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Bioremediation of arsenic in groundwater using residues of the fungus *Suillus luteus*

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ABSTRACT

The present study sought to determine the potential for bioremediation of arsenic in groundwater using the fungus S. luteus. Water samples were taken from two wells in the district of Pacora - Lambayeque, and analysed to determine their As, pH and E.C. content; for bioremediation, we worked with the waste of S. luteus, selected, washed and dehydrated at 50°C/24 hours and 60°C/16 hours, then ground, the obtained from each dehydrated, was applied taking into account the factors of 1, 6 and 12 hours of agitation and doses of 0.6, 1.5 and 2.4 g/300 ml, in triplicate and at 75 RPM; the treated water was filtered and its As concentration was evaluated, and the sludge was analysed by HPLC-UV, to determine its amino acid composition. The results showed that the dose that yielded the highest levels of bioremediation was 0.6g/300 ml with values of 90.63 and 83.33% in the well 'Estadio' and 'Pueblo Viejo', respectively, also, it was evidenced that the pH and conductivity influenced this process, on the other hand, it was identified that the S. luteus has a rich and functionally active amino acid profile (18 amino acids), with glutamic acid (2.08 g/100 g), leucine (1.90 g/100 g) and aspartic acid (1.51 g/100 g) standing out. It is concluded that the depletion of S. luteus has great potential as a bioremediation agent for As in groundwater, although the final values obtained exceeded the MPLs for As in water established by the WHO.

Keywords: Percentage bioremediation, Heavy metals, Groundwater, Fungal Substrate.

Biorremediação de arsênico em águas subterrâneas usando resíduos do fungo *Suillus luteus*

RESUMO (PT-BR)

O presente estudo procurou determinar o potencial de biorremediação do arsênico em águas subterrâneas usando o fungo S. luteus. As amostras de água foram coletadas de dois poços no distrito de Pacora - Lambayeque, e analisadas para determinar seu conteúdo de As, pH e E.C.; para a biorremediação, trabalhamos com os resíduos de S. luteus, selecionado, lavado e desidratado a 50°C/24 horas e 60°C/16 horas, depois moído, o obtido de cada desidratado foi aplicado levando-se em conta os fatores de 1, 6 e 12 horas de agitação e doses de 0,6, 1,5 e 2,4 g/300 ml, em triplicata e a 75 RPM; a água tratada foi filtrada e sua concentração de As foi avaliada, e o lodo foi analisado por HPLC-UV, para determinar sua composição de aminoácidos. Os resultados mostraram que a dose que produziu os níveis mais altos de biorremediação foi de 0,6g/300 ml com valores de 90,63 e 83,33% no poço "Estadio" e "Pueblo Viejo", respectivamente, além disso, foi evidenciado que o pH e a condutividade influenciaram esse processo, por outro lado, foi identificado que o S. luteus tem um perfil de aminoácidos rico e funcionalmente ativo (18 aminoácidos), com destaque para o ácido glutâmico (2,08 g/100 g), a leucina (1,90 g/100 g) e o ácido aspártico (1,51 g/100 g). Conclui-se que a depleção de S. luteus tem um grande potencial como agente de biorremediação para As em águas subterrâneas, embora os valores finais obtidos tenham excedido os MPLs para As em água estabelecidos pela OMS.

Palavras-Chaves: Biorremediação percentual, Metais pesados, Águas subterrâneas, Substrato fúngico.

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Biorremediación de arsénico en aguas subterráneas utilizando residuos del hongo *Suillus luteus*

RESUMEN (SPANISH)

El presente estudio buscó determinar el potencial de biorremediación de arsénico en aguas subterráneas utilizando la merma del hongo S. luteus. Se tomaron muestras de agua de dos pozos (pueblo viejo y estadio) del distrito de Pacora – Lambayeque, siendo analizadas para determinar su contenido de As, pH y C.E; para la biorremediación se trabajó con la merma de *S. luteus*, seleccionada, lavada y deshidratada a 50°C/24 horas y 60°C/16 horas, posteriormente molida, lo obtenido de cada deshidratado, fue aplicado teniendo en cuenta los factores de 1, 6 y 12 horas de agitación y dosis de 0,6, 1,5 y 2,4 g/300 ml, por triplicado y a 75 RPM; el agua tratada, fue filtrada y evaluada su concentración de As, y el lodo se analizó por HPLC-UV, para determinar su composición de aminoácidos. Los resultados arrojaron que, la dosis que arrojó los mayores niveles de biorremediación fue 0,6g/300 ml con valores de 90,63 y 83,33% en el pozo "Estadio" y "Pueblo Viejo", respectivamente, asimismo, se evidenció que el pH y la conductividad influyeron en este proceso, por otro lado, se identificó que la merma de S. luteus, posee un perfil aminoacídico rico y funcionalmente activo (18 aminoácidos), destacando el ácido glutámico (2,08 g/100 g), la leucina (1,90 g/100 g) y el ácido aspártico (1,51 g/100 g). Se concluye que, la merma de S. luteus, tiene un gran potencial como agente biorremediador de As en aguas subterráneas, aunque los valores finales obtenidos superaban los LMP de As en agua establecidos por la OMS.

Palabras clave: Porcentaje de biorremediación, Metales pesados, Aguas subterráneas, Sustrato hongo.

1. Introduction

Groundwater holds fundamental importance due to its natural availability as the primary source of drinking water. It represents approximately 99% of the accessible freshwater on the planet, providing a relatively clean and reliable supply that serves nearly one third of the global population (Rahman et al., 2024; Salehi et al., 2024; Song et al., 2024; Younesi Baneh et al., 2024). However, since 1950, this resource has experienced degradation in both quantity and quality, attributed to natural factors such as climate change and anthropogenic activities including mining, population growth, pollutant runoff, and agricultural development (Asmoay, 2024; Dange et al., 2025; Hao et al., 2024; Irfan et al., 2024). Contamination occurs when harmful substances infiltrate the hydrological system (A. N. Chowdhury et al., 2025), migrating from the soil surface into the subsurface layers (Ihenetu et al., 2024).

Heavy metal contamination (arsenic, mercury, cadmium, and lead) in groundwater poses a significant biological risk and a potential threat to human health, even at extremely low concentrations (A. N. Chowdhury et al., 2025; Latif et al., 2025; Shi et al., 2022; Zhang et al., 2023). Among these, arsenic (As) is one of the most extensively studied metals worldwide due to its high toxicity and environmental hazard. Its occurrence in aquifers and groundwater makes it prone to abrupt variations, raising concern among both the population and authorities (T. N. Chowdhury et al., 2024; Dippong et al., 2024; Velarde-Apaza et al., 2025).

In Peru, the Ministry of Health (MINSA) highlights that exposure to heavy metals and metalloids from contaminated water represents a serious public health concern, particularly for children, who account for 73.5% of reported cases, with an exposure rate of 5 cases per 1,000 inhabitants (MINSA, 2023). In 2019, the Roundtable for the Fight Against Poverty (MCLCP) reported the presence of arsenic in groundwater in the district of Pacora. Eleven sampling points monitored over two months revealed elevated concentrations, identifying critical sites such as Pueblo Viejo (0.160 mg/L), Puente Machuca (0.143 mg/L), and Estadio (0.133 mg/L), all significantly exceeding the maximum permissible limit of 0.01 mg/L, posing a potential risk to the district's population (MCLCP, 2019).

As a result, in 2020, the Presidency of the Council of Ministers (PCM) of Peru declared a 60-day state of emergency in the localities of Pacora, Pueblo Viejo, and Puente Machuca, among others, through Supreme Decree No. 143-2020-PCM (PCM, 2020). Subsequently, in 2021, two extensions were issued under Supreme Decree No. 026-2021-PCM (PCM, 2021) and Supreme Decree No. 084-2021-PCM (PCM, 2021b) to implement mitigation measures. However, the emergency report of the National Institute of Civil Defence (INDECI) (No. 997 – 3/7/2021 / COEN) indicated that despite the interventions of EPSEL, the National Rural Sanitation Programme (PNSR), and the "Nuestras Ciudades" Programme (PNC), the actions remained insufficient in the sectors of Pueblo Viejo and Puente Machuca (INDECI, 2021).

Several techniques have been developed to reduce heavy metal contamination in groundwater. Traditional methods are widely applied but often exhibit limited efficiency and high operational costs (Guerrero et al., 2020). In contrast, effective biological alternatives have emerged through the use of bacteria, fungi, algae, and plants. These techniques, including biosorption, bioaccumulation, bioreduction, phytoremediation, and mycoremediation, employ biomass such as agricultural residues and microorganisms, taking advantage of their antioxidant properties. They represent sustainable and affordable solutions to improve water quality in affected ecosystems (Abd Elnabi et al., 2023; Jeyakumar et al., 2023; Kumar & Dwivedi, 2021).

Within the field of bioremediation, fungal residues have emerged as a sustainable and cost-effective alternative for removing pollutants from aquatic ecosystems. Chaudhary *et al.* (2023) demonstrated the efficiency of mushroom residue (*Agaricus bisporus*) as a biosorbent for the basic dye fuchsine, achieving an 82.6% reduction. Similarly, Ahmed y Ebrahim (2020) evaluated pretreated *Agaricus bisporus* residue for the adsorption of methylene blue and Congo red, obtaining adsorption capacities of 239.818 mg/g and 76.412 mg/g, respectively. In another study, Purnomo *et al.* (2010) showed that *Pleurotus ostreatus* residue could degrade the recalcitrant and toxic pesticide DDT in contaminated soils, achieving an 80% reduction. Singh and Vaishnav (2022) also highlighted the potential of spent mushroom waste (SMW), derived from the production of edible fungi such as *Pleurotus* spp. and *Agaricus bisporus*, for the removal of heavy metals from water. They demonstrated its biosorption capacity for arsenic and lead, attributed to the interaction with metal ions, and reported that the residual biomass derived from the stalks and caps of these fungi, rich in functional groups (carboxyl, amino, and phosphate), significantly contributed to environmental remediation processes. Finally, Ghose *et al.* (2024) determined the effectiveness of laccase extracted from *Pleurotus florida* SMW in degrading micropollutants such as ciprofloxacin and norfloxacin in wastewater, achieving degradation efficiencies of up to 86.12% and 83.27%, respectively. This demonstrates the potential of fungal proteins for removing both organic and inorganic contaminants.

Based on this context, the present research aims to determine the bioremediation potential of arsenic in groundwater using residues of the fungus *Suillus luteus*.

2. Material and Methods

2.1 Preparation of the bio-adsorbent material

The *Suillus luteus* residue used in this study originated from industrial mushroom processing. The preparation process began with the manual removal of impurities. The clean residue was washed by immersion in distilled water and left to stand at room temperature for 20 minutes. Subsequently, 200 g of residue were placed in a dehydrator at 60 °C for 16 continuous hours. Once dried, it was stored in an airtight bag with silica gel. In parallel, another 200 g of residue were dehydrated at 50 °C for 24 hours and packaged in the same manner with silica gel. Finally, both dehydrated batches were ground into a fine powder, which was stored until use.

2.2 Groundwater sampling

Groundwater samples were collected from the wells located in the villages of Pueblo Viejo and Estadio "Arturo Rivadeneira García", Pacora district, in the Lambayeque region, which, according to Report No. 01-2019/SC/MCLCP, exhibit high arsenic concentrations. Samples were collected in properly disinfected and sterilised containers, transported to the laboratory, and preserved in a freezer until subsequent analysis.

2.3 Experimental design

Following the modified methodology of Javaid *et al.* (2011), the experimental design shown in Table 1 was defined.

Table 1 – Defined parameters for the treatments under study
Tabela 1 – Parâmetros definidos para os tratamentos em estudo

Biomass treatment	Agitation time	Biomass dose
50°C x 24 h	1 h	0.6 g
		1.5 g
		2.4 g
	6 h	0.6 g
		1.5 g
		2.4 g
	12 h	0.6 g
		1.5 g
		2.4 g
60°C x 16 h	1 h	0.6 g
		1.5 g
		2.4 g
	6 h	0.6 g
		1.5 g
		2.4 g
	12 h	0.6 g
		1.5 g
		2.4 g

Source: Prepared by the authors
Fonte: Elaborado pelos autores

These parameters were applied to both water samples, defining a total of 36 experimental units. The agitation speed was maintained at 75 rpm. After applying the jar test, the mixture of arsenic-contaminated water and *S. luteus* biomass was filtered using filter paper and a funnel. The solid residue retained on the paper was dried to obtain a sample referred to as "sludge". Both the treated water and the sludge were stored at 3 °C for later analysis Javaid *et al.* (2011).

2.4 Arsenic content in water

To determine the arsenic (As) concentration in the water samples from both wells, Method 3120-B of the APHA, AWWA, and WEF standards was employed: Inductively Coupled Plasma (ICP) Method. The samples were prepared following the standard procedure 3030 prior to analysis.

2.5 Amino acid profile of the sludge

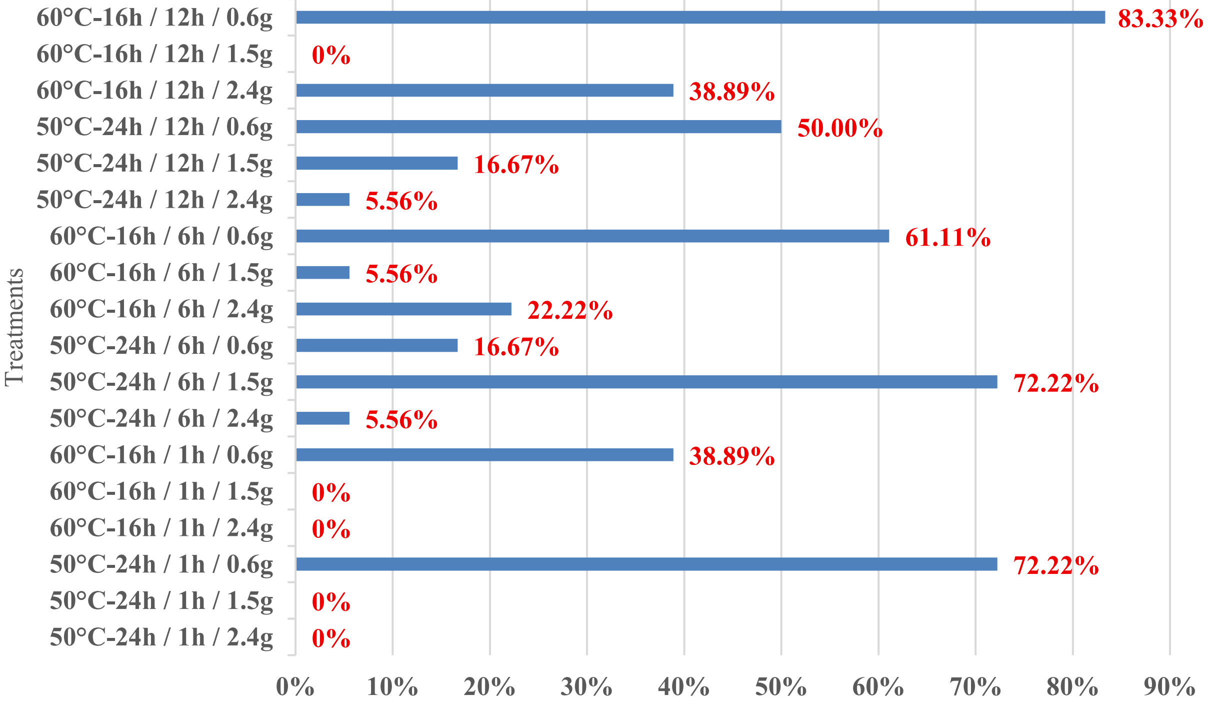
To identify the amino acids present in the *Suillus luteus* sludge, the high-performance liquid chromatography with ultraviolet absorption detection (HPLC-UV) method was applied.

3. Results

3.1 Level of bioremediation of Suillus luteus in the Pueblo Viejo well

In Figure 1, which shows the percentages of bioremediation achieved by *S. luteus* residue in the water from the Pueblo Viejo well, the treatment that yielded the highest value was 60 °C–16 h/12 h/0.6 g, achieving 83.33% biosorption and reducing the As content in water to 0.03 mg/L. In the ANOVA (Table 2), the "Concentration" factor of *S. luteus* was found to be the most influential ($p < 0.000$), showing a significantly greater effect than the others and confirming it as a critical factor in As removal. The "Exposure time" also showed a significant effect ($p < 0.0399$), while its interaction with "Drying" was likewise significant ($p < 0.0405$).

Figure 1 – Percentage of As bioremediation in the water from the Pueblo Viejo well/treatment
Figura 1 - Percentual de biorremediação de As na água do poço Pueblo Viejo/tratamento



As content = 0.18 mg/L, pH = 7.24, and electrical conductivity = 1191 µS

Source: Prepared by the authors
Fonte: Elaboração dos autores

This outcome indicates that the lowest biomass dose provided the highest removal, which points to a more efficient use of accessible binding sites per gram of material. The improvement observed at longer contact times, particularly under low-dose conditions, is consistent with a time-dependent approach to equilibrium, where sorption continues as solute transport reaches less accessible sites. The absence of further gains at higher doses fits a saturation or crowding pattern, where particle aggregation and site masking reduce the fraction of active surface exposed to the water phase. From an operational standpoint, these results support selecting a low dose and extending contact time when the aim is to maximise arsenic removal within the tested conditions.

Table 2 – Analysis of Variance to determine the effect of the analysed factors on the bioremediation capacity of *Suillus luteus* in As-contaminated water

Tabela 2 – Análise de variância para determinar o efeito dos fatores analisados sobre a capacidade de biorremediação de *Suillus luteus* em água contaminada com As

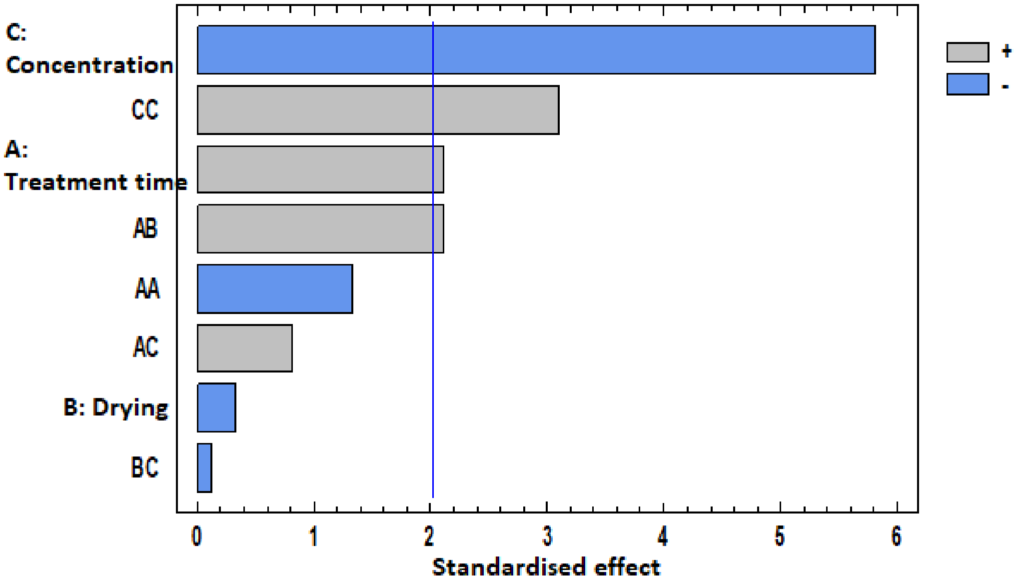
Source	**Sum of Squares**	**df**	**Mean Square**	**F-Ratio**	**p-Value**
A: Exposure time	803.722	1	803.722	4.48	0.0399
B: Drying	19.538	1	19.538	0.11	0.7430
C: Concentration	6074.82	1	6074.82	33.85	0.0000
AB	798.519	1	798.519	4.45	0.0405
AC	119.621	1	119.621	0.67	0.4186
BC	2.89	1	2.89	0.02	0.8996
Total error	8075.76	45	179.461		
Total (corr.)	17958.5	53			

Source: Prepared by the authors
Fonte: Elaboração dos autores

Table 2 reinforces that the concentration term drives the response, with a markedly larger F-ratio than the other effects, while drying alone does not shift the mean response in a consistent direction. The significant A × B term indicates that the effect of exposure time depends on the drying regime, so time optimisation should be interpreted together with the chosen drying condition rather than in isolation. In contrast, the non-significant A × C and B × C terms suggest that, within the studied range, the dominant dose effect remains broadly stable across exposure time and drying levels. The comparatively large residual component also indicates substantial within-treatment variability, which can reflect heterogeneity in the water matrix and small differences in mixing and filtration during jar testing.

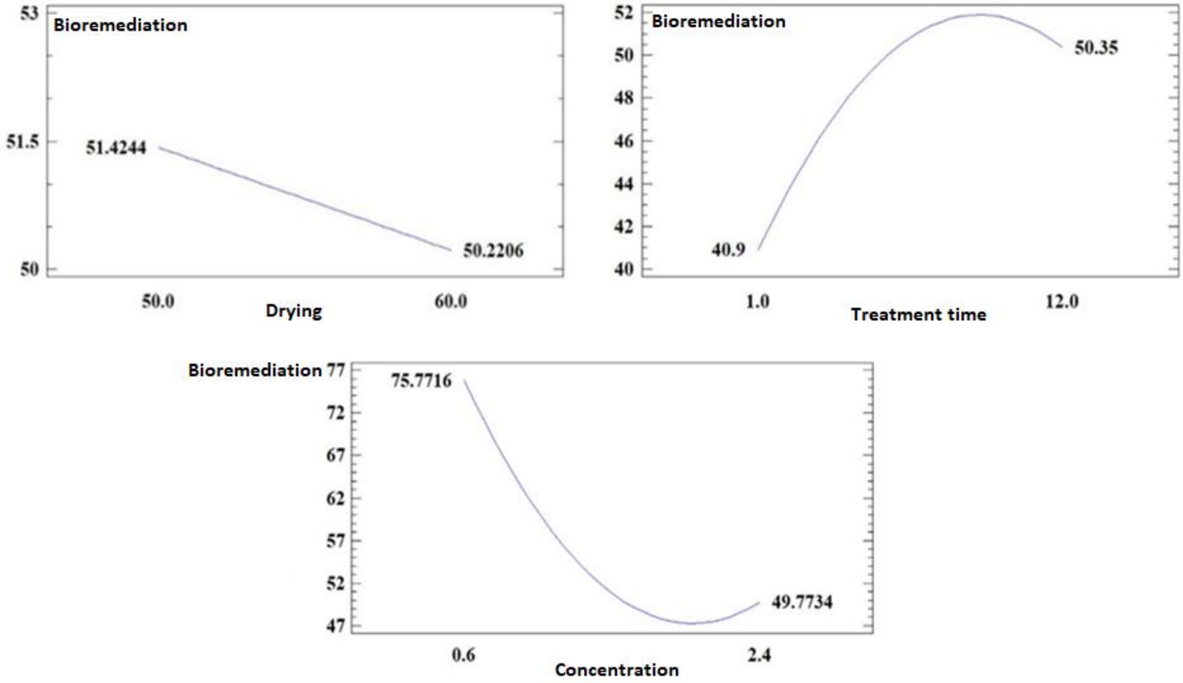
The Pareto diagram (Figure 2) and the main effects plot (Figure 3) support that “Concentration” was the dominant factor, followed by “Treatment time” and the interaction between “Treatment time” and “Drying”. Similarly, in the response surface plot (Figure 4), a longer time and lower concentration increased the efficiency (73–79%), unlike higher concentrations (2.4 g) that reached a plateau more quickly, without significant improvement over time. This suggests that lower fungal substrate concentrations favour a more efficient biosorption process.

Figure 2 - Pareto diagram for the bioremediation factor
Figura 2 – Diagrama de Pareto para o fator de biorremediação



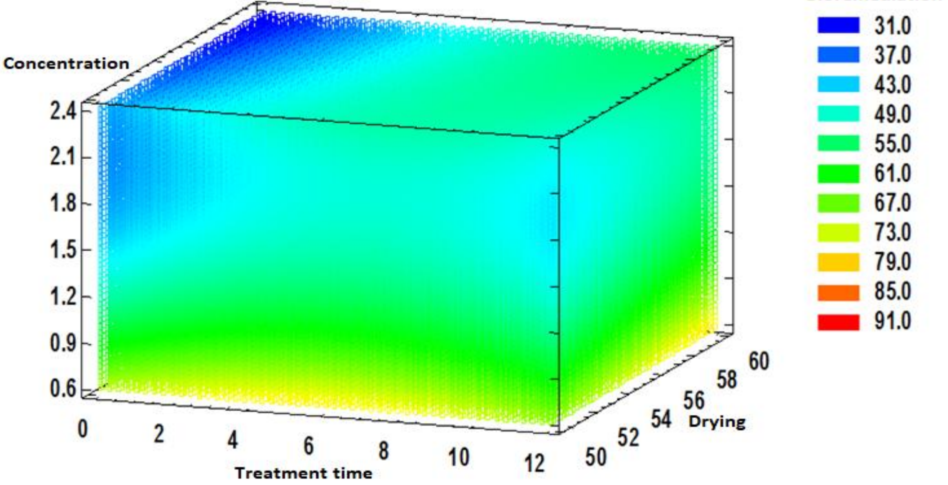
Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 3 - Main effects plot for bioremediation
Figura 3 – Gráfico de efeitos principais para biorremediação



Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 4 - Mesh of the estimated response surface
Figura 4 – Malha da superfície de resposta estimada



Source: Prepared by the authors
Fonte: Elaboração dos autores

In Figure 2, the Pareto chart visually ranks the effects by their standardised magnitude. The “Concentration” bar stands out as the longest, which signals that it drives most of the change in the response. “Treatment time” appears as the next strongest contributor, while the “Treatment time × Drying” interaction shows a smaller but still relevant contribution compared with the remaining terms. The separation in bar lengths indicates a clear dominance of concentration over the other effects within the tested range.

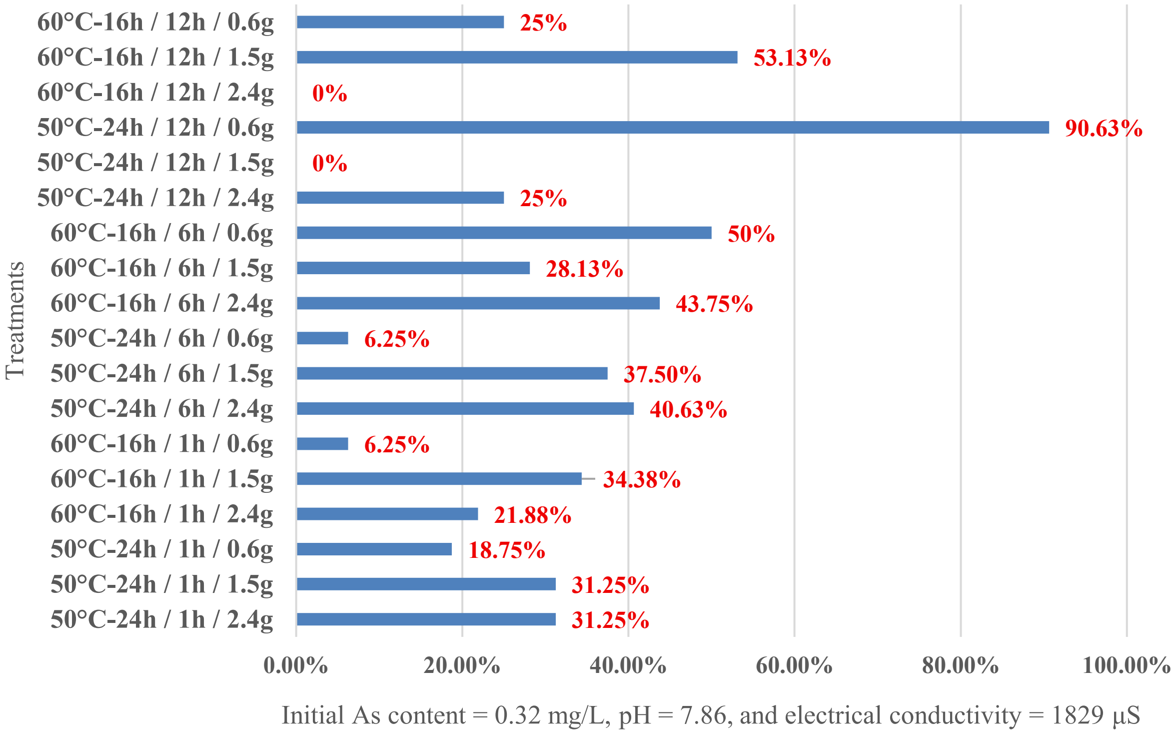
Figure 3 helps you read the direction of these effects. The mean response shifts most strongly across the concentration levels, with a marked change from the lowest to the highest dose, which matches the dominance seen in the Pareto ranking. The time line shows a consistent improvement as treatment time increases, while the drying line changes less across its levels, which suggests a weaker main effect for drying when viewed on its own. The relative steepness of the concentration and time trends supports the idea that these two factors control the response more than drying.

Figure 4 provides a continuous view of the same pattern by mapping efficiency across concentration and time. The surface rises toward longer times at low concentration, forming the high-efficiency region reported (73–79%). At 2.4 g, the surface flattens with time, which is the visual signature of a plateau, where additional time yields minimal gains. The shape of the surface shows that the benefit of increasing time depends on the concentration level, with the strongest time-related gains occurring at the lower doses.

3.2 Level of bioremediation of Suillus luteus in the Estadio well.

In Figure 5, which shows the biosorption percentages of *S. luteus* residue in the water from the Estadio well, the treatment that achieved the highest value was 50 °C–24 h/12 h/0.6 g, with 90.63% biosorption, reducing the As content in water to 0.03 mg/L. In the ANOVA (Table 3), the interaction between “Treatment time” and “Concentration” was found to be the most influential factor in As biosorption ($p < 0.0007$), indicating that the effect of concentration depended on treatment time and vice versa. In contrast, the individual factors (Time, Concentration, and Drying) were not significant.

Figure 5 - Percentage of As bioremediation in the water from the Estadio well/treatment
Figura 5 – Percentual de biorremediação de As na água do Poço Estádio/tratamento



Source: Prepared by the authors
Fonte: Elaboração dos autores

Table 3 – Analysis of Variance to determine the effect of the analysed factors on the bioremediation capacity of *Suillus luteus* in As-contaminated water
Tabela 3 – Análise de Variância para determinar o efeito dos fatores analisados sobre a capacidade de biorremediação de *Suillus luteus* em água contaminada com As

Source	**Sum of Squares**	**df**	**Mean Square**	**F-Ratio**	**p-Value**
A: Treatment time	976.563	1	976.563	2.75	0.1044
B: Drying	58.5313	1	58.5313	0.16	0.6869
C: Concentration	197.684	1	197.684	0.56	0.4598
AB	87.9844	1	87.9844	0.25	0.6213
AC	4746.94	1	4746.94	13.35	0.0007
BC	21.9961	1	21.9961	0.06	0.8047
Total error	16001.8	45	355.595		

Total (corr.)	22437.4	53

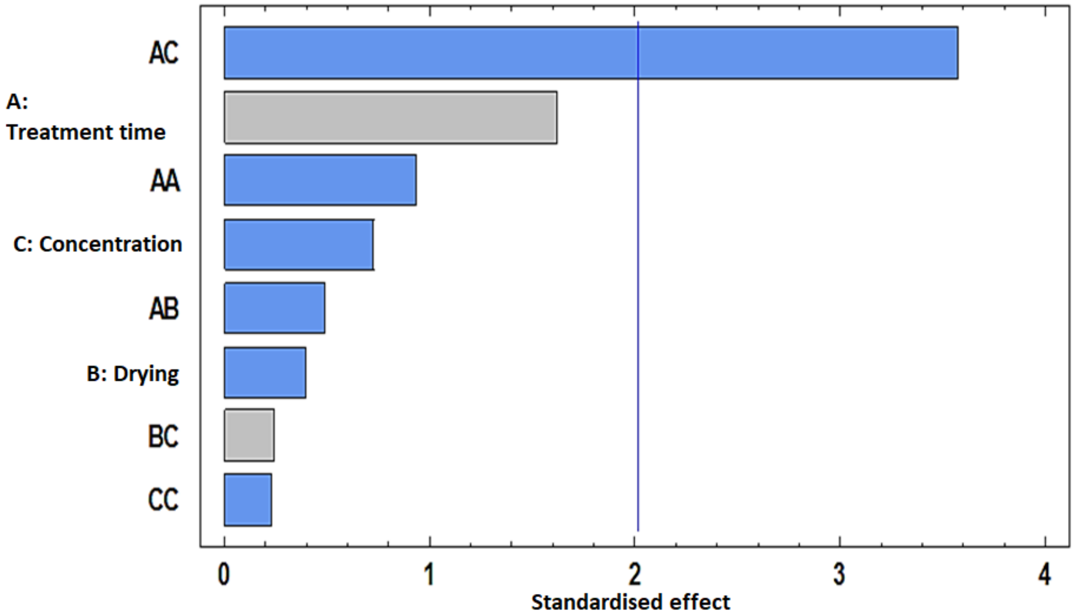
Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 5 highlights a clear peak performance under the combination of drying at 50 °C for 24 h, the longest treatment time, and the lowest dose. The fact that the maximum (90.63%) occurs at 0.6 g indicates that the best outcome does not require a higher biomass load, and the figure makes the contrast between the top treatment and the remaining treatments easy to see. This visual pattern supports that the response changes primarily across specific time and dose settings, rather than improving steadily as you increase biomass.

Table 3 shows that the interaction between Treatment time and Concentration (AC) dominates the model response, with the largest mean square (4746.94), the highest F-ratio (13.35), and a significant p-value (0.0007). In contrast, the main effects for Treatment time, Drying, and Concentration remain non-significant (p = 0.1044, 0.6869, and 0.4598), and the remaining interactions (AB and BC) are also non-significant. This ANOVA structure means that you should interpret time and concentration together, because the effect of one factor depends on the level of the other, rather than expecting a consistent standalone gain from changing only time or only dose.

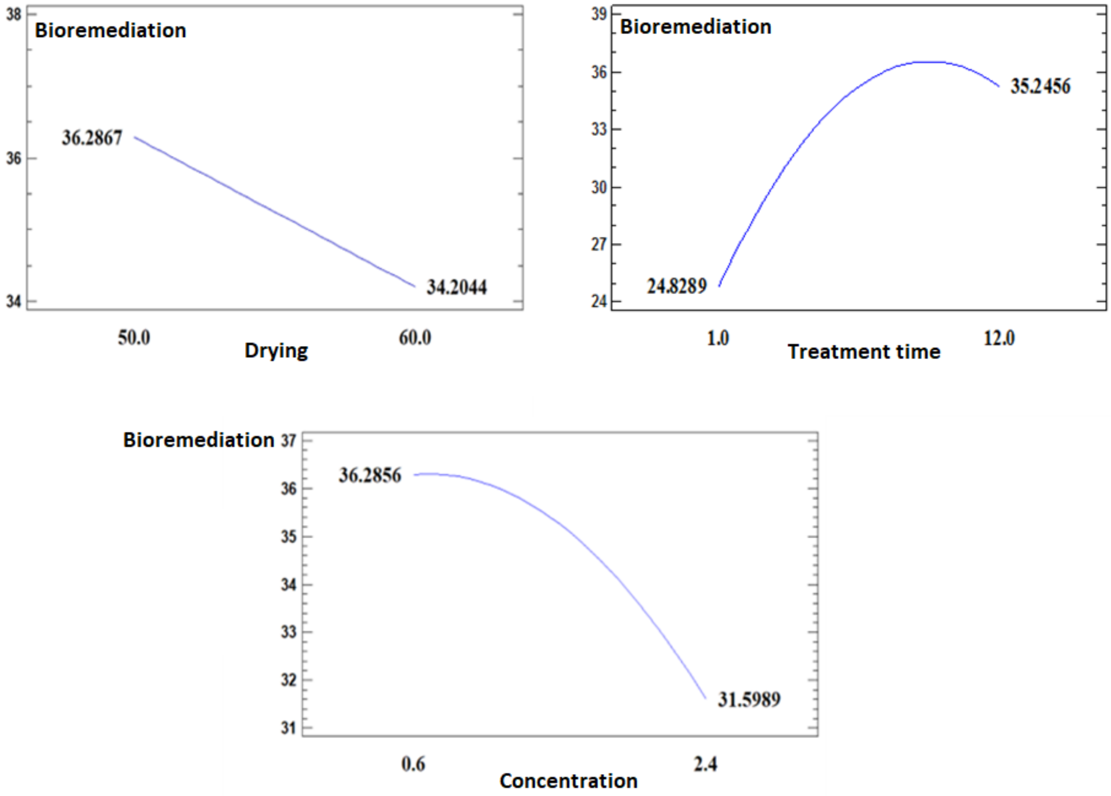
In the Pareto diagram (Figure 6), the dominance of the "Treatment time × Concentration" interaction is evident, followed by both factors individually, suggesting that time and concentration contribute most to the variability in biosorption efficiency. Similarly, in the main effects plot (Figure 7), the individual factors did not show significant effects, although "Treatment time" showed a slight trend towards increased efficiency with longer exposure. In the response surface plot (Figure 8), the highest biosorption efficiency occurred at specific combinations of "Treatment time" and "Concentration", while at high concentrations (2.4 g) there was no improvement, even with longer times. These findings indicate that the optimal intermediate concentration in this context was 1.5 g.

Figure 6 - Pareto diagram for the bioremediation factor
Figura 6 – Diagrama de Pareto para o fator biorremediação



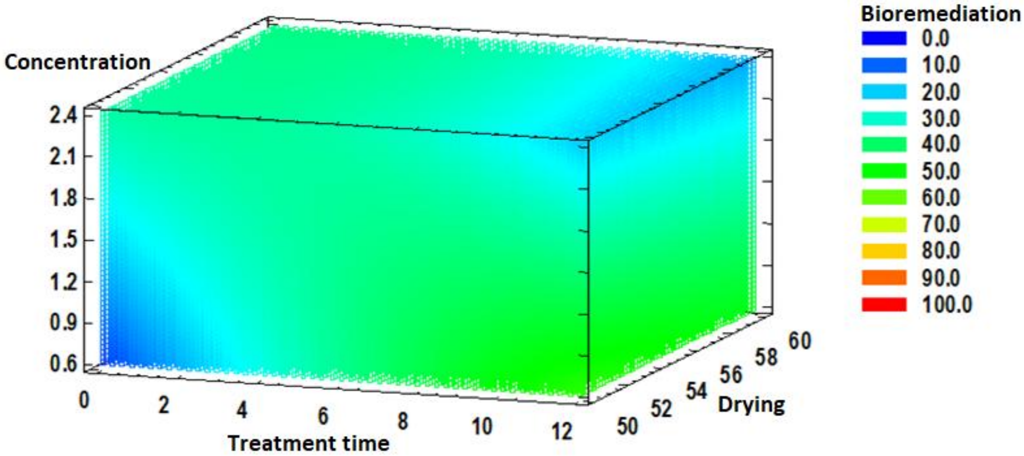
Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 7 - Main effects plot for bioremediation
Figura 7 – Gráfico de efeitos principais de biorremediação



Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 8 - Mesh of the estimated response surface
Figura 8 – Malha da superfície de resposta estimada



Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 6 shows this pattern clearly because the “Treatment time × Concentration” bar stands out as the largest effect on the Pareto chart. The remaining bars sit noticeably lower, which indicates that the interaction explains most of the variation in the response in this dataset. The chart also shows that the single-factor effects for “Treatment time” and “Concentration” remain smaller than their combined effect, so you should read them as secondary compared with the interaction.

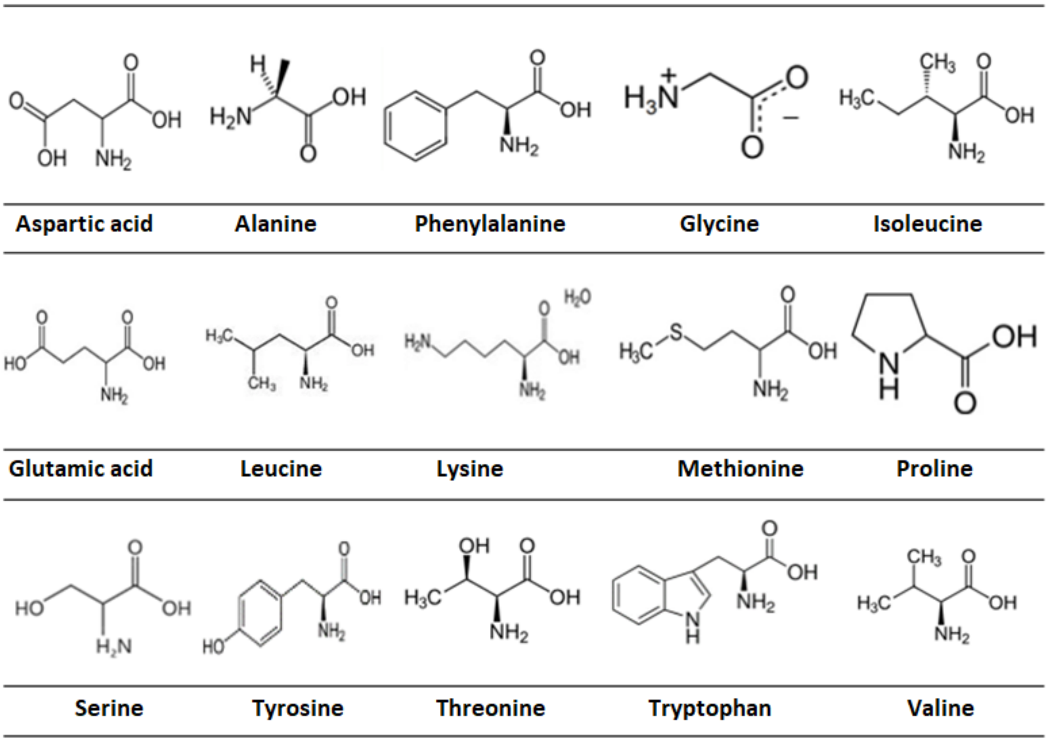
Figure 7 presents near-flat main-effect lines overall, which matches the idea that no single factor shifts the response strongly on its own. You can still see a mild upward change across “Treatment time”, which suggests a small gain as time increases. In contrast, the “Drying” line changes little across its levels, and the “Concentration” line does not show a steady improvement as dose increases, which fits the idea that the response depends on the specific setting rather than on a simple increase in dose.

Figure 8 makes the interaction easy to visualise because the surface changes shape across the time and concentration space. The highest region appears at a specific zone rather than along an edge of the plot, which shows that you reach better efficiency at selected combinations, not by increasing only one factor. The surface also flattens at the high-concentration side, which visually matches the lack of improvement at 2.4 g even when you extend treatment time.

3.3 Characterisation and amino acid content in S. luteus sludge

Figures 9 and 10 show the characterisation and amino acid content in the *S. luteus* sludge, revealing a diverse protein profile with a total of 18 amino acids.

Figure 9 - Structure of the amino acids present in the fungus *Suillus luteus*
Figura 9 – Estrutura dos aminoácidos presentes no fungo *Suillus luteus*

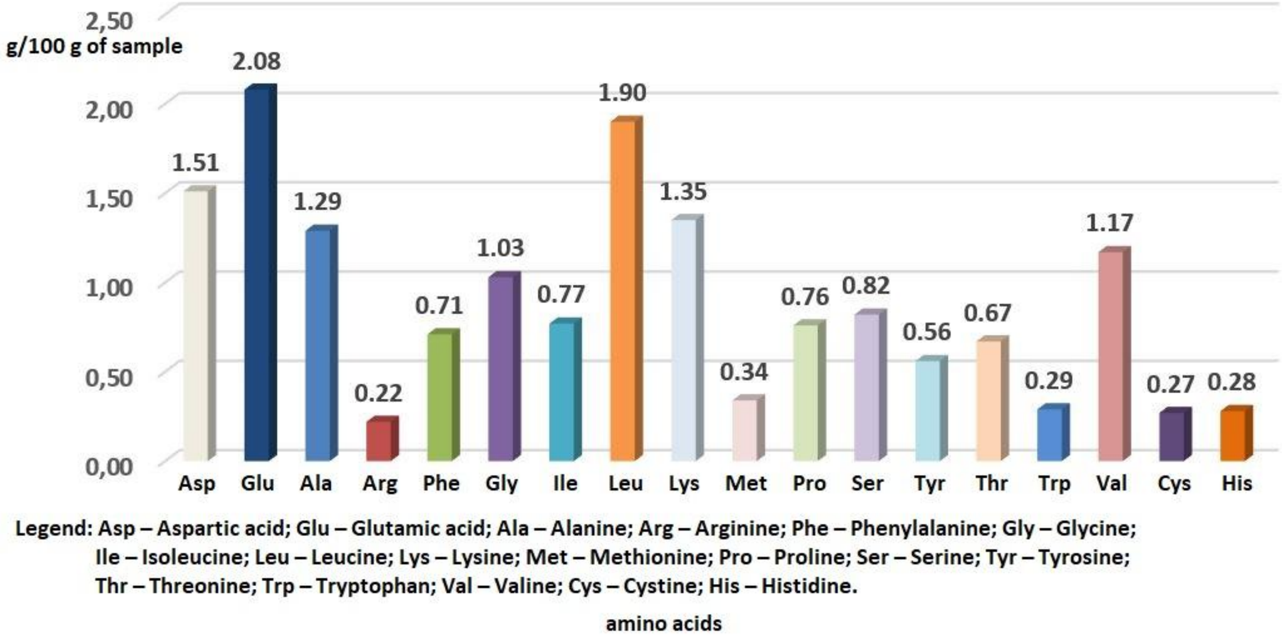


Source: Prepared by the authors
Fonte: Elaboração dos autores

The most predominant were glutamic acid (2.08 g/100 g), leucine (1.90 g/100 g), and aspartic acid (1.51 g/100 g). The presence of glutamic and aspartic acids, due to their carboxyl (-COOH) and amino (-NH_2) groups, allows the formation of stable complexes with metals, reducing their toxicity and mobility. Additionally, leucine (1.90 g/100 g), valine (1.17 g/100 g), and phenylalanine (0.82 g/100 g), hydrophobic amino acids, promote adhesion to lipophilic surfaces (with affinity for fats or lipids), showing low affinity for water and metal ions. T

The presence of basic amino acids such as lysine (1.35 g/100 g), which contains protonated amino groups that enhance ion exchange with metallic anions, was also evident. These results highlight the chemical structural potential of *Suillus luteus* sludge for use as a biosorbent agent for heavy metals.

Figure 10 - Amino acid content in the *Suillus luteus* fungus sample
Figura 10 – Teor de aminoácidos presentes na amostra do fungo *Suillus luteus*



Source: Prepared by the authors
Fonte: Elaboração dos autores

Figure 9 presents the chemical structures for the amino acids detected in the sludge. The figure makes the side-chain differences easy to compare, which is the key point of the panel. Glutamic acid and aspartic acid show side chains with an extra carboxyl group, so their structures carry more oxygen-bearing groups than most others in the set. Lysine shows a long aliphatic chain that ends in an extra amino group, which sets it apart from amino acids that only show one amino group at the alpha position. Leucine and valine show branched aliphatic side chains with no heteroatoms, so the figure groups them visually among the non-polar structures. Phenylalanine shows an aromatic ring in its side chain, which stands out clearly from the aliphatic residues. Taken together, the figure shows that the detected profile includes amino acids with carboxyl-rich side chains, amino-rich side chains, aromatic rings, and purely aliphatic chains. That diversity appears directly in the structures displayed.

Figure 10 reports the amount of each amino acid in the sample in g/100 g and lets you see the distribution at a glance. The chart shows a clear top tier, led by glutamic acid (2.08 g/100 g), leucine (1.90 g/100 g), and aspartic acid (1.51 g/100 g). After these, the bars drop to a second tier, where lysine (1.35 g/100 g) and valine (1.17 g/100 g) sit below the top three but still remain noticeably higher than many of the remaining amino acids. Phenylalanine (0.82 g/100 g) appears as a mid-range component, below the branched-chain residues but still above the lowest bars in the plot. The ranking in Figure 10 also shows that the most abundant amino acids include both acidic residues (glutamic and aspartic acids) and hydrophobic residues (leucine, valine, phenylalanine). This mix appears directly in the bar heights and supports the idea that the sludge contains both polar and non-polar building blocks in meaningful proportions.

4. Discussion

4.1 Concentration and exposure time

The results show that the concentration variable had a statistically significant effect on As biosorption using *S. luteus* residue. In the Pueblo Viejo well, a removal efficiency of 83.33% was achieved, while in the Estadio well, the value reached 90.63%, both obtained with a dosage of 0.6 g/300 ml and 12 hours of exposure. In contrast, when the dosage ranged between 1.5 g and 2.4 g, biosorption efficiency decreased, with some cases registering 0% and a maximum of 72.2%, respectively. This behaviour suggests that lower concentrations favour a greater availability of the biosorbent's functional sites, reducing the risk of saturation and masking of active sites. This agrees with findings by Ahmed and Ebrahim (2020); Chaudhary *et al.* (2023); Sharma *et al.* (2022), who reported that fungal biomass biosorption efficiency tends to be higher when moderate or low dosages are used, since higher concentrations may cause agglomeration or masking of active sites. This phenomenon, known as "overconcentration", reduces effective adsorption capacity, consistent with the observations made in this study.

At the structural level, this efficiency is linked to the presence of functional amino acids in the biomass, such as aspartic acid, glutamic acid, alanine, and leucine, identified in the aminographic analysis. These compounds possess carboxyl (-COOH) and amino ($-NH_2$) groups that, depending on the medium's pH, can promote electrostatic interactions with arsenic anionic species such as $H_2AsO_4^-$. According to Ghose *et al.* (2024); Santos-Domínguez *et al.* (2017), these functional groups are responsible for the high affinity of fungal biomass for heavy metals, highlighting the importance of the biosorbent's chemical profile in its efficiency. Additionally, studies such as that of Oblitas-Jauregui *et al.* (2024) have evaluated the efficiency of *S. luteus* in arsenic removal from acid mine drainage, achieving 30.36% removal using 20 g of biomass and 1 hour of contact, supporting the effectiveness of this fungus in bioremediation processes.

Exposure time is a key parameter in adsorption processes, as it regulates the effective contact between the active sites of the biomass and the contaminants present in the aqueous medium (Ali et al., 2016; Figueroa-Torres et al., 2016; Ma et al., 2021). In a recent study, the biosorption of As(III) using iron-modified *Paecilomyces* sp. biomass showed maximum removal efficiency (64.5%) after 24 hours at pH 6.0 and 30 °C, with no significant improvement at longer exposure times, underscoring the importance of optimising contact duration for effective bioremediation (Ghose et al., 2024). In this study, the p-value for this factor was less than 0.05 ($p = 0.0399$), confirming its statistically significant effect on As removal by *S. luteus* in groundwater from the Pueblo Viejo well. This suggests that contact duration directly influences the progressive saturation of the biosorbent's functional sites, in agreement with Oblitas-Jauregui *et al.* (2024), who observed that the optimal removal efficiency of *S. luteus* occurred after 1 hour of exposure, decreasing when extended to 3 hours, reinforcing the need to optimise this parameter.

In contrast, in the Estadio well, exposure time alone had no significant effect ($p > 0.05$). However, a significant interaction with biosorbent concentration (AC interaction, $p = 0.0007$) was identified, as shown in Table 4 and the Pareto diagram (Figure 6). This interaction indicates that, in this water matrix, exposure time is dependent on the amount of biomass used. The main effects plot (Figure 7) shows that longer exposure times, particularly between 8 and 12 hours, increased bioremediation efficiency, reaching up to 35.25% removal. This increase was most evident when low dosages (0.6 g/300 ml) were applied, reinforcing the synergy between both factors (Figure 8). These findings are consistent with Ahmed and Ebrahim (2020); Sharma *et al.* (2024), who observed that in fungal biosorption systems, adsorption equilibrium is usually reached within the first few hours of treatment, after which the process stabilises or declines due to desorption, saturation, or ionic competition. This behaviour can be explained by an initial rapid adsorption phase, where the active functional groups of the biomass (carboxyl and amino) efficiently interact with As species, followed by a slower phase characterised by the occupation of less accessible sites, gradually reducing the removal rate (Deng et al., 2018; Santos-Domínguez et al., 2017).

Moreover, the adsorption mechanism may depend on As speciation over time, as variations in As(III) and As(V) distribution can occur with increasing exposure, affecting affinity for the fungus's active groups (Arias-Barrantes et al., 2019; Vimala & Das, 2009). Therefore, a well-controlled treatment time not only enhances contaminant removal efficiency but also prevents adverse effects such as partial biomass degradation or desorption of previously adsorbed arsenic (Ghose et al., 2024).

4.2 Agitation time

Agitation time is an important operational factor in biosorption, as it promotes proper dispersion of the biosorbent in the medium and enhances mass transfer between contaminants and the material's active sites. Although this variable did not show a statistically significant individual effect ($p > 0.05$), its inclusion in the experimental design revealed that longer agitation durations, particularly 6 hours, improved process efficiency, especially when combined with low biomass dosages and moderate drying temperatures.

According to Ahmed and Ebrahim (2020); Chaudhary *et al.* (2023); Deng *et al.* (2018), prolonged agitation in bioremediation systems increases effective contact between the biosorbent and dissolved metal or metalloid ions, facilitating saturation of active sites and reducing stagnant zones. However, excessive agitation may have adverse effects such as desorption of previously adsorbed metals or structural degradation of the biosorbent. In this regard, Oblitas-Jauregui *et al.* (2024) reported that intermediate agitation times in *S. luteus* experiments achieved the highest As removal rates without compromising the physical or functional integrity of the biomass.

4.3 Amino acid content

The biomass of *S. luteus* presents an amino acid composition rich in functional groups capable of establishing specific interactions with arsenic species in aqueous media. Among the detected amino acids, those containing carboxyl (-COOH), amino ($-NH_2$), sulfhydryl (-SH), and hydroxyl (-OH) groups were particularly important, mainly found in aspartic acid, glutamic acid, lysine, arginine, cystine, serine, and tyrosine. These structures allow the fungal biomass to participate actively in adsorption processes via mechanisms such as complexation, electrostatic attraction, ion exchange, and the formation of coordinated covalent bonds with As(III) and As(V) species.

Cystine deserves special attention, as its sulfhydryl group forms stable covalent bonds with As(III), greatly increasing biosorbent stability. Meanwhile, the (-OH) groups in serine and tyrosine may contribute through hydrogen bonding, albeit with lower affinity than other groups. The efficiency of *S. luteus* as a biosorbent has been demonstrated in several studies on heavy metal removal, including arsenic, from contaminated solutions (Cárdenas-González et al., 2017; Oblitas-Jauregui et al., 2024). This capacity is largely attributed to amino acids with specific functional properties, whose chemical structures promote active interactions with metal cations (Sajadi, 2010).

The aminographic analysis also revealed a significant abundance of aspartic acid, glutamic acid, alanine, leucine, and lysine - amino acids containing active functional groups (carboxyl and amino) capable of interacting with metallic and metalloid species through mechanisms such as ion exchange, electrostatic attraction, complex formation, and hydrogen bonding (Gao et al., 2008; Sajadi, 2010). Lysine stands out for its free amino group, which exhibits high affinity for negatively charged surfaces, facilitating bonding with metal ions (Absalan & Ghaemi, 2012). Likewise, aspartic and glutamic acids have demonstrated strong coordination capacity with divalent cations such as Cu^{2+}, Zn^{2+}, and Co^{2+} due to the reactive arrangement of their carboxyl groups, reinforcing their key role in adsorption processes (Sajadi, 2010).

This biochemical composition gives the biomass highly efficient biosorption properties. Sharma *et al.* (2022) highlighted that the protein richness of edible mushroom residues produces a strong affinity for heavy metals such as As^{5+}, Pb^{2+}, and Cd^{2+}, due to the diversity of functional groups in their side chains. Similarly Ghose *et al.* (2024) reported that fungal proteins contained in spent mushroom waste (SMW) retain functionality under specific operating conditions, maintaining their capacity for contaminant capture and degradation in aqueous systems.

Numerous authors have emphasised that carboxyl and amino groups are crucial for the adsorption of anionic species such as arsenate ($H_2AsO_4^-$), particularly under neutral or slightly acidic pH conditions where these groups tend to protonate and gain positive charge, favouring electrostatic interactions with anions (Santos-Domínguez et al., 2017). This agrees with the results of the present study, where arsenic removal efficiency reached 90.63% under treatment conditions that preserved the functional integrity of amino acids in the biomass. Differences in removal efficiency between wells could be linked to partial degradation of amino acids, particularly under thermal drying conditions. Ahmed and Ebrahim (2020) reported that excessively high drying temperatures can alter protein tertiary structure, reducing the availability of functional groups for adsorption. This may explain why biomass dried at 60 °C for 16 hours exhibited greater removal capacity than that dried at 50 °C for 24 hours, as its structural and functional configuration was better preserved.

4.4 Electrical conductivity and pH

Although electrical conductivity was not directly evaluated as an experimental variable, its values in the analysed wells allow inferences about its potential influence as a complementary environmental factor. The Pueblo Viejo well showed a conductivity of 1191 µS, while the Estadio well exhibited a higher value of 1829 µS, suggesting a greater concentration of dissolved ions in the aqueous medium (Gören et al., 2020). This difference could have influenced As removal efficiency, as higher ionic strength in solution generates various effects on biosorption mechanisms.

Firstly, ions commonly present in groundwater, both cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}) and anions (phosphate, sulphate, bicarbonate, chloride), may compete directly with As for the adsorption sites, reducing the biosorbent's retention capacity (Gallegos-Garcia et al., 2012; Gören et al., 2020). Moreover, the ionic strength of the medium can alter the biosorbent's surface charge and the configuration of the electrical double layer, affecting electrostatic interactions between the functional groups of *Suillus luteus* (carboxyl, amino, hydroxyl) and arsenic species (Gallegos-Garcia et al., 2012; Miretzky & Cirelli, 2010).

In highly conductive solutions, surface charges may be shielded, decreasing electrostatic attraction towards anions such as $H_2AsO_4^-$. Likewise, metal cations at high concentrations could bind preferentially to the fungus's functional groups, blocking or modifying their availability for interaction with As (Miretzky & Cirelli, 2010). However, certain ions such as Ca^{2+} and Mg^{2+} can also promote alternative processes like coprecipitation or mixed-complex formation, which may in some cases contribute to arsenate removal (Gören et al., 2020). This duality in ionic behaviour shows that the influence of conductivity is not linear but highly dependent on the specific water composition and operating conditions.

The high conductivity recorded in the Estadio well may have created a less favourable environment for effective arsenic bioadsorption due to ionic competition, electrostatic interference, and modulation of the biosorbent's functional groups.

The efficiency of As adsorption is governed by the pH of the medium, which controls both the chemical speciation of the contaminant and the ionisation of functional groups in the biomass (Lu et al., 2022). At pH values between 6.0 and 7.2, carboxyl groups are deprotonated, promoting interaction with As(V) anions such as $H_2AsO_4^-$ and $HAsO_4^{2-}$. At the same time, amino groups from lysine and arginine, in their protonated form, exhibit affinity for As(III) (as H_3AsO_3), facilitating its retention through donor–acceptor interactions. Previous studies Gadd (2009); Wang y Chen (2009) have highlighted the importance of the acid–base surface properties of fungal biopolymers in biosorption processes.

pH is thus a crucial factor that affects As speciation in aqueous solution, directly determining bioremediation performance (G. Sharma et al., 2024). Biomass-based materials rich in functional groups such as carboxyl (-COOH) and amino ($-NH_2$) have demonstrated high adsorption capacity for arsenic through physicochemical interactions (Gadd, 2009). Consequently, pH not only modifies the biosorbent's surface charge but also governs As speciation, emphasising its role in designing more efficient removal strategies (Santos-Domínguez et al., 2017).

The results indicated that the highest As removal efficiency occurred in the Pueblo Viejo well (pH = 7.24), with an 83.33% reduction using 0.6 g/300 ml of *S. luteus* biomass. In contrast, in the Estadio well (pH = 7.86), the removal efficiency was 90.36% with no statistically significant differences ($p > 0.05$). This behaviour suggests that a slightly acidic, near-neutral pH favours the interaction between the biomass and As anionic species by generating a positively charged biosorbent surface, as also reported by (Santos-Domínguez et al., 2017).

The effect of pH on As adsorption can be explained by the protonation or deprotonation of the functional groups in the biomass (Vimala & Das, 2009). At low pH, these groups become protonated, increasing the biosorbent's positive charge and enhancing attraction towards anionic species such as $H_2AsO_3^-$. However, excessive H^+ concentration can compete for active sites, reducing process efficiency (Melgar et al., 2007). At high pH, the biomass acquires a negative charge due to deprotonation, which favours cation adsorption (Cd^{2+}, Pb^{2+}, Cu^{2+}) but hinders anion binding through electrostatic repulsion (Vimala & Das, 2009).

Therefore, the efficiency observed under slightly acidic conditions can be attributed to a favourable electrostatic balance for anion retention, without significant proton competition. A pH range of 6.0–7.2 appears optimal for arsenic bioremediation using *S. luteus* biomass, particularly in groundwater systems similar to those in the Pacora district (Arias-Barrantes et al., 2019; Melgar et al., 2007; Santos-Domínguez et al., 2017; Vimala & Das, 2009). Under alkaline conditions (pH > 7.5), the biomass tends to develop a negative surface charge due to deprotonation, which can lead to electrostatic repulsion with arsenic anions, lowering adsorption efficiency (Mohan & Pittman, 2007). This effect was evident in samples from the Estadio well. Conversely, under slightly acidic conditions, as in the Pueblo Viejo well (pH = 7.24), positive interactions between biosorbent functional groups and arsenic species were enhanced, resulting in higher removal efficiency.

These findings indicate that *S. luteus* biomass possesses suitable structural and functional properties to act as an efficient biosorbent for arsenic-contaminated water. The presence and reactivity of its functional amino acids, modulated by environmental pH, are key determinants of process efficiency, demonstrating the value of fungal residues as a low-cost, widely available biotechnological alternative for treating contaminated effluents.

4.5 Drying time

The drying temperature of the biosorbent is a critical factor that can influence the preservation of active functional groups and the integrity of organic compounds responsible for bioadsorption (Pérez Bou et al., 2018). In this study, although the statistical analysis did not reveal a significant individual effect of this variable ($p > 0.05$) in either well, a significant interaction with exposure time (AB) was observed in the Pueblo Viejo well ($p = 0.0405$). This synergy was evident in treatments that applied biomass dehydrated at 60 °C for 16 hours combined with intermediate exposure times (4–6 hours), achieving bioremediation levels between 55.0% and 79.0%, higher than those obtained with biomass treated at 50 °C for 24 hours. This suggests that a more intense drying process may enhance the availability of active functional groups or prevent premature degradation of key components such as amino acids.

In contrast, in Estadio well, drying showed no statistically significant influence, either individually or in interaction with exposure time. As shown in Figure 6, even under combined conditions of high concentration (2.1–2.4 g/300 ml), prolonged exposure (12 hours), and across the full drying range (50–60 °C), bioremediation remained between 10% and 20%, suggesting that drying did not improve biosorbent performance in this matrix. Several studies have reported the influence of thermal treatment on biomass properties. (Ahmed & Ebrahim, 2020; P. Sharma et al., 2022) noted that controlled drying temperatures can alter the tertiary structure of proteins, modify the availability of carboxyl and amino groups and changing the surface porosity of the biosorbent, which directly affects adsorption capacity. Similarly, (Ghose et al., 2024) demonstrated that functional proteins such as laccase, present in fungal residues, can retain their activity under moderate thermal conditions, promoting interactions between biomass and organic or inorganic contaminants such as arsenic.

4.6 Amino acids and pH / electrical charge

Although the variables "pH" and "amino acid content" were addressed separately, their combined effects may explain part of the differential efficiency observed between samples treated with *S. luteus*. The amino acids identified in the aminogram - aspartic acid, glutamic acid, lysine, leucine, and alanine - contain functional groups such as carboxyl and primary amino moieties whose reactivity depends on the electrochemical environment of the solution, particularly pH and ionic charge. Under slightly acidic or neutral conditions (as in the Pueblo Viejo well, pH 7.24), these functional groups tend to protonate, acquiring positive charges that favour adsorption of anionic species such as arsenate ($H_2AsO_4^-$) through electrostatic interactions (Santos-Domínguez et al., 2017). This behaviour may explain why in that well, removal efficiencies reached up to 72.21%, provided that the amino acids in the biomass remained structurally active.

In contrast, in the Estadio well (pH 7.86), these conditions were less favourable for such interactions, possibly limiting adsorption performance despite the presence of the same bioactive compounds. The medium's electrical charge, influenced by water conductivity, may also modify the availability of active sites. As noted by (Gallegos-Garcia et al., 2012; Miretzky & Cirelli, 2010), high ion concentrations in solution can alter the electrical double layer on the biosorbent surface, producing shielding effects that reduce interactions between amino acid functional groups and arsenic anions. This phenomenon may have occurred in the Estadio well, where conductivity reached 1829 µS - considerably higher than that of the Pueblo Viejo well (1191 µS).

From a practical environmental and territorial planning perspective, this difference in electrical conductivity supports using EC as a rapid screening indicator to prioritise wells and neighbourhoods for intervention. In Pacora, mapping EC together with arsenic concentrations can support a simple risk-zoning layer for groundwater supply planning, indicating where biosorption-based treatments are more likely to face ionic shielding and where you should expect reduced performance without additional controls. Areas that repeatedly show higher EC, such as the Estadio well (1829 µS), can be flagged for stricter monitoring frequency, earlier installation of treatment units, and more conservative design choices. For example, planners can assign these zones to treatment trains that include steps to manage ionic strength or competing ions, and they can use the zoning output to guide the siting of future wells, the protection of recharge areas, and land-use measures that limit salinity and ion inputs that raise conductivity.

5. Conclusions

It was demonstrated that S. luteus residue has high bioremediation potential for As in groundwater, achieving biosorption levels of 83.33% and 90.63% in the Pueblo Viejo and Estadio wells, respectively, using a dosage of 0.6 g/300 ml for 12 hours at 75 rpm, with drying ranges of 50 °C for 24 hours and 60 °C for 16 hours. However, higher biomass concentration reduced the biosorption capacity of S. luteus. Agitation time also proved important, since non-prolonged agitation periods increased efficacy.

The aminogram showed a rich and functionally active amino acid profile in S. luteus biomass, with 18 amino acids identified, notably glutamic acid (2.08 g/100 g), leucine (1.90 g/100 g), and aspartic acid (1.51 g/100 g), whose chemical structures confer bioremediation potential. Regarding As adsorption efficiency, pH and electrical conductivity significantly influenced the process. The highest removal occurred in water from the Estadio well, with pH 7.86 and EC 1829 µS, reaching a 90.63% reduction. In contrast, the Pueblo Viejo well, with pH 7.24 and EC 1191 µS, reached 83.33%. These results confirm that a pH between 7.0 and 8.0 favours interactions between the biomass functional groups, such as carboxyl (-COOH) and amino ($-NH_2$), and arsenic species, positioning S. luteus sludge as an effective biosorbent.

Based on the results, the greatest reduction in As concentration in both wells reached 0.03 mg/L. Even so, the treated water exceeds the WHO maximum permissible limit of 0.01 mg/L. In its current form, treatment with S. luteus shows high bioremediation potential, although it still requires adjustments to align with international quality standards. It is therefore recommended to optimise process conditions, including biomass dose, contact time, and drying temperature, and to consider other factors such as pH, electrical conductivity, and agitation speed to further reduce As levels.

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