

EXPLORING *PICHIA KLUYVERI* K-1 BIOMASS AS A NOVEL BIOSORBENT FOR CHROMIUM REMOVAL FROM AQUEOUS SOLUTION: PROCESS OPTIMIZATION STUDY

Sandra Stamenković Stojanović¹ , Saša Savić¹ , Sanja Petrović¹ ,
Stojan Mančić¹ , Bojana Danilović¹ , Ivana Karabegović¹ 

¹University of Niš, Faculty of Technology in Leskovac, Leskovac, Serbia

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Increased presence of chromium in water resources represents a significant environmental and public health concern due to its high mobility, toxicity, and carcinogenic nature. Biosorption using microbial biomass has emerged as a sustainable alternative to conventional physicochemical treatment methods. In this study, the biosorptive potential of dead *Pichia kluyveri* K-1 biomass was investigated for the first time for chromium removal from aqueous solutions. Process optimization was performed using response surface methodology based on a Box–Behnken experimental design evaluating the effects of solution pH (2–7), initial chromium concentration (20–100 mg/L), and biosorbent dosage (2–10 g/L). Chromium removal efficiency ranged from 7.8% to 42.0%, while final chromium concentrations after biosorption varied between 11.6 and 88.5 mg/L depending on process conditions. Lower initial chromium concentrations and a strongly acidic environment resulted in substantially reduced residual chromium levels, indicating improved biosorption performance under conditions favoring protonation of biomass surface functional groups. Analysis of variance showed that the initial chromium concentration was the dominant factor governing final chromium concentration, whereas pH had the strongest influence on removal efficiency. Response surface analysis confirmed significant nonlinear effects and identified optimal operating conditions at pH 2, an initial chromium concentration of 20 mg/L, and a moderate biosorbent dosage. Under these conditions, predicted removal efficiency was 42.8%, which was also confirmed experimentally. These findings provide a foundation for further optimization and biomass modification for water treatment applications using novel, environmentally safe biosorbents.

Keywords: RSM, experimental design, dead biomass, biosorption, yeast

Introduction

Water is an increasingly threatened and limited resource. Rising global demand and climate change contribute to water shortages and ecosystem degradation, while at the same time, contamination with heavy metals and other toxic pollutants escalates due to increased urbanization and industrialization [1]. Heavy metals represent a serious environmental concern because of their persistence and non-biodegradable nature. In contrast to organic pollutants, they cannot be decomposed through chemical or biological pathways and can only be converted into less toxic forms [2]. As a result, elements such as arsenic, cadmium, chromium, and lead accumulate in the environment and enter the food chain, posing substantial risks to living organisms [3]. Hexavalent chromium is of particular concern because it easily crosses cellular membranes via nonspecific anion transport systems and can cause genotoxic damage, underlining its mutagenic and carcinogenic potential in humans [4].

Traditional methods for removing heavy metals, such

as chemical precipitation, redox treatments, electrochemical processes, and adsorption, are effective but are also often costly, technically complex, and generate secondary waste, primarily chemical sludge [5]. These drawbacks underscore the need for more sustainable and economically viable alternatives. Among such approaches, biosorption has emerged as a promising, cost-effective, and environmentally friendly option [6]. This process is based on the capacity of biological materials, including microorganisms, plants, and their derivatives, to immobilize metal ions through functional groups located on their cell surfaces [5]. A major economic advantage of biosorption lies in the utilization of abundant, low-cost biomass, which in many cases demonstrates competitive performance compared with conventional remediation techniques [3]. Although still largely in the research phase, biosorption already represents a meaningful alternative to traditional metal removal methods, particularly for the treatment of dilute heavy metal solutions [7].

***Author address:** Sandra Stamenković Stojanović, University of Niš, Faculty of Technology in Leskovac, Bulevar oslobođenja 124, 16000 Leskovac, e-mail: sandrastamenkovic@tf.ni.ac.rs.

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Various biological species exhibit affinity toward metal ions. Nevertheless, bacteria and fungi are most frequently employed in biosorption studies [8]. Microbial biomass, which may be bacterial, yeast, or filamentous fungi, has attracted considerable attention due to its high surface area and the relatively easy production [9]. Studies addressing large-scale feasibility have indicated that non-living (dead) microbial biomass offers practical advantages, as it does not require nutrient input, is simpler to manage, and reduces operational expenses [5,6,10]. Fungal biomass, in particular, is regarded as one of the most efficient low-cost adsorbents for chromium removal from wastewater [6,8], partly due to the presence of chitin and chitosan within its cell walls. The deacetylated amino groups of glucosamine in these polymers can function as active binding sites for metal ions [11]. Recent investigations have evaluated biosorptive performance of different fungal species, such as *Aspergillus niger* [12,13], *Pichia pastoris* [14,15], *Pichia stipitis* [16], *Saccharomyces cerevisiae* [17], *Candida tropicalis* [18]. Another study explored the possibility of the application of different wild yeast species, demonstrating that both *Saccharomyces* and non-*Saccharomyces* strains are capable of removing heavy metals from industrial effluents [19]. This insight opens an extensive and largely unexplored pool of potential biosorbents, particularly among non-conventional yeasts that have traditionally been studied for their fermentative or biotechnological applications rather than for environmental remediation. Among them is a wild strain of *Pichia kluyveri*, which is well recognized for its enological and biotechnological relevance [20], although to the best of our knowledge, no studies have yet evaluated its capacity for heavy metal biosorption. Therefore, exploring the biosorptive performance of *P. kluyveri* not only addresses a clear knowledge gap but also contributes to expanding the diversity of novel yeast-based biosorbents for sustainable water treatment applications. Since in batch biosorption systems, process efficiency depends on several operational parameters, such as solution pH, temperature, initial pollutant concentration, biosorbent dosage, contact time, or the presence of competing ions, optimization of the process is the first and essential step for each selected biological species [21].

Therefore, the aim of this study is to evaluate the efficiency of dead *P. kluyveri* K-1 biomass in removing chromium from aqueous solution and to identify the key parameters influencing the process and enabling its optimization.

Materials and methods

Preparation of dry *Pichia kluyveri* K-1 Biomass

The yeast *P. kluyveri* K-1 used in this study was previously isolated from the surface of blackberry fruit (Mančić et al., 2025) and is maintained in the microorganism collection of the Microbiology Laboratory, Faculty of Technology in Leskovac, University of Niš. The inocu-

lum was prepared by aseptically transferring one colony of *P. kluyveri* K-1 into 300 mL of sterile Sabouraud Maltose Broth (SMB) in a 500 mL Erlenmeyer flask. Incubation was carried out at 25 °C for 24 h on a rotary shaker (Multi-functional Orbital Shaker, PSU-20i, Biosan SIA, EU) at 2.5 Hz (150 rpm). For bioreactor cultivation, a 24-hour-old inoculum was used at 1% (v/v) relative to the total volume of previously sterilized SMB medium. Cultivation was performed in a mechanically stirred bioreactor (KLFM, BioEngineering, Wald, Switzerland) with a total volume of 3.7 L (working volume 2.5 L), equipped with two Rushton impellers with six blades each (diameter 48 mm) and four baffles. Aeration was supplied with sterile air from an external compressor at a specific flow rate of 0.3 vvm. The cultivation process lasted 48 hours, after which the fermentation broth was separated by centrifugation (7000 rpm, 10 min). The supernatant was discarded, and the obtained pellet was dried in an oven at 105 °C for 24 h until a constant dry biomass mass was reached.

Preparation of the Stock Chromium (Cr) Solution

All chemicals used were of analytical grade, and distilled water was used as the solvent. The aqueous chromium solution was prepared by dissolving an appropriate mass of $K_2Cr_2O_7$ (Merck, Germany) in 1 L of water to obtain the desired concentrations of 20, 50, and 100 mg/L.

Biosorption Process

The biosorption process using dead *P. kluyveri* K-1 cell biomass was carried out at room temperature (26 ± 1 °C) by mixing the appropriate amount of biosorbent in a 250 mL Erlenmeyer flask containing 100 mL of chromium solution at concentrations of 20, 50, and 100 mg/L, according to the Box–Behnken experimental design. The solution was mixed on a rotary shaker at 150 rpm for 24 h, after which the solid residues were filtered using filter paper. Chromium concentration in the supernatant was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, model ARCOS FHE12, SPECTRO Analytical Instruments, Germany), following the manufacturer's instructions. A calibration standard was prepared to evaluate chromium content. For preparation of the standard solution, Multi-Standard IV (Merck) was used. Argon 5.0 (purity 99.999%) was used as the carrier gas.

The wavelength detection of Cr in the samples, correlation coefficient (R), and limit of detection (LOD), are shown in Table 1.

The chromium removal efficiency (%) was calculated using the equation:

$$E = \left(\frac{C_0 - C_{eq}}{C_0} \right) \times 100 \dots\dots\dots (1)$$

where C_0 and C_{eq} are the initial and the final Cr concentration, respectively.

Table 1. Calibration parameters: λ (nm), R, LD ($\mu\text{g}/\text{dm}^3$)

Element	Detection wavelength (nm)	Correlation coefficient (R)	Detection limit ($\mu\text{g}/\text{dm}^3$)
Cr	283.563	0.99989	0.2859

Table 2. Coded and uncoded values of the monitored process factors

Symbol	Factor	Low level	Middle level	High level
		(-1)	(0)	(+1)
A	pH	2	4.5	7
B	Initial Cr concentration, mg/L	20	60	100
C	Biosorbent concentration, g/L	2	6	10

Experimental Design

To optimize the biosorption conditions using dead *P. kluyveri* K-1 cell biomass, a Box–Behnken experimental design with three factors was applied. Each factor was varied at three levels, with five replicates at the central point, resulting in a total of 17 experiments (Table 2). The factors selected based on literature data and preliminary studies were: biosorbent concentration (2–10 g/L), solution concentration (20–100 mg/L), and pH (2–7). The monitored system response was the final chromium concentration and chromium removal efficiency.

Statistical analysis

The software Expert Design 7.0 (Stat Ease Inc) was used for the statistical processing of experimental data. RSM and Derringer's desirability function were used for modeling and optimization. The adequacy of the response surface model was assessed using the analysis of variance (ANOVA). The *F*-test, *p*-values, coefficient of determination R^2 , adjusted coefficient of determination $Adj R^2$, lack of fit, and coefficient of variation (CV %) values obtained by the ANOVA were used to estimate the statistical significance of linear, quadratic, and interactive effects of each parameter on the responses, as well as the quality of the proposed models. The *p*-values lower than 0.05 were considered statistically significant. The optimal biosorption conditions were proposed using a desirability function approach and validated by conducting experiments on the chosen optimal conditions [22].

Results and discussion

Screening of the significant factors for the biosorption process

Biosorption of pollutants is affected by various physical and chemical factors that determine the efficacy and the success of the process. Therefore, the first step when setting up a biosorption experiment is the determi-

nation of the individual and combined effects of various factors on biosorption performance [21]. In this study, the effects of chosen process conditions on biosorption efficiency for chromium removal using dead *P. kluyveri* K-1 biomass were evaluated using multifactor optimization based on response surface methodology (RSM). Three independent factors (initial Cr concentration, biosorbent concentration, and pH of the solution) were varied at three levels using a Box–Behnken experimental design, and their effects on the final chromium concentration and biosorption efficiency after 24 h are presented in Table 3.

The results show that chromium removal efficiency ranged from 7.8% to 42.0%, with the highest efficiency achieved at pH 2.0, low initial Cr concentration (20 mg/L), and 6 g/L biosorbent. On the other hand, the final chromium concentration ranged between 11.6 and 88.5 mg/L. In general, lower initial chromium concentrations resulted in higher percentage removal, while higher concentrations (100 mg/L) reduced efficiency. The replicated central points indicated good experimental reproducibility. Changes in biosorbent concentration did not cause a corresponding increase in removal efficiency, suggesting a non-linear relationship between biomass dosage and Cr removal. Multiple nonlinear regression analysis generated a quadratic polynomial equation that mathematically describes the effect of independent factors on the biosorption using dead biomass of *P. kluyveri* K-1 on the final chromium concentration (Y_1 , mg/L):

$$Y_1 = 14.53934 - 0.70500 \cdot A + 0.41952 \cdot B - 3.28410 \cdot C + 0.017 \cdot A \cdot B + 0.14625 \cdot A \cdot C - 0.143 \cdot B \cdot C - 0.00357 \cdot B^2 + 0.2337 \cdot C^2 \quad (2)$$

Table 3. Box-Behnken experimental design for Cr biosorption using dead *P. kluyveri* K-1 biomass with actual system responses

No	Uncoded values of the factor			Response	
	pH	Initial Cr concentration (mg/L)	Biosorbent concentration (g/L)	Final Cr concentration (mg/L)	Cr removal efficiency (%)
1	7.0	60.0	10.0	46.44	22.6
2	4.5	60.0	6.0	41.15	31.4
3	4.5	60.0	6.0	42.2	29.7
4	4.5	20.0	2.0	18.28	8.6
5	2.0	100.0	6.0	76.9	23.1
6	2.0	20.0	6.0	11.6	42.0
7	4.5	60.0	6.0	40.65	32.2
8	7.0	60.0	6.0	37.19	38.0
9	7.0	100.0	6.0	85.9	14.1
10	2.0	60.0	2.0	46.6	22.3
11	4.5	60.0	6.0	41.85	30.0
12	4.5	20.0	10.0	18.44	7.8
13	7.0	60.0	2.0	50.0	16.7
14	7.0	20.0	6.0	13.8	31.0
15	4.5	100.0	10.0	79.5	20.5
16	4.5	60.0	6.0	42.55	29.1
17	4.5	100.0	2.0	88.5	11.5

To assess the statistical significance of the effects of the selected factors and their interactions on the final chromium concentration and removal efficiency after the biosorption process, an analysis of variance (ANOVA) was performed based on the quadratic model obtained by RSM. The results of ANOVA analysis (Table 4) showed that the quadratic model for the final chromium concentration was statistically highly significant, with an overall F -value of 771.96 and $p < 0.0001$, confirming that the model reliably describes the relationship between the investigated factors and the response. The lack-of-fit test was not significant, indicating good agreement between the model and the experimental data and the absence of systematic errors. The statistically significant factors identified were pH (A), initial chromium concentration (B), biosorbent concentration (C), as well as the interactions AB, BC, and AC, and the quadratic terms B^2 and C^2 . The initial chromium concentration contributed most strongly to the model, which is explained by the increased driving force for mass transfer depending on the metal concentration [23], while pH and biosorbent concentration exhibited moderate but statistically significant effects. The

quadratic terms B^2 and C^2 indicated nonlinear influences of these factors, suggesting the presence of optimal levels in their effects.

During the modeling of chromium removal efficiency, the initial quadratic model was further refined to improve its statistical validity and predictive capability. Model refinement was carried out using the backward elimination method, with the alpha level set at 0.10, a criterion commonly used in stepwise regression procedures to prevent the exclusion of potentially influential terms and to improve model adequacy in RSM [24]. This enabled the gradual removal of statistically insignificant terms while preserving the hierarchical structure of the model, and additional higher-order interaction terms were added to the model when they significantly improved model adequacy and eliminated lack of fit. The resulting modified quadratic model included significant linear, interaction, and quadratic effects of pH (A), initial chromium concentration (B), and biosorbent concentration (C), namely A, B, C, AC, BC, A^2 , B^2 , C^2 , A^2B , and A^2C . Similar modifications have been performed across multiple biosorption studies employing Box–Behnken and Central Compos-

ite Designs [12,18,25,26].

After the modifications, the model explaining removal efficiency exhibited a highly significant overall F -value (109.23), indicating that the selected terms successfully describe the variability of chromium removal efficiency. The lack-of-fit test was not significant, confirming that the model adequately represents the experimental data and that no systematic deviations are present. Additionally, the high coefficients of determination R^2 and

adj R^2 (0.9952 and 0.9867, respectively) indicate excellent agreement between the experimental and predicted values, confirming the reliability of the model (Table 4). The following equation is proposed for the mathematical model for chromium efficiency removal:

$$Y_2 = 51.8319 - 28.5064 \cdot A + 0.46785 \cdot B - 11.296 \cdot C + 0.4676 \cdot A \cdot B - 1.451 \cdot A \cdot C + 0.015312 \cdot B \cdot C + 3.0696 \cdot A^2 - 0.004916 \cdot B^2 + 0.65719 \cdot C^2 - 0.0514 \cdot A^2 \cdot B + 0.134 \cdot A^2 \cdot C \dots\dots\dots(3)$$

Table 4. Analysis of variance of the model for predicting final Cr concentration (mg/L) and chromium removal efficiency (%) after biosorption using dead *Pichia kluyveri* biomass

Parameter	Cr concentration		Removal efficacy	
	F value	p -value	F value	p -value
Model	933.72	< 0.0001	109.23	< 0.0001
A	56.48	<0.0001	157.44	< 0.0001
B	7168.16	< 0.0001	45.59	0.0011
C	47.23	0.0001	12.60	0.0164
AB	9.18	0.0163	0.75	0.4263
AC	6.80	0.0313	17.99	0.0082
BC	16.66	0.0036	17.99	0.0082
A^2	-	-	76.84	0.0003
B^2	109.99	< 0.0001	195.16	< 0.0001
C^2	46.76	0.0001	348.82	< 0.0001
A^2B	-	-	247.45	< 0.0001
A^2C	-	-	16.82	0.0093
Lack of fit	3.20	0.1431	0.076	0.7960
R^2	0.9989		0.9959	
Adj R^2	0.9979		0.9867	
Pred R^2	0.9916		-	
C.V., %	2.44		4.78	
PRESS	79.24		-	

Among the investigated variables, pH was the most significant factor for chromium removal efficiency, as it directly affects both the speciation of Cr in solution and the surface charge binding sites on the biosorbent. Changes in pH can modify the protonation state of surface groups, affecting their affinity for chromium species and significantly affecting the overall adsorption process [23]. This behavior is consistent with previous fungal Cr (VI) biosorption studies, where pH exhibited the highest F -values among all investigated factors in the Box-Behnken optimization of Cr (VI) removal by *Aspergillus niger* live biomass [13]. Similar conclusions were drawn in another study for dead *A. niger* biomass, which reported significant linear and quadratic terms for pH alongside non-significant interactions [12]. In contrast, in the multi-metal biosorption study using *Chlorella coloniales*,

pH was not included as a variable, and reaction time and algal biomass concentration emerged as the most influential factors for Cr, Cd, Co, Fe, and As removal [25]. This difference can be attributed to the nature of the system investigated. In multi-metal systems, competitive interactions among different cations for the same active binding sites occur, making the availability and density of biosorption sites the dominant factor for removal efficiency [23]. As a result, biosorbent dose is often among the strongest predictors of biosorption efficiency due to the increased availability of active binding sites. This is also evident in the present study, particularly in the model describing Cr removal efficiency, where the quadratic term of biosorbent concentration was a highly significant factor, indicating a nonlinear relationship between biosorbent concentration and removal efficiency, with an optimal

dose at which maximum efficiency is achieved.

Optimization of the biosorption process

The obtained model was used for graphical interpretation of the results through 3D response surface plots and contour diagrams, in order to visually present the effects of individual factors and their interactions on the biosorption process, as well as to identify the optimal conditions to achieve minimum Cr concentration after biosorption. At a lower initial Cr concentration (Fig. 1a), the influence of biosorbent dose and pH is less significant. In fact, at biosorbent doses below approximately 3.5 g/L, pH shows no effect on the final chromium concentration. Increasing the biosorbent dose contributes to a decrease in final Cr concentration, but only up to an optimal value. Beyond this point, further increases in biosorbent dosage do not result in additional improvement in chromium removal efficiency. In contrast, at a higher initial Cr concentration (Fig. 1b), the combination of low to moderately acidic pH values and biosorbent doses above 6 g/L results in lower final Cr concentrations. The effect of these two factors is considerably more pronounced at higher initial Cr levels, as a relatively small change in pH and biosorbent dose leads to a greater reduction in residual chromium concentration.

Figure 2 represents the generated three-dimensional response surface plots and their corresponding contour projections that illustrate how simultaneous changes in the examined variables influence Cr removal efficiency. Across all pH conditions, the contour plots describing Cr removal efficiency display mostly elliptical closed curves, while the existence of a central region with maximum predicted removal suggests the presence of an optimum zone. At pH 2, the model predicts the highest Cr removal efficiency, with the response maximum located in the region of moderate to high biosorbent concentration (6–8 g/L) and moderate initial Cr concentration (35–50 mg/L). The plot indicates a predicted peak of approximately 44% removal, with response values decreasing substantially toward the boundaries of the experimental domain. This behavior is consistent with the well-established effect of strongly acidic conditions on the biosorption of chromium. Chromium exists in several oxidation states, although the most common forms are the trivalent and hexavalent species. Hexavalent chromium, which is the most toxic one, is usually present in the aqueous solution as $\text{Cr}_2\text{O}_7^{2-}$, CrO_4^{2-} , H_2CrO_4 , and HCrO_4^- , with pH largely influencing the amount of each species. In the pH range from one to six, Cr(VI) is mainly present as HCrO_4^- , with some contribution of $\text{Cr}_2\text{O}_7^{2-}$ [8]. This leads to strong electrostatic interactions between negatively charged chromium species and positively charged functional groups located on the biomass [21].

To determine the optimal combination of factor values that enables the highest biosorption efficiency and the lowest final Cr concentration, it is necessary to apply statistical methods capable of simultaneously processing multiple variables. Using Derringer's desirability func-

tion, a multi-criteria optimization was performed to obtain the maximum value for removal efficiency and the minimum value for remaining Cr concentration. As the overall optimal solution, the following combination of factors was proposed: pH (2), initial chromium concentration (20 mg/L), and biomass concentration (7 g/L). This combination of factors is predicted to yield a chromium removal efficiency of 42.8% and a final Cr concentration of 11.94 mg/L, with overall desirability of 0.9977. The experimentally obtained value under those conditions was 41.5%, which is in good agreement with the predicted values.

The comparative analysis of previously published studies indicates that Cr and other heavy metal removal efficiencies achieved using yeast biosorbents vary depending on the species employed and experimental conditions. Several studies have reported comparable removal efficiencies under similar operational ranges. Particularly, Chen and coworkers observed a maximum Cu(II) removal efficiency of 41.1% using *P. pastoris* biomass at pH 6.0 and 25 °C, with biosorption predominantly associated with cell wall functional groups [15]. In another study, *Candida albicans* removed 76% of Cr(VI) at pH 1, although lower efficiencies were observed for other metals (57% for Pb, 31% for Cu, and 22% for Zn) [27]. Comparable variability has also been reported for *S. cerevisiae*: Cu(II) removal efficiencies were 34% at an initial concentration of 25 mg/L [28], whereas another group of authors achieved 48.69% Cd(II) and 77.91% Pb(II) removal under pH conditions in the range of 4.0–5.5 [29]. About 40% chromium removal was also observed by growing mutant cells of *Bacillus cereus* at an initial Cr concentration of 50 ppm, although the same bacteria achieved up to 98% of removal at lower initial Cr concentration, and optimal values being pH 6.5 and 30 °C [30]. Medina-Armijo and coworkers explained that the reason for lower biosorption rates may be attributed to the presence of an outer cell wall barrier that limits metal penetration. In microbial cells, the complex and multilayered structure of the cell wall, which is composed of polysaccharides, proteins, and in some cases, lipopolysaccharides, can act as a diffusional barrier, limiting access of metal ions to high-affinity functional groups [31].

Still, many of the published optimization studies reported much higher removal efficiencies. Zinicovscaia and coworkers report 97.7% removal of Cr using *S. cerevisiae* at pH 2, explaining that the main mechanism of removal is the adsorption via cell wall functional groups [32]. Mondal and coworkers achieved approximately 90% Cr(VI) removal using *A. niger* under RSM optimized conditions (pH 4.63, initial Cr concentration of 32.98 mg/L, biosorbent dose of 1 g/L, and contact time of 47.88 min) [13]. Another group of authors also used *A. niger* biomass for Cr removal, reporting an 80% efficiency under optimized conditions with strong model performance (adj R^2 0.996; pred R^2 0.990) [12]. RSM study on Cr(VI) removal using *Prosopis cineraria* leaf powder reported a maximum chromium removal efficiency of about 89.65% under optimized conditions, which were biosorbent dose

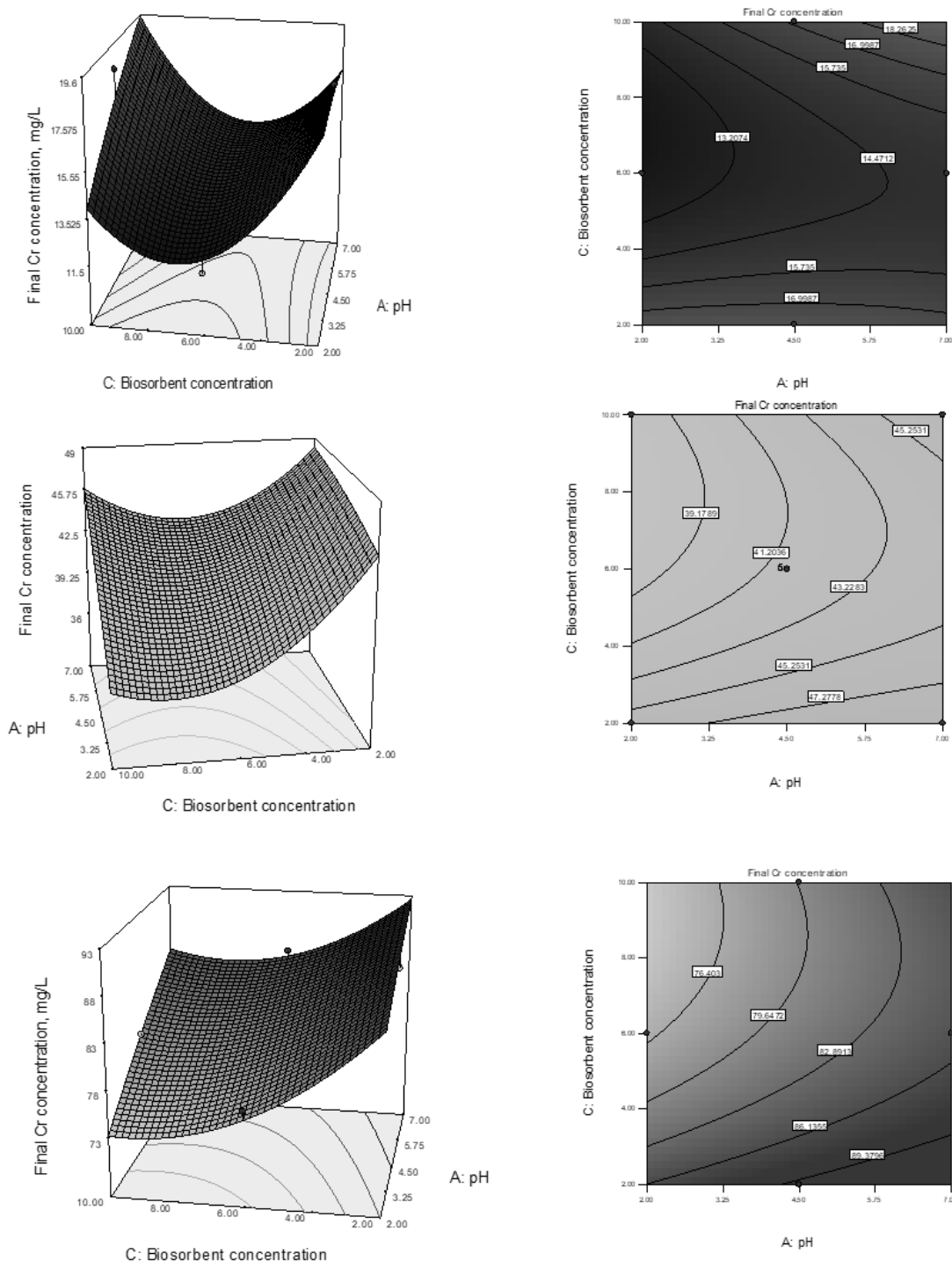


Figure 1. Response surface plots for final Cr concentration as a function of pH and biosorbent dose at initial Cr concentration of: a) 20 mg/L, b) 60 mg/L, c) 100 mg/L

of 3.98 g/L, pH 2.02, contact time 60 min, and initial Cr concentration of 25 mg/L [33]. This discrepancy among achieved results suggests that further optimization is needed in order to improve removal efficiency. The present system with dead *P. kluyveri* K-1 biomass may require consideration of additional independent variables, such as temperature and contact time, both of which

are known to significantly affect metal uptake kinetics and equilibrium capacity. Moreover, modification of the biomass may enhance biosorption performance. For example, in fungal systems, exposure to sublethal heavy metal stress has been shown to induce adaptive responses that modify cell wall composition and enhance metal-binding affinity [31]. Thus, despite the moderate

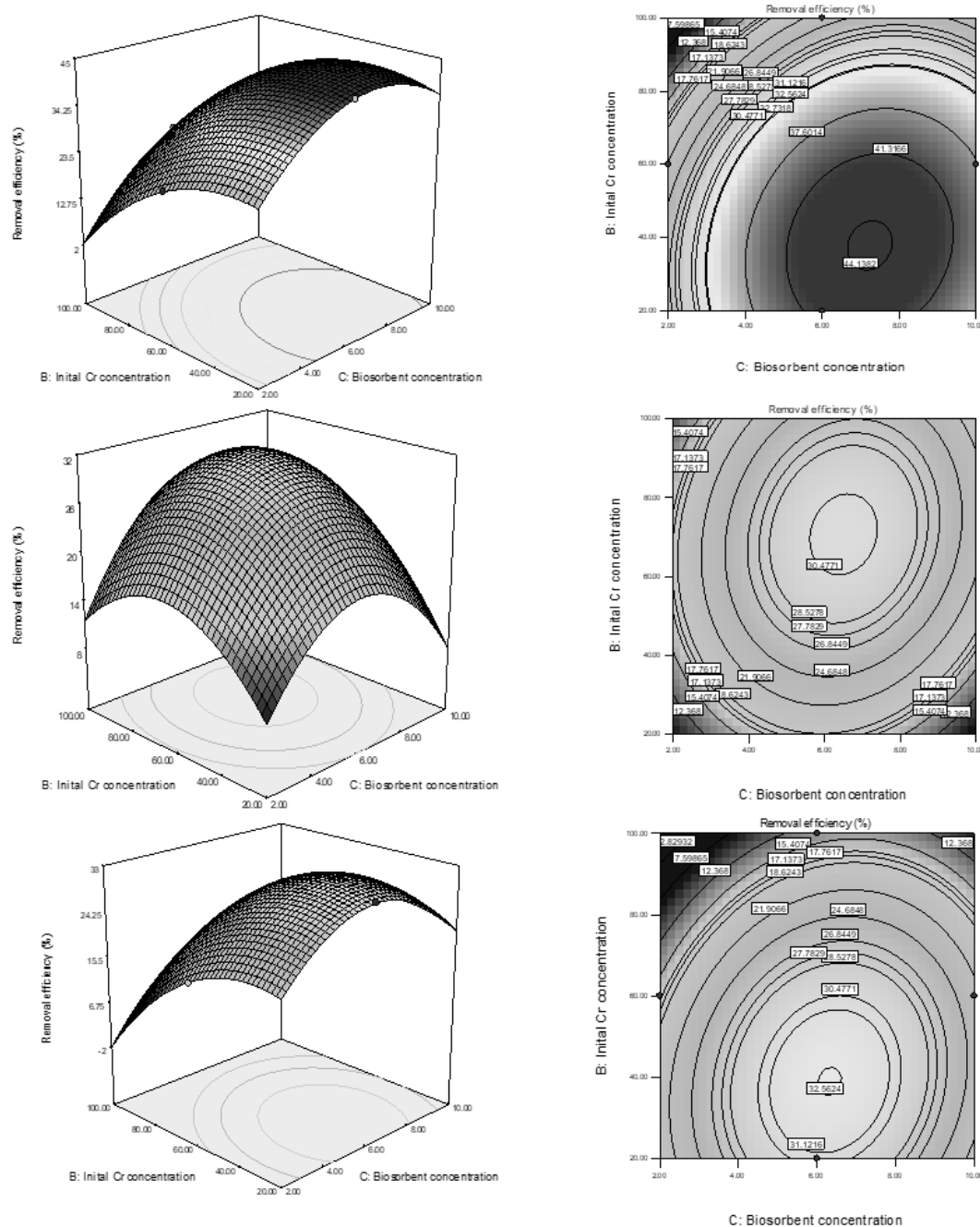


Figure 2. Response surface and contour plots for removal efficiency as a function of initial Cr concentration and biosorbent dose at: a) pH 2, b) pH 4, c) pH 7

removal efficiency obtained in the present study, the results demonstrate that *P. kluyveri* possesses biosorptive potential, representing a promising starting point for future investigations aimed at optimizing its performance and expanding the range of yeast-based biosorbents for chromium removal.

Conclusion

The results presented in this study represent the first investigation of *P. kluyveri* K-1 as a biosorbent for Cr

removal. The obtained results confirm that RSM is an efficient approach for modelling and optimization of bio-sorption using dead biomass of wild yeast *P. kluyveri* K-1. This approach allows efficient identification of key process variables, reduces the resources needed for optimization, and provides a foundation for future research. Individual and combined effects of initial metal concentration, biosorbent dose, and pH on final Cr concentration and Cr removal efficiency were assessed and explained. Analysis of variance (ANOVA) indicated that the developed model was statistically significant, while the

lack of fit was not significant, confirming that the model adequately describes the experimental data. Among the studied variables, pH and the initial chromium concentration had the strongest influence on the monitored responses.

The optimal removal efficiency was achieved at pH 2, initial chromium concentration 20 mg/L, and biomass concentration 7 g/L. The experimental validation of the model confirmed a good agreement with the predicted values. Although the removal efficiency achieved is moderate compared to some optimized systems, the findings clearly demonstrate that *P. kluyveri* K-1 has biosorption potential and constitutes a promising candidate for further development. Also, unlike controlled laboratory conditions, industrial waters contain various pollutants, indicating the need for investigation of the biosorption effect in the presence of competing ions. Therefore, the obtained results represent an important first step in evaluating the biosorptive potential of *P. kluyveri* K-1.

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Reference

- [1] Yaashikaa PR, Kumar PS, Saravanan A. Advances in biosorbents for removal of environmental pollutants: A review on pretreatment, removal mechanism and future outlook. *Journal of Hazardous Materials*. 2021, 420, 126596. <https://doi.org/10.1016/j.jhazmat.2021.126596>
- [2] Ayangbenro AS, Babalola OO. A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *International Journal of Environmental Research and Public Health*. 2017, 14, 94. <https://doi.org/10.3390/ijerph14010094>
- [3] Aslam A, Kanwal F, Javied S, Nisar N, Torriero AAJ. Microbial biosorption: a sustainable approach for metal removal and environmental remediation. *International Journal of Environmental Science and Technology*. 2025, 22, 13245–13276. <https://doi.org/10.1007/s13762-025-06611-1>
- [4] Gorny J, Billon G, Noiriell C, Dumoulin D, Lesven L, Madé B. Chromium behavior in aquatic environments: A review. *Environmental Reviews*. 2016, 24, 503–516. <https://doi.org/10.1139/er-2016-0012>
- [5] Aryal M, Liakopoulou-Kyriakides M. Bioremoval of heavy metals by bacterial biomass. *Environmental Monitoring and Assessment*. 2015, 187, 4173. <https://doi.org/10.1007/s10661-014-4173-z>
- [6] Park D, Yun YS, Park JM. Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp. *Chemosphere*. 2005, 60, 1356–1364. <https://doi.org/10.1016/j.chemosphere.2005.02.020>
- [7] Beni AA, Esmaeili A. Biosorption, an efficient method for removing heavy metals from industrial effluents: A review. *Environmental Technology and Innovation*. 2020, 17, 100503. <https://doi.org/10.1016/j.eti.2019.100503>
- [8] Islam MM, Mohana AA, Rahman MA, Rahman M, Naidu R, Rahman MM. A comprehensive review of the current progress of chromium removal methods from aqueous solution. *Toxics*. 2023, 11, 252. <https://doi.org/10.3390/toxics11030252>
- [9] Volesky B. Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*. 2001, 59, 203–216. [https://doi.org/10.1016/S0304-386X\(00\)00160-2](https://doi.org/10.1016/S0304-386X(00)00160-2)
- [10] Karchiyappan T, Ettiyagounder P, Selvaraj PS, Ramanujam K. Transforming waste into value: Advancements in protein recovery and functional product development from wastewater sources. *Desalination and Water Treatment*. 2025, 321, 101044. <https://doi.org/10.1016/j.dwt.2025.101044>
- [11] Cervantes C, Campos-García J, Devares S, Gutiérrez-Corona F, Loza-Tavera H, Torres-Guzmán JC, et al. Interactions of chromium with microorganisms and plants. *FEMS Microbiology Reviews*. 2001, 25, 335–347. <https://doi.org/10.1111/j.1574-6976.2001.tb00581.x>
- [12] Gupta M, Gupta SS. Application of Box-Behnken model to study the biosorption of chromium Cr(VI) ion by *Aspergillus niger* isolated from polluted site. *International Journal of Current Microbiology and Applied Sciences*. 2021, 10, 414–424. <https://doi.org/10.20546/ijcmas.2021.1007.045>
- [13] Mondal NK, Samanta A, Dutta S, Chattoraj S. Optimization of Cr(VI) biosorption onto *Aspergillus niger* using 3-level Box-Behnken design: Equilibrium, kinetic, thermodynamic and regeneration studies. *Journal of Genetic Engineering and Biotechnology*. 2017, 15, 151–160. <https://doi.org/10.1016/j.jgeb.2017.01.006>
- [14] Elahian F, Heidari R, Charghan VR, Asadbeik E, Mirzaei SA. Genetically modified *Pichia pastoris*, a powerful resistant factory for gold and palladium bioleaching and nanostructure heavy metal biosynthesis. *Artificial Cells, Nanomedicine, and Biotechnology*. 2020, 48, 259–265. <https://doi.org/10.1080/21691401.2019.1699832>
- [15] Chen X, Tian Z, Cheng H, Xu G, Zhou H. Adsorption process and mechanism of heavy metal ions by different components of cells, using yeast (*Pichia pastoris*) and Cu²⁺ as biosorption models. *RSC Advances*. 2021, 11, 17080–17091. <https://doi.org/10.1039/d0ra09744f>
- [16] Yilmazer P, Saracoglu N. Bioaccumulation and biosorption of copper(II) and chromium (III) from aqueous solutions by *Pichia stipitis* yeast. *Journal of Chemical Technology and Biotechnology*. 2009, 84, 604–610. <https://doi.org/10.1002/jctb.2088>
- [17] El-Bestawy EA, El-Batouti MM, Zaberemawi NM, Zaghlol HM. Removal of heavy metals, turbidity and coliform from contaminated raw drinking water using *Saccharomyces cerevisiae*, the baker's yeast. *Sustainable Chemistry and Pharmacy*. 2023, 33, 101131. <https://doi.org/10.1016/j.scp.2023.101131>
- [18] Gönen F, Aksu Z. Single and binary dye and heavy metal bioaccumulation properties of *Candida tropicalis*: Use of response surface methodology (RSM) for the estimation of removal yields. *Journal of Hazardous Materials*. 2009, 172, 1512–1519. <https://doi.org/10.1016/j.jhazmat.2009.08.021>
- [19] García-Béjar B, Arévalo-Villena M, Guisantes-Batan E, Rodríguez-Flores J, Briones A. Study of the bioremediatory capacity of wild yeasts. *Scientific Reports*. 2020, 10, 11265. <https://doi.org/10.1038/s41598-020-68154-4>

- [20] Mančić S, Stojanović SS, Malićanin M, Danilović B, Karabegović I. Optimization of nutrient medium and process conditions for cell growth of yeast strain *Pichia kluyveri* K-1. *Advanced Technologies*. 2025, 14, 57–70. <https://doi.org/10.5937/savteh2501056M>
- [21] Park D, Yun YS, Park JM. The past, present, and future trends of biosorption. *Biotechnology and Bioprocess Engineering*. 2010, 15, 86–102. <https://doi.org/10.1007/s12257-009-0199-4>
- [22] Karabegović I, Stamenković-Stojanović S, Lazić M, Đorđević N, Danilović B. Antimicrobial activity and overall sensory acceptance of fermented goat whey beverage: Process conditions optimization using response surface approach. *Advanced Technologies*. 2022, 11, 26–35. <https://doi.org/10.5937/savteh2202026k>
- [23] Vijayaraghavan K, Yun YS. Bacterial biosorbents and biosorption. *Biotechnology Advances*. 2008, 26, 266–291. <https://doi.org/10.1016/j.biotechadv.2008.02.002>
- [24] Myers RH, Montgomery DC, Anderson-Cook CM. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. 3rd ed. John Wiley & Sons, Hoboken, New Jersey, 2009.
- [25] Jaafari J, Yaghmaeian K. Optimization of heavy metal biosorption onto freshwater algae (*Chlorella coloniales*) using response surface methodology (RSM). *Chemosphere*. 2019, 217, 447–455. <https://doi.org/10.1016/j.chemosphere.2018.10.205>
- [26] Ferreira N, Viana T, Henriques B, Tavares DS, Jacinto J, Colónia J, Pinto K., Pereira E. Application of response surface methodology and Box–Behnken design for the optimization of mercury removal by *Ulva* sp. *Journal of Hazardous Materials*. 2023, 445, 130405. <https://doi.org/10.1016/j.jhazmat.2022.130405>
- [27] Rodríguez IA, Cárdenas-González JF, Juárez VMM, Pérez AR, Zarate MG, Castillo NCP. Biosorption of heavy metals by *Candida albicans*. In: *Advances in Bioremediation and Phytoremediation*. IntechOpen, 2017. <https://doi.org/10.5772/intechopen.72454>
- [28] do Nascimento JM, de Oliveira JD, Rizzo ACL, Leite SGF. Biosorption Cu(II) by the yeast *Saccharomyces cerevisiae*. *Biotechnology Reports*. 2019, 21, e00315. <https://doi.org/10.1016/j.btre.2019.e00315>
- [29] Dong X, Ye B, Xiang H, Yao M. Kinetic and isotherm of competitive adsorption cadmium and lead onto *Saccharomyces cerevisiae* autoclaved cells. *Environmental Geochemistry and Health*. 2023, 45, 4853–4865. <https://doi.org/10.1007/s10653-023-01540-9>
- [30] Paul S, Bera D, Ray L. Biosorption of chromium (VI) by a mutated strain of *Bacillus cereus* M116. *Journal of Hazardous Substance Research*. 2007, 7, 1. <https://doi.org/10.4148/1090-7025.1010>
- [31] Medina-Armijo C, Isola D, Illa J, Puerta A, Viñas M, Prenafeta-Boldú FX. The metallotolerance and biosorption of As(V) and Cr(VI) by black fungi. *Journal of Fungi*. 2024, 10, 47. <https://doi.org/10.3390/jof10010047>
- [32] Zinicovscaia I, Grozdov D, Yushin N, Abdusamadzoda D, Gundorina S, Rodlovskaya E, Kristavcuha O. Metal removal from chromium containing synthetic effluents by *Saccharomyces cerevisiae*. *Desalination and Water Treatment*. 2020, 178, 254–270. <https://doi.org/10.5004/dwt.2020.24987>
- [33] Soleimani M, Jamali HA, Mousazadehgavan M, Ghanbari R. Chromium adsorption using powdered leaves of *Prosopis cineraria*: Kinetic, isotherm, and optimization by response surface methodology. *Desalination and Water Treatment*. 2024, 317, 100286. <https://doi.org/10.1016/j.dwt.2024.100286>

Izvod

ISTRAŽIVANJE MOGUĆNOSTI PRIMENE BIOMASE *PICHIA KLUYVERI* K-1 KAO NOVOG BIOSORBENTA ZA UKLANJANJE HROMA IZ VODENOG RASTVORA: STUDIJA OPTIMIZACIJE PROCESA

Sandra Stamenković Stojanović¹, Saša Savić¹, Sanja Petrović¹,
Stojan Mančić¹, Bojana Danilović¹, Ivana Karabegović¹

¹Univerzitet u Nišu, Tehnološki fakultet u Leskovcu, Leskovac, Srbija

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Povećano prisustvo hroma u vodenim resursima predstavlja značajan ekološki i javnozdravstveni problem zbog njegove visoke pokretljivosti, toksičnosti i kancerogenog potencijala. Biosorpcija pomoću mikrobne biomase predstavlja održivu alternativu konvencionalnim fizičko-hemijskim metodama tretmana vode. U ovom radu je po prvi put ispitivan biosorpcioni potencijal nevjabilne biomase kvasca *Pichia kluyveri* K-1 za uklanjanje hroma iz vodenih rastvora. Optimizacija procesa sprovedena je primenom metodologije odzivne površine zasnovane na Boks–Benken eksperimentalnom dizajnu, pri čemu su ispitivani uticaji pH vrednosti rastvora (2–7), početne koncentracije hroma (20–100 mg/l) i doze biosorbenta (2–10 g/l). Efikasnost uklanjanja hroma kretala se u opsegu od 7,8% do 42,0%, dok su konačne koncentracije hroma nakon biosorpcije varirale između 11,6 i 88,5 mg/l u zavisnosti od procesnih uslova. Niže početne koncentracije hroma i izrazito kisela sredina rezultovale su značajno nižim rezidualnim koncentracijama hroma, što ukazuje na poboljšanu biosorpcionu efikasnost u uslovima koji pogoduju protonaciji funkcionalnih grupa na površini biomase. Analiza varijanse pokazala je da je početna koncentracija hroma dominantan faktor koji utiče na konačnu koncentraciju hroma, dok pH ima najveći uticaj na efikasnost uklanjanja. Analiza odzivne površine potvrdila je prisustvo značajnih nelinearnih efekata i identifikovala optimalne radne uslove pri pH 2, početnoj koncentraciji hroma od 20 mg/l i umerenoj dozi biosorbenta, pri čemu je predviđena efikasnost uklanjanja iznosila 42,8%, što je i eksperimentalno potvrđeno. Dobijeni rezultati predstavljaju osnovu za dalju optimizaciju procesa i modifikaciju biomase u cilju primene novih, ekološki bezbednih biosorbenata u tretmanu voda.

Cljučne reči: metoda odzivnih površina, eksperimentalni dizajn, biomasa, biosorpcija, teški metali, kvasac

CRedit authorship contribution statement:

Sandra Stamenković Stojanović: Conceptualization, Supervision, Writing original draft. Writing – review & editing.

Saša Savić: Formal analysis, Laboratory analysis.

Sanja Petrović: Data curation, Laboratory analysis, Visualization.

Stojan Mančić: Methodology, Investigation

Bojana Danilović: Resources, Validation, Project administration.

Ivana Karabegović: Software, Supervision

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