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BIOREMEDIATION IN CIRCULAR ECONOMY: CASE STUDY OF Cu(II) REMOVAL VIA LIGNIN-BASED BIOMASS

BY

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Abstract. In the context of sustainable development, the present study proposes a novel approach to minimise the harmful effects of pollutant sources, such as heavy metal ions, and to effectively utilise bioresources in the same time. The study proposes the retention of Cu(II) ions in static conditions using Sarkanda grass lignin, a biomass residue, representing a renewable natural resource and a versatile biomaterial. This complex compound demonstrates thermodynamic, and biological efficiency. The experimental data obtained suggest potential applications in the treatment of wastewater, offering a sustainable alternative to the already established, but more expensive, technologies. This research supports the expansion of the use of biomaterials for environmental remediation, highlighting the importance of minimising waste and conserving natural resources in support of a circular bioeconomy. Future research will focus on optimizing the retention process, evaluating the long-term stability of the material, and exploring the scalability of this technology for industrial wastewater treatment.

Keywords: bioremediation, lignin, Cu(II) ions, retention, *Lypercosium esculentum*, circular bioeconomy.

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1. Introduction

The circular economy has been identified as a potentially effective tool for initiating a sustainable development process (Zavos *et al*., 2024). With a focus on the recovery of resources to minimise waste and the impact on the environment, the circular bioeconomy highlights the potential that biomass residues can bring (Tofănică *et al*., 2024).

Lignocellulosic biomass is a renewable and abundant biomass feedstock, mainly composed of polysaccharides (cellulose-40-50% and hemicellulose-25-35%) and aromatic polymer (lignin-15-20%) (Baruah *et al*., 2018). Lignin, a macromolecule that is second only to cellulose in abundance, is found in all parts of plant cells and is estimated to be produced by plants at a rate of approximately 20 billion tons per year (Balk *et al*., 2023). Its effective utilisation could therefore offer a means of achieving the sustainable development of the circular economy in the future (Balk *et al*., 2023).

However, the presence of heavy metal ions, originating from both point and nonpoint sources, has been observed to contaminate a wide range of natural resources, including air, water, sediments, and soil. This phenomenon has been demonstrated to have significant environmental and health implications, as evidenced by studies conducted on humans (Okereafor *et al*., 2020), animals (Singh *et al*., 2024) and plants (Ungureanu *et al*., 2023).

In this context, lignin can be considered a type of convenient biomass. Its porous structure and the presence of a considerable number of functional groups, particularly carboxyl and hydroxyl groups, mean that it is able to bind and concentrate heavy metals by complexation, forming stable lignocomplexes. This process mitigates the destructive activity of these chemical species (Vasile *et al*., 2023; Mondal *et al*., 2023).

Copper, a metal that is categorised as a heavy metal, is the third most widely used metal on the global scale. It exhibits a dualistic behaviour towards plants, exhibiting essentiality at optimal levels and toxicity at elevated concentrations. This necessitates the development of methodologies for its elimination from natural environments (Shabbir *et al*., 2020).

From a redox perspective, copper is classified as an active metal under biological conditions and can exist in two oxidation states, namely the unstable cuprous ion: Cu^{+} or Cu(I) and stable cupric ion: Cu^{2+} or Cu(II). This intense redox activity can render copper a harmful metal, causing cellular damage through a series of possible mechanisms, often claimed to be the main mechanism of toxicity with serious health consequences (Zhang *et al*., 2023). These mechanisms damage the brain, muscles, liver, heart, and can lead to neurodegenerative diseases, including Alzheimer's, Parkinson's, Wilson's, and Huntington's diseases. It is therefore vital that the copper is effectively protected inside cells in order to avoid both indiscriminate binding to biomolecules and excessive formation of reactive oxygen species through redox cycling of weakly bound copper (Scheiber *et al*., 2014).

The process of copper production is a complex one, involving several stages: mining, crushing, milling, ore concentration, smelting, refining and waste management (Izydorczyk *et al*., 2021). The most common method of waste management is deposition, but it is not the most appropriate due to the potential for contamination of terrestrial and aquatic environments with polluting species, such as heavy metals (Timofeev *et al*., 2018).

Currently, techniques such as chemical precipitation, ion exchange or membrane filtration are effective for Cu(II) retention, but present a series of disadvantages that cannot be neglected, mainly being energy-consuming and secondary waste generators (Ungureanu *et al*., 2024).

It is acknowledged that biosorption is associated with the capacity of specific biomolecules or categories of living or dead biomass to attract and accumulate specific ions or other molecules from aqueous solutions. This process is characterized by a passive behaviour and is primarily dependent on the "affinity" between the sorbent and the sorbate, as well as factors like concentration and removal efficiency (Volesky, 2007).

Lignin, a by-product or non-toxic waste derived from plant biomass, has been found to be a reservoir rich in carboxyl, amino or hydroxyl active sites (Garcia-Valls *et al*., 2003; Vasile *et al*., 2023; Ungureanu *et al*., 2024). These sites are easily available for complexation with metal ions, thus recommending it for the adsorption of polluting species, such as heavy metal ions.

The majority of biosorption applications are focused on laboratory studies, with limited development on an industrial scale or in separation technologies. There is also insufficient exploration of biomaterials such as biomass residues. This requires current and future research to be directed in this direction (Torres, 2020).

In this context, supporting the principle of sustainability implemented by the circular bio economy, the objective of the present study is to assess the retention capacity of lignin isolated from Sarkanda grass (*Tripidium bengalense*) for the removal of cupric ion under batch conditions. This evaluation will be conducted through a comprehensive approach that incorporates spectral, thermodynamic, and biological analyses. This follows a previous study that demonstrated the kinetic efficiency of the biopolymer (Ungureanu *et al*., 2022).

2. Materials and Methods

The following materials have been used:

– Lignin isolated from Sarkanda grass (*Tripidium bengalense*) provided by Granit Recherche Development S.A. Lausanne, Switzerland and

$CuSO_4 \cdot 5H_2O$ provided by ChimReactiv S.R.L., Bucharest (Ungureanu *et al*., 2022).

– Tomato seeds (*Lypercosium esculentum*), San Marzano variety, supplied by "Ion Ionescu de la Brad" Iasi University of Life Sciences, Iasi, Romania (Fortună *et al*., 2022).

– The stock solutions (0.001 mg/L) was prepared by dissolving of analytical-grade $CuSO_4 \cdot 5H_2O$ in distilled water. The working solutions were obtained by diluting an accurately measured volume of the stock solution in the distilled water. The resulting concentrations (mg/mL) are as follows: 6.355, 12.71, 19.065, 25.42, 32.665, 38.13, 44.485, 50.84, 57.195, 63.5. A total of 5 g of Sarkanda grass lignin were added in 20 mL of $CuSO_4 \cdot 5H_2O$ solutions with previously specified concentrations. The samples were then subjected to an interaction process at a temperature of 20 ± 0.5°C at three reaction times: 30, 60 and 90 minutes (Ungureanu *et al*., 2022).

Work procedure: The concentration of Cu(II) was determined using the rubeanic acid method, which exhibited a maximum absorption at 390 nm. The cupric ion concentration in the filtrate from the aqueous solutions was determined by analysing a precisely measured 2 mL sample, following the established experimental procedure. The concentration for each sample was calculated using the regression equation derived from the calibration curve (Ungureanu *et al*., 2022).

For the spectrophotometric analysis, a Visible Spectrophotometer for laboratory, model VS-721N, 300-1000 nm, manufactured by JKI, Shanghai, China, was utilized. Scanning electron microscopy (SEM) was performed on platinum (Pt)-coated samples to improve contrast, using a Quanta 200 scanning electron microscope (5 kV) from Brno, Czech Republic.

The retention of Cu(II) on Sarkanda grass lignin was thermodynamically tested by evaluating the thermodynamic parameters recommended by the literature: free energy change (ΔG), enthalpy (ΔH) and entropy (ΔS), applying van't Hoff laws (Banerjee *et al*., 2016; Sahmoune *et al*., 2019; Lima *et al*., 2020; Ciobanu *et al*., 2023).

The present series of experiments was designed to investigate the effects of different concentrations of cupric ions on the germination of seeds from the tomato plants. The seeds from the San Marzano variety were used for this study, and they were germinated in the presence of aqueous copper solutions in a range of concentrations, from 6.355 to 53.5 mg/mL. The experiment included three different contact times for the seeds to germinate, and the seeds were also germinated in the filtrates from the experiments, as well as in uncontaminated adsorbent. The use of distilled water and the control samples of uncontaminated adsorbent and filtrates allowed for the determination of the impact of these factors on the germination rate of the seeds. The experiment was conducted for a period of seven days, in accordance with the protocol outlined by Ungureanu et al. 2004.

3. Results and Discussions

The thermodynamic interpretation of Cu(II) retention from aqueous media on lignin extracted from Sarkanda grass was evaluated using several thermodynamic parameters, namely: free energy of variation (ΔG), enthalpy (ΔH) and entropy (ΔS), (Table 1).

At both pH values, the change in Gibbs free energy (ΔG) exhibits negative values, thereby suggesting a spontaneous retention of Cu(II) from aqueous solutions on lignin. For the ionic adsorption of Cu(II) on lignin at both pH values of the initial solution, the experimentally determined ΔG values range from -26.17 kJ/mol to -37.99 kJ/mol. These values suggest that the adsorption mechanism is primarily driven by electrostatic interactions, as a Gibbs free energy value greater than -30 kJ/mol indicates charge transfer between the metal ion and the adsorbent surface (Table 1).

Certainly in the case of lignin, the functional groups have the greatest availability to interact with Cu(II) ions in aqueous media, an observation that is in total agreement with the experimental results presented (Table 1).

Table 1

Thermodynamic parameters calculated for the adsorption of ions of Cu (II) from aqueous media on lignin isolated from Sarkanda grass

pH	Time (minutes)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol K)
2.11	30	- 26.17	13.07	97.76
	60	- 28.04	11.98	88.06
	90	- 29.13	12.92	92.89
5.02	30	- 31.31	15.02	110.28
	60	- 36.18	13.95	131.47
	90	- 37.99	14.93	123.86

The values of the enthalpy change (ΔH) indicate that the process of Cu(II) adsorption on Sarkanda grass lignin is endothermic under both pH values of the initial solution (Table 1). The endothermic nature of Cu(II) sorption on lignin is also demonstrated by the positive values of (ΔH), the functional groups of the biopolymer showing willingness to interact with Cu(II) in aqueous systems, an observation consistent with the experimental results presented (Table 1). In the case of Cu(II) sorption on lignin, the ΔH values varied between 11.98 and 15.02 kJ/mol, which would indicate a physical character for the adsorption, according to the literature that assigns a range of values between 2 and 20 kJ/mol (Menezes *et al.*, 2020). The findings presented herein indicate that biosorption cannot be regarded as a physical phenomenon. The probability of ion-exchange type electrostatic interactions between metal ions and functional groups of adsorbents appears to be significant, thereby explaining the values obtained for both (ΔH) and (ΔG) in the present study.

The existence of predominantly electrostatic interactions in Cu(II) sorption is also supported by and positive values of (ΔS), which show the affinity of Cu(II) ions for the functional groups in the lignin structure (Table 1).

Figure 1 illustrates the morphology of Sarkanda grass lignin before and after Cu(II) adsorption at a concentration of 63.5 mg/L and a contact time between phases of 60 minutes, as obtained by scanning electron microscopy (SEM). The micrograph SEM of the untreated Sarkanda grass lignin displays a concentration of dirtied micrometer particles that are well separated in relation to the surface morphology of the lignin treatetd with Cu(II). This observation confirms the contact between the two phases, followed by the migration and retention of the metal ion in the lignin pores.

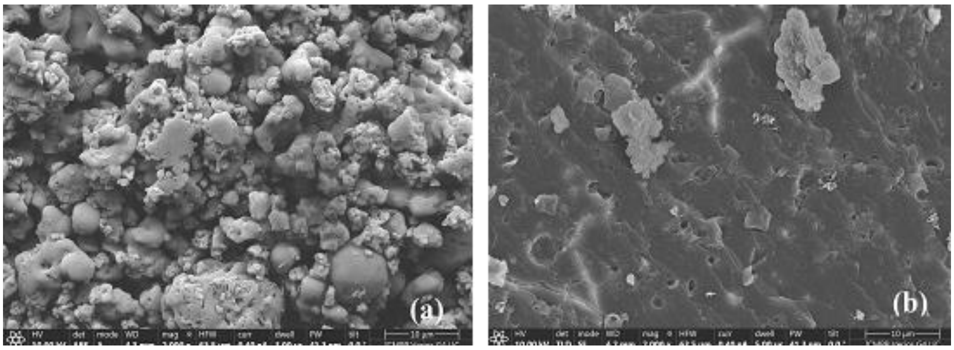


Fig. 1 − The SEM images for Sarkanda grass lignin before adsorption (a) and after Cu(II) retention ions (b), contact time of 60 minutes.

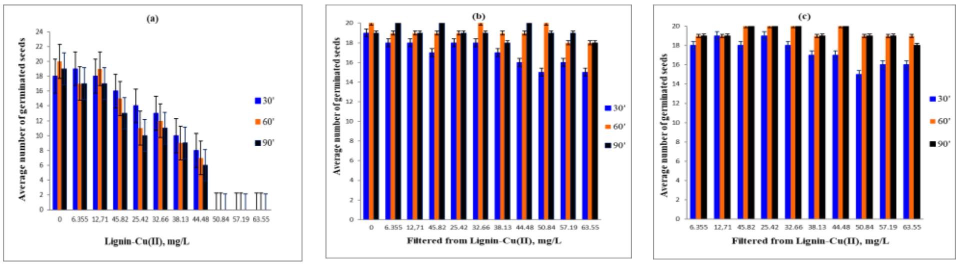
The copper ions are essential for plants when they are present in smaller amounts, while in excessive amounts they exert harmful effects because they intervene in morphological, physiological and biochemical processes, but the optimal dose for good plant growth and development can be appreciated by the permanent monitoring of the biogeochemical behavior of copper in the soil-plant system (Kumar *et al*., 2022). The literature unanimously summarizes the adverse effects of excess copper on germination, growth, photosynthesis and antioxidant response in agricultural crops, and its inhibitory effect on mineral nutrition, chlorophyll biosynthesis and antioxidant enzyme activity has been demonstrated (Mir *et al*., 2021).

In consideration of this aspect, the biological stability of the tomato seeds (*Lypercosium esculentum*) in contact with samples contaminated with Cu(II) was monitored over a period of seven days.

Figure 2 (a, b, and c) presents the average number of wheat seeds germinated after 3 days for samples treated with Cu(II), as well as the average number of germinated seeds after 3 and 7 days for the filtrates resulting from Cu(II) adsorption at the three contact times. As shown in Fig. 2 (a, b, and c), the number of *Lypercosium esculentum* germinated seeds decreases with the increase in Cu(II) concentration and the contact time between the phases. At a concentration of 50.84 mg/L, no germination is observed.

Out of the 20 seeds used, 18 germinated in lignin, and 19 germinated in distilled water. For the filtrates, the number of germinated seeds after 3 days, as well as the number of seedlings after 7 days, were similar to those observed in the control group at contact times of 60 and 90 minutes. However, at the 30-minute contact time, the number of germinated seeds was lower, indicating an instability in the adsorption equilibrium. This is consistent with the thermodynamic results, which suggest that the optimal contact time is 60 minutes. Seven days after germination, no seeds germinated in any of the treated lignin samples, and the existing seedlings died at all concentrations of Cu(II) and contact times, confirming the fixation of Cu(II) ions in the capillaries of Sarkanda grass lignin.

The clear conclusion of this study, supported by the observations derived from the surface analysis, thermodynamic data and biological stability tests, is that Sarkanda grass lignin can serve as a biosorbent of Cu(II) from aqueous systems, thanks to its porous structure and rich in functional groups available to participate in ion exchange with the polluting and form stable lignocomplexes, perfectly fitting the concept of "zero waste".



a) b) c)

Fig. 2 − The average number of tomato (*Lypercosium esculentum*) seeds germinated at 3 days for the treated samples (a) and for the filtrates resulting from Cu(II) adsorption at 3 days (b) and 7 days (c).

4. Conclusions

In this study, lignin isolated from Sarkanda grass (*Tripidium bengalense*) was used as a biosorbent to retain Cu(II) ions from aqueous solutions.

Thermodynamic data and SEM analysis indicate that the biosorption process is spontaneous and feasible, the retention of Cu(II) ions occurring through electrostatic interactions at the lignin surface, the abundance of superficial functional groups and the surface morphology of the biosorbent playing a crucial role in the progress of biosorption.

Biological tests have indicated that Cu(II) is retained by Sarkanda grass lignin, as demonstrated by its toxic impact on tomato seed germination, which is associated with its inhibition of antioxidant enzyme activity.

Therefore, this study adds pertinent information to the segment dedicated to the expansion of biomaterials for environmental remediation, offering new insights into the biosorption mechanisms of some polluting species, such as heavy metal ions, with the recommendation that in the near future residual biomass could be explored for large-scale use.

Future research will aim to further optimize the biosorption process through the investigation of other environmental factors, including pH, temperature, concentration, ionic strength and so on. Additionally, studies will explore the regeneration and reusability of Sarkanda grass lignin for multiple cycles of Cu(II) removal. Efforts will also be directed towards understanding the interactions between Cu(II) ions and other potential pollutants, as well as evaluating the performance of this biosorbent in real-world wastewater treatment scenarios. Ultimately, research will focus on scaling up the process for industrial applications and assessing the economic feasibility of using Sarkanda grass lignin in large-scale environmental remediation projects.

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EXPLORAREA REZIDUURILOR BIOMASICE PE BAZĂ DE LIGNINĂ PENTRU RETENȚIA Cu(II) DIN MEDII APOASE ÎN CONTEXTUL BIOECONOMIEI CIRCULARE

(Rezumat)

În contextul dezvoltării durabile, prezentul studiu propune o abordare inovativă pentru a minimiza efectele dăunătoare ale surselor de poluanți, cum ar fi ionii de metale grele, și în același timp utilizarea eficientă a bioresurselor. Studiul propune reținerea ionilor de Cu(II) în condiții statice utilizând lignina din iarba Sarkanda, un reziduu de biomasă, care reprezintă o resursă naturală regenerabilă și un biomaterial versatil. Acest compus complex demonstrează eficiență termodinamică și biologică. Datele experimentale obținute sugerează aplicații potențiale în tratarea apelor uzate, oferind o alternativă sustenabilă față de tehnologiile deja existente, dar mai costisitoare. Această cercetare susține extinderea utilizării biomaterialelor pentru remedierea mediului, subliniind importanța minimizării deșeurilor și conservării resurselor naturale în sprijinul unei economii circulare bioeconomice. Cercetările viitoare se vor concentra pe optimizarea procesului de reținere, evaluarea stabilității pe termen lung a materialului și explorarea scalabilității acestei tehnologii pentru tratarea apelor uzate industriale.