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Phytoremediation of nickel and chromium-containing industrial wastewaters by water lettuce (*Pistia stratiotes*)

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ABSTRACT

This study was conducted to assess the phytoremediation potential of *Pistia stratiotes* for post-treatment of Ni(II) and Cr(III)-containing industrial wastewater effluents in mono (synthetic wastewater) and bimetallic systems (real wastewater). Differences were seen in metal uptake, growth performance, and metal accumulation of the plants. In the monometallic system, the highest removal efficiency was calculated as 77.50% for Cr(III) and 70.79% for Ni(II) at 5 mg L^{-1} concentration. At 1.25 mg L^{-1} concentration, the bioconcentration factor of *P. stratiotes* was calculated as 734.2 for Ni(II) and 799.0 for Cr(III). To assess the effects of metal stress on plants, photosynthetic pigments and percent growth rates were also investigated. The percent growth rate increased from 38.22 to 81.74% for Ni and decreased from 87.53 to 43.18% for Cr(III) when the metal concentrations increased from 1.25 to 5 mg L^{-1}. Toxicity symptoms were less severe in plants exposed to low Ni concentrations. The greatest reduction in chlorophyll was observed at 5 mg L^{-1} Ni concentration. *P. stratiotes* showed better performance in the monometallic system. It was concluded based on present findings that *P. stratiotes* could potentially be used for the post-treatment of wastewaters containing Ni and Cr.

NOVELTY STATEMENT

Previous phytoremediation studies were mostly conducted only in either mono- or multi-metallic systems. In this study, mono- and bimetallic systems were assessed together and the feasibility of research findings on a large scale was investigated in detail. Present findings may also aid in the development of phyto-remedial strategies and the identification of Ni and Cr toxicity in macrophytes. *Pistia stratiotes* are already known for its incredible potential in removing metals and other contaminants from wastewater effluents. However, most studies only present data regarding the plant performance in laboratory studies (synthetic wastewater), while this study provides some important additional information on natural effluent conditions, which transform the presented data more interesting from a practical point of view.

KEYWORDS

A post-treatment process; Aquatic macrophytes; green technology; heavy metals; mono- and bimetallic systems; plant growth performance; tolerance

Introduction

Industrial residues, tanneries, textile and chemical industry, mining and military operations, fertilizer and pesticide applications, fuel production, and urban wastewaters constitute the primary sources of metals encountered in aquatic environments (Alalwan *et al*. 2020). Metals are highly toxic to animals and aquatic biota due to their persistence and bioaccumulation. Negative impacts of metals on flora and fauna have largely been observed in recent years (del Carmen 2022). Several methods, such as adsorption, reverse-osmosis, ion-exchange, chemical precipitation, electrocoagulation, and biological treatment are used to remove metals from wastewater effluents. However, these methods are usually uneconomic, energy-intensive, metal-specific, troublesome methods and generate secondary waste (Mishra and Tripathi 2008). In addition, in many industries, post-treatment processes are preferred to remove toxic and non-biodegradable pollutants and pathogenic microorganisms that are not fully removed by primary and secondary treatments (Andrade *et al.* 2013). Phytoremediation applied through plants is a common post-treatment process to remove metals from wastewater effluents (Schwantes *et al.* 2019). Constructed wetlands planted with aquatic or terrestrial plants are generally used to enhance the wastewater treatment potential of the system and improve the quality of wastewater before it is discharged into a water body. These plants can be used to remove metals, nutrients, and other pollutants from industrial wastewaters (Rezania *et al.* 2016). Macrophytes are aquatic plants used in the post-treatment of industrial wastewaters (Andrade *et al.* 2013).

Aquatic macrophytes are of great interest in the phytoremediation of water pollutants due to their rapid growth, high biomass, high tolerance, and accumulation ability (Lu *et al*. 2011). Phytoremediation via aquatic macrophytes is an

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Table 1. Composition of Hoagland nutrient solution (Hoagland and Arnon 1950; Buta *et al.* 2014).

Chemical	Concentration
KNO_3	1.25 mM
$Ca(NO_3)_2{\cdot}4H_2O$	1.25 mM
$MgSO_4{\cdot}7H_2O$	0.5 mM
KH_2PO_4	0.25 mM
H_3BO_3	11.6 μM
$MnCl_2{\cdot}4H_2O$	4.5 μM
$ZnSO_4{\cdot}7H_2O$	0.19 μM
$Na_2MoO_4{\cdot}2H_2O$	0.12 μM
$CuSO_4{\cdot}5H_2O$	0.08 μM
Fe(III)-EDTA, $C_{10}H_{13}FeN_2O_8$	10 μM

environment-friendly, cost-effective, and innovative green technology and offers efficient conservation of aquatic ecosystems (Buta *et al.* 2014; Das *et al.* 2014; Robles-Pliego *et al.* 2015). This technology includes a series of methods, such as reduction, removal, degradation, or immobilization of environmental pollutants without secondary waste generation using green plants. It is possible to accumulate and tolerate the targeted metal significantly with low-cost plants that are easily available (Lakra *et al.* 2017). There are several studies conducted on metal accumulation through aquatic plants (Das *et al.* 2014; Victor *et al.* 2016; Sricoth *et al.* 2018).

Free-floating aquatic macrophytes, *P. stratiotes*, chosen for the present work are widely used in the treatment of agricultural, domestic, and industrial wastewaters. These macrophytes are tolerant to various environmental conditions, have a wide application area, biomass production potential, exhibit rapid growth, and are easily harvested (Das *et al.* 2014; Galal and Farahat 2015; Ansari *et al.* 2020; Mustafa and Hayder 2021). *Pistia stratiotes* species can accumulate or absorb the contaminants by their whole body (Kumar *et al.* 2017).

Pistia stratiotes can grow in different industrial wastewaters, such as rubber, dairy, chromate mine, tannery, steel foundry, sugar mill, paper mill, etc. (Kumar *et al.* 2018a). Thus, the use of *P. stratiotes* for phytoremediation of industrial wastewater offers a low-cost and solar energy-driven cleanup practice (Kumar and Chopra 2016). Furthermore, plant biomass after the phytoremediation can be used as the raw material for making biogas since *P. stratiotes* contain 49.4% carbohydrates, 16.5% protein, 3.6% lipid, 17.8% fiber, and 23.8% ash, and furthermore (5.38%) (Pantawong *et al.* 2015; Kumar *et al.* 2017).

Pistia stratiotes have also been used for metal remediation and metal detoxification (Hanafiah *et al.* 2021; Saralegui 2021; Chan *et al.* 2022). However, there are few research results involving the use of *P. stratiotes* in the phytoremediation as a post-treatment process for the treatment of Ni and Cr in industrial wastewaters. Thus, the novelty of this work is to evaluate the efficiency of the potential use of *P. stratiotes* as a post-treatment process for Ni(II) and Cr(III)-containing industrial wastewaters through phytoremediation. Previous phytoremediation studies were mostly conducted only in either mono- or multi-metallic systems. In the present study, mono- and bimetallic systems were assessed together. In addition, in this study, plant growth performance, metal uptake, and accumulation were assessed through plant tissue analysis of aquatic macrophytes.

Materials and methods

Chemicals and materials

All the chemicals used were of high purity grade and were used without further purification. KNO_3 (≥99.0%), $Ca(NO_3)_2{\cdot}4H_2O$ (≥99.5%), H_3BO_3 (≥99.9%), HNO_3 (65%), CH_3COCH_3 and HCl (37%) were procured from Merck, Germany; $MgSO_4{\cdot}7H_2O$ (≥97.0%), KH_2PO_4 (≥99.0%) and NaOH (98–100.5%) were procured from Sigma-Aldrich, Germany; $MnCl_2{\cdot}4H_2O$ (≥99.0%) and $Na_2MoO_4{\cdot}2H_2O$ (≥99.5%) from Roth, Germany; $ZnSO_4{\cdot}7H_2O$ (>99%) from Fluka, Germany; $CuSO_4{\cdot}5H_2O$ (≥99.0%) from Acros, Organics, USA; H_2O_2 (30%) from Carlo Erba, Germany; and Fe(III)-EDTA from Alfa Chemistry, Germany.

Experimental design

Uniform and healthy *P. stratiotes* plants were collected from the Research and Application Center of Botanical Garden and Herbarium of Ege University, İzmir, Turkey (38°27′32.0″N and 27°13′59.3″E) and from the Botanical Garden named "Palm Center," Muğla, Turkey (36°57′40.0″N and 28°39′54.7″E). Collected macrophytes were brought to the laboratory, carefully washed through tap water to remove dust and other organic matter, and rinsed through deionized water. The macrophytes were then placed into a Hoagland nutrient solution medium for 10 days before the beginning of the experiment to acclimatize them to laboratory conditions before being exposed to metal contaminants for phytoremediation experiments. The Hoagland solution is a hydroponic nutrient solution. It is one of the most popular solution compositions for growing plants. The Hoagland solution contains all essential elements at proper levels for the growth of a wide range of plants. The chemical composition of the Hoagland solution prepared is provided in Table 1.

The phytoremediation experiments for the removal of Ni(II) and Cr(III) metals from wastewaters in mono and bimetallic system using *P. stratiotes* was conducted between June and July 2019, for 28 days in a plant growth chambers in the laboratory belonging to the Department of Environmental Engineering, Sivas Cumhuriyet University, located at Sivas, Turkey (39°42′23.7″N and 37°01′48.0″E). *Pistia stratiotes* were cultured under 24–28 °C temperatures and 12:12 light:dark photoperiods. The same conditions were also applied to the experimental studies. Following the acclimatization period, only young healthy plants that were not at the stage of senescence with similar size and weights were selected for phytoremediation experiments. This selection was made to avoid interference of the plant phenological stage in the phytoremediation performance (Aurangzeb *et al.* 2014). For phytoremediation experiments, 10 L polyethylene reactors (27 × 27 × 13 cm) were used (Figure 1).

Synthetic metal solutions were prepared by dissolving certain quantities of metals (Cr in the form of $CrCl_3{\cdot}6H_2O$ and Ni in the form of $NiCl_2{\cdot}6H_2O$) in the Hoagland solution (Table 2). About 40 g of fresh macrophytes were put separately into the reactors with 4 L of prepared Hoagland's solution and Ni (Set 1) and Cr (Set 2) solutions, respectively. The ratio of 10 g L^{-1} for phytoremediation reactors was determined based on literature findings (Maine *et al.* 2016; Victor *et al.* 2016; Lakra *et al.* 2017). Following the preparation of metal solutions, pH values were measured instantly. In Cr(III) reactors, pH values were arranged between 5.4 and 5.8 (Ma and Tobin 2004; Maine *et al.* 2016), and in Ni(II) reactors, pH values were kept below 5.0 to prevent chemical precipitation (Ji and Cooper 1996) based on both Mineql modeling and earlier speciation data (Baes and Mesmer 1986). Solution pH values were continuously monitored and pH arrangements were made with the use of 0.1 M HCl and NaOH. The daily maximum ±0.3-unit change was monitored.

Metal uptake was investigated in monometallic and bimetallic systems. In a monometallic system, macrophytes were subjected to separate absorption of each metal. In the bimetallic system, on the other hand, macrophytes were subjected to absorption of real wastewater mixtures containing both metals. The experiments were conducted in three polyethylene reactors, batch operating with a retention time of 24 h for 18 days. Hoagland's solution without the addition of $NiCl_2{\cdot}6H_2O$ or $CrCl_3.6H_2O$ was used for the biological control experiments. The same amount of sampling was done each time and solution volumes of experimental reactors were maintained constant.

Pistia stratiotes were exposed to different Cr(III) and Ni(II) concentrations (5, 3, 1.25, 0 mg L^{-1}). Ni and Cr concentrations were also monitored throughout the experiments. Morning hours were always preferred for the sampling. Sampling processes were continued until the metal concentration of polyethylene reactors did not decrease and the experiments were terminated when the concentration reached equilibrium. However, plant observations were as effective as water sampling in determining the duration of experiments. Throughout the study period, plant condition (necrosis, wilting, chlorosis, dying, shedding of leaves, etc.) was observed and recorded, and experiments were continued until decay, leaf, and root ruptures were observed in the plant. Experiments were terminated when the toxicity symptoms were encountered (Figure 2).

Figure 1. Experiments were prepared in three parallels for *P. stratiotes*.

The water samples were collected into glass tubes and the sample pH was brought below 2.0 with concentrated (65%) HNO_3. Samples were then stored in a fridge at 4 °C until the analyses.

Metal removal efficiency (%) of *P. stratiotes* was determined with the use of the standard formula, Equation (1) (Hurst 2007):

$$\%\text{removal efficiency} = (C_i - C_n/C_i) * 100 \tag{1}$$

where;

C_i = Initial concentration of metal in the wastewater (mg L^{-1})

C_n = Final concentration of metal in the wastewater (mg L^{-1})

Analysis of plant tissue

Plant samples (composed of leaves and roots) were carefully taken from each reactor with different media concentrations at the beginning and end of the experiments. Samples were rinsed through distilled water and oven-dried at 60 °C for 24 h (APHA/AWWA/WPCF 2005). Dry samples were ground in a grinder and a 200 mg ground sample was subjected to wet-digestion (Milestone Srl—ETHOS EASY model) in 2 mL H_2O_2 (30% purity) and 6 mL HNO_3 (65% purity) at 200 °C for 40 min. Samples were then cooled, supplemented with 20 mL distilled water, and filtered through Whatman No 42 filter paper. The digested samples were analyzed for Ni(II) and Cr(III) metals in an atomic absorption spectrophotometer (Shimadzu—AA-7000) (Nazreen *et al.* 2017). The metal detection limit of the device was 0.0001 mg L^{-1} for effluent and 0.0005 mg kg^{-1} DW. Standard solutions were used for device calibration before the analyses. Samples were analyzed three times for the accuracy of the analytical procedure. Any analysis result exceeding ±5% of the original measurement was reiterated immediately.

Metal concentrations measured at the beginning and the end of experiments were used to calculate the bioconcentration factor (BCF) and metal bioaccumulation (MB, %) of the macrophytes. A plant's ability to accumulate trace metals in contaminated environments can be estimated with the use of the bioconcentration factor. Present BCF values were determined with the use of Equation (2) (Victor *et al.* 2016; Galal *et al.* 2018).

Table 2. Metal concentrations.

Chemical	Metal	Study concentrations (mg L^{-1})
$NiCl_2{\cdot}6H_2O$ (Merck-Supelco; Grade:ACS; Quality Level: MQ300)	Ni(II)	0.75–1.25–3.00–5.00
$CrCl_3{\cdot}6H_2O$ (Sigma-Aldrich; Grade:CAS 10060-12-5; Quality Level: MQ200)	Cr(III)	1.25–3.00–5.00

Figure 2. Visible symptoms of toxicity.

$$\mathrm{BCF} = \frac{C_{macrophyte}}{C_{w\ initial}} \quad (2)$$

where;

$C_{\mathrm{macrophyte}}$ = Metal content in whole plant tissue at harvest (mg kg^{-1} DW)

$C_{w\ \mathrm{initial}}$ = Initial metal content in the growing medium (mg L^{-1})

The percentage of metal bioaccumulation (MB, %) by *P. stratiotes* was determined with the use of Equation (3) (Lakra *et al.* 2017).

$$\mathrm{MB}(\%) = (B_1 - B_2/B_1) * 100 \quad (3)$$

where;

B_1 = Plant metal concentration before phytoremediation

B_2 = Plant metal concentration after phytoremediation

Pistia stratiotes were harvested and dried in an oven at 60 °C for 24 h to remove the moisture content. The dry biomass weight (DW) was then measured. Chlorophyll a and b concentrations (mg g^{-1} DW) were determined spectrophotometrically to see the response of Ni(II) and Cr(III) on photosynthetic pigments (Arnon 1949). Ground samples (0.1 g) were dissolved in 10 mL 80% (v/v) acetone and kept overnight in dark conditions at 4 °C. Samples were centrifuged the next day at 5000 rpm and absorbance readings were performed for chlorophyll a (Chl-*a*) at 663 nm and chlorophyll b (Chl-*b*) at 645 nm in a UV-Vis spectrophotometer (Lakra *et al.* 2017). The chlorophyll values were calculated with the use of Equation (4) (Kumar *et al.* 2018b):

$$\text{Total chlorophyll:}\ \ 20.2(\mathrm{A645}) + 8.02(\mathrm{A663}) \quad (4)$$

$$\text{Chlorophyll } a\text{:}\ \ 12.7(\mathrm{A663})–2.69(\mathrm{A645})$$

$$\text{Chlorophyll } b\text{:}\ \ 22.9(\mathrm{A645})–4.68(\mathrm{A663})$$

Relative growth rate (RGR) is a critical parameter used in the assessment of the physiological response of plants to toxic chemicals (Maine *et al.* 2016). Present RGR values (g DW g^{-1} d^{-1}) were calculated with the use of Equation (5) (Hunt 1978):

$$\mathrm{RGR} = (\ln\ W_2 - \ln\ W_1)/(t_2 - t_1) \quad (5)$$

where;

W_1 = Initial plant dry weight (g)

W_2 = Final plant dry weight at harvest (g)

t_2 and t_1 = Planting periods after and before harvest (day).

The RGR value was then converted to a percent growth rate (% PGR) by Equation (6). Percentage growth rate (PGR) values were used to compare plant growth performance and metal tolerance of plants in solution (Saengwilai *et al.* 2017; Woraharn *et al.* 2021).

$\mathrm{PGR} = (W_2 - W_1)$/Total days of plant growth $\times$ 1000(6)

Real industrial wastewater

Experiments were also carried out with real industrial wastewater (RW) to evaluate the effect of *P. stratiotes* on post-treatment of wastewater from a nickel and chromium plating industry. The industrial effluent was obtained from wastewater pools of an industrial facility dealing with nickel and chromium plating in the Organized Industrial Region of Sivas province, Turkey. Due to high metal concentrations, wastewater samples were diluted to a concentration of 5 mg L^{-1}, then used in the preparation of the RW (Ni + Cr) mixture. For mixtures, wastewaters diluted to 5 mg L^{-1} separately were mixed in a 1:1 ratio (Bimetallic system) and used in treatment experiments (Figure 3). Wastewater characteristics are provided in Table 3. The pH was adjusted again to prevent chemical precipitation during the industrial wastewater treatment process.

Statistical analysis

Experimental findings were subjected to analysis of variance with the use of SPSS 23.0. Significant means were compared with the use of Tukey's test at $p < 0.05$. In addition, correlations between investigated parameters were determined. All experiments were conducted in triplicate; values are presented as means ± standard deviation (*SD*).

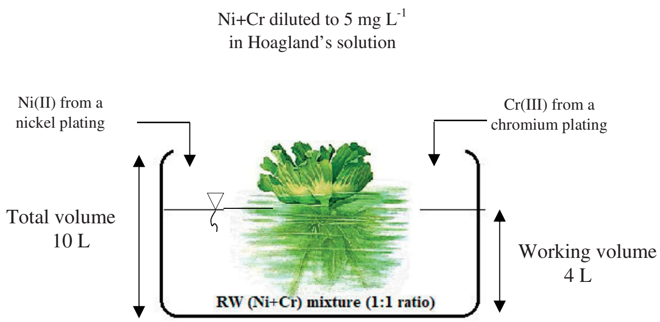

Figure 3. Industrial wastewater test setup.

Table 3. Characteristics of industrial wastewater.

Parameters	Ni (II)	Cr (III)
Concentration (mg L^{-1})	2735	25
pH	6.77	7.86
Conductivity (mS cm^{-1})	11.21	2.38
Total suspended solid (TSS) (mg L^{-1})	120	155
Oil-grease (mg L^{-1})	10	10

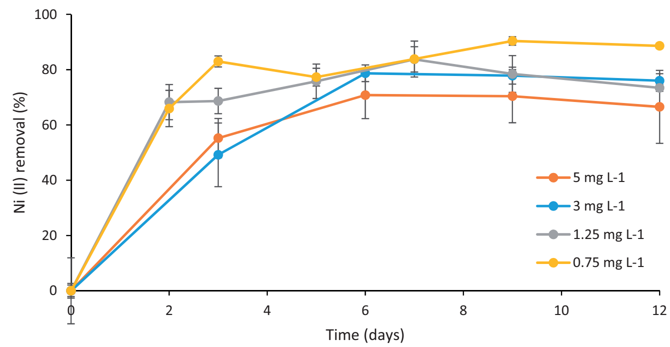

Figure 4. Reduction percentage of Ni(II) by *P. stratiotes* with time ($pH_{ort} = 4.5$, $T_{ort} = 22\,°C$).

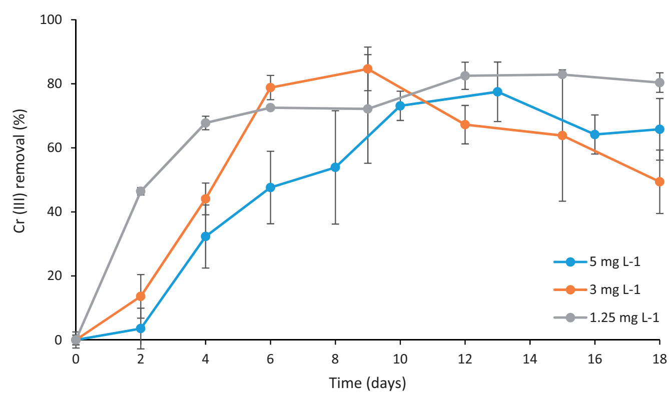

Figure 5. Reduction percentage of Cr(III) by *P. stratiotes* with time (pH = 5.4–5.8, $T_{ort} = 22\,°C$).

Results and discussion

Ni(II) and Cr(III) removal in water after phytoremediation

Pistia stratiotes was exposed to different Ni(II) concentrations (5, 3, 1.25, 0.75, 0 mg L^{-1}). The 70.79% removal was achieved at the 5 mg L^{-1} Ni concentration for the first 6 days in wastewater treated by *P. stratiotes*. Quite efficient findings were also observed in the other 3 concentrations (Figure 4). Maximum reductions in Ni concentrations were calculated as 90.39, 83.73, 78.70, and 70.79% for 0.75, 1.25, 3.0, and 5.0 mg L^{-1} concentrations, respectively. *Pistia stratiotes* were seen to be more effective at lower nickel concentrations. Kumar and Chopra (2016) conducted phytoremediation experiments with water caltrop (*Trapa natans*) and reported that aquatic plants worked best when contaminant levels were low because high concentrations may limit plant growth and take a longer time to clean up. The Ni(II) toxicity-induced morphological changes were encountered in aerial parts and roots of *P. stratiotes* especially at 3 and 5 mg L^{-1} concentrations. Toxicity symptoms encountered in aerial parts included chlorosis and necrosis in leaves. Toxicity symptoms encountered after the 7th day of experiments reduced removal performance.

Metal concentrations of the reactors on different days of the experiments for *P. stratiotes* exposed to different Cr(III) concentrations (5, 3, 1.25, and 0 mg L^{-1}) are presented in Figure 5.

The metal removal efficiency of *P. stratiotes* increased in time, reached a peak value, and decreased then. While removal efficiency continued to increase at 5 and 1.25 mg L^{-1} Cr(III) concentrations between days 9–12, it decreased after the 9th day at 3 mg L^{-1} concentration. During the initial 9 days of experiments, removal efficiency (84.65%) was greater at the 3 mg L^{-1} Cr(III) concentration than at the other concentrations. However, decreases in removal efficiency were encountered at all concentrations after the 12th day. Mishra and Tripathi (2008) conducted experiments for Cu, Cd, Fe, Cr, and Zn (1, 2, and 5 mg L^{-1}) removal performance of *P. stratiotes*, *Spirodela polyrhiza*, and *Eichhornia crassipes* and reported 81, 75, and 70% Cr removal efficiencies at 1, 2, and 5 mg L^{-1} concentrations, respectively. It was also reported that greater removal efficiencies were achieved at 2 mg L^{-1} concentration and except for a couple of treatments, metal removal efficiencies decreased at 5 mg L^{-1} concentration. Present findings were similar to those findings.

Removal efficiencies varied with metal concentrations. Increasing removal efficiencies were observed with increasing days and concentrations during the initial stages. *Pistia stratiotes* exhibited quite a good performance in removing metals from wastewater. Average removal performance during the 12-day experimental period was calculated as 75%. The study was terminated on the 12th day for Ni and the 18th day for Cr. An increase was observed in metal concentrations because some plants got into the senescent process, returning to the environment half of all that was absorbed. Similarly, Victor *et al.* (2016) reported decreasing Cr concentrations during the initial 10 days, but increasing concentrations later on. This result suggests the necessity of removing plants in senescence, so the system could operate properly (De Castro *et al.* 2017). According to the results presented, the management of these plants should be made between 12 and 18 days after their senescence in the environment. Older and/or metal-saturated plants should be removed from the environment so that younger plants do not lose their role in removing contaminants. Therefore, it is necessary to remove plants from the reactor at the beginning of the process of senescence (Schwantes *et al.* 2019).

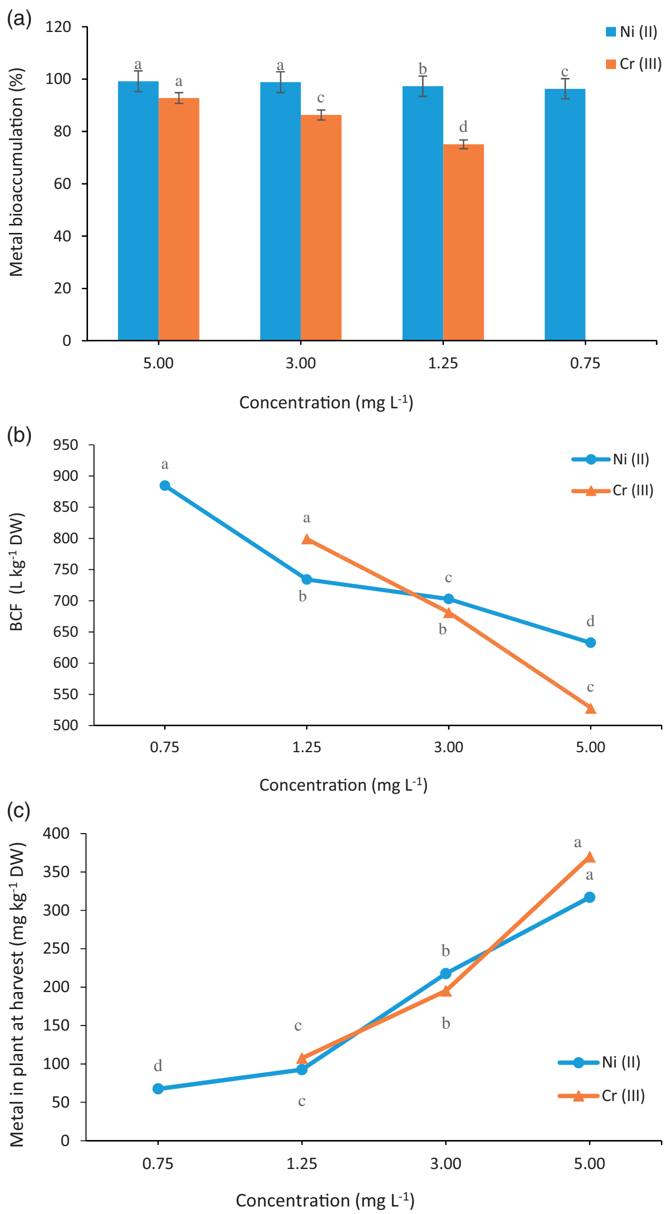

Figure 6. (a) Ni(II) and Cr(III) bioaccumulation of *P. stratiotes* (different letters in the same metal indicate a significant difference between each concentration at the level of $p < 0.05$). (b) Bioconcentration factor of metals in plants [different letters indicate the significant difference in BCF affected by different concentrations of metals ($p < 0.05$)]. (c) Metal concentrations in *P. stratiotes* at harvest after the experimental period (different letters are significantly different at $p < 0.05$).

Metal accumulation in plants after phytoremediation

Since metal removal efficiency is proportional to bioaccumulation capacity of macrophyte and metal concentration of liquid phase, Cr (III) and Ni (II) bioaccumulation percentage (MB, %) of *P. stratiotes*, bioconcentration factor (BCF) of metals in the plant and metal concentrations in the plant tissue of *P. stratiotes* at harvest were calculated and results are presented in Figures 6a–c, respectively.

Percentage metal bioaccumulation (MB, %) is calculated to assess metal tolerance and the potential use of selected species for phytoremediation (Ribeiro *et al.* 2020). It is an indicator of a plant's capacity to accumulate heavy metals (Aladesanmi *et al.* 2019). Thus, MB (%) was used to quantify the bioaccumulation effect of *P. stratiotes* on metal uptake from aqueous solutions. In *P. stratiotes* under Ni(II) and Cr(III) stress, increasing bioaccumulation quantities were encountered with increasing concentrations. When the metal concentration increased from 1.25 to 5.0 mg L^{-1}, metal bioaccumulation increased from 75.08 to 92.76% for Cr(III) and from 97.3 to 99.21% for Ni(II).

Leblebici and Aksoy (2011) applied different Ni(II) concentrations (1–5–10–20 mg L^{-1}) to *Spirodela polyrhiza* L. and investigated bioaccumulation quantities on 1, 3, 5, and 7th days. Increasing bioaccumulation percentages were seen with increasing metal concentrations. Present findings comply with those findings. Lakra *et al.* (2017) conducted a similar study and reported metal accumulation as 46% for Ni(II) and 82% for Cr(III), which were lower than the present values (Figure 6a). As can be inferred from Figure 6a, *P. stratiotes* had a high bioaccumulation level for Cr(III) and Ni(II).

The bioconcentration factor (BCF) is an important index for evaluating metal phytoremediation potential (Mendez and Maier 2008). In a hydroponic system, a high BCF ($\geq$ 1000) for whole plant tissue indicates the phyto-stabilization potential of plants for heavy metals (Syuhaida *et al.* 2014). Present BCF values decreased with increasing metal concentrations. When Ni(II) concentrations increased from 0.75 to 5 mg L^{-1}, BCF values decreased from 884.7 to 632.7 L kg^{-1} DW. When Cr(III) concentrations increased from 1.25 to 5 mg L^{-1}, BCF values decreased from 799 to 527.5 L kg^{-1} DW (Figure 6b). Present findings on BCF values revealed that *P. stratiotes* have phyto-stabilization potential for Ni(II) and Cr(III).

Different findings have been reported in similar studies. For instance, Zayed *et al.* (1998) worked with *Lemna minor*, six trace elements (Cd, Cr, Cu, Pb, Ni, and Se), and different concentrations of each trace element (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 mg L^{-1}) and reported BCF value as 200 for Ni and 600 for Cr. Odjegba and Fasidi (2004) worked with *P. stratiotes*, eight metals (Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) and different concentrations (0, 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0 mM) of each metal and reported BCF values as 605 for Ni and 1,607 Cr. Galal *et al.* (2018) used *P. stratiotes* for 2.0 mg L^{-1} of Fe, 0.05 mg L^{-1} of Mn, 0.1 mg L^{-1} of Cu and Pb, 0.01 mg L^{-1} of Zn, 0.05 mg L^{-1} of Cd and Cr, 0.15 mg L^{-1} of Co, and 0.2 mg L^{-1} of Ni. BCF values were reported to be >1,000 for the majority of the metals, except for Cr and Pb.

Accumulation of metals in total biomass of *P. stratiotes* after phytoremediation is presented in Figure 6c. There were linear relationships between the metal concentration of wastewater and the amount of metal translocated to *P. stratiotes*. Increasing quantities of metals were translocated to plants with increasing metal concentrations. Metal concentration in *P. stratiotes* at 5.0 mg L^{-1} concentration was measured as 0.317 g kg^{-1} DW for Ni(II) and 0.370 g kg^{-1} DW for Cr(III) (Figure 6c).

Maine *et al.* (2004) reported important linear relationships between the amount of Cr to be treated and Cr quantities translocated to roots and aerial parts of *P. stratiotes* and *Salvinia herzogii*. Similar linear relationships were also reported in Cu and Cd removal studies with *Eichhornia crassipes*, *Azolla filiculoides*, and *P. stratiotes* (Maine *et al.*

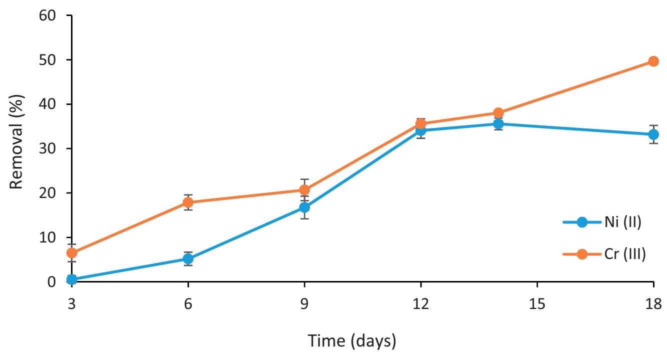

Figure 7. Cr(III) and Ni(II) removal efficiency of *P. stratiotes* from real wastewaters (Values are mean ± standard deviation of three replicates).

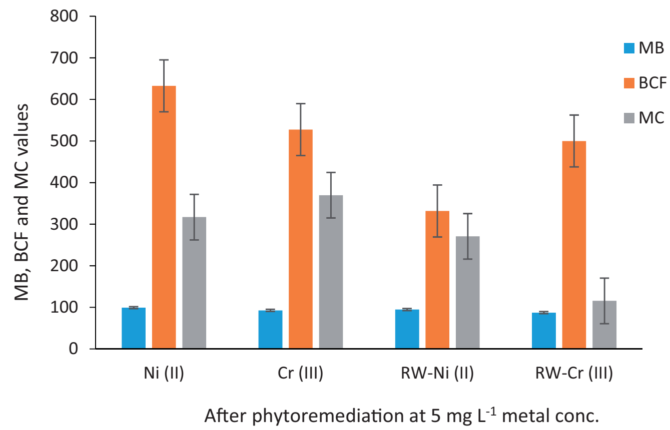

Figure 8. MB (%), BCF (L kg^{-1} DW), and MC (mg kg^{-1} DW) values after phytoremediation in mono- and bimetallic systems by *P. stratiotes* (Values are mean ± standard deviation of three replicates).

2001; Mufarrege *et al.* 2010). Lakra *et al.* (2017) reported that metal uptake of plants increased with an increased concentration of metal present in the aquatic environment.

Metal removal from industrial wastewater mixture after phytoremediation

Real wastewaters supplied from wastewater pools of an industrial facility dealing with metal plating experimented for this part of the study. Wastewaters from Ni and Cr plating operations were diluted separately to have 5 mg L^{-1}, then mixed in a 1:1 ratio (v/v) and used in experiments. Plants in experimental reactors were monitored throughout the experiments. On the 6th day of experiments, while there was wilting and necrosis in plant leaves and slight deformations in roots; new leaf and shoot formations were seen. Plant aging was encountered in subsequent days and intense necrosis, ruptures, wilting, and chlorosis were encountered in leaves and roots on the 18th day. Experiments were then terminated by taking such changes into account. Time-dependent Cr(III) and Ni(II) removal efficiency of *P. stratiotes* are presented in Figure 7.

While removal efficiencies exhibited an increasing trend throughout the study, a decrease was encountered after the 14th day for Ni(II). However, Cr(III) removal efficiency increased throughout the experiments. In measurements made on the 12th day of the bimetallic system, Cr(III) and Ni(II) removal efficiencies were respectively measured as 35.59 and 34.05%. In measurements made on the 12th day of the monometallic system under the same conditions, removal efficiencies at 5 mg L^{-1} concentration were respectively measured as 66.53% for Ni(II) (Figure 4) and 75.40% for Cr(III) (Figure 5). In this sense, for both metals, *P. stratiotes* exhibited twice as much performance in the monometallic systems prepared with synthetic wastewaters. In a bimetallic system prepared with the real wastewaters, wastewater mixtures had toxic impacts on plants and thus reduced treatment performance. Similar findings were also reported by Mufarrege *et al.* (2010) and Shmaefsky (2020).

Accumulation of metals in plants after phytoremediation of real wastewater

Metal bioaccumulation (MB, %), bioconcentration factor of *P. stratiotes* (BCF, L kg^{-1} DW), and metal concentrations in the plant at harvest (MC, mg kg^{-1} DW) in the synthetic monometallic system and bimetallic system prepared with real wastewater mixture are presented in Figure 8. In both systems, 5 mg L^{-1} metal concentrations were experimented.

When the accumulation levels of the plants harvested after contact with the *P. stratiotes* during an 18-day experimental period were examined, it was seen that metal bioaccumulation percentages of the plants in the bimetallic system were lower as compared to accumulation levels in synthetic metal solutions alone. As can be seen from Figure 8, Cr(III) bioaccumulation of *P. stratiotes* in industrial wastewater was lower (87.36%) than in the others. Present findings revealed that Cr(III) and Ni(II) could be bioaccumulated by *P. stratiotes* both in synthetic and real wastewaters. Within the selected range of concentration, *P. stratiotes* effectively bioaccumulated both metals and highly reduced metal levels in the water.

The bioconcentration factor is an important index for the screening of candidate plants for use in phytoremediation (Hasnaoui *et al.* 2020). BCF values were greater in synthetic wastewater samples (Figure 8). The greatest value was observed in the 5 mg L^{-1} Ni(II) reactor (632.7 L kg^{-1} DW) and the lowest value in the RW-Ni reactor (331.8 L kg^{-1} DW). For both metals, BCF values decreased in the bimetallic system. From the wastewater mixture, the Cr(III) uptake capacity of the plant (500.14 L kg^{-1} DW) was greater than Ni(II) uptake capacity (331.77 L kg^{-1} DW). Such greater BCF values made *P. stratiotes* more suitable for Cr(III) phytoremediation rather than Ni(II) for the bimetallic system. The increase in BCF value is an important criterion showing the increase in the phytostabilization potential of the plant for heavy metals.

Pistia stratiotes accumulated a significant quantity (mg kg^{-1} DW) of both metals. However, the amount of metal translocated into the plant was greater in synthetic single metal solutions. On the other hand, less quantities of metals were translocated into the plants in real wastewater samples and less reductions were encountered in Cr(III) reactors. Plant Cr(III) content at harvest was measured as 0.370 g kg^{-1} DW in synthetic wastewater and 0.116 g kg^{-1} DW in real wastewater. Similarly, plant Ni(II) content at harvest

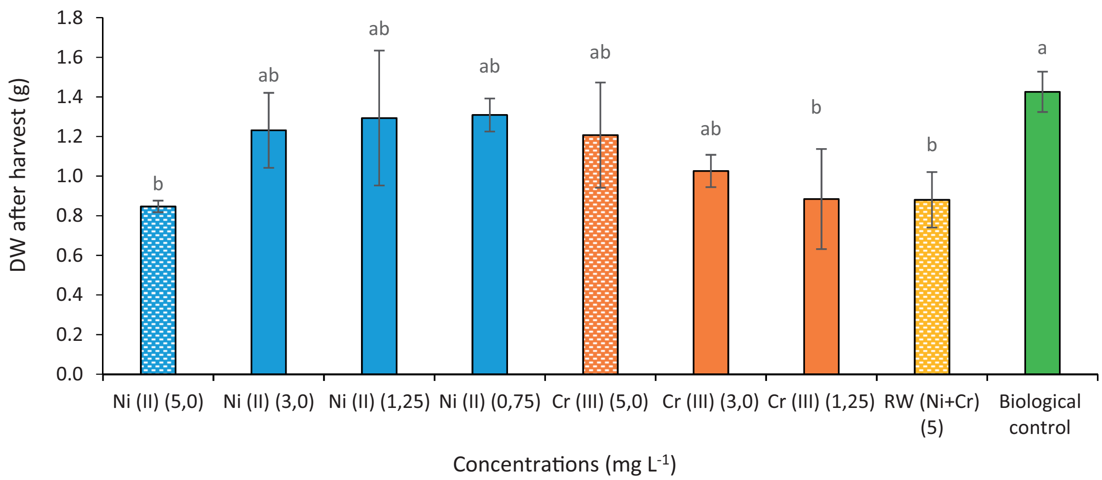

Figure 9. Plant dry weights (DW) measured after metal treatments in mono- and bimetallic systems (values are mean ± standard deviation of three replicates) (different letters are significantly different at $p < 0.05$).

was measured as 0.317 g kg^{-1} DW in synthetic wastewater and 0.271 g kg^{-1} DW in real wastewater (Figure 8).

Toxic impacts of metals

Plant dry weight at harvest

Plant samples with an initial fresh weight of 40 g had a significant loss of biomass at harvest. As compared to the control, significant decreases were observed in *P. stratiotes* biomass at harvest and the greatest reduction in plant biomass was seen in the 5 mg L^{-1} Ni treatment.

Alacabey and Zorer Çelebi (2020) conducted a study with switchgrass (*Panicum virgatum*) and reported increasing plant Cr(III) contents with increasing wastewater chromium concentrations. It was also reported that plant growth continued and the plant could tolerate increased concentrations. It was reported in another study conducted with *P. stratiotes* that plant dry weights increased with increasing chromium concentrations and metal removals increased with increasing plant biomass (Tabinda *et al.* 2020). Present findings comply with those earlier ones. Plant dry weight at harvest increased from 0.88 to 1.21 g when Cr(III) concentrations increased from 1.25 to 5.0 mg L^{-1}. Plant dry weights at harvest decreased in real wastewater mixture (RW (Ni + Cr)) prepared at 5 mg L^{-1} concentration with the effect of Ni(II). As can be seen in Figure 9, plant weight losses were greater in real wastewater mixtures than in synthetic wastewaters and greater weight losses were encountered as compared to the control set.

Estimation of relative growth rate

The relative growth rate (RGR) is the most widely used method for estimating plant growth rate during the phytoremediation studies (Kumar *et al.* 2018a). RGR is calculated to assess the effects of toxic chemicals on plants (Farnese *et al.* 2014). *Pistia stratiotes* were kept in synthetic and real wastewaters for 12 days for Ni and 18 days for Cr. Samples were taken at the beginning and end of the experiments. To specify the toxic effects of both metals on *P. stratiotes*, relative growth rates were calculated by Equation (5) and results (% PGR) are presented in Figure 10. In plants treated with different concentrations of effluent, the relative growth rates decreased as compared to the biological control. Percent growth rate (% PGR) can be used as an indicator of the level of heavy metal stress on plant growth performance of different species and growing periods (Meeinkuirt *et al.* 2013; Sricoth *et al.* 2018). In this study, *P. stratiotes* showed lower relative growth rates (87%) in the treatment of mixture (RW (Ni + Cr)) as compared to the biological control. High heavy metal concentrations can result in inhibitory effects on the plant's metabolic and physiological processes and decreases in growth parameters of the aquatic plants as determined by Woraharn *et al.* (2021).

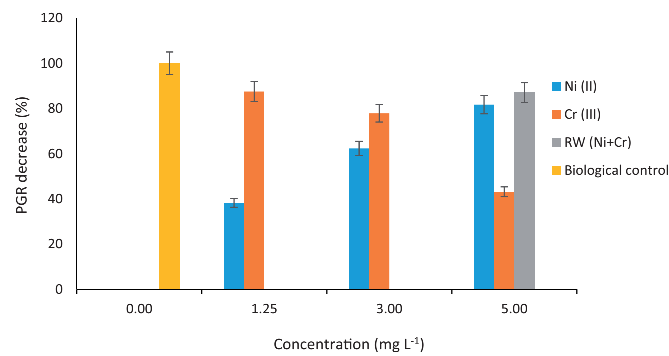

Figure 10. PGR decreases (%) after metal treatments in mono- and bimetallic systems (values are mean ± standard deviation of three replicates).

Decreasing growth rates were seen with increasing Ni(II) concentrations. At 5 mg L^{-1} Ni(II) concentration, *P. stratiotes* had significantly lower RGR values than the other concentrations (Figure 10). Leblebici and Aksoy (2011) applied different Ni concentrations (1–5–10–20 mg L^{-1}) to *Spirodela polyrhiza L.* and investigated the relative growth rates on the 1, 3, 5, and 7th days. Decreasing relative growth rates were reported with increasing metal concentrations of the growth media. Present findings comply with those earlier ones. In the present study, the PGR reduction value as compared to the control set increased from 38.22 to 81.74% when Ni concentrations increased from 1.25 to 5 mg L^{-1}. Odjegba and Fasidi (2004) also reported an inverse relationship between metal concentrations and plant growth rates.

Increasing growth rates were seen with increasing Cr(III) concentrations. The percent growth rate decreased from 87.53 to 43.18% when the Cr(III) concentrations increased from 1.25 to 5 mg L^{-1}. PGR reduction value was calculated as 81.74% for synthetic wastewater prepared with 5 mg L^{-1} Ni and 87.06% for real wastewater mixture (RW (Ni + Cr)) and these values were the highest values calculated throughout the experiments (Figure 10). Present findings revealed that Ni had the greatest toxic effects on plants. RGR values measured in experimental treatments were significantly lower than the values of biological control treatments. Such a case demonstrated the growth-inhibiting effect of metals. Morphological signs of toxicity were recorded for both metals throughout the experiment. Symptoms, such as yellowing, chlorosis, and necrosis of leaves and roots were also very clear at 5 mg L^{-1} Ni and Cr(III) concentrations. Veselý *et al.* (2011) indicated a 40% growth reduction for *P. stratiotes* grown in 10 mg L^{-1} Pb as compared to 5 mg L^{-1} Pb in solution.

Photosynthetic pigments

Metals result in various changes in plant physiological and biochemical characteristics. Among these changes, photosynthetic pigment concentrations are the most important ones. Kolotov *et al.* (2003) and Hadad *et al.* (2007) pointed out chlorophyll concentration as a precise measure of metal toxicity. Plant response to metals largely depends on metals and macrophyte species used in phytoremediation (Di Luca *et al.* 2014). Therefore, rather than the relative growth rate, chlorophyll-*a* can be used as a more precise indicator of metal toxicity (Mufarrege *et al.* 2010). RGR and chlorophyll values should be assessed together for an accurate assessment of the toxic impacts of metals. Therefore, chlorophyll values of *P. stratiotes* were determined at harvest and the results are provided in Table 4. Chlorophyll (Chl) contents decreased in treated plants as compared to the control groups due to alteration in the physiological processes of the plants as a result of metal accumulation. It was seen that Cr(III) and Ni(II) toxicity reduced chlorophyll concentration and plant growth. Present evidence complies with the results of previous studies conducted with *Azolla pinnata* (Bharti and Kumar Banerjee 2012), *Lemna minor* (Vaseem and Banerjee 2012), and *Hydrilla verticillata* (Kumar and Banerjee 2015). Lakra *et al.* (2017) reported significantly decreasing Chl-*a* contents of Ni-treating plants as compared to the control plants.

Mishra and Tripathi (2008) investigated metal (Fe, Zn, Cu, Cr, and Cd) removal efficiencies of three macrophyte species (*P. stratiotes*, *Spirodela polyrrhiza*, and *Eichhornia crassipes*) and reported toxicity symptoms (chlorosis in leaves and shedding in roots) in plants exposed to 5.0 mg L^{-1} Cr and Cd. Decreases were seen in chlorophyll values of the plants and such decreases were associated with inhibition of chlorophyll synthesis and resultant loss of photosynthetic activity. Similar findings were seen in the present study. Decreases were seen in Chl-*a*, Chl-*b,* and total Chl contents at high Cr(III) concentrations. The greatest reduction was encountered at 5 mg L^{-1} Cr (III) and Ni(II) concentrations.

In addition to the chlorophyll content, plants in experimental reactors were monitored throughout the study. Morphological toxicity signs were recorded for both metals during the experimental period. Following the exposure to nickel and chromium, symptoms, such as yellowing, chlorosis, and necrosis of leaves and roots were also very clear from the 7th day in Ni-containing reactors and the 5th day in Cr-containing reactors at all concentrations. In addition, a small number of new leaves and shoots were also observed. The water in the test reactors was clear and clean. Intense necrosis and rupture were encountered in leaves and roots on the 12th day of Ni exposure and the 18th day of Cr exposure. The reduction in biomass and growth of the plants in this study may well be due to the inhibitory effect of Ni and Cr on the synthesis of photosynthetic pigments that negatively impacts photosynthesis processes. Similar results were also reported by John *et al.* (2009) for Cd treatment by *Brassica juncea* L.

In the present study, Chl-*b* values were more influenced by metals than Chl-*a* values. Such a case indicated that Chl-*b* was more sensitive to Ni concentrations. Increasing Chl-*a*/*b* ratios were observed with increasing Ni and Cr concentrations. Similar to the present findings, Pandey and Sharma (2002) reported greater reductions in Chl-*b* contents than in Chl-*a* contents of cabbage leaves exposed to Ni. Vajpayee *et al.* (2001) also reported higher reductions in leaf Chl-*b* contents with exposure to Ni and Cr toxicity. Hadad *et al.* (2007) determined Cr, Ni, and Zn tolerance of *Salvinia herzogii* and identified Ni as the most toxic to plants. Similar findings were also observed in the present study. Toxicity symptoms mostly included bleaching and chlorosis in plant leaves and browning and ruptures in plant roots. Such symptoms were quite similar to the symptoms of nickel toxicity. Toxicity symptoms primarily resulted from the high level of Ni accumulation in plant tissues. Toxicity symptoms were less severe in plants exposed to 0.75 and 1.25 mg L^{-1} Ni concentrations. Ni toxicity may alter plant metabolism, have negative impacts on photosynthetic activity and result in loss of water.

Maine *et al.* (2004) reported decreasing chlorophyll contents of *P. stratiotes* with exposure to 4 mg L^{-1} Cr, but unchanged chlorophyll contents of *S. herzogii* with exposure to 6 mg L^{-1} Cr. In the present study, the effects of metals on chlorophyll contents varied with the concentrations, and decreases were observed in chlorophyll pigment at all Cr(III) and Ni(II) concentrations. As compared to the control group, the greatest reduction in chlorophyll was observed at 5 mg L^{-1} Ni concentration (Table 4). Moreover, present findings confirmed that Ni(II) and Cr(III) had significant impacts on the photosynthetic activity of macrophytes in both mono- and bimetallic systems. Correlations between entire traits are provided in Table 5.

Plant Ni(II) removal efficiencies were highly correlated with wastewater Ni(II) concentrations. Plant Ni(II) contents increased with increasing wastewater Ni(II) concentrations ($p < 0.01$). On the other hand, decreases were encountered in

Table 4. Chl-*a*, Chl-*b* and total Chl content of *P. stratiotes* after metal treatments in mono- and bimetallic systems.

	Chl-*a* (mg g^{-1} DW)			Chl-*b* (mg g^{-1} DW)			Total Chl ($a+b$) (mg g^{-1} DW)		
Concentration (mg L^{-1})	Ni (II)	Cr (III)	RW (Ni + Cr)	Ni (II)	Cr (III)	RW (Ni + Cr)	Ni (II)	Cr (III)	RW (Ni + Cr)
5.0	0.415 ± 0.069^d	0.704 ± 0.062^c	1.101 ± 0.108^c	0.305 ± 0.067^c	0.419 ± 0.056^d	0.228 ± 0.000^e	0.720 ± 0.137^e	1.122 ± 0.118^d	1.328 ± 0.109^d
3.0	1.014 ± 0.218^c	1.439 ± 0.072^b	–	0.638 ± 0.151^b	0.778 ± 0.036^c	–	1.651 ± 0.368^{bc}	2.217 ± 0.081^c	–
1.25	1.308 ± 0.071^{bc}	1.677 ± 0.139^a	–	0.652 ± 0.025^b	1.103 ± 0.152^b	–	1.960 ± 0.046^{ab}	2.779 ± 0.013^b	–
0.75	1.589 ± 0.263^{ab}	–	–	0.660 ± 0.119^b	–	–	2.249 ± 0.144^b	–	–
Control	1.693 ± 0.122^a			1.514 ± 0.040^a			3.207 ± 0.153^a		

Values are presented as means of three replicates ± *SD*, means indicated with the same letters are not significantly different at $p < 0.05$.

Table 5. Correlation table for the parameters tested in experiments.

Metal	Parameters	Dosage	Removal	Ni/Cr in water	Ni/Cr in plant	BCF	Dry weight	Bioac. in plant	RGR	Chl-*a*	Chl-*b*
Ni(II)	Ni dosage	1									
	Ni removal	0.591**	1								
	Ni in water	0.113	−0.177	1							
	Ni in plant	0.431	0.822**	0.395	1						
	BCF	0.571*	0.999**	−0.149	0.837**	1					
	Dry weight	0.140	−0.037	−0.753**	−0.437	−0.055	1				
	Bioac. in plant	0.386	0.189	0.858**	0.633**	0.207	−0.775**	1			
	RGR	0.311	−0.301	−0.859**	−0.748**	−0.324	0.795**	−0.972**	1		
	Chl-*a*	−0.649**	−0.246	−0.642**	−0.544*	−0.251	0.701**	−0.896**	0.835**	1	
	Chl-*b*	−0.262	−0.527*	−0.726**	−0.898**	−0.549*	0.695**	−0.833**	0.925**	0.689**	1
	Total Chl ($a+b$)	−0.498*	−0.419	−0.743**	−0.782**	−0.433	0.760**	−0.941**	0.957**	0.921**	0.916**
Cr(III)	Cr dosage	1									
	Cr removal	0.628*	1								
	Cr in water	0.494	−0.001	1							
	Cr in plant	0.396	0.882**	0.456	1						
	BCF	0.381	0.951**	0.090	0.848**	1					
	Dry weight	−0.005	−0.598*	−0.231	−0.585*	−0.731**	1				
	Bioac. in plant	0.808**	0.678**	0.103	0.727**	0.455	−0.111	1			
	RGR	−0.041	−0.748**	−0.436	−0.804**	−0.889**	0.777**	−0.194	1		
	Chl-*a*	−0.389	−0.320	−0.531*	−0.609*	−0.145	−0.014	−0.824**	0.099	1	
	Chl-*b*	−0.107	−0.489	−0.798**	−0.833**	−0.442	0.344	−0.642**	0.558*	0.830**	1
	Total Chl ($a+b$)	−0.244	−0.432	−0.709**	−0.765**	−0.322	0.191	−0.756**	0.368	0.947**	0.965**

*Correlation is significant at 0.05 level (two-tailed).
**Correlation is significant at 0.01 level (two-tailed).

dry matter contents. Increasing wastewater metal concentrations reduced plant Chl-*a* and Chl-*b* contents ($p < 0.01$). In this sense, Ni(II) removal positively correlated with Ni(II) in plant and BCF ($p < 0.01$), and water Ni(II) concentration positively correlated with plant metal concentration ($p < 0.01$). There were negative correlations between plant metal uptake and dry matter contents ($p < 0.01$). Plant Cr(III) accumulation correlated with Cr(III) removal ($p < 0.01$). Decreasing plant dry weights were observed with increasing plant Cr(III) contents. Decreasing plant Chl-*a* and Chl-*b* were observed with increasing plant Cr(III) removals (Table 5). RGR values were negatively correlated with Ni and Cr concentrations in wastewater. There is also a negative correlation between RGR values and Ni/Cr concentrations in the plant ($p < 0.01$). However, there is a positive correlation between total Chl ($a+b$) and RGR values. Total Chl ($a+b$) values decreased with decreasing RGR values. Therefore, RGR values may follow the same trends as total chlorophyll values, which can be used to assess metal toxicity.

Conclusions

It was concluded based on present findings that *P. stratiotes* could potentially be used for the post-treatment of Ni(II) and Cr(III)-containing industrial wastewaters. Under 5 mg L^{-1} concentration, 77.5% removal was achieved for Cr(III) and 70.8% for Ni(II). Results indicated that *P. stratiotes* could be used in phytoremediation for the post-treatment of Ni(II) and Cr(III) contaminations at low concentrations (≤ 5 mg L^{-1}). Plant chlorophyll levels decreased under metal stress in mono- and bimetallic systems. Metals also had toxic effects on relative growth rate (RGR) and photosynthetic pigments.

The phytoremediation potential of *P. stratiotes* could be enhanced through the periodical harvest of macrophytes. Harvested macrophytes could be ashed and the metals accumulated in the plant structure can be recovered for commercial purposes. Bioenergy could be used as an alternative renewable energy source. *Pistia stratiotes* biomass after the phytoremediation process has a high potential for use in biogas production. Further research is recommended for the appropriate recycling of metal-enriched biomass of *P. stratiotes*.

Author contributions

İlknur Şentürk: supervision, investigation, visualization, resources, data collection, methodology, and writing original draft—review and editing. Nur Sena Eyceyurt Divarcı : investigation, resources, data collection, and experiment. Mustafa Öztürk: statistical analysis, AAS analysis, and review and editing.

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