

Taguchi model optimization for Cu(II) removal using aqueous polyphenolic plant extract

Trésor Lukusa^{1*}, Eddy Mbuyu², Michel Kananda², Papy Musampa¹, André Nonda², Albert Kanangila², Marsi Mbayo², Emery Kalonda², John Kabuba¹

¹ Department of Chemical and Metallurgical Engineering, Faculty of Engineering and Technology, Vaal University of Technology, VanderbijlPark, South Africa

² Department of Chemistry, Faculty of Sciences, University of Lubumbashi, Lubumbashi, Democratic Republic of Congo

* Corresponding author E-mail: lukusaseth@gmail.com

Article info

Received 19/4/2024; received in revised form 5/09/2024; accepted 20/9/2024

DOI: [10.6092/issn.2281-4485/19404](https://doi.org/10.6092/issn.2281-4485/19404)

© 2025 The Authors.

Abstract

Removal of Cu(II) using polyphenolic compounds was investigated under batch settings. The influence of extract concentration, initial concentration of metal ion, and contact time on Cu(II) removal was evaluated by Taguchi's L9 OA; and Larger-the-better analysis and ANOVA was used to find the optimum experimental setup and the impact of various variables. The most significant factor was contact time. Maximum Cu(II) removal of 86.2% experimentally was achieved under the optimal conditions (5 mg/ml; 100 mg/L, and 3 hours) polyphenolic compounds of *Phragmites Australis* (Cav.) Trin. Steud. root.

Keywords

Taguchi, optimization, polyphenols, Copper removal

Introduction

One of the main economic uses of phytoremediation is the photoextraction of heavy metals (Ghori *et al.* 2016; Anoopkumar *et al.* 2020; Kafle *et al.* 2022; Miranda *et al.* 2022). This is due to the size and severity of the environmental issues caused by soil and water that have been poisoned by metals, as well as the competitive advantage provided by technology based on plants. By employing plants to reduce, degrade, or immobilize environmental toxins, primarily those with anthropogenic origins, phytoremediation aims to clean polluted areas. Indeed, some plants have thrived in metal-enriched environments. Some of these species have accumulated unusually high concentrations of toxic metals, sometimes exceeding trace metal levels. They are known as accumulators of lead, nickel, zinc, selenium, cobalt,

copper, manganese and many other metals present in the soil (Kumar *et al.* 1995). Metal-accumulating substances are plant bioactive compounds such as tannins, polyphenols, flavonoids and saponins present in these plants, which are responsible for the phytoextraction of metal ions. Environmental protection is severely hampered by the large-scale discharge of wastewater that contains toxins like Ni, As, Zn, Co, Cu, Cr, Cd, and Pb. Environmental pollution is generally linked to anthropogenic activities related to mining, agriculture, and industrial production (Saikia *et al.* 2017; Matias *et al.* 2023). Nature is becoming unable to purify itself (Yadolahi *et al.* 2020). Heavy metals ions have the potential to enter the food chain, cause serious health issues for humans, and have a considerable deal of solubility in aquatic settings and the capacity to be absorbed by living species (Kabuba

and Lukusa, 2021). Under these conditions, it is imperative to remove heavy metals from wastewater before they are released into the environment by using polyphenolic compounds as adsorbents (Pandey & Bajpai, 2019). Thus, numerous research efforts are directed towards reducing environmental degradation and the spread of pollution. To achieve this, several materials are used as adsorbents for the metals contained in water and/or effluents. This includes activated carbon (Alatabe, 2018), hydrogels (Kabuba & Lukusa, 2021), hydroxyapatites (Ulucan-Altuntas *et al.* 2020; Ayodele *et al.* 2021), chitosans (Dinu & Dragan, 2010; Ji *et al.* 2012; Matias *et al.*, 2023), activated clays (Al-Saydeha *et al.* 2017), and natural organic substances such as polyphenols extracted from plants (Rodríguez-Arce & Saldías, 2021; Hu *et al.* 2022). Polyphenols are the most widespread phytochemical compounds today, among which are flavonoids (Jańczak-Pieniążek *et al.* 2023). They are biologically active and important substances for humans (Han *et al.* 2007; Kumar & Pandey, 2013; Cano-Avendaño *et al.* 2021). Polyphenolic compounds are good materials for removing heavy metal ions from wastewater due to their ability to bind and complex metal ions with functional groups such as OH (Raskin *et al.* 1997).

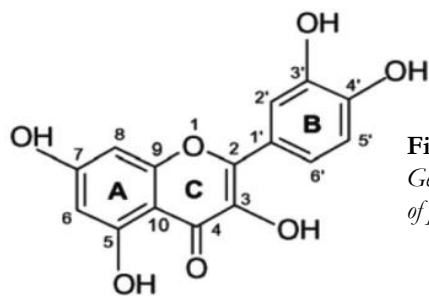


Figure 1
General structure
of polyphenols

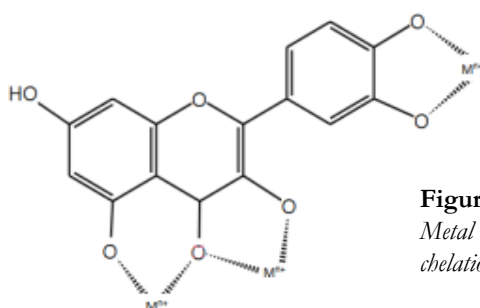


Figure 2
Metal ion flavonoid
chelation sites

The chelating properties polyphenolic compounds (Flavonoids and tannins) for metals such as copper, cobalt, lead, iron and aluminum, have long been known (Massoudi *et al.* 2007; Karamać, 2009; Symonowicz & Kolanek 2012; Říha *et al.* 2014; Rodríguez-Arce & Saldías, 2021; Hu *et al.* 2022). The catechol

motif serves as a crucial structural element for the creation of chelates (flavonoid-metals), which can be used to account for this action. The catechol motif, a bidentate chelation site, has been shown to be responsible for Fe(III) complexation (Fernandez *et al.* 2002; Mira *et al.* 2002). The aim of this study was to evaluate the possibility of depolluting wastewater loaded with Cu(II) ions by adsorption using polyphenolic compounds as adsorbents. These compounds were extracted from five plant species such as *Ceratophyllum demersum* L. (Ceratophyllaceae), *Equisetum hyemale* L. (Equisetaceae), *Phragmites australis* (Cav.) Trin. Steud. (Poaceae), *Spartina maritima* (Curtis) Fernald. (Poaceae) and *Typha latifolia* L. (Typhaceae) and to determine their optimal adsorption conditions using a Taguchi L9 experimental design.

Material and Methods

Extraction of Polyphenols compounds

30 g of dried leaves were macerated in a 70:30 ethanol:water mixture for 24. The resultant hydro-ethanolic extract was filtered, and then it was evaporated at 45°C under reduced pressure. 300 millilitres of distilled water were used to dissolve the resulting hydroethanolic extract. The resultant aqueous phase was extracted liquid-by-liquid using progressively more polar solvents, such as petroleum ether, dichloromethane, and ethyl acetate. To obtain solid extracts, each acquired organic phase was vacuum-evaporated (Rajbhar *et al.* 2016; Alara *et al.* 2021).

Experimental set up

For each trial, 50 ml of copper solution of given concentration (50, 100, 150 ppm) was added to 200 ml reactors (Beaker of 500 ml). The polyphenolic extract of given concentration was added to the reaction medium and the reactors were stirred at 100 rpm and at room temperature (25 ± 2 °C). After a certain period of time, the samples were taken, filtered and subjected to atomic absorption spectrometry analysis to determine the residual copper concentration. The percentage removal of copper was calculated using Equation [1].

$$R (\%) = \frac{(C_0 - C_f)}{C_0} \times 100 \quad [1]$$

where C_0 is the initial concentration of Cu ions in solution, and C_f is the final concentration of metal in solutions at time t .

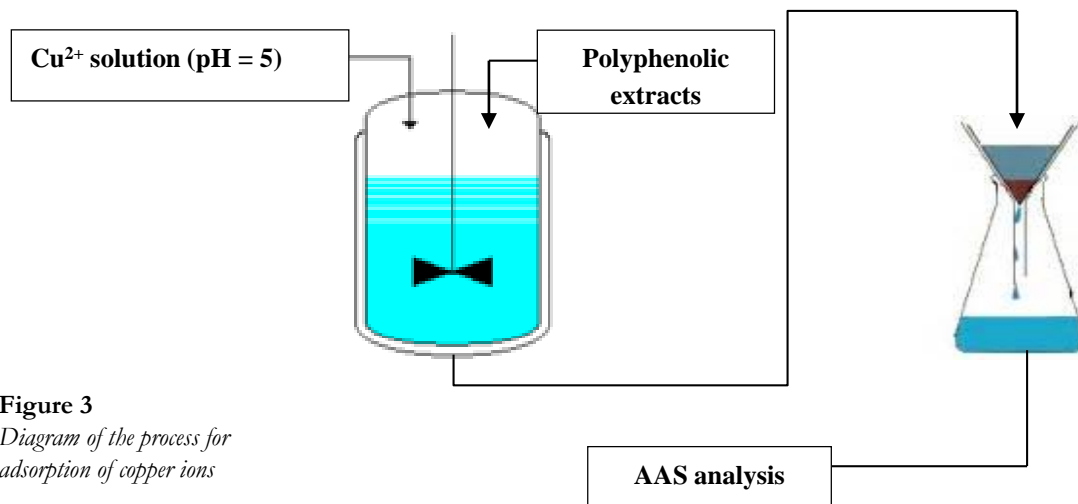


Figure 3
Diagram of the process for
adsorption of copper ions

Optimization set up

Under any relevant constraints, one or more functions are maximized or minimized using a mathematical programming technique called optimization. Minimizing both desired and undesired variables while still adhering to the constraints is an alternative method for identifying the optimal performance. The main goal of using optimization techniques is to improve performance by running fewer tests, which lowers the overall cost of the experimental effort (Saini *et al.* 2019; Kabuba and Lukusa, 2023).

Taguchi Method

Additive cause-effect is the fundamental concept behind Taguchi techniques. Let's say we have two variables that affect a process (A and B). Let a and β represent the impact of factors A and B, respectively, on the response variable Y . The major impacts, as described by Taguchi, can often be modeled using an additive cause-and-effect model in real-world applications (Taguchi and Konishi, 1987; Krishnaiah and Shababudeen, 2012). Equation [2] is how the additive model is expressed.

$$Y_{ij} = \mu + a_i + \beta_j + e_{ij} \quad [2]$$

where, μ = mean value of Y in the region of experiment; a_i and β_j = individual or main effects of the influencing A and B and, e_{ij} = error term.

Mathematical Model

The Taguchi technique is an effective quantitative and standardized Design of Experiment (DOE) approach to study the effects of numerous parameters at once in order to identify the best outcome. The Taguchi model needs fewer tests than the Response Surface

Methodology (RSM) to identify a process' ideal conditions (Saini *et al.* 2019; Yadolahi *et al.* 2020). Taguchi devised an exclusive design known as Orthogonal Arrays (OA) to study the whole parameter space with a small number of tests. The results of the experiment are then used to calculate the signal to noise (S/N) ratio. To determine how far or close quality parameter values are from the desired values, the S/N ratio is used (Vankanti & Ganta, 2014; Svilović *et al.* 2019). The S/N ratio study classifies quality attributes into three groups: bigger is better, smaller is better, and nominal. The formula for calculating S/N ratio is given in equations [2] through 4. When a smaller value is desired, the phrase "The Smaller the Better" is employed

$$\frac{S}{N} \text{ ratio } (\eta) = -10 \log_{10} \frac{1}{n} \sum_{i=1}^n y_i^2 \quad [3]$$

where y_i is the observed response value and n the of replication.

Namely the best is applied in situations where the fluctuation around the nominal or goal value is at a minimum.

$$\frac{S}{N} \text{ ratio } (\eta) = -10 \log_{10} \frac{\mu^2}{\sigma^2} \quad [4]$$

where μ is the mean and σ is the variance.

In cases where a larger value is required, higher is better is utilized.

$$\frac{S}{N} \text{ ratio } (\eta) = -10 \log_{10} \frac{1}{n} \sum_{i=1}^n \frac{1}{y_i^2} \quad [5]$$

where y_i is the observed response value and n the number of replication.

The variance of the data was calculated using Equa-

tion [5], which states that the bigger the S/N, the better. ANOVA was used to improve the S/N results even more. The Taguchi design of experiments is used in this study to alter the process parameters for copper ion adsorption, and the ANOVA analysis is used to assess the effects of each parameter. The main objective of the current work is to apply the Taguchi method design to optimize the adsorption process parameters and the ANOVA to assess the significance of each process parameter (Hasan & Setiabudi, 2019; Razmi & Ghasemi-Fasaee, 2018). The statistical analysis MINITAB version 19 softwa-

are was used to perform Taguchi and ANOVA analyses as well as to build regression models. By considering the three factors listed in Tables 1 and 2, the Robuste Taguchi model has been connected to an analysis of variance (ANOVA). The Taguchi's L9 orthogonal array (Table 1), which contains three columns for the controllable factors and nine rows for the total number of tests, was used to set up the trials. The concentration of polyphenolic compounds (A), initial concentration of Copper ion (B), and contact time (C) all had an impact on the removal of copper.

Table 1. Taguchi L9 design of experiments

Trial number	Factors								
	Level 1 3	Level 2 5	Level 3 10	Level 1 50	Level 2 100	Level 3 150	Level 1 1	Level 2 2	Level 3 3
	A (mg/mL)				B (mg/L)			C (hour)	
1		3			50			1	
2		3			100			2	
3		3			150			3	
4		5			50			2	
5		5			100			3	
6		5			150			1	
7		10			50			3	
8		10			100			1	
9		10			150			2	

Results and Discussion

Taguchi experiment

Batch experiments using polyphenolic compounds of *C. demersum*, *E. hyemale*, *P. australis*, *S. maratina*, and *T. latifolia* extract for copper ions adsorption. Experiments were conducted according to the Taguchi L9 design (Table 1) in order to find out the optimum combination for variables for the adsorption of copper ions using polyphenolic compounds extracts. The experimental domains for different variables with their codes is shown in the Table 1. Taguchi Robust plan was applied to analyse the interactive effect of three factors on adsorption capacity. The three factors studies were polyphenolic compounds concentration, initial concentration in copper ion, and contact time for copper ions adsorption in aqueous solution. Nine trials were carried out for each organ plant species studied, for a total of 63 experiments according to design (Table 1). Table 2 summarizes the optimal operating conditions for removal copper by the polyphenolic compounds of different plants The optimum of copper adsorption efficiencies were 66.8

and 83.2% (Table 2) for root and stem of *T. latifolia*, respectively and 86.2% with *P. australis* root. These optimum yields were obtained under the operating conditions of 5mg/mL polyphenolic extract (*T. latifolia* root, *T. latifolia* stem, and *P. australis* root), 100mg/L initial Copper ion concentration and 3 hours of contact time. The higher removal efficiency of copper ions was observed with root's polyphenolic compounds as adsorbent (Yadav et al. 2021). Optimums for *P. australis* stem (69.4%) and *E. Hyemale* (82.2%) were obtained under the same operating conditions of 3 mg/ml polyphenolic extract, 150 mg/l initial metal ion concentration for a contact time of 3 hours. With *S. maratina* polyphenolic extract, the optimum yield was 44% for 10 mg/mL extract, 50 mg/L and 3 hours contact time, while with *C. Demersum* extract, the optimum yield was 70.7% for 5 mg/ml polyphenolic extract, 150 mg/L initial metal ion concentration and 1 hour of contact time. This scenario of ions being adsorbed observed by many plant species is confirmed by some anterior research (Sarwar et al. 2016; Muthusaravanan et al. 2018).

Table 2. Cu removal efficiency by polyphenolic compounds

Trial	A	B	C	Response (%)
T. latifolia root				
1	3	50	1	64.0
2	3	100	2	12.2
3	3	150	3	61.8
4	5	50	2	20.2
5	5	100	3	66.8
6	5	150	2	33.3
7	10	50	3	43.9
8	10	100	1	61.0
9	10	150	2	29.3
T. latifolia stem				
1	3	50	1	15.4
2	3	100	2	9.6
3	3	150	3	34.8
4	5	50	2	20.7
5	5	100	3	83.2
6	5	150	1	30.0
7	10	50	3	65.0
8	10	100	1	61.0
9	10	150	2	33.3
P. australis root				
1	3	50	1	62.0
2	3	100	2	25.7
3	3	150	3	58.7
4	5	50	2	35.3
5	5	100	3	86.2
6	5	150	1	33.3
7	10	50	3	48.0
8	10	100	1	64.0
9	10	150	2	20.0

Compared with some other studies on the removal of copper ions from water, polyphenols extracted from the metallophytic plants species in our study showed low copper ion removal efficiencies. These efficiencies ranged from 2 to 83.2% for contact times varying from 1 to 3 hours. The use of fungal pellets achieved 95% removal efficiency at 25°C, pH 5.5, for an initial copper concentration of 100 ppm and stirring at a speed of around 250 rpm for 72 h (Al-Shammari *et al.* 2023). Furthermore, in their study,

Trial	A	B	C	Response (%)
P. australis stem				
1	3	50	1	42.0
2	3	100	2	58.9
3	3	150	3	69.4
4	5	50	2	33.3
5	5	100	3	62.5
6	5	150	1	43.3
7	10	50	3	44.0
8	10	100	1	51.0
9	10	150	2	12.0
S. maratina				
1	3	50	1	44.0
2	3	100	2	12.6
3	3	150	3	43.3
4	5	50	2	26.8
5	5	100	3	34.7
6	5	150	1	33.3
7	10	50	3	41.4
8	10	100	1	43.0
9	10	150	2	10.7
E. hyemale				
1	3	50	1	10.0
2	3	100	2	67.4
3	3	150	3	82.2
4	5	50	2	21.8
5	5	100	3	58.1
6	5	150	1	6.7
7	10	50	3	65.6
8	10	100	1	8.0
9	10	150	2	9.5
C. demersum				
1	3	50	1	12.0
2	3	100	2	12.5
3	3	150	3	18.0
4	5	50	2	24.5
5	5	100	3	15.8
6	5	150	1	70.7
7	10	50	3	49.4
8	10	100	1	2.0
9	10	150	2	6.7

Ulucan-Altuntas *et al.* (2020) achieved copper removal rates higher than 90% using 514.0 mg l⁻¹ nanohydroxyapatite for a contact time of 60.4 minutes. On the other hand, using hydroxyapatite from eggshells, Ayodele *et al.* (2021) achieved an adsorption efficiency of 99.99% after 360 minutes of contact. They also observed that hydroxyapatite could be reused after ten cycles of use. However, copper adsorption efficiency was totally reduced 95.88 and 62.13% respectively for the first and tenth cycle of use.

These studies were carried out under totally different conditions of pH, contact time, temperature and initial copper ion concentration in the water. So, for our study, the copper removal yield could increase if we also adjust the operating conditions.

Taguchi analysis of main effect plot S/N ratio and ANOVA

The S/N analysis was carried out by examining the differences between the means of the means of the factor levels using the graphs of the main effects (Fig- 4). This

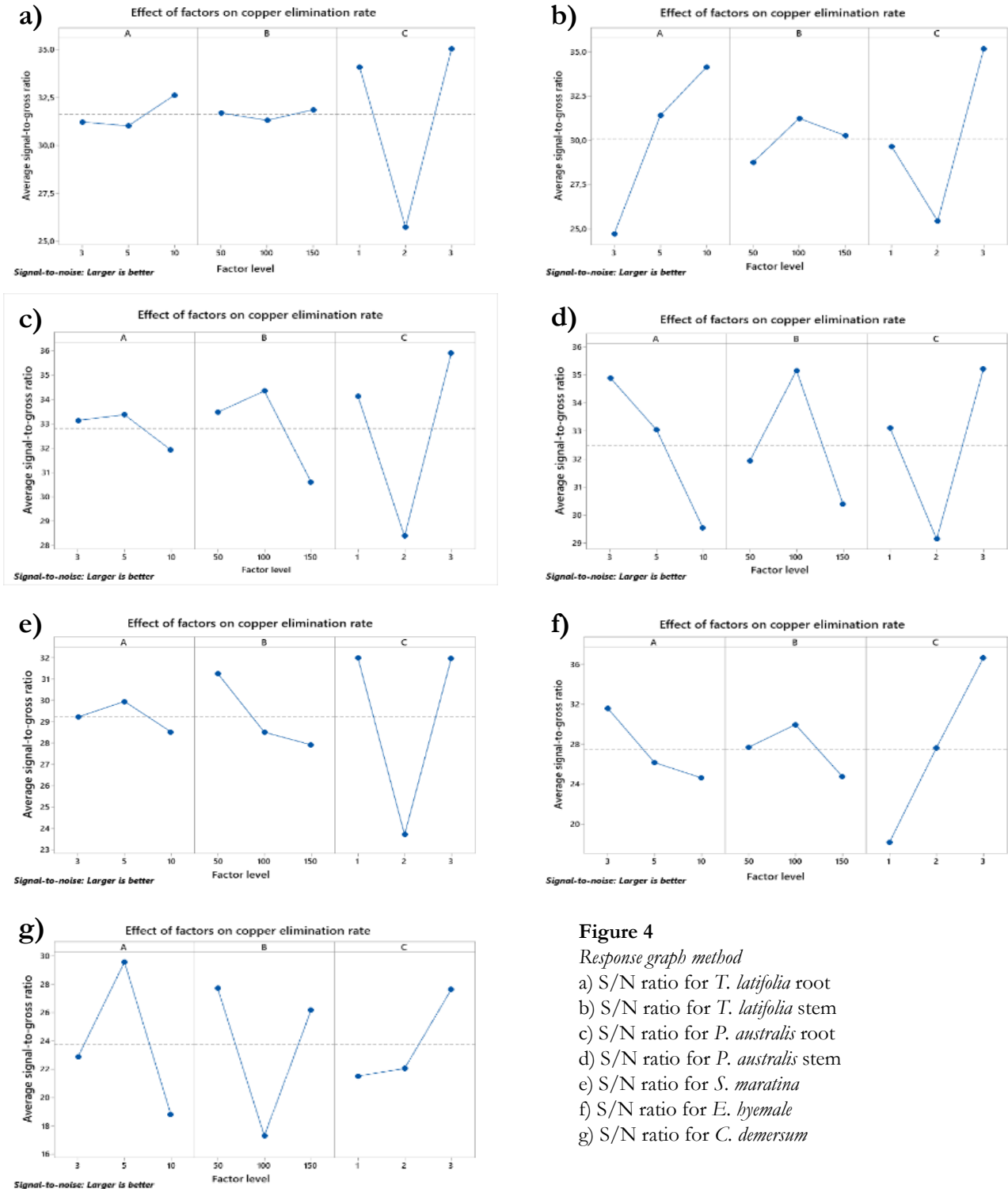


Figure 4

Response graph method

- a) S/N ratio for *T. latifolia* root
- b) S/N ratio for *T. latifolia* stem
- c) S/N ratio for *P. australis* root
- d) S/N ratio for *P. australis* stem
- e) S/N ratio for *S. maritima*
- f) S/N ratio for *E. hyemale*
- g) S/N ratio for *C. demersum*

analysis according to the Taguchi method considers the greatest difference as the most influential parameter (Kouassi *et al.* 2018). The experimental results collected from Taguchi/Orthogonal Array (Table 2) using the response graph method were linked to analysis of variance (ANOVA) in order to determine the impact of each parameter (A, B, and C) on copper removal efficiency. This response graph method is very easy to understand and apply. The regression analysis for this optimization of the

synthesis parameters is shown in terms of the coefficients, degree of freedom (DF), sum of squares (SS), mean square (MS), *F* and probability *p*-value in Table 3 (Maity & Ray, 2017). The adequacy and significance of the model were examined using ANOVA for removal of metal ions. The significance of the factors can be examined by the *p*-value and the smaller magnitude of *p*-value <0.05 portray as the more significant factor (Saini *et al.* 2019; Hasan & Setiabudi, 2019).

Table 3
ANOVA
analysis with
polyphenolic
compounds

<i>T. latifolia</i> root					
Source	DF	SS	MS	F-value	p-value
A	2	57	29	0.06	0.940
B	2	44	22	0.05	0.953
C	2	2419	1210	2.7	0.272
Residual Error	2	905	453	-	-
Total	8	3427	-	-	-
<i>T. latifolia</i> stems					
A	2	1784	892	14,21	0,066
B	2	655	328	5,22	0,161
C	2	2437	1219	19,42	0,049
Residual Error	2	126	63	-	-
Total	8	5002	-	-	-
<i>P. australis</i> root					
A	2	89	44	0,12	0,890
B	2	681	341	0,95	0,512
C	2	2198	1099	3,07	0,245
Residual Error	2	715	358	-	-
Total	8	3683	-	-	-
<i>P. australis</i> root					
A	2	668	334	2,59	0,279
B	2	569	284	2,20	0,312
C	2	861	430	3,33	0,231
Residual Error	2	258	129	-	-
Total	8	2356	-	-	-
<i>S. maratina</i>					
A	2	6	3	0,04	0,961
B	2	122	61	0,89	0,528
C	2	1081	541	7,88	0,113
Residual Error	2	137	69	-	-
Total	8	1346	-	-	-
<i>E. hyemale</i>					
A	2	1245	622	1,93	0,341
B	2	282	141	0,44	0,696
C	2	5535	2768	8,58	0,104
Residual Error	2	645	323	-	-
Total	8	7707	-	-	-
<i>C. demersum</i>					
A	2	857	429	0,44	0,694
B	2	823	411	0,42	0,703
C	2	361	181	0,19	0,843
Residual Error	2	1944	972	-	-
Total	8	3985	-	-	-

Statistical analysis (ANOVA) for adsorbing of copper are presented in Table 3 and the effect of the three main factors in the design are presented briefly. Also, because with 9 experiments, the effect of the interaction of factors cannot be investigated, the effect of the interaction of factors between AB, AC, and BC has been discarded. Based on the design of ANOVA, the more the p -value of the investigated factor is smaller (close to zero), the more effective it is. In the same condition, the sum of squares (SS) will be decisive, so larger the SS number, more effective it will be. As it can be seen, the result of ANOVA analysis for adsorbing copper from aqueous solution by polyphenolic plant compounds are consistent with the results of the analysis of the effect of the factor considered in the previous section (Razmi & Ghasemi-Fasaei, 2018). Thus, based on the response graph method (Figures 5) connected to ANOVA analysis (Table 3); it was observed that the contact time (C) was the most influential parameter on the S/N (35.0, 35.0, 36, 35.4, 32, 36.4, and 29.8) ratio during the Cu removal process by polyphenols from different plants with the exception of *C. Demersum*, which depends on the ini-

tial concentration of copper ion. The lowest p -values were: 0.272, 0.049, 0.245, 0.231, 0.113, 0.104 and 0.703 for *T. latifolia* root, *T. latifolia* stem, *P. australis* root, *P. australis* stem, *S. maritima*, *E. Hyemale*, and *C. demersum*. It was found that the regression was statistically significant at F -value of 2.7, 19.42, 3.07, 3.33, 7.88, 8.58, and 0.44 (S/N = 29.8) (A) for copper ions indicate model terms are significant. The highest optimal Cu(II) removal yield (Table 4) is 86.2% with polyphenolic compounds of *P. australis* root, followed by *T. latifolia* stem (83.2%) and *E. hyemale* (82.2%). *C. demersum* and *P. australis* stem had a similar optimal copper removal yield of 70% and are followed by *T. latifolia* root (66.8%). Only *S. maritima* had an optimum yield of less than 50%. The plants under study are typically found by rivers or on their banks, and their concentration of polyphenolic chemicals has been shown to be appropriate. By obstructing metal ions in their roots, stems, and leaves which would prevent them from migrating towards watergrounds or assimilating into the food chain, cultivating it next to mining areas or riverbanks would be advantageous in reducing soil and water pollution.

Plant species	A	B	C	Response (%)
<i>C. demersum</i>	5	150	1	70.7
<i>E. hyemale</i>	3	150	3	82.2
<i>P. australis</i> root	5	100	3	86.2
<i>P. australis</i> stem	3	150	3	69.4
<i>S. maritima</i> ,	3	50	1	44.0
<i>T. latifolia</i> root	5	100	3	66.8
<i>T. latifolia</i> stem	5	100	3	83.2

Table 4

Optimal operating conditions for removal copper by the polyphenolic compounds

Conclusions

The aim of this study was to determine the optimal conditions for the removal of Cu(II) in aqueous solution using polyphenolic extracts from different plants such as: *T. Latifolia*, *P. Australis*, *S. Maratima*, *E. Hyemale* and, *C. Demersum*. The optimization was performed using Taguchi's L9 orthogonal array, which was associated with ANOVA to assess the significance of each process parameter. Thus, the highest efficiency of Cu(II) removal was 86.20% with polyphenolic compound compounds of *P. australis* root. The contact time was the most influential parameter of the copper removal process and those, for all polyphenolic compounds of the different plants and the S/N ratio was 36 with *P. australis* root

and whose optimal conditions were: 5 mg/mL, 100 mg/L, and 3 hours.

Conflict of interest

The authors declared that there is no conflict of interest.

References

- ALARA O.R., ABDURAHMAN N.H. & UKAEGBU C.I. (2021) Extraction of phenolic compounds: A review. *Current Research in Food Science*, (4), 200 – 214. <https://doi.org/10.1016/j.crfs.2021.03.011>.
- ALATABE, M. J. (2018) Adsorption of Copper (II) Ions from Aqueous Solution onto Activated Carbon Prepared from Cane Papyrus. *Pollution*, 4(4), 649-662 [doi: 10.22059/poll.2018.249931.377](https://doi.org/10.22059/poll.2018.249931.377)

- AL-SAYDEHA S.A., EL-NAASA M.H. & ZAĪDĪB S.J. (2017) Copper removal from industrial wastewater: A comprehensive review. *Journal of Industrial and Engineering Chemistry* 56, 35–44. <http://dx.doi.org/10.1016/j.jiec.2017.07.026>
- AL-SHAMMARĪ R.H.H., SHAĪMAA S.M.A. & HUSSĪN M.S. (2023) Efficient Copper Adsorption from Aqueous Solution by Dictyochus sterile Pellets. *Nature Environment and Pollution Technology* 22 (2), 905-912. <https://doi.org/10.46488/NEPT.2023.v22i02.033>
- ANOOPKUMAR A.N., REBELLO S., DEVASSY E., KAJ K.K., PUTHUR S., ANEESH E.M., SĪNDHU R., BĪNOD P. & PANDEY A. (2020) Phytoextraction of Heavy Metals. *Methods for Bioremediation of Water and Wastewater Pollution*, 267–276. http://dx.doi.org/10.1007/978-3-030-48985-4_12
- AYODELE O., OLUSEGUN S.J., OLUWASĪNA O.O., OKORONKWO E.A., OLANĪPEKUN E.O., MOHALLEM N.D.S., GUĪMARAES W.G., DE M. GOMES B.L.F., DE O. SOUZA G. & DUARTE H.A. (2021). Experimental and theoretical studies of the adsorption of Cu and Ni ions from wastewater by hydroxyapatite derived from eggshells. *Environmental Nanotechnology, Monitoring & Management* 15, 100439. <https://doi.org/10.1016/j.enmm.2021.100439>
- CANO-AVENDAÑO B.A., CARMONA-HERNANDEZ J.C., RODRĪGUEZ R.E., TABORDA-OCAMPO G. & GONZÁLEZ-CARREA C.H. (2021) Chemical properties of polyphenols: a review focused on anti-inflammatory and anti-viral medical application. *Biomedicine* 41 (1), 3–8. <https://doi.org/10.51248/v41i1.524>
- DĪNU M.V. & DRAGAN ES (2010) Evaluation of Cu²⁺, Co²⁺ and Ni²⁺ ions removal from aqueous solution using a novel chitosan/clinoptilolite composite: Kinetics and isotherms. *Chemical Engineering Journal* 160, 157–163. [doi:10.1016/j.cej.2010.03.029](https://doi.org/10.1016/j.cej.2010.03.029)
- FERNANDEZ M.T., MĪRA M.L., FLORÊNCIO M.H. & JENNĪNGS K.R. (2002) Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. *Journal of Inorganic Biochemistry*, 2 (11), 105–111. [https://doi.org/10.1016/S0162-0134\(02\)00511-1](https://doi.org/10.1016/S0162-0134(02)00511-1)
- GHORĪ Z., IFTĪKHAR H., BHATTĪ M.F., NASAR-UM-MĪNULLAH, SHARMA I., KAZĪ A.G., AHMAD P. (2016) Phytoextraction: The use of Plants to Remove Heavy Metals from Soil. *Plant Metal Interaction*, 385–409. <https://doi.org/10.1016/B978-0-12-803158-2.00015-1>
- HAN X., SHEN T. & LOU H. (2007) Dietary Polyphenols and Their Biological Significance. *International Journal of Molecular Sciences*, (8), 950–988. ISSN 14422 – 0067.
- HASAN R. & SETĪABUDĪ (2019) Removal of Pb(II) from aqueous solution using KCC-1. Optimization by response surface methodology (RSM). *Journal of King Saud University – Science*, (31), 1182–1188. <https://doi.org/10.1016/j.jksus.2018.10.005>
- HU N., QĪN H., CHEN X., HUANG Y., XU J. & HE H. (2022) Tannic acid assisted metal-chelate interphase toward highly stable Zn metal anodes in rechargeable aqueous Zinc-ion batteries, *Frontiers in Chemistry*, (10), 10–98. <https://doi.org/10.3389/fchem.2022.981623>.
- JAŃCZAK-PIENIAZĒK M., CICHONSKI J., MICHALIK P. & CHRZANOWSKI G. (2023) Effect of Heavy Metal Stress on Phenolic Compounds Accumulation in Winter Wheat Plants. *Molecules*, 28 (241), 1–15. <https://doi.org/10.3390/molecules28010241>.
- JĪ G., BAO W., GAO G., AN B., ZOU H. & GAN S. (2012) Removal of Cu (II) from Aqueous Solution Using a Novel Crosslinked Alumina-Chitosan Hybrid Adsorbent. *Chinese Journal of Chemical Engineering*, 20(4), 641-8.
- KABUBA J. & LUKUSA T. (2021) Synthesis of Gelatin-Cellulose Nanocrystals Hydrogel Membrane For Removal of Cu (II) and Co (II) From Mining Processes Wastewater. *Research Square*, <https://doi.org/10.21203/rs.3.rs-383692/v1>.
- KABUBA J. & LUKUSA T. (2023) Process Optimization Using Response Surface Methodology for the Removal of Cu(II) and Co(II) from Aqueous solution Using Gelatin-Cellulose Nanocrystals Hydrogel Membrane. *International Journal of Environment Science and Development*, 14 (4), 252 – 258. <https://doi.org/10.18178/ijesd.2023.14.4.1441>.
- KAFLE A., TĪMĪLSĪNA A., GAUTAM A., ADHĪKARĪ K., BHATTARĪ A. & 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>
- KARAMAĆ M. (2009) Chelation of Cu(II), Zn(II), and Fe(II) by Tannin Constituents of Selected Edible Nuts. *International Journal of Molecular Sciences*, 10 (12), 5485–5497. <https://doi.org/10.3390%2Fijms10125485>.
- KOUASSĪ K.E., ABOLLE A., YAO K.B., BOA D., ADOUBY K., DROGUĪ P. & TYAGĪ R.D. (2018) Optimization of Rubber Seed Oil Transesterification to Biodiesel Using Experimental Designs and Artificial Neural Networks. *Green and Sustainable Chemistry*, (8), 39–61. <https://doi.org/10.4236/gsc.2018.81004>.
- KRĪSHNAĪAH K. & SHAHABUDEEN (2012) Applied Design of Experiments and Taguchi Methods, PHI Learning Private Limited, New Delhi, ISBN–978–81–203–4527–0.

- KUMAR P., DUSHENKOV V, MOTTO H, RASKIN I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. *Environ.Sci.Tech*, (29),1232-1238. <https://pubs.acs.org/doi/10.1021/es00005a014>
- KUMAR S. & PANDEY A. (2013) Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, 1 – 16. <http://dx.doi.org/10.1155/2013/162750>.
- MAÍTY J. & RAY S.K. (2017) Removal of Cu(II) ion from water using sugar can bagasse cellulose and gelatin based composite hydrogels. *International Journal of Biological Macromolecules*, 97, 238–248. <https://doi.org/10.1016/j.ijbiomac.2017.01.011>.
- MASSOUDI NEJAD M.R., REZAZADEH AZARY M. & KHATÍBY M. (2007) Treatment of wastewater in Planting Industry by chelate extraction method. *Journal of Environmental Health Science & Engineering*, 4 (1), 13–20.
- MATÍAS P.M.C., SOUSA J.F.M., BERNARDINO E.F., VAREDA J.P., DURÃES L., ABREU P.E., MARQUES J.M.C., MURTINHO D. & VALENTE A.J.M. (2023) Reduced Chitosan as a Strategy for Removing Copper Ions from Water. *Molecules* 28, 4110. <https://doi.org/10.3390/molecules28104110>
- MIRA L., FERNANDEZ M.T., SANTOS M., ROCHA R., FLORÊNCIO M.H. & JENNINGS K.R. (2002) Interactions of Flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radical research*, 36 (11), 1199–208. <https://doi.org/10.1080/1071576021000016463>.
- MIRANDA R.D.S., BOECHAT C.L., BOMFIM M.R. GONZAGA SANTOS J.A., COETHO D.G., ASSUNÇÃO S.J.R., CARDOSO K.M. & CARDOSO E.B. (2022) 3-Phytoremediation: A Sustainable green approach for environmental cleanup, *Phytoremediation Technology for the Removal of Heavy Metal and Other Contaminants from Soil and Water*, 49–75. <https://doi.org/10.1016/B978-0-323-85763-5.00017-9>
- MUTHUSARAVANAN S., SIVARAJASEKAR N., VIVEK J. S., PARAMASIVAN T., NAUSHAD M., PRAKASHMARAN J., GAYATHRI V. & AL-DUAJI O.K. (2018) Phytoremediation of heavy metals: mechanisms, methods and enhancements. *Environmental Chemistry Letters*. <https://doi.org/10.1007/s10311-018-0762-3>.
- PANDEY V.C. & BAJPAI O. (2019) Phytoremediation: From Theory Toward Practice, *Phytomanagement of Polluted Sites*, 1–49. <https://doi.org/10.1016/B978-0-12-813912-7.00001-6>.
- RAJBHAR K., DAWDA H. & MUKUNDAN U. (2016) Comparative analysis of extraction and estimation of tea polyphenols, flavonoids and antioxidant in commercially available tea powders. *Indian Journal of Plant Sciences*, 5 (1), 11–26. ISSN: 2319–3824. ù
- RASKIN I, SMITH R.D., SALT D.E. (1997) Phytoremediation of metals: Using plant to remove pollutants from environment. *Current Opinion in Biotechnology*, (8), 221–226. [http://dx.doi.org/10.1016/S0958-1669\(97\)80106-1](http://dx.doi.org/10.1016/S0958-1669(97)80106-1)
- RAZMI B.. GHASEMI-FASAEI R. (2018) Investigation of Taguchi optimization, equilibrium, isotherms, and kinetic modeling for phosphorus adsorption onto natural zeolite of clinoptilolite type. *Adsorption Science & Technology*, 36 (7-8): 1470–1483. <https://doi.org/10.1177/0263617418779738>.
- ŘÍHA M., KARLÍČKOVÁ J., FILÍPSKÝ T., MACÁKOVÁ K., ROCHA L., BOVÍCELLI P., SILVESTRI I.P., SASO L., JAHODÁŘ L., HRDINA R. & MLADĚNKA P. (2014) *In vitro* evaluation of Copper – chelating properties of flavonoids. *The Royal Society of Chemistry Advances*, (4), 32628 – 32638. <https://doi.org/10.1039/C4RA04575K>.
- RODRÍGUEZ-ARCE E. & SALDÍAS M. (2021) Antioxidant properties of flavonoid metal complexes and their potential inclusion in the development of novel strategies for the treatment against neurodegenerative diseases. *Biomedicine & Pharmacotherapy*, (14), 1112236. <https://doi.org/10.1016/j.biopha.2021.112236>.
- SAIKIA R., GOSWAMI R., BORDOLOI N., SENAPATI K.K., PANT K.K., KUMAR M. & KATAKI R. (2017) Removal of arsenic and fluoride from aqueous solution by biomass based activated biochar: Optimization through response surface methodology. *Journal of Environmental Chemical Engineering*, 5: 5528–39
- SAINI S., CHAWLA J. KUMAR R. & KAUR I. (2019) Response Surface Methodology (RSM) for optimization of cadmium ions adsorption using C₁₆₋₆₋₁₆ incorporated mesoporous MCM-41. *SN Applied Science*, (1), 894. <https://doi.org/10.1007/s42452-019-0922-5>.
- SARWAR N., IMRAN M., SHAHEEN M.R., ISHAQ W., KAMRAN A., MATLOOB A., REHIMB A., HUSSAIN S. (2016) Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, <http://dx.doi.org/10.1016/j.chemosphere.2016.12.116>.
- SVILOVIĆ S. MUŽEK M.N., NUIĆ I. & VUČENOVIĆ. (2019) Taguchi design of optimum process parameters for sorption of copper ions using different sorbents. *Water Science & Technology*, 80 (1), 98–108. <https://doi.org/10.2166/wst.2019.249>.
- SYMONOWICZ M. & KOLANEK M. (2012) Flavonoids and their properties to form chelate complexes. *Biotechnology and Food Sciences*, 76 (1), 35–41.

TAGUCHI G. & KONISHI S. (1987). Taguchi Methods, Orthogonal Arrays and linear graphs: Tools for quality, American Supplier Institute, American Supplier Institute, Dearborn, Michigan.

ULUCAN-ALTUNTAS K., UZUN H.I., USTUNDAG C.B., DEBIK E. (2020) Adsorption of copper ion from aqueous solutions by well-crystallized nanosized hydroxyapatite. Mater. Res. Express 6, 125545

VANKANTI V.K., GANTA V. (2014) Optimization of process parameters in drilling of GFRP composite using Taguchi method. Journal of Materials Research and Technology, 3(1):35–41. <https://doi.org/10.1016/j.jmrt.2013.10.007>.

YADAV M., SINGH G., JADEJA R.N. (2021) Phytoremediation for Heavy Metal Removal. Pollutants and Water Management: Resources, Strategies and Scarcity, (6):128 – 150.

YADOLAHÍ K., GHADÍ A., ALAVÍ S.A. (2020) Optimization of copper adsorption process from aqueous solution by *Descurainia Sophia* plant stem using Taguchi Method. Journal of Water and Environmental Nanotechnology, 5 (1):68–80. <https://doi.org/10.22090/jwent.2020.01.006>