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Helophyte *Typha latifolia* L. as an Underestimated Biomonitor and Phytoremediator at Ultra-High Copper Concentrations: An *In-Situ* and *Ex-Situ* Study of Adaptive Responses

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Abstract. *Typha latifolia* (broadleaf cattail) is a helophyte widely used for the treatment of wastewater and surface waters contaminated with multiple metals, including copper. At the same time, its potentials as a biomonitor and phytoremediator in habitats with ultra-high levels of copper pollution have not been sufficiently studied. To deal with this issue, the morphophysiological parameters of cattail were studied *in situ* at the site with extreme polymetallic pollution near the Karabash Copper Smelter (Karabash, the Chelyabinsk Region, Russia). In addition, pot-scale (*ex-situ*) experiment spiked with 2000 and 6000 mg Cu/kg of soil was carried out to study its seed progeny. Plants from the natural population exhibited a fairly high copper resistance and viability of seeds (germination index 40 %). The copper content in cattail organs was positively correlated with its concentration in the substrate. *T. latifolia* showed predominant Cu accumulation in underground organs, especially in the roots, which was 179.9 mg/kg of dry weight under natural conditions, surging to 1407.5 mg/kg of dry weight under model conditions. Helophyte seed progeny also demonstrated high copper tolerance, with biomass, leaf length, and plant height only decreasing at a copper concentration as high as 6000 mg Cu/kg soil. The content of photosynthetic pigments remained constant. An increase in the contents of hydrogen peroxide and malondialdehyde (30 % on average) was accompanied by the accumulation of non-enzymatic antioxidants

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and a significant decrease in the activities of catalase and ascorbate peroxidase. The highest correlation with Cu concentration in soil was observed for protein and non-protein thiols (0.76 on average, $p < 0.05$), which puts them forward as potential biomarkers of copper contamination. Thus, *T. latifolia* can be used for biomonitoring and phytoremediation (phytostabilization and rhizofiltration) not only at high, but also at ultra-high levels of copper pollution.

Keywords: broadleaf cattail, heavy metals, seed progeny, pot-scale experiment, photosynthetic pigments, redox reactions, biomonitoring, phytostabilization, rhizofiltration.

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Гелофит *Typha latifolia* L. как недооцененный биомонитор и фиторемедиатор при сверхвысоком содержании меди: исследование адаптивных реакций *in-situ* и *ex-situ*

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Аннотация. *Typha latifolia* (рогоз широколистный) – гелофит, широко использующийся для очистки сточных и поверхностных вод при загрязнении металлами, включая медь. В то же время возможности его использования в качестве биомонитора и фиторемедиатора местообитаний со сверхвысоким уровнем загрязнения медью изучены недостаточно. Для решения данной проблемы были исследованы морфофизиологические параметры рогоза *in-situ* на участке с экстремальным полиметаллическим загрязнением вблизи Карабашского медеплавильного комбината (г. Карабаш, Челябинская область, Россия), а также его семенного потомства в горшечных культурах (*ex-situ*) при добавлении 2000 и 6000 мг Cu/кг почвы. Растения из природной популяции отличались достаточно высокой устойчивостью и жизнеспособностью семенного материала (индекс прорастания 40 %). Содержание меди в органах рогоза положительно коррелировало с ее концентрацией в субстрате. Рогоз показал преимущественное накопление меди в подземных органах, особенно в корнях: в естественных условиях ее содержание составляло 179,9 мг/кг сухого веса, в то время как в модельных условиях достигало 1407,5 мг/кг сухого веса. Семенное потомство гелофита также продемонстрировало высокую устойчивость к меди: снижение биомассы, длины листьев и высоты растений наблюдалось лишь при 6000 мг Cu/кг почвы. При этом содержание фотосинтетических пигментов оставалось на постоянном уровне. Увеличение содержания пероксида водорода и малонового диальдегида (в среднем на 30 %) сопровождалось накоплением неэнзиматических антиоксидантов и существенным снижением активности каталазы и аскорбатпероксидазы. Наиболее высокая корреляция с концентрацией меди в почве была отмечена для белковых и небелковых тиолов (в среднем 0,76, $p < 0,05$), что позволяет рекомендовать их в качестве биомаркеров загрязнения этим металлом. Таким образом, рогоз может быть использован для биомониторинга и фиторемедиации (фитостабилизации и ризофильтрации) не только при высоком, но и сверхвысоком уровне загрязнения медью.

Ключевые слова: рогоз широколистный, тяжелые металлы, семенное потомство, горшечные эксперименты, фотосинтетические пигменты, редокс-реакции, биомониторинг, фитостабилизация, ризофильтрация.

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Introduction

There is a rapid and continuous increase in the emission of pollutants, including heavy metals (HMs). Metals can accumulate in various components of aquatic and terrestrial ecosystems, resulting in toxic effects on living organisms and increasing risks to human health (Kabata-Pendias, Mukherjee, 2007; Ashraf et al., 2019; Amir et al., 2020; Kumar et al., 2022). Water pollution by trace elements is an important factor for both geochemical cycling of the elements and environmental health (Kabata-Pendias, 2010). Helophytes (wetland plants) play an important role in purification of aquatic ecosystems due to their direct contact with both the aquatic environment and bottom sediments and ability to transform and/or accumulate the pollutants entering the hydro-ecosystems (Grisey et al., 2012; Vroom et al., 2018; Rana et al., 2022). Thus, the search for plant species with high HM tolerance is relevant to biomonitoring and phytoremediation.

Typha latifolia L. (better known as broadleaf cattail) is one of the wetland plants that can grow in habitats with high levels of toxic elements and under low pH (Klink et al., 2013; Kumari, Tripathi, 2015). It is a large, perennial, rhizomatous plant, which belongs to helophytes with a wide habitat. Studies of the *T. latifolia* accumulative ability with respect to different HMs were carried out in natural habitats including constructed wetlands both in Russia (Kurilenko, Osmolovskaya, 2006; Brekhovskikh et al., 2009; Sviridenko et al., 2015; Maleva et al., 2019; Petrov et al., 2023; Shiryaev et al., 2024) and other countries (Grisey et al., 2012;

Klink, 2017; Parzych et al., 2016; Aucour et al., 2017; Ben Salem et al., 2017; Bonanno, Cirelli, 2017; Rana, Maiti, 2018; Hejna et al., 2020; Haghnazar et al., 2021; Rana et al., 2022). Many researchers showed the high accumulative ability of *T. latifolia* in relation to HMs (Lyubenova, Schröder, 2011; Parzych et al., 2016; Bonanno, Cirelli, 2017; Yang, Shen, 2020; Abbas et al., 2021). However, most of the studies investigated *T. latifolia* plants growing in water bodies with low or moderate levels of pollution. Several authors carried out studies in model systems using *T. latifolia* plants treated with relatively low concentrations of HMs. For example, for copper (Cu), the range of concentrations was 0.5–10 mg L^{-1} (Saygİdeger et al., 2009) or 10–100 µmol L^{-1} (Lyubenova et al., 2015); for lead (Pb), it was 5.0–7.5 mg L^{-1} (Alonso-Castro et al., 2009), 10–250 µmol L^{-1} (Lyubenova, Schröder, 2011) or 1–5 mmol L^{-1} (Amir et al., 2020); and for cadmium (Cd), it was 0–30 mg kg^{-1} (Yang, Shen, 2020) or 10–250 µmol L^{-1} (Lyubenova, Schröder, 2011).

Among HMs, copper plays an important and ambiguous role. In small concentration, it is an essential element for living organisms, while its excessive amount negatively affects the growth and metabolism of plants, changing the processes of photosynthesis and respiration (Kumar et al., 2021; Mir et al., 2021). A major source of copper pollution is the non-ferrous metal mining and processing industries. Their activities result in the formation of artificial geochemical provinces (Yurkevich et al., 2015; Ashraf et al., 2019). One of these artificial geochemical provinces was formed in the vicinity of the city of Karabash (Russia, Chelyabinsk Region) because of the impact of the Karabash Copper Smelter (KCS) (Yurkevich et al., 2015; Tripti et al., 2021). For more than a century of KCS operation, emissions into the atmosphere and discharges of untreated industrial, mining, and domestic wastewater into the river system have led to considerable pollution of the soil, water bodies, bottom sediments, and the atmosphere, soil degradation, and the disappearance of the surrounding vegetation (Minkina et al., 2018; Kumar et al., 2020; Shiryaev et al., 2024).

Previously, local populations of *T. latifolia* were studied in water bodies with varying degrees of polymetallic pollution, at different distances from the KCS, as compared to relatively clean natural habitats (Maleva et al., 2019; Shiryaev et al., 2024). A high positive correlation was found between the concentration of HMs in sediments and their content in aboveground and, especially, in underground organs of *T. latifolia*, which indicated the high stabilizing capacity of this helophyte and its potential as a bioindicator. Moreover, a significant increase was noted in the level of non-enzymatic antioxidants such as soluble phenolic compounds, free proline, and soluble thiols in helophyte leaves, which presumably contributed to an increase in the resistance of this species to anthropogenic impacts.

Reproductive ability is one of the most important functions for maintaining the viability of plant populations. Preserving seed productivity is especially important in conditions of high HM pollution. The danger of HM accumulation in plants lies not only in a decrease in their growth and physiological functions, but also in a significant change in the viability and quality of seed progeny. According to Vasilyev (2021), the reliability of plant adaptation mechanisms to excess HMs should be judged not only by the development indicators of treated plants, but also by the quality of their progeny, which is a guarantor of population stability.

Seeds of helophytes are material for redevelopment of plant communities and can be valuable in the wetland conservation and restoration if the seeds germinate and survive (Meng et al., 2016). There are a few works aimed at identifying optimal conditions for germination of *T. latifolia* seeds (Bonnewell et al., 1983; Meng et al., 2016). Of particular interest is the research of the responses of metal-stressed cattail seed progeny grown in laboratory conditions from seeds of plants initially growing in habitats with extreme levels of polymetallic pollution. However, such studies are very limited. For example, Ye et al. (1997) investigated tolerance to lead, zinc, and cadmium of *T. latifolia* grown from seeds collected in metal-contaminated and non-contaminated sites, but the metal concentrations used in the experiments were low and reached 20 mg L^{-1} (for Pb), 5.0 mg L^{-1} (for Zn), and 0.5 mg L^{-1} (for Cd).

Thus, despite numerous publications on HM accumulation by *T. latifolia*, there is still insufficient information about the ability of this species to grow under extreme polymetallic stress. The adaptive physiological responses that allow this helophyte to survive at ultra-high concentrations of metals, including copper, are not well understood. In addition, there are virtually no data on the ability of the seed progeny of *T. latifolia* to accumulate copper and survive at ultra-high Cu concentrations.

The specific objectives of the study include: (a) estimation of metal accumulation and morphophysiological characteristics of *T. latifolia* plants growing *in situ* at a natural heavily polluted site close to copper smelter tailing, (b) evaluating the tolerance of *T. latifolia* seed progeny plants grown *ex situ* in pot-scale experiment at high and ultra-high concentrations of copper (2000 and 6000 mg kg^{-1} of dry soil, respectively) and the effect of copper on the growth and physiological parameters of plants, and (c) assessment of the possibility of using some physiological traits of the plant as biomarkers of contamination.

Materials and methods

The helophyte *T. latifolia* has formed natural local populations near the tailings dump of the KCS (JSC "Karabashmed"), which has been operating for more than 100 years in the city of Karabash, the Chelyabinsk Region, Russia. The local population of *T. latifolia* growing at the tailings water site (55°27′15″ N 60°12′48″ E) with an extremely high level of polymetallic pollution, which subsequently forms the Ryzhiy stream (Shiryaev et al., 2024), was selected for the study (Fig. 1a).

The *in-situ* surveys of *T. latifolia* population were conducted in mid-July 2019. The morphological characteristics of 20 plants in the flowering–fruiting phase (length of shoots, length and width of leaves) were examined under natural conditions. In addition, five randomly selected plants were carefully uprooted with part of the sediments to preserve the underground organs (rhizome and roots). Surface water samples were taken in 5 replicates from the cattail site in 0.5-L plastic bottles pre-cleaned with dilute HNO_3. Plant samples with sediments were placed in separate sterile 10-L plastic containers so that their underground organs remained completely immersed in water from the study site to avoid drying and immediately transferred to the laboratory for further analysis. In addition, in early September 2019, ripe seeds were collected from *T. latifolia* growing at the study site.

The water samples (0.5 L) were fixed by 70 % HNO_3 (by lowering the pH below 2.0), digested to reduce the final volume to 10 mL by warm evaporation, and stored at 4 ºC until analysis for metals. Sediment samples were taken from each *T. latifolia* root zone, air dried, homogenized, passed through a sieve (< 2 mm), and preserved for further metal analysis. The plant samples were cleaned to remove soil particles, washed with running water, treated with ultrasonication (UM-4, Unitra Unima, Olsztyn, Poland), and finally washed in deionized water (Milli-Q system, Millipore SAS, Molsheim, France). One part of the fresh plant material (leaves, rhizome, and roots)

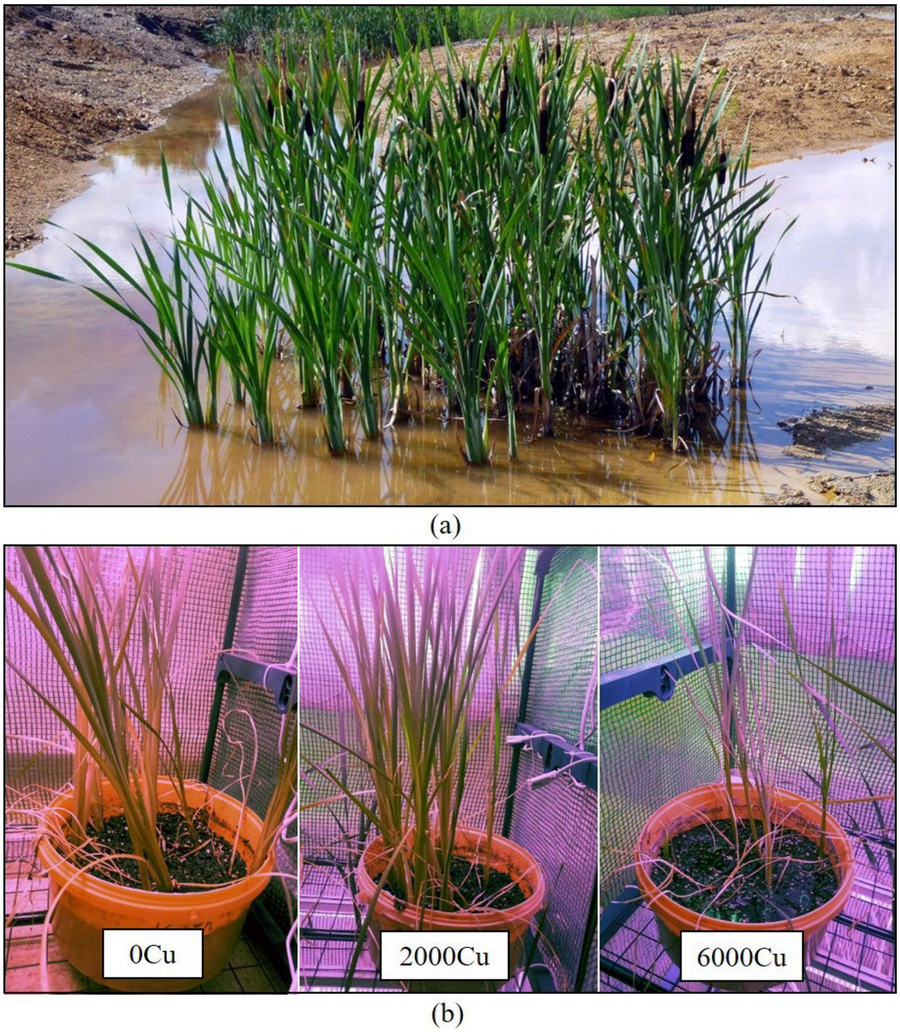

Fig. 1. The local population of *Typha latifolia* at the natural contaminated site (a) and the plant seed progeny in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations (b)

was fixed at 105 °C for 2 h and dried at 75 °C for 24 h for further metal analysis. The other part of the plant material (fresh leaves) was weighed, frozen in liquid nitrogen, and stored at –80 °C to further measure physiological and biochemical parameters such as the content of photosynthetic pigments, lipid peroxidation products (malondialdehyde, MDA), free proline, soluble phenolic compounds, thiols, and protein. For the estimation of dry weight (DW), weighed fresh leaves (FW) were dried in a hot air oven at 75 °C for 24 h, and the ratio of FW/DW was calculated.

T. latifolia seeds previously collected *in situ* from a natural population exposed to long-term anthropogenic impact were tested for germination index in Petri dishes (100 seeds in four replicates). Then, the seedlings were grown for a year (from March 2020 to April 2021) on a watered peat substrate under natural light and at room temperature, with the addition of Hoagland's nutrient solution (200 mL per two-liter plastic container once every three weeks). After that, they were transplanted into larger (4-L) containers, to which copper (sulfate form) was added at concentrations of 2000 and 6000 mg per kg of dry soil (Fig. 1b). The peat-based substrate containing 250 mg L^{-1} nitrogen (NH_4+NO_3), 280 mg L^{-1} phosphorus (P_2O_5), and 400 mg L^{-1} potassium (K_2O) with pH 5.5 (NORD PALP, LLC, Russia) was used for pot-scale experiment. Plants grown on the Cu-free substrate were used as the control. Each treatment included 8 plants in two independent replicates. *T. latifolia* plants were grown for 4 months in a growth chamber at a temperature of 23 ± 2 °C, photoperiod of 14:10 (day: night), and lighting of 150 ± 20 µmol m^{-2} s^{-1} (using plant grow lights ULI-P10–18V/SPFR IP40). At the end of experiment, plants were carefully uprooted, cleaned to remove substrate particles, cut into organs (leaves, rhizome, and roots), and washed with running water and twice in deionized water. The growth parameters such as plant height, leaf length and width, and fresh biomass of aboveground and underground organs were measured. Part of the plant material and substrate samples were dried for further metal analysis. Some of the weighed fresh leaves were frozen in liquid nitrogen and stored at –80 °C for further biochemical analysis.

The dried *in-situ* and *ex-situ* plant samples were weighed and digested with concentrated HNO_3 (analytical grade) using a MARS 5 Digestion Microwave System (CEM, U.S.A.). The available forms of metals in sediments and peat substrates were analyzed by extracting the soil sample (5 g) using 10 mL of 0.5 M nitric acid solution as described elsewhere (Filimonova et al., 2020). The Fe, Zn, Mn, Cu, Ni, Pb, Cd, and Co concentrations in all samples were determined using an AA240FS flame atomic absorption spectrometer (Varian Australia Pty Ltd., Australia). The bioconcentration factor (BCF) was calculated as the ratio of metal concentration in the *T. latifolia* leaves/rhizome/roots to its available concentration in the sediment/substrate. The metal translocation factor (TF) was calculated as the ratio of metal content in the aboveground part (leaves) to its content in the underground organs (rhizome or roots).

Physiological and biochemical parameters in *T. latifolia* leaves were determined spectrophotometrically using an Infinite M200 PRO multimode plate reader (Tecan, Austria). The photosynthetic pigment contents were determined at 470, 647, and 663 nm after preliminary homogenization of fresh leaf cuttings (about 50 mg) and their extraction in 10 mL of 80 % cold acetone; calculations were done according to Lichtenthaler (1987). The chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid contents were expressed as mg per g DW. The content of lipid peroxidation products was measured based on the level of MDA in the reaction with thiobarbituric acid by the absorption at 532 and 600 nm (Heath and Packer, 1968). The concentration of hydrogen peroxide (H_2O_2) was measured using a method based on the peroxide-mediated oxidation of Fe^{2+} followed by the reaction of Fe^{3+} with xylenol orange according to Bellincampi et al. (2000). The accumulation of peroxides was expressed as nmol H_2O_2 per g DW.

The content of free proline was determined after 10-min extraction of fresh leaf cuttings in boiling water (100 °C) using a modified method (Kalinkina et al., 1990) at a wavelength of 520 nm. Staining was carried out with a ninhydrin solution with the addition of glacial acetic acid in an equivalent ratio, using exact concentrations of proline (Sigma-Aldrich Chemie GmbH, Germany) as a standard, and expressed as mg per g DW.

The amount of soluble phenolic compounds (total phenolics and flavonoids) was determined after 24-h extraction of fresh leaf cuttings with an 80 %-ethanol solution (in darkness). The total phenolic content was measured in the reaction with the Folin–Ciocalteu reagent at 725 nm. Gallic acid (Sigma-Aldrich Chemie GmbH, Germany) was used as a standard (Singleton et al., 1999).

The amount of flavonoids was measured at 412 nm after reacting with 10 % aluminum chloride, using rutin (Sigma-Aldrich Chemie GmbH, Germany) as a standard (Chang et al., 2002).

The extraction and determination of protein and non-protein thiols were described in detail elsewhere (Maleva et al., 2009; Borisova et al., 2016). The total content of soluble thiols (–SH) was determined after reaction with Elman's reagent (5.5'-dithiobis (2-nitrobenzoic) acid) at 412 nm. The content of protein thiols was calculated by subtracting the amount of non-protein thiols previously obtained by precipitation of proteins with 50 % trichloroacetic acid (*w/v*) from the total soluble thiol fraction. Reduced glutathione (Sigma-Aldrich Chemie GmbH, Germany) was used as a standard. The content of protein thiols (PT) and non-protein thiols (NPT) was expressed in μmol of –SH per g DW. The content of soluble protein was determined at a wavelength of 595 nm according to Bradford (1976) using bovine serum albumin (Sigma-Aldrich Chemie GmbH, Germany) as a standard.

For the determination of antioxidant activity of enzymes such as catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and guaiacol peroxidase (GPX, EC 1.11.1.7), fresh leaf cuttings (0.5 g) frozen in liquid nitrogen were homogenized with a mortar and pestle and then placed into a cool 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged for 20 min at 15000×*g* at 4 °C, and the supernatant was collected for enzyme assay (Maleva et al., 2016). The activity of CAT was measured by tracking the decrease in H_2O_2 quantity in time at 240 nm (extinction coefficient of 0.036 mmol^{-1} cm^{-1}) and was expressed as mmol of H_2O_2 decomposed per mg of protein per min. The activity of APX was measured through ascorbate oxidation in time at 290 nm (extinction coefficient of 2.8 mmol^{-1} cm^{-1}) and was expressed as mmol of oxidized ascorbate per mg of protein per min. The activity of GPX was determined by an increase in the absorbance of the reaction medium at 470 nm as a result of guaiacol oxidation (extinction coefficient of 26.6 mmol^{-1} cm^{-1}) and was expressed as mmol of oxidized tetraguaiacol per mg of protein per min.

Statistical processing of the results was carried out using STATISTICA 10.0 and Excel 16.0 software. After checking the normality by the Shapiro–Wilk test and the homogeneity of variance by Levene's test, the differences between the treatments were determined with the non-parametric Kruskal–Wallis H test and Mann–Whitney U test at $p < 0.05$. The relationship between different parameters was estimated by Spearman's rank correlation coefficient. The figures and the tables show the arithmetic mean values (Means) and their standard errors (SE); significant differences between the treatments are indicated by different letters.

Results and discussion

Determination of the chemical composition of surface water at the site where the local *T. latifolia* population was growing showed high concentrations of all studied HMs, which exceeded their maximum permissible concentrations (MPCs) for fishery water bodies (Water quality standards …, 2016). The greatest excess was noted for Cu (2600 times), Zn, Mn (1330 times), Fe (460 times), and Ni (79 times), while Pb, Cd, and Co concentrations were 10 times higher than their MPCs on average (Table 1). The average concentrations of HMs were significantly higher compared to the data reported in other studies carried out in anthropogenically disturbed habitats of this helophyte species. For example, the concentrations of Cu, Mn, Zn, and Fe in water were several hundred times higher compared to their maximal levels in mixed samples from five ponds in the southwest of Poland (Klink et al., 2013).

Table 1. Heavy metal contents in surface water and sediments at the natural contaminated site and in the organs of *T. latifolia*

Metal	Contaminated site		*T. latifolia* organs		
	Water, g L^{-1}	Sediments*, g kg^{-1} DW	Leaves, g kg^{-1} DW	Rhizome, g kg^{-1} DW	Roots, g kg^{-1} DW
Fe	0.046 ± 0.003	78.20 ± 3.17	0.67 ± 0.013	12.10 ± 0.42	30.81 ± 0.64
Zn	0.013 ± 0.001	9.35 ± 0.46	0.27 ± 0.013	0.46 ± 0.01	3.37 ± 0.16
	mg L^{-1}	mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW
Mn	13.32 ± 0.94	4472.32 ± 213.76	774.03 ± 12.02	272.28 ± 10.84	515.76 ± 16.14
Cu	2.58 ± 0.11	2965.46 ± 85.66	94.62 ± 2.06	146.35 ± 8.21	179.86 ± 7.05
Ni	0.79 ± 0.03	536.98 ± 11.79	34.05 ± 0.76	139.87 ± 2.87	69.15 ± 2.27
Pb	0.061 ± 0.003	451.50 ± 17.02	8.39 ± 0.48	27.11 ± 1.26	53.58 ± 1.93
Cd	0.024 ± 0.001	23.82 ± 1.31	0.60 ± 0.03	7.99 ± 0.89	45.07 ± 2.29
Co	0.173 ± 0.004	95.39 ± 0.88	1.62 ± 0.04	2.55 ± 0.15	10.85 ± 0.48

*Available form of metals. Data are presented as Means ± SE (n = 5).

The average concentrations of HMs in sediments from the natural contaminated site (Table 1) were also considerably higher compared to the data for other contaminated habitats of *T. latifolia* reported by other authors. For example, in the present research, the concentrations of Cu and Cd were 35, Ni and Zn 28, and Mn and Pb 6 times higher than their concentrations in sediments of urban wetland (Sicily, Italy) subjected to municipal wastewater and metal contaminations (Bonanno, Cirelli, 2017). The contents of Zn and Cd in sediments were 100 times higher, Cu 40 times higher, Mn, Ni and Pb on average 10 times higher compared to the data reported for 10 sites of Bahmanshir River, Iran (Haghnazar et al., 2021). The concentrations of all studied metals in water and sediments were also considerably higher than their maximal levels measured in small water bodies of Saint Petersburg (Kurilenko, Osmolovskaya, 2006). The previous estimation of the geoaccumulation index (I_{geo}) in the water body near the study site (the Ryzhiy stream) revealed its extreme contamination ($I_{geo} > 5$) by Cu and Zn (5.3 and 6.6, respectively); heavy contamination ($3 > I_{geo} < 5$) by Ni and Cd (3.5 and 4.5, respectively); and moderate to heavy contamination ($2 < I_{geo} < 3$) by Pb (2.8) (Shiryaev et al., 2024).

The contents of the studied HMs in *T. latifolia* organs could be arranged in the following sequences: Mn>Fe>Zn>Cu>Ni>Pb>Co>Cd in leaves and Fe>Zn>Mn>Cu>Ni>Pb>Cd>Co in both rhizome and roots (Table 1). The cattail plants accumulated HMs mainly in rhizome and roots. The contents of most metals were higher in the roots than in the rhizome: 1.9 times for Fe, Mn, Cu, and Pb, and 5.7 times for Zn, Cd, and Co, on average. The exception was nickel, the content of which in the roots was half of that in the rhizome. The preferential accumulation of HMs in the roots, compared with the rhizome, was also noted by other authors who studied their accumulation in *T. latifolia* organs (Klink et al., 2013; Bonanno, Cirelli, 2017).

The leaves of *T. latifolia* accumulated twice more Mn than the underground organs (Table 1), while the TF for other HMs was less than one. That could be explained by the developed barrier mechanisms demonstrated for cattail previously (Grisey et al., 2012; Yang, Shen, 2020). The results obtained in the current study are consistent with the data of other authors, who found that *T. latifolia* accumulates HMs predominantly in its underground part (Alonso-Castro et al., 2009; Grisey et al., 2012; Parzych et al., 2016; Rana, Maiti, 2018; Haghnazar et al., 2021). Moreover, some researchers reported that the TF for manganese was higher than one (Rana et al., 2022). TF values below one indicate that the plant has low efficiency of metal translocation from roots to leaves and, thus, can be used for phytostabilization purposes (Elizareva et al., 2016; Bonanno, Cirelli, 2017).

According to Chaney (1989) and Kabata-Pendias (2010), the following metal concentrations are considered critical for plants: Cu: 20–100; Ni: 10–100; Zn: 500–1500; Mn: 300–500; Pb: 30–300; Cd: 5–7 mg kg^{-1} DW. The concentrations of Cu, Ni, Zn, and Pb in the rhizome and/or roots of *T. latifolia* were higher than their critical values by 1.6 times on average (Table 1). At the same time, the contents of most studied HMs in the leaves did not exceed critical values; the only exception was Mn, whose content was significantly (1.5 times) higher than its maximum critical concentration.

Although concentrations of metals in plant organs were higher than the critical ones, the morphological characteristics of *T. latifolia* corresponded to the usual sizes of this helophyte in a natural habitat (Table 2). For example, Lisitsyna and Papchenkov (2000) reported that *T. latifolia* height varied from 100 to 200 cm and leaf width from 1.5 to 2.0 cm. The variability of these parameters in plants from the study site was not considerable: the coefficient of variation (CV) was no more than 16 % (Table 2). The remarkable ability of this helophyte to form aboveground biomass regardless of environmental conditions and its well-developed root system are the reasons for recommending *T. latifolia* for rhizofiltration of contaminated wastewater (Lyubenova, Schröder, 2011; Elizareva et al., 2016).

As is known, excess metals can initiate the formation of reactive oxygen species (ROS), which cause oxidative degradation of lipids, resulting in the accumulation of thiobarbituric acid reaction products, such as MDA, in plant cells (Sharova, 2016; Hasanuzzaman et al., 2020;

Table 2. Morphophysiological parameters of *T. latifolia* plants from the natural contaminated site

Parameters	Means ± SE	CV, %	Lim (min–max)
Plant height, cm	124.50 ± 4.08	10.36	109.10–145.04
Leaf length, cm	55.6 ± 1.75	9.97	45.01–63.02
Leaf width, cm	1.34 ± 0.07	15.81	1.10–1.80
MDA, nmol g^{-1} DW	151.60 ± 3.53	5.70	140.91–162.53
Chlorophyll *a*, mg g^{-1} DW	2.88 ± 0.23	22.74	2.12–3.77
Chlorophyll *b*, mg g^{-1} DW	0.97 ± 0.08	22.66	0.65–1.20
Carotenoids, mg g^{-1} DW	0.88 ± 0.07	22.58	0.68–1.18
Proline, mg g^{-1} DW	0.61 ± 0.01	10.68	0.45–0.68
Total phenolics, mg g^{-1} DW	19.34 ± 0.68	6.86	16.38–20.83
Flavonoids, mg g^{-1} DW	7.46 ± 0.30	10.92	6.48–8.03
PT, µmol g^{-1} DW	5.44 ± 0.39	17.61	4.38–7.12
NPT, µmol g^{-1} DW	0.95 ± 0.09	22.09	0.73–1.26
Soluble protein, mg g^{-1} DW	35.69 ± 2.61	17.94	28.30–42.40

Data are presented as means ± SE (for morphological parameters n = 20, for physiological and biochemical parameters n = 5). SE – standard error; CV – the coefficient of variation; Lim – the minimum and maximum parameter values; MDA – malondialdehyde; PT –protein thiols; NPT – non-protein thiols.

Mir et al., 2021). In a previous study (Shiryaev et al., 2024), it was reported that the MDA content in *T. latifolia* significantly correlated with the degree of sediment contamination (r_s = 0.987 at $p < 0.01$) and reached its maximum – 149.8 nmol g^{-1} DW – at the most polluted site (the Ryzhiy stream). The MDA content in the leaves of *T. latifolia* growing at the study site was approximately at the same level (Table 2). The total chlorophyll content in cattail leaves was 3.9 mg g^{-1} DW (Table 2), which approximately corresponded to its amount in *T. latifolia* watered with metalliferous water (Manios et al., 2003). The Chl *a* to Chl *b* ratio was close to 3.0 while the ratio of total chlorophyll to carotenoids was 4.4 on average. The previous study showed that the content of free proline varied from 0.43 to 0.61 mg g^{-1} DW, soluble phenolics – from 17.5 to 35.1 mg g^{-1} DW, and soluble thiols – from 3.0 to 6.9 µmol g^{-1} DW. At the same time, a high positive correlation was found between these parameters and the degree of sediment contamination (Shiryaev et al., 2024). The content of these non-enzymatic antioxidants in the leaves of *T. latifolia* plants from the natural contaminated site (Table 2) was close to the previously reported values (Shiryaev et al., 2024).

The germination index of *T. latifolia* seeds collected from the natural contaminated site averaged 40 %, which indicates its fairly high viability. As is known, this index directly depends on external factors and can vary in cattail from 10 to 55 % (Bonnewell et al., 1983; Meng et al., 2016). Of particular interest is the comparison of the accumulative capacity and some morphophysiological traits of *T. latifolia* plants from the natural contaminated site (Table 2) with the responses of their seed progeny to high (2000 mg kg^{-1} of soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of soil, 6000Cu) copper concentrations in pot-scale experiment.

With the addition of 2000Cu and 6000Cu, copper content in cattail leaves was found to be lower than in the roots by a factor of 10 and 37, respectively (Fig. 2). Compared to the control (0Cu), the copper content in plants treated with 6000Cu increased by 83 times in the roots and 3 times in the leaves. A high positive correlation was found between copper content in the soil and its accumulation in cattail organs (r_s = 0.86, $p < 0.001$).

Such calculated parameters as BCF and TF for copper treatments were below one (Table 3). Their values were close to those in plants from

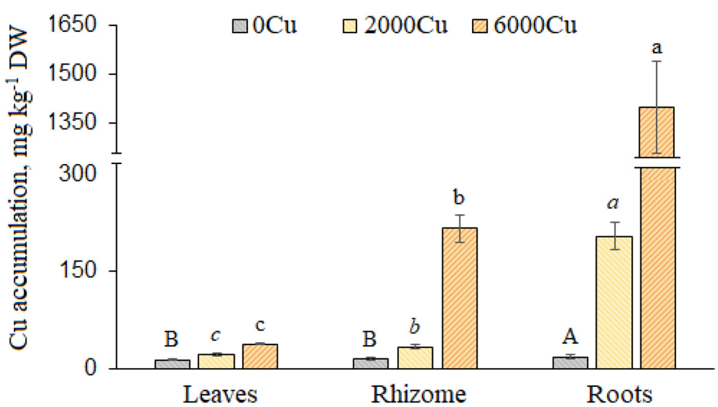

Fig. 2. Copper accumulation in different organs of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations. Data are presented as Means ± SE (n = 5). Different letters indicate significant differences between the treatments at $p < 0.05$

Table 3. Available Cu content in soil, bioconcentration factor (BCF), and translocation factor (TF) of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations

Parameters		Treatments		
		0Cu	2000Cu	6000Cu
Available Cu content, mg kg^{-1} of dry soil*		5.20 ± 0.22	1609.42 ± 90.01	3374.93 ± 45.75
Bioconcentration factor (BCF)	Leaves	2.495	0.013	0.011
	Rhizome	2.668	0.021	0.064
	Roots	3.352	0.127	0.417
Translocation factor (TF)	Leaves/Rhizome	0.935	0.640	0.175
	Leaves/Roots	0.741	0.104	0.027

*Data are presented as Means ± SE (n = 5).

Table 4. Morphophysiological parameters of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations

Parameters		Treatment		
		0Cu	2000Cu	6000Cu
Plant height, cm		57.90 ± 5.12 b	72.37 ± 5.51 a	48.86 ± 5.65 c
Leaf length, cm		70.20 ± 6.80 b	86.50 ± 6.98 a	56.29 ± 6.45 c
Leaf width, cm		0.74 ± 0.06 a	0.64 ± 0.06 a	0.49 ± 0.05 b
Plant fresh biomass, g	Aboveground	7.27 ± 1.78 a	7.39 ± 1.14 a	4.49 ± 0.64 b
	Underground	6.96 ± 0.88 a	9.58 ± 1.18 a	2.07 ± 0.29 b
Chlorophyll *a* content, mg g^{-1} DW		5.96 ± 0.34 a	6.10 ± 0.29 a	6.26 ± 0.32 a
Chlorophyll *b* content, mg g^{-1} DW		1.97 ± 0.09 a	2.04 ± 0.11 a	2.17 ± 0.10 a

Data are presented as Means ± SE (n = 8).

the natural contaminated site: 0.05 and 0.59, on average, for BCF and TF, respectively. However, the copper BCF values calculated for *in-situ* and *ex-situ T. latifolia* plants were lower than the data reported by other authors (Rana, Maiti, 2018; Haghnazar et al., 2021). This fact can be explained by the considerable concentrations of copper in the substrate, which was tens of times higher than the copper concentrations in sediments of polluted water bodies reported by other authors (Bonanno, Cirelli, 2017; Haghnazar et al., 2021).

Copper at 2000 mg kg^{-1} stimulated plant growth while 6000 mg kg^{-1} inhibited it (Table 4). The negative effect of the highest copper concentration (6000Cu) was also exhibited as the development of more necrotic lesions of *T. latifolia* leaves and their partial drying (Fig. 1).

The addition of 2000Cu increased leaf length (by 25 % of the control) and underground biomass (by 37 %), but no effect on the aboveground biomass of cattail was detected (Table 4). At ultra-high copper concentration, the length and width of the leaves, as well as the aboveground and underground biomass, significantly decreased (by half on average).

The study showed that there were no significant differences in the contents of Chl *a* and Chl *b* and in the Chl *a*/Chl *b* ratio between the treatments with and without Cu (Table 4). Interestingly, the total chlorophyll content in pot-scale experiment was higher by a factor of two compared with plants from the natural contaminated site (Table 2), but the Chl *a*/Chl *b* ratios were similar (about 3.0 on average). The resistance of this parameter to environmental pollution by HMs was previosly revealed by Shiryaev et al. (2024).

Since Cu is a redox active metal, it catalyzes the formation of ROS, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which are involved in free radical chain reactions of membrane lipids and proteins, thus causing oxidative degradation (Mir et al., 2021). ROS and antioxidants interacting with them are considered as functionally related compounds and are defined as redox active molecules, which are the main components of redox processes involving oxygen (Pradedova et al., 2017).

In *T. latifolia* plants treated with high and ultra-high Cu concentrations, the H_2O_2 content increased by 40 % on average and MDA by 20 %, compared with the control (Fig. 3a, b), and the H_2O_2 and MDA contents were positively correlated ($r_s = 0.59$ and 0.68, $p < 0.05$) with copper accumulation in cattail leaves. Nevertheless, the MDA level in *T. latifolia* seed progeny from pot-scale experiment was 24 % lower than in the plants from the natural contaminated site (Fig. 3b, Table 2). The MDA content is generally considered as an indicator of cell oxidative damage (Amir et al., 2020; Hasanuzzaman et al., 2020). A significant (30–70 %) increase in its content in cattail plants treated with different HMs was also noted by other authors (Tang et al., 2005; Mamine et al., 2022). What is more, the treatment of *T. latifolia* plants with Pb and Hg increased the MDA content by 2.3–3.5 times depending on the concentration of metals (Amir et al., 2020).

Proline accumulation is a general physiological response of plants subjected to various abiotic stresses (Kaur, Asthir, 2015; Aslam et al., 2017). The content of free proline in *T. latifolia* treated with 2000Cu was the same as in the control, but at 6000Cu it increased by 20 % (Fig. 4a). However, a positive correlation ($r_s = 0.58$, $p < 0.05$) was found between soil copper concentration and proline content in cattail leaves. The rise in *T. latifolia* proline content with the increase in copper concentration to 10 mg L^{-1} was also noted by other authors (Saygİdeger et al., 2009).

The application of 2000Cu non-significantly increased the content of carotenoids in cattail leaves, whereas at 6000Cu it was higher by

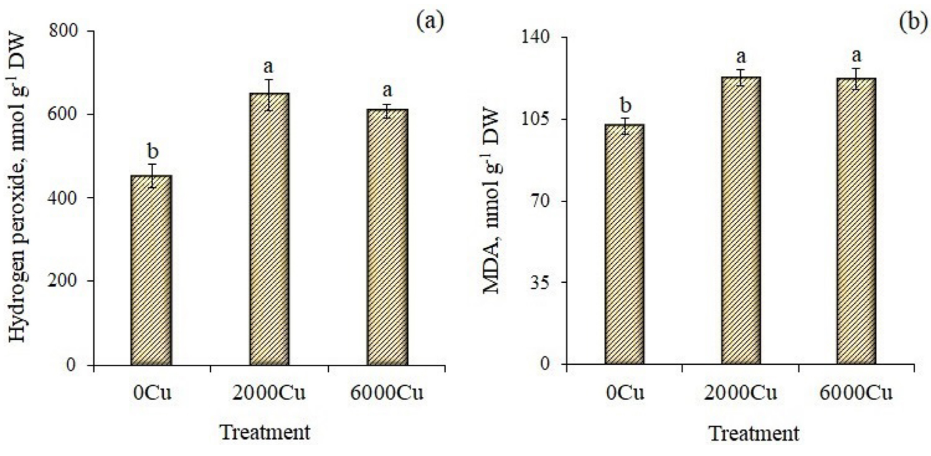

Fig. 3. Prooxidant content in the leaves of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations: (a) hydrogen peroxyde and (b) malondialdehyde (MDA)

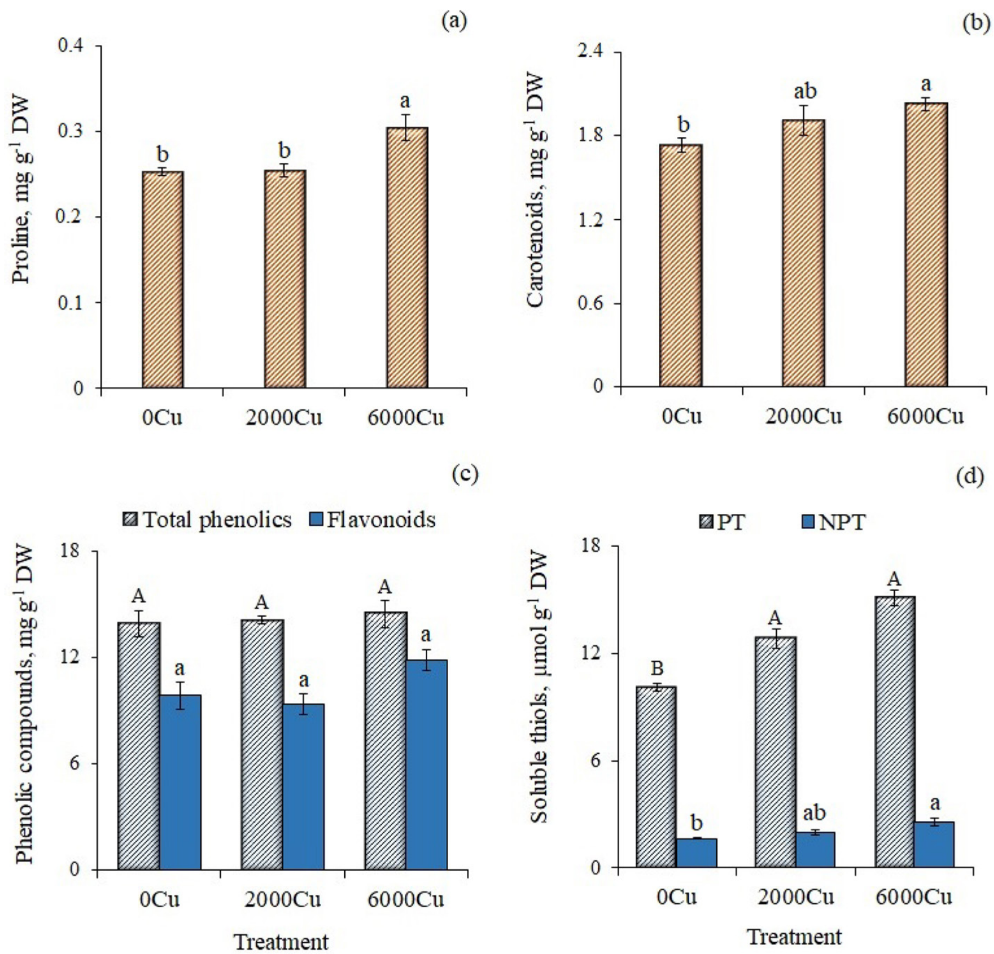

Fig. 4. Non-enzymatic antioxidant content in the leaves of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations: (a) free proline; (b) carotenoids; (c) soluble phenolics and flavonoids; (d) soluble protein thiols (PT) and non-protein thiols (NPT). Data are presented as means ± SE (n = 9). Different letters indicate significant differences between the treatments at $p < 0.05$

16 % (Fig. 4b). The Chl ($a + b$)/carotenoids ratio remained approximately at the same level and was slightly decreasing (from 4.6 to 4.2) with the addition of copper. In general, these values corresponded to those of *T. latifolia* from the *in-situ* site (Table 2). As is known, carotenoids are not only auxiliary photosynthetic pigments, but also important antioxidants. The antioxidant effect of carotenoids is associated with quenching of triplet chlorophyll and singlet oxygen. In addition, there is evidence of the ability of carotenoids to inhibit lipid peroxidation (Sharova, 2016).

No significant differences were found between the treatments in the total content of soluble phenolic compounds, including flavonoids in *T. latifolia* (Fig. 4c). The total content of phenolics in plants from pot-scale experiment was lower by a factor of 1.7 compared to plants in the natural contaminated site (Table 2, Fig. 4c). The opposite trend was observed for the content of flavonoids: their amount in seed progeny plants was 1.5 times higher than in the *in-situ* plants. Thus, the proportion of flavonoids in the total amount of soluble phenolics increased from 28 %

(natural contaminated site) to 74 % (pot-scale experiment).

The treatment of *T. latifolia* with copper led to an increase in the content of soluble thiols, both protein and non-protein ones, by 1.4 times on average compared to the control (Fig. 4d). At the same time, the proportion of protein thiols in their total amount was quite stable (about 86 %). There was a high positive correlation between copper concentration in leaves and soluble thiols (r_s = 0.82 and 0.69, $p < 0.05$ for protein and non-protein thiols, respectively). The important role of Cu and other metals in thiol synthesis was confirmed by our previous studies, which showed a high positive correlation between HM accumulation and soluble thiol content in several submerged and floating macrophytes (Borisova et al., 2016). The results of the present study are also consistent with research of Mamine et al. (2022), who found an increase in glutathione content by 40 % in *T. latifolia* treated with HM-polluted wastewaters. Lyubenova et al. (2015) also noted an increase in glutathione level in cattail treated with copper sulfate and cupric nitrate (by 20 and 60 %, respectively). Thus, the soluble thiols can be used as biomarkers of Cu contamination of water and sediment.

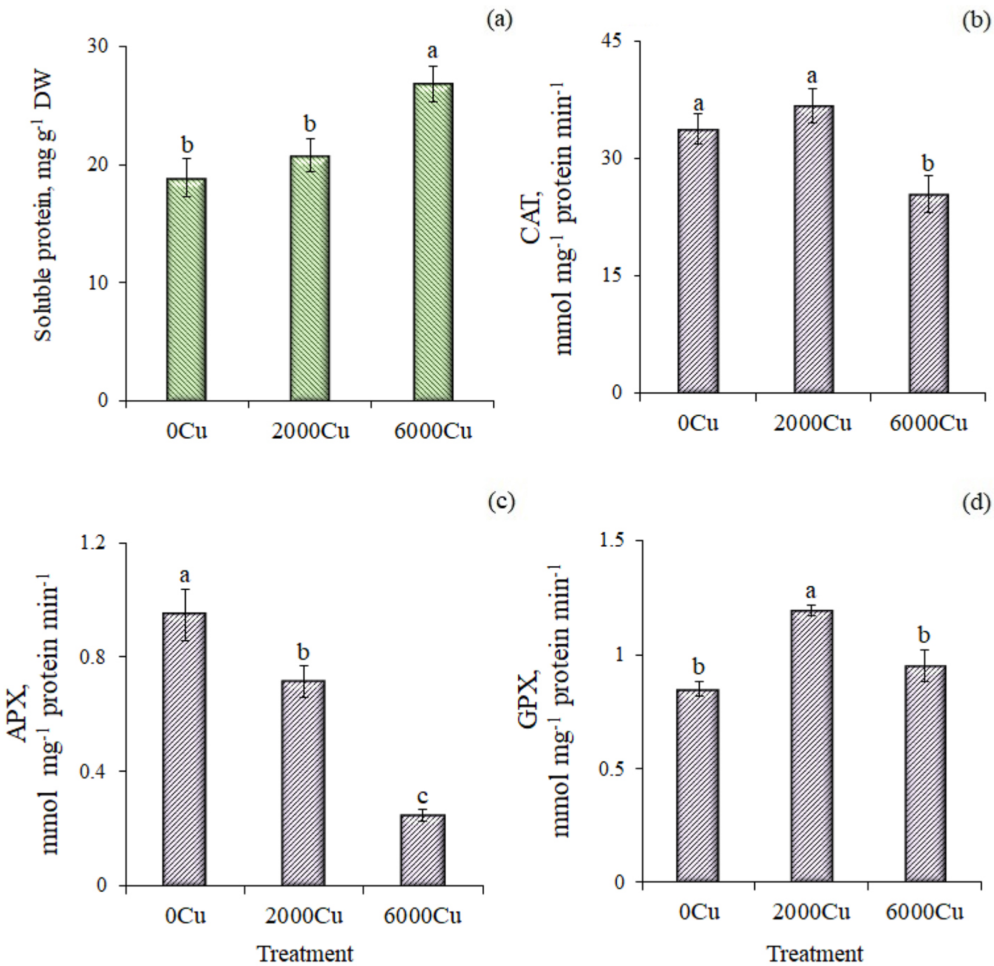

Fig. 5. Soluble protein content (a) and enzymatic antioxidant activity: (b) catalase (CAT), (c) ascorbate peroxidase (APX), and (d) guaiacol peroxidase (GPX) in the leaves of *T. latifolia* in pot-scale experiment at high (2000 mg kg^{-1} of dry soil, 2000Cu) and ultra-high (6000 mg kg^{-1} of dry soil, 6000Cu) copper concentrations. Data are presented as Means ± SE (n = 4). Different letters indicate significant differences between the treatments at $p < 0.05$

There were no significant differences in soluble protein content between control plants and plants treated with 2000Cu. At 6000Cu, though, the content of soluble proteins increased 1.4 times compared to the control (Fig. 5a). An increase in the soluble protein content in the HM-contaminated habitats was also observed in our previous study of the *T. latifolia* responses (Shiryaev et al., 2019). The same trend was found when studying submerged and floating macrophytes (Chukina, Borisova, 2010; Shiryaev et al., 2021). Various proteins are involved in the antioxidant defense system of plants. They are capable of both directly chelating HMs and acting as enzymes, catalyzing the reactions of ROS neutralization (Kulaeva, Tsyganov, 2011).

The activity of CAT in *T. latifolia* plants treated with 2000Cu changed insignificantly, whereas at 6000Cu it decreased by 25 % compared to the control (Fig. 5b). Both copper concentrations significantly reduced the APX activity: by 25 and 74 %, respectively, for high and ultra-high concentrations (Fig. 5c). The treatment with 2000Cu stimulated GPX activity increasing it by 40 % compared with the control, while 6000Cu did not affect the activity of this enzyme (Fig. 5d). Data on changes in the activity of antioxidant enzymes in metal-stressed *T. latifolia* plants are limited. It was previously shown that activity of CAT in *T. latifolia* shoots increased with rising Cu concentrations from 10 μmol to 50 μmol, but this inductive effect was almost lost at the highest concentration (100 μmol). At the same time, APX activity in cattail shoots was significantly lower compared to the control while POX activities remained at the same level (Lyubenova et al., 2015). Lyubenova and Schröder (2011) reported that with an increase in the concentrations of Pb, Cd, and As from 10 μmol to 250 μmol, the activities of CAT and POX in the leaves of *T. latifolia* increased.

Thus, comparison of the results of *ex-situ* and *in-situ* studies did not reveal same-direction trends in changes of most parameters under polymetallic stress and single copper action. The most sensitive biomarkers of copper stress were the contents of lipid peroxidation products (MDA) and soluble thiols.

Conclusions

The present study focused on copper accumulative ability and adaptive responses of *T. latifolia* in a natural multi-metal contaminated site (*in situ*) and in pot-scale experiment (*ex situ*) at high (2000 mg kg^{-1} of dry soil) and ultra-high (6000 mg kg^{-1} of dry soil) copper concentrations.

In addition, the study estimated the ability of plants from the natural contaminated site to accumulate other metals.

Results of the study demonstrated that *T. latifolia* is highly resistant to long-term anthropogenic impact in the area close to the Karabash Copper Smelter, which is heavily contaminated by copper. Furthermore, the seed progeny of this helophyte in pot-scale experiment also showed resistance to high copper concentrations, which neither affected the stability of the photosynthetic pigment complex nor produced any considerable inhibitory effect on plant growth. A decrease in plant biomass was observed only at the ultra-high copper concentration in the soil, but no plant death occurred. *T. latifolia* predominantly accumulated copper in roots and rhizomes: its content was tens of times higher than phytotoxic concentrations. That was accompanied by the generation of hydrogen peroxide and accumulation of malondialdehyde and other lipid peroxidation products. The high plant resistance to Cu was apparently achieved by increasing the level of free proline and soluble thiols. Hence, helophyte *T. latifolia* is a promising species for biomonitoring and phytoremediation processes (such as phytostabilization and rhizofiltration) in wastewater and water bodies not only at high, but also at ultra-high copper concentrations.

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