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LIGNIN – A PROMISING STRATEGIC RESOURCE FOR AQUEOUS MEDIA CONTAMINATED WITH As(III)

BY

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Abstract. The current study proposes to evaluate the adsorption capacity of Sarkanda grass lignin regarding the retention of As(III) from aqueous media from a thermodynamic, spectral and biological point of view, considering the need for rational use of resources and the danger of the As(III) ion derived from its potentially toxic function. The state of chemical equilibrium was assessed by determining the enthalpy, entropy and Gibbs free energy of adsorption, and the diffusion and retention of the polluting species in the lignin pores were highlighted using scanning electron microscopy (SEM). The biostability was analyzed by means of germination tests applied to barley seeds (*Hordeum vulgare L.*, Amethyst variety) incorporated in the lignin infested with As(III) and in the filtrates collected, after phase separation. The experimental results obtained recommend Sarkanda grass lignin for potential applications in the sorption of polluting species from aqueous environments, in particular As(III), offering a sustainable solution also in terms of the valorization of secondary agricultural products, in the context of the circular bioeconomy.

Keywords: sorption, toxic, lignin, As(III), *Hordeum vulgare L.*

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1. Introduction

Due to its toxicity in both organic and especially inorganic form, arsenic is considered a dangerous metalloid that affects natural resources and public health, so that its contaminating action has become a global preoccupation (Bhat *et al.*, 2024). The exposure to arsenic occurs easily through drinking water which, in addition to its main purpose, is used for irrigation or food processing, which is a real threat to living systems (Mandal and Suzuki, 2002; Sevak and Pushkar, 2024). Moreover, the complex interaction of volcanic rocks and geological formations combined with the variation in the characteristics of certain geographical regions, anthropogenic activities (mining, excessive use of arsenic-based fertilizers) and natural biochemical reactions, create environments conducive to high metalloid concentrations, leading to the infestation of groundwater mainly and generating a wide range of serious health complications (skin, kidney, liver, bladder and lung cancers), which prioritizes the issue of its contamination (Kumar *et al.*, 2021; Ungureanu *et al.*, 2022; Bhat *et al.*, 2024; Sevak and Pushkar, 2024).

The forms in which arsenic exists have different mobility, which explains its toxicity, and its most stable forms: As(III) (arsenite) and As(V) (arsenate) are the most toxic. If As(V) is frequently detected in an environment with moderate to high redox potential, As(III) is observed in a predominantly reducing environment, being very soluble compared to As(V) which makes it extremely bioavailable and extremely toxic (Singh *et al.*, 2023).

To attenuate the concentration of As(III) and reduce potential sources of pollution, mainly with As(III), researchers are increasingly proposing biosorption, a simple to implement technique, with high efficiency and cost-effective yield, based on the property of some biomaterials, such as secondary agricultural products (agricultural waste) to sequester polluted species, such as heavy metals and toxic metalloids, such as trivalent arsenic As(III) (Singh *et al.*, 2023; Ungureanu *et al.*, 2025).

One such biomaterial is lignin, strategic resource rich in carbon, cheap, renewable, biodegradable and underexploited, but which can be an ecological and sustainable alternative to polymers and chemicals derived from fossil fuels (Tofănică *et al.*, 2024; Dong *et al.*, 2025; Georgs *et al.*, 2025).

The heterogeneous surface and chemical structure of lignin, with a high density of hydrophilic functional groups, especially carboxyl, hydroxyl and carbonyl, promote a variety of interfacial interactions, including ion exchange and complexation interactions. These are essential for the adsorption various toxic metallic and metalloids species (Supanchaiyamat *et al.*, 2019). Also, a major advantage of lignin is its versatility, which allows it to undergo chemical transformations, such as: grafting functional groups or forming composites with other materials, to significantly increase its adsorption capacity and selectivity for specific metal ions (Bergamasco *et al.*, 2024).

The current research tests the efficiency of lignin from Sarkanda grass extracted industrially from *Triplidium bengalense* on the static adsorption of As(III) from aqueous media, using morphological, thermodynamic and biological analyses, which are instructive for elucidating the optimal adsorption equilibrium and reproducibly complete the conclusions of a previous study that demonstrated, from a kinetic point of view, the good adsorption capacity of the bioresource, opening new directions for specific and optimized solutions in environmental remediation in accordance with the principles of the circular economy (Ungureanu *et al.*, 2022; Tofănică *et al.*, 2024).

Previous investigations, including our earlier kinetic assessment (Ungureanu *et al.*, 2022), have confirmed the rapid uptake of As(III) by Sarkanda grass lignin. However, several critical questions remain unresolved, particularly regarding the thermodynamic stability of the adsorption process and the biological safety of the resulting filtrates. A high adsorption capacity does not necessarily ensure either a stable chemical equilibrium or a non-toxic effluent. In this context, the present study addresses these gaps by providing a comprehensive evaluation of thermodynamic parameters and morphological changes, complemented by ecotoxicity assessment. This integrated approach extends beyond simple removal efficiency, supporting the feasibility of lignin-based remediation within a circular economy framework.

2. Material and Method

The following materials have been used:

– Sarkanda Grass lignin, supplied by Granit Recherche Development S.A. Lausanne, Switzerland and NaAsO₂ supplied by ChimReactiv S.R.L., Bucharest (Ungureanu *et al.*, 2022).

– *Hordeum vulgare* L. seeds (Ametist variety) offered by “Vasile Goldis” West University of Arad, Arad, Romania (Ungureanu *et al.*, 2021).

– Stock solutions - The preparation (0.001 mg/L) consisted of the dissolution of NaAsO₂ in distilled water. The working solutions were obtained by diluting an accurately measured volume of the stock solution with distilled water, and the arsenic ion concentrations in the aqueous medium (mg/L) are as follows: 7.492, 14.984, 22.476, 29.968, 34.46, 44.952, 52.444, 59.936, 67.428, 74.92. Subsequently, 20 mL of solution (in the mentioned concentrations) were added over 5 g of lignin, followed by a rest period of 30, 60 and 120 minutes, under laboratory conditions (20 ± 0.5°C).

Work procedure: The As(III) concentration was determined by hydride generation inductively coupled plasma optical emission spectrometry (HG-ICP-OES), applying the method with 0.6% NaBH₄ in 0.5% NaOH at an absorption maximum at 189.042 nm, Avio 220 Max ICP-OES equipment (Ungureanu *et al.*, 2022). The quantity of As(III) following filtration and phase separation was obtained for a volume of 2 mL, and the concentration value for each sample was

calculated based on the regression equation of the calibration curve. All adsorption experiments, including the interphase contact times (30, 60, 120 min), were performed in triplicate to ensure reproducibility.

Scanning electron microscopy (SEM) was performed using a Quanta 200 scanning electron microscope (5 kV) (Brno, Czech Republic).

By applying van't Hoff's laws, it was possible to calculate the variation of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), critical indices for evaluating the thermodynamics of adsorption in order to establish chemical equilibrium. (Singh *et al.*, 2024).

Toxicity: The germination tests were carried out over a period of seven days according to the procedures described by Ungureanu et al. (Ungureanu *et al.*, 2025) and included as biological material, seeds of *Hordeum vulgare* L. and, as culture media, lignin infested with aqueous solutions of As(III), in the concentration range used (7.492-74.92 mg/L), at the three experimental interphase times, respectively the filtrates resulting from the separation of the phases involved. For contaminated lignin, unmodified lignin was used as a control, and for the filtrates, the control was represented by distilled water. All experiments, were performed in triplicate to ensure reproducibility.

3. Results and Discussions

The thermodynamics is essential for understanding the practicality of adsorption in wastewater treatment and for knowing the reusability of the adsorbent in industrial applications (Girish, 2025). Therefore, the importance of chemical affinity, surface coordination, and thermodynamic parameters—such as free energy, enthalpy and entropy, which directly determine the feasibility of the process, can be deduced from thermodynamic analysis (Elkady *et al.*, 2011; Ungureanu *et al.*, 2025).

For example, the value variations for free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), which represent the driving forces, are very important for evaluating the spontaneity of a process, providing clear information about the thermal stability of pollutant adsorption on the adsorbent in the wastewater treatment system and to the same extent the efficiency of a treatment system, pollutants, being able to attract pollutants (Lemma *et al.*, 2023; Yaqub *et al.*, 2023).

The thermodynamic parameters: ΔG , ΔH and ΔS obtained in the case of As(III) adsorption from aqueous media on Sarkanda grass lignin are presented in Table 1.

Table 1

The thermodynamic parameters (Gibbs free energy, Enthalpy, and Entropy) calculated for the adsorption of As(III) from aqueous media onto Sarkanda grass lignin at different pH values and contact times (Temperature = 293 K, n=3)

| pH | Time (minutes) | ΔG (kJ/mol) | ΔH (kJ/mol) | ΔS (J/mol K) |
|------|----------------|---------------------|---------------------|----------------------|
| 2.18 | 30 | -29.82 | 13.64 | 100.24 |
| | 60 | -30.46 | 12.71 | 92.86 |
| | 120 | -32.05 | 13.09 | 98.43 |
| 6.0 | 30 | -33.24 | 16.92 | 121.97 |
| | 60 | -38.13 | 15.56 | 142.16 |
| | 120 | -42.01 | 16.21 | 136.27 |

As can be seen (Table 1), the variation of the Gibbs free energy (ΔG) has negative values, from (-29.82 to -42.01), at both pH values, which indicates the probability of spontaneous adsorption of As(III) from aqueous solutions onto lignin, but also a possible charge transfer between the metalloid and the adsorbent surface. Given that the Gibbs free energy is smaller than -30 kJ/mol, this suggests a chemisorption mechanism dominated by surface complexation rather than simple physical or electrostatic adsorption (Lemma *et al.*, 2023; Ungureanu *et al.*, 2025). Although As(III) exists predominantly as the neutral species H_3AsO_3 at the experimental pH values, the significantly negative ΔG values (-29.82 to -42.01 kJ/mol) indicate a spontaneous adsorption process. The magnitude of these values suggests that the mechanism transcends simple physisorption, likely involving surface complexation and chemical coordination between the arsenic species and the oxygen-containing functional groups (particularly phenolic hydroxyls) of the lignin matrix (Jekel and Amy, 2006).

The positive values of the enthalpy factor variation (ΔH), ranging from (12.71-16.92) suggest an endothermic character of As(III) adsorption on lignin from Sarkanda grass, indifferent of the pH of the initial solution (Table 1). In the case of the entropic factor (ΔS) variation, positive results are also recorded in the range (92.86-142.16), as observed in (Table 1), which indicates an increase in the randomness at the solid-liquid interface during the adsorption process. This is primarily attributed to the displacement of water molecules from the hydration shells of the As(III) ions and the lignin surface functional groups. When the arsenic species are adsorbed, the water molecules previously 'organized' around the ion are released into the bulk solution. The gain in translational entropy of these released water molecules outweighs the loss of entropy of the adsorbed arsenic, providing the necessary driving force to compensate for the endothermic nature ($\Delta H > 0$) of the process (Ciopec *et al.*, 2021). In a previous study, Ungureanu *et al.* (Ungureanu *et al.*, 2022), demonstrated, based on adsorption isotherms and kinetic modeling, that the adsorption of As(III) from aqueous media on Sarkanda grass lignin has an optimum, at an interphase time of 60 minutes and pH 6, which also results from

the analysis of the obtained thermodynamic parameters (Table 1), thus outlining the feasibility of the process.

To confirm the contact and migration of As(III) towards the pores of Sarkanda grass lignin, scanning electron microscopy (SEM) was used, a complementary and non-destructive technique that allows obtaining information about the degree of rugosity of a surface, about particles attached to a surface/contaminants, particle size and the distribution of atomic elements, more precisely details regarding the homogeneity or heterogeneity of a material (Sacco *et al.*, 2025).

Figure 1 (a and b) illustrates the morphology of lignin from Sarkanda grass before and after As(III) adsorption at a concentration of 74.92 mg/L, contact time 60 minutes and show agglomerations of delimited micrometric particles in the case of uncontaminated lignin, compared to the surface morphology of lignin infested with As(III) which confirms the achievement of adsorption between the species involved.

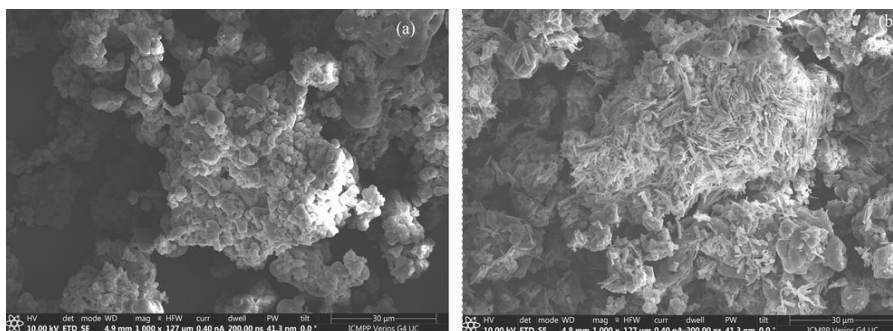


Fig. 1 – The SEM images for Sarkanda grass lignin before adsorption (a) and after As(III) adsorption (b), contact time of 60 minutes.

The morphological changes observed in the SEM images (Fig. 1) provide a direct visual correlation with the thermodynamic data. The transition from the relatively smooth, well-defined micrometric particles of the pristine lignin (Fig. 1a) to the rougher, more irregular surface after contact with As(III) (Fig. 1b) indicates the formation of surface complexes. These structural modifications are consistent with the high negative values of ΔG , suggesting that the sequestration of arsenic is not merely a surface coating but a robust interaction that alters the surface topography of the bioresource.

The arsenic is not essential for plant growth and development, but it can accumulate in plants to toxic levels, especially since it is the 20th most ubiquitous element in the environment, with an estimated concentration in the earth's crust between 1.5-3 mg kg⁻¹, becoming lethal, especially if it is in its two extremely toxic forms: As(III) and As(V) (Farooq *et al.*, 2016; Akoijam and Ram, 2026). Considering these premises, for 7 days the germination degree of

Hordeum vulgare L. (barley) seeds introduced into uncontaminated lignin and contaminated with As(III) solutions in the studied concentration range (7.492–74.92 mg/L), in the filtrates obtained after phase separation and in distilled water was tested, monitoring both the degree of retention of the polluting species in the tested adsorbent, as well as the behavior of the plants towards the well-known toxic action of As(III).

The Fig. 2 (a, b and c) shows the average number of barley seeds, after 3 repetitions germinated at 3 days for uncontaminated lignin and for the samples of lignin contaminated with As(III) and the average number of barley seeds, after 3 repetitions germinated at 3 and 7 days for distilled water and the filtrates resulting from the adsorption of the polluting species at the three contact times. As expected, the increase in the As(III) concentration and the contact time between the phases led to a decrease in the germination capacity of *Hordeum vulgare* L. seeds, so that from the concentration of 52.444 mg/L, germination was zero in all samples (Fig. 2).

The ecotoxicological data presented in Fig. 2 serves as a biological validation of the adsorption efficiency and environmental safety of the process. Control samples showed high viability, with 19–20 seeds germinating in the unmodified lignin and 20 in distilled water, confirming the inherent biocompatibility of the Sarkanda grass lignin. In contrast, all seeds incorporated directly into the As(III)-contaminated lignin matrix (Fig. 2a) failed to survive by the end of the 7-day period, regardless of the initial pollutant concentration. This outcome emphasizes the high toxicity of As(III) on plant systems and reinforces the conclusion that the metalloid is strongly sequestered and concentrated within the porous structure of the adsorbent. Regarding the filtrates (Fig. 2b and 2c), the germination rates and subsequent seedling development at 60 and 120 minutes were comparable to the distilled water control, whereas the 30-minute interval yielded significantly lower survival rates. This indicates that the 30-minute duration is insufficient for the system to reach adsorption equilibrium, a finding consistent with our previous kinetic modeling (Ungureanu *et al.*, 2022) which demonstrated that the pseudo-second-order equilibrium plateau is fully approached only toward the 60-minute mark. Ultimately, these biological results match the kinetic and thermodynamic states discussed earlier, confirming that the adsorption process effectively reduced the bioavailable concentration of As(III) in the aqueous medium to non-lethal levels, thus closing the gap between chemical equilibrium and environmental remediation. From the perspective of the thermodynamic, morphological and biological analyses performed in the current study, lignin from Sarkanda grass, under precisely established experimental conditions, is capable of efficiently attracting As(III) from aqueous environments, thanks to its good complexation capacity (Ungureanu *et al.*, 2024) and becomes a sustainable candidate that can be involved in the development of a non-polluting dynamic technology involves biosorption systems (Çelebi *et al.*, 2024).

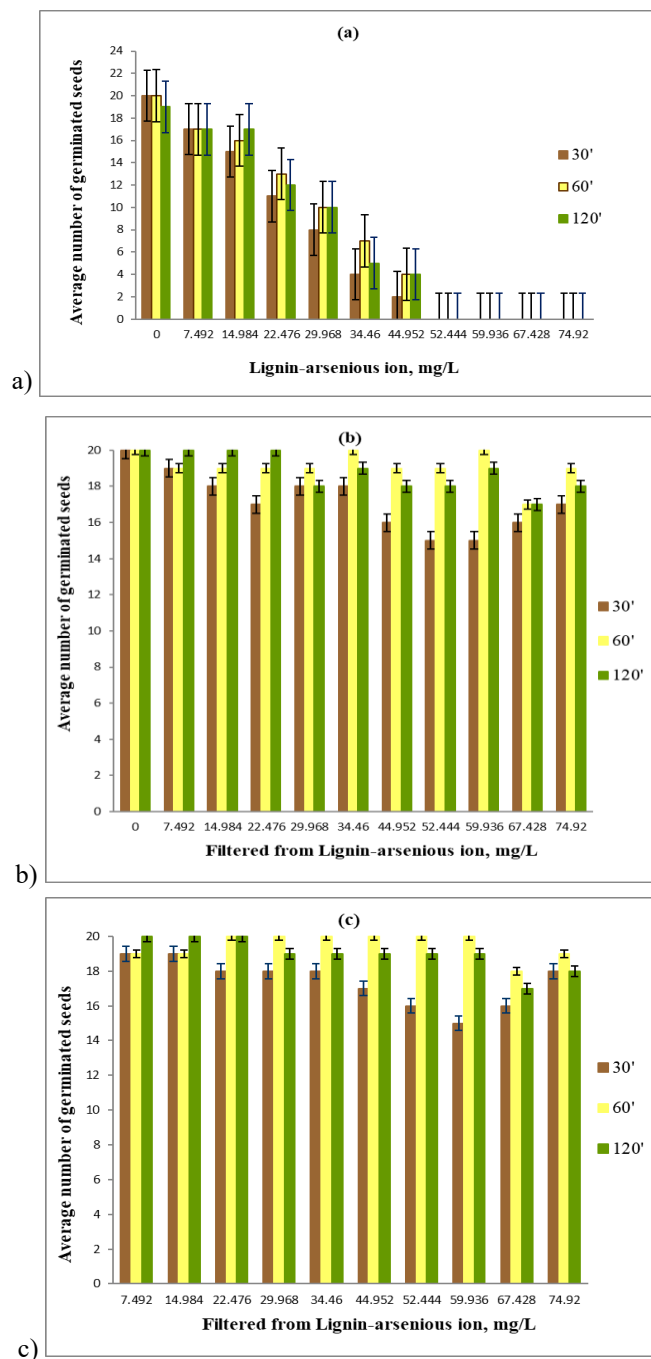


Fig. 2 – The average number of *Hordeum vulgare L.* seeds germinated at 3 days for the contaminated samples (a) and for the filtrates resulting from As(III) adsorption at 3 days (b) and 7 days (c). The results are presented as the mean value \pm standard deviation (SD).

4. Conclusions

Given the great interest in implementing cheap, biocompatible, biodegradable, renewable and versatile adsorbent materials, the present study proposes lignin from Sarkanda grass as a potential biosorbent for As(III) from aqueous environments, which could be speculated in wastewater treatment and would lend itself to scalability, in accordance with the principles of the circular economy.

The calculated thermodynamic parameters: the variation of free energy, enthalpy and entropy, directly responsible for the feasibility of adsorption, indicate a spontaneous and endothermic process. The results suggest that the adsorption of As(III) onto Sarkanda grass lignin is driven by chemical coordination and surface functional group complexation, leading to a stable thermodynamic equilibrium in direct interdependence with the efficiency and implicit practicality of the process.

The scanning electron microscopy captured the diffusion of As(III) in the pores of Sarkanda grass lignin, and toxicity tests performed on *Hordeum vulgare L.* seeds, incorporated into lignin contaminated with the polluting species and in the filtrates obtained following phase isolation, confirmed the toxic impact of As(III) on plant systems and implicitly the fixation of the metal ion in the lignin matrix, a fact that certifies the good adsorption capacity of the bioresource.

This work offers a promising research direction regarding the valorisation of biomaterials as adsorption agents for toxic metalloids from aqueous media, in the context in which industrialization generates increasingly pronounced contaminating actions and may create an opportunity for the design of high-performance lignin-based adsorbents that can provide major economic and ecological benefits in terms of stability, biocompatibility and abundance in the plant kingdom combined with its agricultural waste character, attributes that transform the biomass fraction into a feasible strategic resource. (Zhang *et al.*, 2024).

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LIGNINA – RESURSĂ STRATEGICĂ PROMIȚĂTOARE
PENTRU DEPOLUAREA MEDIILOR APOASE CONTAMINATE CU As(III)

(Rezumat)

Studiul evaluează capacitatea de adsorbție a ligninei din iarba Sarkanda în ceea ce privește reținerea As(III) din medii apoase, din perspectivă termodinamică, morfologică și biologică. Cercetarea este motivată de necesitatea valorificării sustenabile a subproduselor agricole și de toxicitatea ridicată a arsenului trivalent. Starea de echilibru chimic a fost evaluată prin determinarea entalpiei, entropiei și energiei libere Gibbs de adsorbție, iar procesele de difuzie și retenție ale speciilor poluante în porii ligninei au fost evidențiate prin microscopie electronică de baleiaj (SEM). Biostabilitatea a fost analizată prin teste de germinație aplicate semințelor de orz (*Hordeum vulgare* L., soiul Amethyst), încorporate în lignina contaminată cu As(III), precum și în filtratele colectate după separarea fazelor. Rezultatele experimentale obținute recomandă lignina din iarba Sarkanda pentru aplicații potențiale în sorbția speciilor poluante din mediile apoase, în special a As(III), oferind totodată o soluție sustenabilă din perspectiva valorificării produselor secundare agricole, în contextul bioeconomiei circulare.