leaves, at a rate of 10 g/l by fresh weight) was added to them and kept for 30 min. The control was a test in which the plants were kept in distilled water, i.e. without adding toxicants. After being kept in the tested solutions of pollutants (in the control – distilled water), the duckweed was removed and thoroughly, repeatedly washed with distilled water. This made it possible to remove residual toxicants from the surface of plants. Washed *L. minor* biomass in an amount of 0.5 g was placed in a 50 ml cell filled with distilled water. Every 30 minutes for 1.5 hours, a UEP-P-S submersible sensor was placed in the cell and the electrical conductivity was measured using an Expert-002 conductometer (Econix-Expert, Russia).

A higher (compared to the control) yield of electrolytes from plants indicated increased toxicity of the tested solutions.

In parallel, UV-VIS spectra of solutions of phenolic compounds, metals and their mixtures were assessed using a pHotoLab 6600 spectrophotometer (WTW, Germany). Also in the experiments, changes in the pH of toxicant solutions were recorded using an Expert-001-4(0.4) liquid analyzer (Econix-Expert, Russia) and ESK-1 electrodes.

All digital values given in the work were obtained in 3-5 independent experiments performed in triplicate. The figures show the results of a typical experiment. Statistical data processing was carried out using the Excel Windows software package. The graphs show the values of the arithmetic mean and the standard deviation of the arithmetic mean (or mean square error). The conclusions were made with the probability of an error-free forecast $P \geq 0.95$. Differences between experimental variants were determined using Student's t-test.

3 Results and discussion

Experiments showed that the dihydric phenol under study caused an increase in the yield of electrolytes from plant tissues: at a concentration of 0.001 n – 1.7 times, at 0.1 n – 5.8 times compared to the control. Thus, the electrical conductivity of distilled water into which duckweed, untreated with toxicants, was placed increased from 2.0 to 3.5 μS (control) over 1.5 hours of exposure. Over the same time, the electrical conductivity of water containing duckweed treated with pyrocatechol in concentrations of 0.001 and 0.1 n reached 5.9 and 20.0 μS, respectively (Fig. 2).

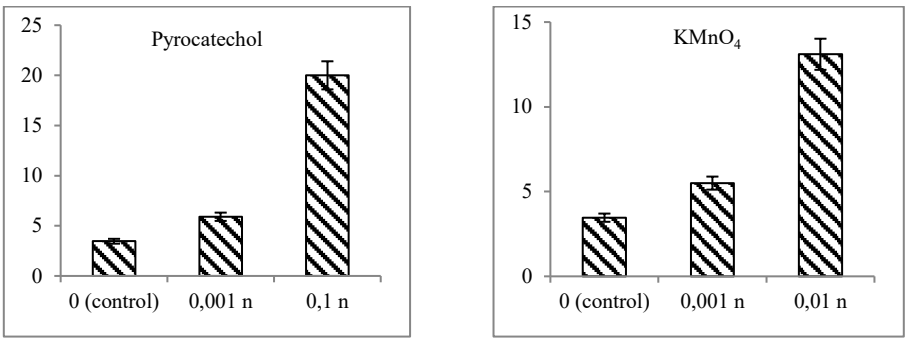
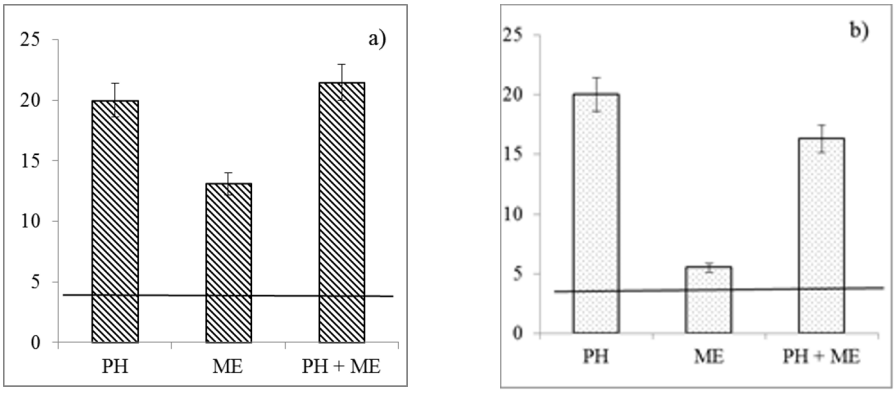


Fig. 2. The influence of individual solutions of pyrocatechol (0.001 n; 0.1 n) and $KMnO_4$ (0.001 n; 0.01 n) on the yield of electrolytes from duckweed after its treatment with toxicants for 30 minutes. Y axis – electrical conductivity of the solution (μS).

The solutions of potassium permanganate tested in our experiments (0.001 n; 0.01 n) had an effect on duckweed similar to pyrocatechol. Treatment of duckweed with these solutions led to an increase in the yield of electrolytes from its tissues: at 0.001 n $KMnO_4$ – 1.6 times, at 0.01 n – 3.8 times relative to the control (see Fig. 2). Thus, individual solutions of 0.1 n pyrocatechol and 0.01 n $KMnO_4$ showed the most aggressive effect.

An assessment of the damaging effect of mixtures of pyrocatechol (0.001 n; 0.1 n) and $KMnO_4$ (0.001 n; 0.01 n) on duckweed revealed the following.

The addition of potassium permanganate (0.001 n; 0.01 n) to a solution containing 0.1 n pyrocatechol did not have a significant effect on the toxicity of the polyphenol. The yield of electrolytes from duckweed tissues treated with mixtures of pyrocatechol + permanganate (in the indicated concentrations) corresponded to what was observed under the action of individual solutions (Fig. 3a, 3b).



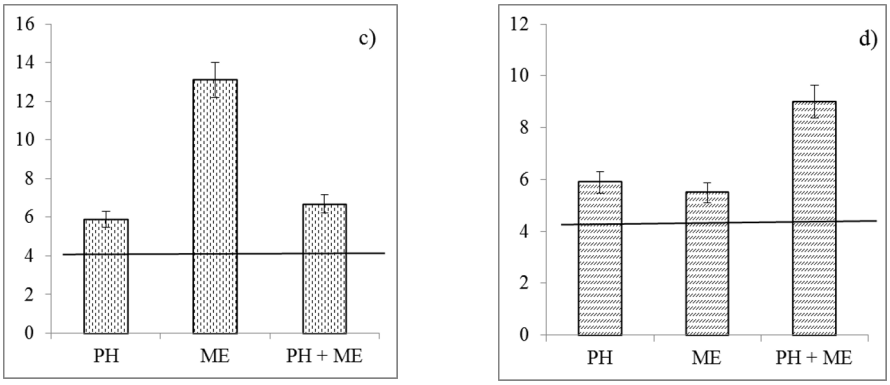


Fig. 3. Effect of pyrocatechol (phenol – PH), $KMnO_4$ (metal – ME) and their mixtures (PH + ME) on the yield of electrolytes from duckweed after treatment with toxicants for 30 min. Y axis – electrical conductivity of the solution (μS); horizontal line – level of electrical conductivity in the control (duckweed without treatment with toxicants). a) $KMnO_4$ 0.01 n + pyrocatechol 0.1 n; b) $KMnO_4$ 0.001 n + pyrocatechol 0.1 n; c) $KMnO_4$ 0.01 n + pyrocatechol 0.001 n;

Thus, the toxicity of mixtures of pyrocatechol (0.1 n) with permanganate (0.001 n; 0.01 n) practically corresponded to the toxicity of its individual solutions (see Fig. 3). In experiments with a lower concentration of pyrocatechol (0.001 n), a slightly different picture was observed. When mixed with 0.001 n $KMnO_4$, the toxicity of the resulting mixture was higher than the toxicity of each of these substances separately (see Fig. 3d). In contrast, in a system containing $KMnO_4$ (0.01 n) + pyrocatechol (0.001 n), a decrease in the toxicity of manganese by pyrocatechol was noted (see Fig. 3c).

Thus, in the experiments carried out in the range of concentrations and ratios of reagents used, it was possible to reduce the toxicity of both pyrocatechol and a salt solution in the highest degree of oxidation of the metal.

The stability, reactivity and behavior in solutions of both metal ions and organic compounds, as a rule, depend on the active reaction of the medium. Therefore, pH values were determined in solutions of individual substances (pyrocatechol and $KMnO_4$) and their mixtures. From Fig. 4 it follows that in individual solutions of the studied compounds there were no significant shifts in the pH of the medium compared to distilled water. However, after mixing the reagents, the pH of the solutions increased in all four systems (Fig. 4). At the same time, the pH increased to a greater extent in the mixture with a higher concentration of $KMnO_4$ 0.01 n and a relatively low concentration of pyrocatechol 0.001 n. The addition of 0.001 n pyrocatechol increased the pH of the 0.01 n $KMnO_4$ solution to a value of 8.5. There was reason to believe that in all four mixtures reduction of MnO_4^- occurred, but in systems with 0.001 n $KMnO_4$ the increase in pH was less pronounced due to the lower salt concentration. The reduction of MnO_4^- to MnO_2 was probably accompanied by partial oxidation of catechol to 1,2-benzoquinone, which itself is very unstable and led to the formation of oxidative condensation products, which, as a rule, are less toxic than the original monomers, because the functional groups of the latter during polymerization are spent on the formation of bonds.

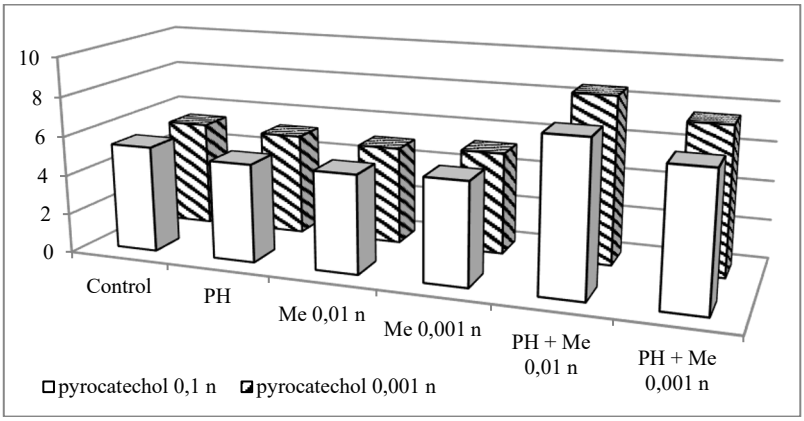
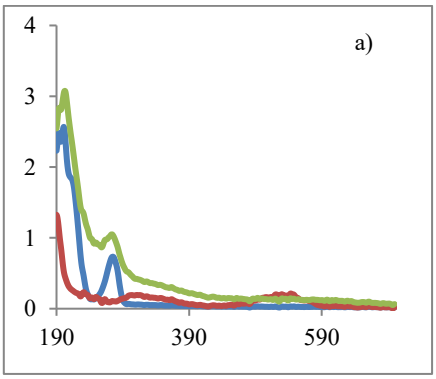
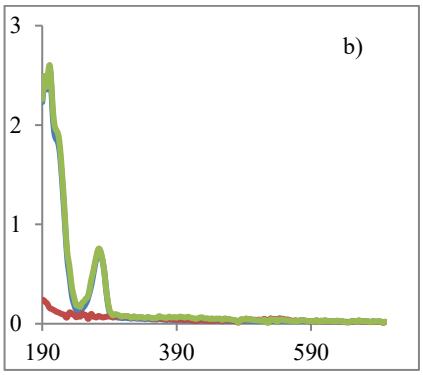


Fig. 4. pH values of solutions of pyrocatechol (PH), $KMnO_4$ (ME) and their mixtures (PH + ME). Y axis – solution pH (units).

When preparing solutions in equinormal mixtures and in systems with an excess of an equivalent concentration of pyrocatechol, discoloration of the permanganate immediately occurred. This evidenced its reduction presumably to Mn(II). When mixed with a 10-fold excess of metal salt, the solution acquired a rich orange color. In redox reactions, MnO_4^- in an acidified environment becomes discolored, turning into Mn^{+2}, in a neutral environment it turns into a brown precipitate of MnO_2, and in an alkaline environment into a green solution of MnO_4^{2-}. It is possible that a colored complex was formed under the conditions of our experiment. Further research is needed to explain the nature of the obtained material.

Figure 5 shows UV-VIS spectra of solutions and mixtures containing Mn(VII) in concentrations of 0.0006 n, 0.00006 n, 0.000006 n and 0.002 n and pyrocatechol 0.0006 n and 0.0002 n. The ratios of equivalent concentrations of metal ion and catechol were 1:1, 1:10, 1:100 and 10:1. Thus, the ratios of the reagents in the mixtures corresponded to those used to study the combined effect of pyrocatechol with MnO_4^- ions on the aquatic plant *L. minor*.





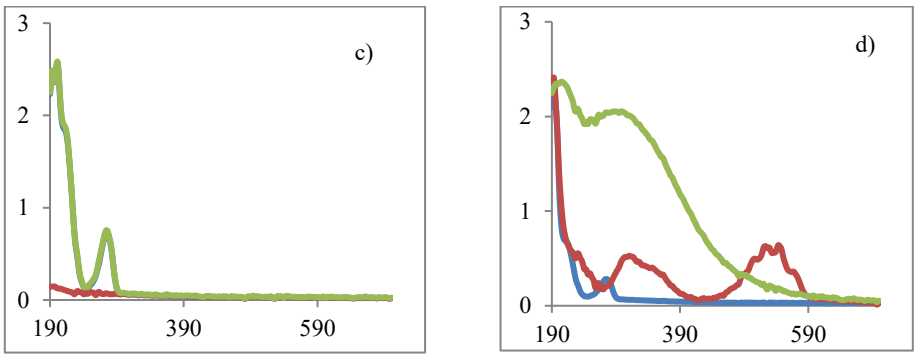


Fig. 5. UV-VIS spectra of solutions of $KMnO_4$, pyrocatechol and their mixtures (X axis – Wave length λ, nm; Y axis – Absorption, A; ——— – Pyrocatechol, ——— – $KMnO_4$, ——— – Pyrocatechol + $KMnO_4$) a) $KMnO_4$ 0,0006 n + pyrocatechol 0,0006 n (1:1); b) $KMnO_4$ 0,00006 n + pyrocatechol 0,0006 n (1:10); c) $KMnO_4$ 0,000006 n + pyrocatechol 0,0006 n (1:100); d) $KMnO_4$ 0,002 n + pyrocatechol 0,0002 n (10:1).

Consideration of the UV-VIS spectra of the solutions under study allows us to formulate the following. In mixtures with a predominance of equivalent concentrations of catechol of 10 (Fig. 5b) and 100 times (Fig. 5c), no significant changes in the spectrum of catechol occurred. Therefore, it can be assumed that either no significant chemical interactions occurred between the components of the mixture (which is unlikely), or a small amount of reaction product was formed (for example, a reaction with the reduction of Mn(VII) presumably to Mn(II), as evidenced by the discoloration of the permanganate solution), but only a small part of pyrocatechin is consumed, so its spectrum is practically no different from the spectrum of an individual polyphenolic compound.

In the spectrum of an equinormal mixture (Fig. 5a), the disappearance of max potassium permanganate in the region of 519-550 nm indicates the reduction of Mn(VII). This is clearly visible from the discoloration of the $KMnO_4$ solution. Compared to the spectrum of pyrocatechol, a number of changes are also present in the spectrum of the mixture: the min of pyrocatechol at 197 nm is converted into a shoulder, the shoulder disappears in the region of 212-214 nm, but slightly appears at 229 nm, the min is less pronounced in the region of 240 nm, shifts to the region 257-258 nm, – intensity max at 273-275 nm increases, but no new max appeared in the region of 400 nm. This may indicate that the dihydric phenol reacts chemically with an equivalent concentration of the metal, and a new compound, possibly a complex, is likely formed. This is accompanied by a change in the violet color of the permanganate solution to orange. In the spectrum of the mixture (Fig. 5d), the disappearance of the broad max of potassium permanganate in the region of 519-550 nm may indicate the reduction of Mn(VII). All characteristic lines of pyrocatechol also disappeared: max at 273-275 nm, shoulder in the region of 212-214 nm, min at 197 nm.

The results obtained do not contradict the data obtained when studying the toxic effects of solutions and mixtures of toxicants (the concentrations of solutions for UV-VIS studies were chosen so that the optical density of the spectral peaks characteristic of these solutions and their mixtures did not exceed 1.0).

When studying the toxicity of systems, it was shown that in equinormal mixtures of diatomic phenol (0.001 n) and $KMnO_4$ (0.001 n), compared with their individual solutions, the yield of electrolytes significantly increased. Changes in the spectra indicate the likely occurrence of redox reactions with the reduction of potassium permanganate and partial oxidation of catechol, which can increase the toxicity of the mixture.

The weakening of the damaging effect of a 0.01 n $KMnO_4$ solution by polyphenol was shown under conditions of excess metal. Analysis of UV-VIS spectra in a system with also a 10-fold excess of the equivalent concentration of $KMnO_4$ leads to the conclusion about the possible formation of a new compound, probably a complex, the aggressive effect of which is lower compared to that of individual solutions.

4 Conclusion

Thus, the combined effect of pyrocatechol (0.001 n and 0.1 n) with $KMnO_4$ (0.001 n and 0.01 n) on the aquatic plant *L. minor* was studied. The range of ratios of equivalent concentrations of metal ion and pyrocatechol was from 1:100 to 10:1. It was shown that individual solutions of pyrocatechol exhibited a pronounced damaging effect against *L. minor*, starting from a concentration of 0.1 n, and $KMnO_4$ – from 0.01 n.

It was revealed that the toxic effect of a 0.1 n solution of pyrocatechol decreased with the addition of 0.01 n and, to a greater extent, 0.001 n solutions of $KMnO_4$. A significant reduction in the toxic effect of 0.01 n $KMnO_4$ solution was established when 0.001 n pyrocatechol was added to a 10-fold excess of the equivalent concentration of potassium permanganate (2.0-2.4 times). In equinormal 0.001 n mixtures of solutions of pyrocatechol and a metal salt, in comparison with individual solutions of diatomic phenol and $KMnO_4$, the yield of electrolytes, on the contrary, increased.

Acknowledgements

This research was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP19677698)". The work used the equipment of the Center for Collective Use "Scientific Expeditionary Center" Baikal "(https://ckp-rf.ru/catalog/ckp/3213559/). The authors are grateful to PhD E. V. Stolpovskaya for participation in the experiments.

References

1. J. Briffa, E. Sinagra, R. Blundell, Heliyon **6** (2020)
2. N. Patel et al. *Contamination and Health Impact of Heavy Metals,* In: Inamuddin, Ahamed, M.I., Lichtfouse, E. (eds) Water Pollution and Remediation: Heavy Metals. Environmental Chemistry for a Sustainable World. (Springer, 2021).
3. S. Rajendran, T.A.K. Priya, K.S. Khoo, T.K.A. Hoang, H.-S. Ng et al., Chemosphere **287(4)** (2022)
4. C.R. Vanisree, M. S. Sankhla, P. Singh, E. B. Jadhav, R. K. Verma, et al., *Environmental Impact and Remediation of Heavy Metals*, IntechOpen (2022)
5. N. Verma, M. Rachamalla, P. K. Sravan, K. Dua, *Chapter 6 - Assessment and impact of metal toxicity on wildlife and human health*, In Advances in Environmental Pollution Research, Metals in Water (Elsevier, 2023)
6. Md. Ahmaruzzaman, S. R. Mishra, V. Gadore, G. Yadav, S. Roy, B. Bhattacharjee, A. Bhuyan, et al., Journal of Environmental Chemical Engineering **12(3)** (2024)
7. R. L. Ramos, V. R. Moreira, M. C. S. Amaral, Journal of Environmental Management **351** (2024)
8. O. B. Otitoju, M. O. Alfred, O. O. Ogunlaja et al. Environ. Sci. Pollut. Res. **30** (2023).

9. G. B. Adebayo, B. O. Orimolade, H. K. Okoro, M. A. Banwo, Nigerian Journal of Pharmaceutical and Applied Science Research **8(1)** (2019)
10. K. T. Kim, Y. G. Lee, S. D. Kim, *Environ. Int.* **32(4)** (2006)
11. E. V. Stolpovskaya, V. O. Sukhovnina, G. O. Zhdanova, O. A. Barkhatova, A. D. Stom, M. N. Saksonov, et al., Uchenye Zapiski Kazanskogo Universiteta. Seriya Estestvennye Nauki **166(1)** (2024)
12. D. I. Stom, T. A. Geel, G. V. Shahova, N. F. Aprelkova, S. E. Medvedeva, O. A. Menshikova, Acta hydrochim. hydrobiol. **19(1)** (1991)
13. K. S. Sree, K. J. Appenroth, *Chapter 12 - Duckweeds: Bioremediation of surface wastewater and biorefinery*, in Bioremediation and Bioeconomy Editor(s): Majeti Narasimha Vara Prasad (Elsevier, 2024)
14. Y. Zhou, A. Stepanenko, O. Kishchenko, J. Xu, N. Borisjuk, Water. Plants. **12(3)** (2023)
15. D. Sosa, F. M. Alves, M. A. Prieto, M. C. Pedrosa, S. A. Heleno, L. Barros, M. Feliciano, M. Carocho, Foods **13(10)** (2024)
16. M. Mohanty. *Remediation of Heavy Metals by Different Aquatic Macrophytes.* In: Kumar, S., Bauddh, K., Singh, R., Kumar, N., Kumar, R. (eds) Aquatic Macrophytes: Ecology, Functions and Services (Springer, 2023)
17. M. Aslanzadeh, A. Saboora, O. Moradlou, Int. J. Environ. Sci. Technol. (2024)
18. N. Moreno-Rubio, D. Ortega-Villamizar, W. Marimon-Bolívar et al., Environ Monit Assess **195** (2023)
19. J. Yang, X. Zhao, X. Wang, M. Xia, S. Ba, B. L. Lim, H. Hou, Environmental Research **245** (2024)
20. ISO 20079:2005. Water quality – determination of the toxic effect of water constituents and waste water to duckweed (Lemna minor) – Duckweed growth inhibition test (2005)