

EFFECTIVENESS OF YELLOW VELVETLEAF (*Limnocharis Flava*) AND WATER CLOVER (*Marsilea Crenata*) AS PHYTOREMEDIATORS OF CHROMIUM (CR) IN TANNERY WASTEWATER

Yumna Alya Az-Zahro¹, Fida Rachmadiarti²

^{1,2} Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Negeri Surabaya, Surabaya, Indonesia

*Corresponding author, e-mail: yumnaalva04@gmail.com

Abstract

Tannery wastewater contains chromium, a persistent heavy metal that requires effective and affordable treatment. This study evaluated the effectiveness of yellow velvetleaf and water clover, individually and in combination, in reducing chromium from tannery wastewater and examined changes in leaf chlorophyll and fresh biomass. The experiment used a factorial randomized block design with two factors, namely plant treatment and chromium concentration, with three replications. Plant treatments consisted of yellow velvetleaf, water clover, and their combination, while chromium concentrations were 0, 5, and 10 ppm. Plants were acclimatized for seven days and exposed to treatment media for ten days. Chromium concentration was measured by atomic absorption spectrophotometry, chlorophyll by spectrophotometry, biomass by weighing, and water quality parameters were monitored. Data were analyzed by two-way ANOVA followed by Duncan test at 5% significance level. Plant treatment and chromium concentration significantly affected chromium reduction and fresh biomass, while plant treatment also affected chlorophyll. The interaction between both factors significantly affected chromium reduction but not chlorophyll or biomass. The highest reduction at 5 ppm was achieved by water clover ($93.07 \pm 1.93\%$), whereas the plant combination at 10 ppm reduced chromium by $80.35 \pm 10.92\%$. Biomass and chlorophyll generally decreased as chromium concentration increased. Water clover and the combined treatment have potential as aquatic phytoremediators for chromium-contaminated tannery wastewater

Keywords: aquatic macrophytes, heavy metal, leaf chlorophyll, plant biomass, wastewater treatment

Article History

Received: September 2026

Reviewed: September 2026

Published: September 2026

Plagiarism Checker:

No 234.GT8.,35

.800

Copyright : Author

Publishby : Trigonometri



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/)

INTRODUCTION

The leather tanning industry is economically important because it converts raw hides into products such as bags, shoes, jackets, and other leather crafts. However, its production process uses large volumes of water and chemical reagents, particularly during soaking, liming, tanning, retanning, dyeing, and finishing. Wastewater generated from these steps may contain organic matter, suspended solids, salts, and heavy metals. Chromium salts are widely associated with the tanning process because they improve leather stability and quality, but residual chromium in wastewater can become an environmental pollutant when treatment is insufficient (Putri, 2022; Astuti et al., 2022).

Chromium pollution is of particular concern because this metal is persistent and may accumulate in aquatic environments. Chromium occurs mainly as trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}). Cr^{6+} is generally more soluble, mobile, and toxic than Cr^{3+} , and exposure can disrupt plant growth, damage aquatic ecosystems, and pose risks to human health (Pereira et al., 2021; Berniyanti, 2020). In plants, chromium toxicity may inhibit enzyme activity, disturb nutrient absorption, alter chloroplast structure, reduce chlorophyll content, and decrease biomass production (Sharma et al., 2020; Restiani et al., 2024). Therefore, wastewater containing chromium requires treatment before being released into the environment.

Several treatment methods can be applied to reduce chromium in wastewater, including chemical precipitation, adsorption, membrane separation, and biological treatment. Among biological approaches, phytoremediation is considered a promising low-cost and environmentally friendly technology. Phytoremediation uses plants and associated rhizosphere processes to absorb, accumulate, transform, or stabilize contaminants in water, soil, or sediment. The success of phytoremediation depends on plant tolerance, root morphology, biomass production, contaminant concentration, water quality, and contact time (Kafle et al., 2022; Widyasari, 2021). Aquatic macrophytes are suitable for wastewater treatment because their roots are directly exposed to contaminated water and can interact with dissolved metal ions.

Yellow velvetleaf (*Limnocharis flava*) and water clover (*Marsilea crenata*) are aquatic plants that are abundant in wetlands and shallow waters in Indonesia. *Limnocharis flava* has a fibrous root system and has been reported to absorb heavy metals from contaminated media (Amalia et al., 2023; Ramagita et al., 2025). *Marsilea crenata* has spreading rhizomes and adventitious roots that increase contact between the plant and wastewater, and previous studies have shown its potential in improving wastewater quality (Musapana et al., 2020; Rulitasari and Rachmadiarti, 2021). However, information comparing these two species and their combination for chromium

removal from tannery wastewater, while also evaluating chlorophyll and biomass responses, remains limited.

This study aimed to evaluate the effects of plant treatment, chromium concentration, and their interaction on chromium reduction in tannery wastewater, leaf chlorophyll content, and fresh biomass of *Limnocharis flava* and *Marsilea crenata*. The study also monitored pH, temperature, dissolved oxygen, and carbon dioxide as supporting environmental parameters during the phytoremediation process.

MATERIALS AND METHODS

This experimental study was conducted from November 2025 to January 2026 at the Biology Greenhouse, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya. Tannery wastewater was obtained from UPT Industri Kulit & Produk Kulit Magetan, Magetan Regency, East Java. *Limnocharis flava* samples were collected from rice-field areas in Pertapan Maduretno, Taman District, Sidoarjo, while *Marsilea crenata* samples were obtained from Kampung Semanggi, Surabaya. Chromium analysis was carried out at UPT Laboratorium Lingkungan DLHK Kabupaten Sidoarjo. Chlorophyll analysis was performed at the IDB Biology Laboratory, and supporting water quality analysis was performed at the Ecology Laboratory C14, Department of Biology, Universitas Negeri Surabaya.

The experiment used a factorial randomized block design with two factors and three

replications. The first factor was plant treatment consisting of *Limnocharis flava*, *Marsilea crenata*, and a combination of both species. The second factor was chromium concentration in the treatment medium, namely 0 ppm as control, 5 ppm, and 10 ppm. Thus, there were nine treatment combinations and 27 experimental units. Each unit contained 5 L of treatment medium and 100 g initial fresh biomass. In the combination treatment, the biomass ratio was 1:1, consisting of 50 g *Limnocharis flava* and 50 g *Marsilea crenata*.

Before treatment, plants were washed with clean water and acclimatized for seven days. Tannery wastewater was adjusted to the required chromium concentrations. When the initial chromium content of wastewater was lower than the target concentration, a chromium stock solution prepared from potassium dichromate ($K_2Cr_2O_7$) was added. The treatment media consisted of 4 L distilled water and 1 L tannery wastewater for the control, with additional chromium stock solution for the 5 ppm and 10 ppm treatments. Plants were exposed to the treatment media for ten days.

Chromium concentration in water was measured at the beginning and at the end of the treatment using atomic absorption spectrophotometry. The percentage of chromium reduction was calculated from the difference between initial and final chromium concentration divided by the initial concentration. Leaf chlorophyll was extracted

using 96% alcohol and measured using spectrophotometry at 649 nm and 665 nm. Chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the Wintermans and De Mots equations as described by Sumiati (2021). Fresh biomass was measured by weighing whole plants after harvesting, cleaning, and draining. Supporting parameters including pH, temperature, dissolved oxygen, and carbon dioxide were measured at the beginning and end of the treatment.

Data for chromium reduction, chlorophyll content, and fresh biomass were tested for normality using the Shapiro-Wilk test and for homogeneity using the Levene test. Normally distributed and homogeneous data were analyzed using two-way ANOVA at a 5% significance level. Significant effects were followed by Duncan test at $\alpha = 0.05$. The physicochemical parameters were analyzed descriptively by comparing the initial and final values and by considering applicable water quality standards.

RESULTS

The data met the assumptions for parametric analysis. Shapiro-Wilk tests showed that the chromium reduction, chlorophyll, and fresh biomass data were normally distributed, with significance values of 0.370, 0.875, and 0.478, respectively. Levene tests also showed homogeneous variances, with significance values of 0.146, 0.243, and 0.298. The two-way ANOVA results showed that plant treatment significantly affected all observed response

variables. Chromium concentration significantly affected chromium reduction and fresh biomass, but did not significantly affect chlorophyll. The interaction between plant treatment and chromium concentration significantly affected chromium reduction, but did not significantly affect chlorophyll or fresh biomass (Table 1).

Table 1. Significance values of two-way ANOVA for chromium reduction, chlorophyll content, and fresh biomass.

Source of variation	Chromium reduction	Chlorophyll content	Fresh biomass
Plant treatment	0.001*	0.012*	0.001*
Chromium concentration	0.041*	0.469ns	0.014*
Plant treatment × concentration	0.012*	0.902ns	0.752ns

Note: * = significant at $\alpha = 0.05$; ns = not significant.

Chromium reduction differed among plant treatments and concentrations (Table 2). At 5 ppm, *Marsilea crenata* showed the highest mean chromium reduction, followed by the combination treatment and *Limnocharis flava*. At 10 ppm, the combination of both plants showed the highest reduction, followed by *Marsilea crenata*, whereas *Limnocharis flava* showed the lowest reduction. The control treatment did not show chromium reduction because chromium was absent from the treatment medium.

Table 2. Interaction between plant treatment and chromium concentration on chromium reduction after ten days of phytoremediation.

Plant treatment	0 ppm	5 ppm	10 ppm	Mean
<i>Limnocharis flava</i>	0.00 ± 0.00Aa	64.97 ± 19.57Ab	19.00 ± 16.06Ac	27.99
<i>Marsilea crenata</i>	0.00 ± 0.00Ba	93.07 ± 1.93Bb	62.62 ± 20.60Bc	51.90
Combination	0.00 ± 0.00Ba	86.24 ± 5.03Bb	80.35 ± 10.92Bc	55.53
Mean	0.00	81.43	53.99	

Note: Values are mean ± standard deviation. Different uppercase letters in the same concentration and different lowercase letters in the same plant treatment indicate significant differences based on Duncan test at $\alpha = 0.05$.

Leaf chlorophyll content and fresh biomass showed different response patterns (Table 3).

Marsilea crenata had higher chlorophyll content than *Limnocharis flava* across chromium concentrations. Chlorophyll tended to decrease as chromium concentration increased, but the concentration effect and the interaction effect were not significant. Fresh biomass also decreased with increasing chromium concentration. The combination treatment maintained the highest biomass at all concentrations, whereas *Limnocharis flava* showed the lowest biomass under chromium exposure.

Table 3. Leaf chlorophyll content and fresh biomass of aquatic plants after ten days of exposure to chromium-containing tannery wastewater.

Plant treatment	Cr concentration (ppm)	Total chlorophyll (mg/L)	Fresh biomass (g)
<i>Limnocharis flava</i>	0	10.66 ± 0.66Aa	104.33 ± 3.21Ab
<i>Limnocharis flava</i>	5	9.16 ± 2.31Aa	83.00 ± 16.64Aa
<i>Limnocharis flava</i>	10	7.61 ± 2.68Aa	76.67 ± 10.41Aa
<i>Marsilea crenata</i>	0	14.09 ± 2.21Ba	107.67 ± 7.37Ab
<i>Marsilea crenata</i>	5	13.87 ± 5.89Ba	96.00 ± 1.00Aa
<i>Marsilea crenata</i>	10	12.58 ± 2.59Ba	93.67 ± 1.15Aa
Combination	0	12.38 ± 1.43Aa	120.67 ± 19.50Bb
Combination	5	11.51 ± 3.75Aa	112.67 ± 4.73Ba
Combination	10	10.09 ± 1.87Aa	110.00 ± 20.07Ba

Note: Values are mean ± standard deviation. Different letters indicate significant differences based on Duncan test at $\alpha = 0.05$.

The supporting water quality parameters remained within ranges that allowed plant survival during the experiment. The final pH ranged from 6.47 to 7.60, while the final temperature ranged from 26.00°C to 26.67°C. Dissolved oxygen at the end of treatment ranged from 3.23 mg/L to 4.63 mg/L. Carbon dioxide increased during the experiment, with final values ranging from 4.00 ppm to 48.94 ppm, and higher chromium concentrations generally produced higher final carbon dioxide values.

DISCUSSION

The significant effect of plant treatment on chromium reduction indicates that phytoremediation performance was strongly influenced by plant species and plant structure. *Marsilea crenata* and the combination treatment showed higher chromium reduction than *Limnocharis flava* in most chromium-containing treatments. This response may be related to the growth habit of *Marsilea crenata*, which forms spreading rhizomes and adventitious roots. Such morphology increases the contact area between the root system and contaminated water, enabling more sites for metal adsorption, absorption, and rhizosphere interaction. In contrast, *Limnocharis flava* has fibrous roots that can absorb dissolved metals, but its effectiveness in this study was lower under higher chromium stress.

The interaction between plant treatment and chromium concentration was significant for chromium reduction, showing that the effectiveness of each plant treatment depended on the initial chromium level. At 5 ppm, *Marsilea crenata* achieved the highest reduction. At 10 ppm, the combination treatment showed the greatest reduction, suggesting that the presence of two plant species may broaden the rhizosphere contact area and improve tolerance under higher chromium exposure. Similar principles have been described in phytoremediation studies, where plant selection, root development, biomass, and contaminant concentration affect

metal uptake and removal efficiency (Kafle et al., 2022; Safarrida and Ngadiman, 2023).

Additional evidence from BioLink Indonesia strengthens the relevance of biological treatment for contaminated water. Magfirah and Hartati (2026) emphasized that bioremediation performance depends on biological capacity to bind, tolerate, and detoxify metals, especially when pollutants persist in aquatic systems. Syarifah et al. (2026) also showed that simple ecological treatment units can improve wastewater quality by stabilizing pH and reducing visible pollution indicators. These findings support the present study because plant-based remediation similarly relies on biological interaction between organisms, pollutants, and water quality conditions, while remaining affordable for small-scale wastewater management in areas affected by tannery activities in Indonesian industrial regions.

Chromium reduction in the media may occur through rhizofiltration and accumulation in plant tissues. Metal ions in water can be adsorbed onto the root surface, absorbed through root tissues, and transported to other organs through vascular tissues. Once inside the plant, chromium can be stored in cell walls, cytoplasm, or vacuoles as part of plant tolerance strategies. This mechanism reduces the dissolved chromium concentration in the media but may also cause physiological stress when the accumulated metal exceeds the plant tolerance capacity (Alwi et al., 2018; Sharma

et al., 2020). Therefore, the decrease in chromium concentration should be interpreted together with plant performance indicators such as chlorophyll and biomass.

The decrease in chlorophyll with increasing chromium concentration was consistent with the known physiological effects of heavy metals. Chromium can disrupt chloroplast structure, inhibit chlorophyll biosynthesis, reduce the uptake of essential nutrients such as magnesium and iron, and trigger oxidative stress. These processes may reduce photosynthetic pigment content and photosynthetic efficiency (Sharma et al., 2020; Kumar et al., 2022). In this study, the concentration effect on chlorophyll was not statistically significant, but the descriptive trend indicated that both plant species experienced pigment reduction at higher chromium levels. This suggests that the plants were still able to maintain chlorophyll stability during the ten-day exposure period, although early signs of stress were observed.

Fresh biomass declined significantly as chromium concentration increased. The reduction in biomass reflects growth inhibition caused by chromium toxicity. Heavy metals can interfere with nutrient absorption, water balance, enzyme activity, cell division, and energy metabolism. As a result, the plant allocates more energy to defense and detoxification mechanisms than to growth. The combination treatment had the highest biomass at all concentrations, which may have

increased its overall capacity to interact with the contaminated medium. However, the absence of a significant interaction effect on biomass indicates that the reduction in growth was mainly determined by plant type and chromium concentration rather than by a specific combination of both factors.

The supporting physicochemical parameters showed that the media remained suitable for aquatic plant survival. The pH values stayed within a near-neutral range, which is important because pH affects chromium solubility and metal availability to plants. Dissolved oxygen remained above 3 mg/L at the end of treatment, indicating that the media still supported aerobic biological activity. The increase in carbon dioxide may reflect respiration by plant roots and microorganisms as well as organic matter decomposition. These conditions suggest that the phytoremediation process occurred in biologically active media, allowing plant uptake and rhizosphere processes to contribute to chromium reduction (Astuti and Sugianti, 2018; Rini, 2017).

Overall, *Marsilea crenata* showed stronger potential for chromium removal at moderate concentration, while the combination of *Limnocharis flava* and *Marsilea crenata* was more effective at the higher concentration. The decline in chlorophyll and biomass with increasing chromium indicates that remediation effectiveness must be balanced with plant tolerance. Longer exposure periods, wider concentration ranges, and analysis of

chromium accumulation in roots, stems, and leaves are needed to determine uptake distribution and to evaluate the practical application of these plants in tannery wastewater treatment systems.

CONCLUSION

Plant treatment, chromium concentration, and their interaction significantly affected chromium reduction in tannery wastewater. *Marsilea crenata* was the most effective treatment at 5 ppm chromium, while the combination of *Limnocharis flava* and *Marsilea crenata* maintained the highest reduction at 10 ppm. Plant treatment and chromium concentration also affected fresh biomass, and plant treatment affected leaf chlorophyll, but the interaction between both factors did not significantly affect chlorophyll or biomass. The decline in chlorophyll and biomass at higher chromium concentrations indicated physiological stress caused by chromium exposure. *Marsilea crenata* and the combined plant treatment have potential to be developed as environmentally friendly phytoremediation agents for chromium-contaminated tannery wastewater.

ACKNOWLEDGEMENTS

The authors express gratitude to the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, UPT Laboratorium Lingkungan DLHK Kabupaten Sidoarjo, and UPT Industri Kulit & Produk Kulit Magetan for facilities and support during this research.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Alwi NA, Asmawati A and Liong S, 2018. Phytoaccumulation of chromium (VI) metal ion by snake plant (*Sansevieria trifasciata* Prain). *Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta)*: 1-10.
- Amalia S, Fasya AG, Hasanah U and HS MRA, 2023. Fitoremediasi Logam Tembaga oleh Tanaman Genjer (*Limnocharis flava*) dan *Hydrilla verticillata* berdasarkan Variasi Konsentrasi. *ALCHEMY: Journal of Chemistry* 11(2): 43-50. <https://doi.org/10.18860/al.v11i2.18341>.
- Astuti D, Sukmawati N, Asyfiradayati R and Darnoto S, 2022. Kajian Literatur Tentang Reduksi Kromium dalam Air Limbah Penyamakan Kulit dengan Fitoremediasi. *Syntax Literate: Jurnal Ilmiah Indonesia* 7(1): 146. <https://doi.org/10.36418/syntax-literate.v7i1.5723>.
- Astuti L and Sugianti Y, 2018. Respon Oksigen Terlarut Terhadap Pencemaran dan Pengaruhnya Terhadap Keberadaan Sumber Daya Ikan di Sungai Citarum. *Jurnal Teknologi Lingkungan*.
- Berniyanti T, 2020. Biomarker Toksisitas: Paparan Logam Tingkat Molekuler. Surabaya: Airlangga University Press.
- Kafle A, Timilsina A, Gautam A, Adhikari K, Bhattarai A and Aryal N, 2022. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances* 8: 100203. <https://doi.org/10.1016/j.envadv.2022.100203>.
- Kumar S, Wang M, Fahad S, Qayyum A, Chen Y and Zhu G, 2022. Chromium induces toxicity at different phenotypic, physiological, biochemical, and ultrastructural levels in sweet potato (*Ipomoea batatas* L.) plants. *International Journal of Molecular Sciences* 23(21): 13496.
- Magfirah N and Hartati H, 2026. Microalgae metallothionein optimization through genetic engineering for bioremediation and biology education. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)* 12(2): 195-205. <https://doi.org/10.31289/biolink.v12i2.16729>.
- Musapana S, Dewi ERS and Rahayu RC, 2020. Efektivitas Semanggi Air (*Marsilea crenata*) Terhadap Kadar TSS pada Fitoremediasi Limbah Cair Tahu. *Florea: Jurnal Biologi dan Pembelajarannya* 7(2): 92-97. <https://doi.org/10.25273/florea.v7i2.7645>.
- Pereira SC, Oliveira PF, Oliveira SR, Pereira MDL and Alves MG, 2021. Impact of Environmental and Lifestyle Use of Chromium on Male Fertility: Focus on Antioxidant Activity and Oxidative Stress. *Antioxidants* 10(9): 1365. <https://doi.org/10.3390/antiox10091365>.
- Putri AE, 2022. Strategi Pemerintah Daerah dalam Pemberdayaan Industri Kerajinan Kulit Magetan. Thesis.
- Ramagita MA, Ie JJYP, Kasmiyati S and Kristiani EBE, 2025. Dualisme Pemanfaatan Gulma Genjer (*Limnocharis flava*): Peluang Sebagai Agen Fitoremediasi dan Pangan Nutrasetika. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*: 191-203. <https://doi.org/10.24002/biota.v10i2.10270>.
- Restiani R, Kaban S, Sekar A and Matheos J, 2024. Respon Pertumbuhan dan Toleransi Kalus *Talinum paniculatum* terhadap Cekaman Logam Berat Krom (Cr). *Jurnal Biologi Papua* 16(1): 71-78. <https://doi.org/10.31957/jbp.3590>.

- Rini PP, 2017. Pengaruh Waktu Kontak Terhadap Potensi Tanaman Genjer (*Limnocharis flava*) dalam Menyerap Logam Chromium (Cr) Limbah Cair Industri Penyamakan Kulit. Doctoral dissertation, Universitas Brawijaya.
- Rulitasari D and Rachmadiarti F, 2021. Semanggi Air (*Marsilea crenata*) Sebagai Agen Fitoremediasi LAS Detergen. LenteraBio: Berkala Ilmiah Biologi 9(2): 99-104.
<https://doi.org/10.26740/lenterabio.v9n2.p99-104>.
- Safarrida A and Ngadiman WJ, 2023. Fitoremediasi kandungan kromium pada limbah cair menggunakan tanaman air. Jurnal Bioteknologi dan Biosains Indonesia 2: 55-59.
- Sharma A, Kapoor D, Wang J, Shahzad B, Kumar V, Bali AS and Yan D, 2020. Chromium bioaccumulation and its impacts on plants: an overview. Plants 9(1): 100.
- Sumiati S, 2021. Penggunaan Pelarut Etanol dan Aseton pada Prosedur Kerja Ekstraksi Total Klorofil Daun Jati (*Tectona grandis*) dengan Metode Spektrofotometri. Indonesian Journal of Laboratory 4(1): 30.
<https://doi.org/10.22146/ijl.v4i1.65418>.
- Syarifah, Javani TM, Hidayat LA, Sughani K, Maratis J, Hidayat AK and Irfandi A, 2026. Design of a homemade biofilter for domestic wastewater treatment in the Situ Rawa Besar. BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan) 12(2): 206-218.
<https://doi.org/10.31289/biolink.v12i2.16464>.
- Widyasari NL, 2021. Kajian Tanaman Hiperakumulator Pada Teknik Remediasi Lahan Tercemar Logam Berat. Jurnal Ecocentrism 1(1): 17-24.
<https://doi.org/10.36733/jeco.v1i1.1748>.