Optimization of Bioleaching Process for Arsenic Extraction from Abandoned Mine Soils by Oxalic Acid

Sun Lul Kwon,[a] Yong-Seok Choi,[a,]* Jae-Jin Kim,[b] and Gyu-Hyeok Kim[b]

Soils contaminated with heavy metals, such as arsenic (As), pose significant environmental risks and can cause serious ecological and health-related damage. Biological methods, such as bioleaching (organic acids), biosorption (lignocellulosic materials, microbial biofilms), and bioaccumulation (microbial biofilms), have shown potential for remediating of heavy metal pollution sites. However, research optimizing the bio-based solutions remains limited. This study aimed to optimize As leaching from two abandoned mine soils using oxalic acid (OA). Response surface methodology (RSM) with a second-order central composite design (CCD) was employed to determine the optimal conditions, focusing on OA concentration and soil weight. Additionally, OA production by the brown-rot fungus *Antrodia albida* was monitored, revealing significant synthesis under low pH conditions, with peak production observed on the eighth day of incubation. The bioleaching efficiency of fungal OA was compared with commercial OA, showing comparable As extraction rates exceeding 95% in both soil types. However, the extraction efficiency for other heavy metals varied: fungal OA was more effective for cadmium, nickel, and lead, whereas commercial OA demonstrated higher efficiency for chromium, copper, and zinc. These findings underscore the potential of fungal OA as a sustainable alternative for remediating soils contaminated with heavy metals, particularly in low-pH environments.

DOI: 10.15376/biores.21.1.2186-2201

Keywords: Abandoned mine; Antrodia albida; Arsenic; Bioleaching; Low-pH; Oxalic acid

*Contact information: a: Wood Engineering Division, Forest Products and Industry Department, National Institute of Forest Science, Seoul 02455, Republic of Korea; b: Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea; *Corresponding author: choikfri@korea.kr*

INTRODUCTION

Soils contaminated with heavy metals can induce a significant threat to ecosystems and human health due to the persistence, non-degradability, and toxicity of these elements. Prolonged exposure of heavy metals, even at low concentrations, can lead to neurological, renal, and carcinogenic effects (Jomova *et al.* 2025). Given these risks, the remediation of heavy metal-contaminated soils has become a critical issue in environmental science.

Conventional remediation methods, such as soil washing, excavation, and chemical immobilization, often involve high costs and can disrupt the environment (Wuana and Okieimen 2011; Khalil and Hassan 2024). As an alternative, biological approaches have gained attention for their sustainability and low environmental impact. Lignocellulosic materials and microbial biofilms are widely studied due to their ability to bind or uptake heavy metals, which enable mechanisms such as biosorption or bioaccumulation (Salman *et al*. 2015; Syed *et al.* 2023).

The bioleaching method has emerged as a promising strategy that utilizes microorganisms or their metabolic products, such as organic acids, to solubilize metals. Bioleaching processes are generally categorized as either direct or indirect, depending on the involvement of living microorganisms. In direct bioleaching, active microbial cells interact directly with metal-bearing substrates to promote solubilization. In contrast, indirect bioleaching relies on microbial metabolites—such as organic acids—that facilitate metal leaching without the presence of live cells. Low-molecular-weight organic acids (LMWOAs) are one of the promising bioleaching agents for remediating heavy-metal-contaminated soils in abandoned mines and coastal wetlands (Wang and Mulligan 2013; Sun *et al*. 2021). LMWOAs can directly mobilize heavy metals and enhance metal mobility by lowering pH (Renella *et al*. 2004; Schwab *et al*. 2005).

In nature, LMWOAs are derived from various sources, including bacteria, fungi, and plants (Cruz-Rodríguez *et al*. 2022; Seregin and Kozhevnikova 2024). Fungi are valuable biomaterials that produce numerous secondary metabolites, including organic acids such as acetic acid, citric acid, formic acid, gluconic acid, isocitric acid, lactic acid, malonic acid, oxalic acid (OA), and pyruvic acid, during their growth phase (Islam 2008). Fungal organic acid production depends on several factors, including biomass pretreatment, nitrogen source, pH, and temperature (Lapeyrie *et al*. 1987; Gharieb and Gadd 1999; Zhang *et al*. 2020). Zhang *et al*. (2020) compared the organic acid production of *Penicillium oxalicum*, an alkali-resistant, acid-producing fungus isolated from bauxite residue disposal sites, under different biomass pretreatment methods. Their study found that steam-exploded biomass was the most effective in enhancing organic acid production, specifically oxalic acid, formic acid, and acetic acid. Similarly, Lapeyrie *et al*. (1987) demonstrated increased oxalate production in fungi when the fungus was grown with nitrate compared to ammonium. This increased oxalate production has been observed in 11 different ectomycorrhizal strains (Lapeyrie *et al*. 1987) and four saprotrophic fungi, including *Aspergillus niger*, *Coriolus versicolor* (= *Trametes versicolor*), *Penicillium* sp., and *Phanerochaete chrysosporium* (Gharieb and Gadd 1999).

Several studies have examined the efficiency of organic acid-producing fungal isolates in removing heavy metals (Chaerun *et al*. 2017; Din *et al*. 2020; Nasab *et al*. 2020; Zhang *et al*. 2020). Ren *et al*. (2009) investigated the bioleaching efficiency of four LMWOAs (citric acid, gluconic acid, malic acid, and OA) produced by the fungus *Aspergillus niger* for extracting four heavy metals: copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) from the soil. The safety of the bioleaching process was also confirmed by analyzing the remaining metals in the soil, which were predominantly found in stable fractions and therefore unavailable to the surrounding environment (Ren *et al*. 2009). The impact of LMWOAs on heavy metal mobility varies depending on the specific metal, the type of organic acid, and environmental conditions (Schwab *et al*. 2008). Citric acid exhibited the highest R_f values for Zn, followed by malic, tartaric, fumaric, and glutaric acids. Similarly, for Cd, citric acid showed the highest R_f value, followed by fumaric acid. Citric and tartaric acids also enhanced Pb transport to the greatest extent (Schwab *et al*. 2008).

Oxalic acid demonstrated a lower leaching capacity for Cd and iron (Fe) compared to citric and malic acids and a lower leaching capacity for Cr and Cu compared to malic acid (Geng *et al*. 2020). However, OA exhibited the highest arsenic (As) leaching efficiency in soil compared to citric and acetic acids (Ash *et al*. 2016).

OA is a weak organic acid commonly used in the leaching of heavy metals, primarily due to its ability to dissolve metal-containing minerals. Upon dissociation, oxalic acid from oxalate ions ($C_2O_4^{2-}$), which readily coordinate with metal cations such as Cu^{2+}, Fe^{3+}, and Pb^{2+}, resulting in the formation of stable metal-oxalate complexes (Krishnamurty and Harris 1961). These complexes are generally more soluble in aqueous solutions, facilitating the extraction of metal ions from solid matrices (Peters 1999). Additionally, OA acts as a reducing agent, promoting the reduction of higher oxidation states (*e.g.*, Fe^{3+} to Fe^{2+}), thereby further enhancing metal leaching efficiency (Arantes *et al*. 2009).

The metal leaching process can be optimized by considering various factors. Previous studies have examined the effects of the bioleaching method, leaching agent concentration, environmental properties (*e.g.*, pH, soil texture, and temperature), fungal species, and treatment duration on heavy metal removal rates by OA (Mandal and Banerjee 2004; Kim *et al*. 2009; She-Bardan *et al*. 2012; Zhang *et al*. 2024). Kim *et al*. (2009) demonstrated that OA produced by brown rot fungi is a potent bioleaching agent for Cu, Cr, and As in CCA-treated wood. In their study, *Antrodia albida* KUC88020, *Polyporales* sp. LAS6497, and *Fomitopsis palustris* TYP0507 exhibited higher As removal efficiencies than for Cu or Cr, while *Daedalea dickinsii* KUC8811 showed greater bioleaching ability for Cu (Kim *et al*. 2009). Onireti and Lin (2016) investigated the effects of OA concentration and treatment duration on arsenic (As) mobilization. Their findings indicated that As mobility was significantly influenced by OA concentration.

This approach offers several advantages, including selective metal extraction, lower environmental toxicity, and reduced corrosivity compared to traditional mineral acids such as sulfuric acid. However, potential challenges include the precipitation of metal oxalates under certain conditions, as well as the higher cost of oxalic acid compared to other acids commonly used in leaching. Therefore, investigating OA-producing organisms and optimizing the heavy metal bioleaching process is crucial for efficiently managing heavy metal contamination in abandoned mine soils.

Another important consideration is the environmental risk associated with mobilized metals. If not properly managed, the use of oxalic acid could convert solid-phase contaminants into mobile aqueous forms, increasing the likelihood of leaching into groundwater and surround ecosystems. This would pose a serious risk of secondary pollution, underscoring the need for integrated treatment approaches, including containment, stabilization, or reuse of extracted metals, to ensure long-term environmental safety.

This study aimed to optimize the heavy metal leaching process by determining the optimal OA concentration and the amount of contaminated soil using response surface methodology (RSM) and a second-order central composite design (CCD) (RSM-CCD). Additionally, this study compared the As leaching efficiency of OA derived from commercial sources and the brown rot fungus *Antrodia albida* while simultaneously evaluating the leaching efficiency for other heavy metals in abandoned mine soils.

EXPERIMENTAL

Soil Sampling and Characterization

Soil samples were collected from two abandoned mine sites, Bok Su (BS) and Dae Yang (DY), located in Jecheon, Korea. Samples were taken at a depth of 20 cm near the mine entrances, air-dried, and sieved through a 2 mm mesh. The soil properties, including pH, soil texture, and concentrations of heavy metals (As, Cd, Cr, Cu, Ni, Pb, and Zn), were analyzed and are summarized in Table 1.

Table 1. Selected Characteristics of Soils Used in this Study

Soil[a]	Acidity (pH)	Size texture (%)			Metal concentration (mg/kg)[b]						
		Sand	Silt	Clay	As	Cd	Cr	Cu	Ni	Pb	Zn
BS	3.9	79.0	13.1	7.9	10069 ± 759	29 ± 4	27 ± 1	2944 ± 35	13 ± 1	34117 ± 2495	4561 ± 390
DY	3.3	57.3	28.4	14.3	21591 ± 395	17 ± 1	39 ± 3	247 ± 8	20 ± 2	6415 ± 240	1694 ± 151

[a] BS, soil collected from the Bok Su abandoned mine; DY, Soil collected from the Dae Yang abandoned mine.
[b] Values represent the average of 5 replicates

Leaching of Arsenic by OA

Extraction process and arsenic determination

A commercial OA solution (Showa Chemicals Inc., Tokyo, Japan; 99.5% purity) was used to extract As from the two abandoned mine soils, following the procedure described in a previous study (Kim *et al*. 2009). Soil samples were placed in flasks containing 100 mL of the OA solution. The flasks were subjected to ultrasound treatment for 1 hour at a frequency of 40 kHz (4,000 W) and then agitated for 24 hours at 150 rpm on a rotary shaker at 27°C. The extracted As content was analyzed using an Agilent 730 inductively coupled plasma optical emission spectrometer (ICP-OES).

Central composite design and response surface methodology

A second-order CCD was employed with RSM to optimize the concentration of OA (4–40 g/L) and the amount of added soil (3–15 g/100 mL) for As extraction from soils (Eq. (1)). Eleven experimental runs were conducted, consisting of four cube points (Runs 1–4), four star points (Runs 5–8), and three central points (Runs 9–11). The CCD included two variables and five levels (Table 2).

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \quad (1)$$

In Eq. 1, Y is the predicted response, and $\beta_0, \beta_i, \beta_{ii}$, and β_{ij} represent the constant, linear, quadratic, and interaction coefficients, respectively. x_i and x_j are the coded factors (Aghaie *et al*. 2009).

Table 2. Coded and Assigned Variables of Different Levels of the Central Composite Design

Factors	Levels				
	-1.5	-1	0	1	1.5
Oxalic acid concentration (g/L)	4	10	22	34	40
The amount of added soil (g/100 mL)	3	5	9	13	15

Bioleaching of Arsenic Based on the Evaluated Model

Fungal oxalic acid solution

The brown-rot fungus *Antrodia albida* KUC8802 was obtained from the Korea University Culture Collection (KUC). OA production by this fungal strain was confirmed through a screening test (Kim *et al*. 2009). In this study, the rate of OA production was examined to optimize the preparation of a fungal OA solution. Ten fungal discs (5 mm in diameter) were inoculated into a 4 L bioreactor containing 2% malt extract (Difco) broth medium with an initial pH of 4.97–5.00. The culture was incubated at 27 °C with agitation at 150 rpm for 22 days. Samples of the fungal culture broth were collected every two days, and fungal hyphae were removed by filtration to measure the pH change and OA production. OA production was quantified using high-performance liquid chromatography with an ion-exchange column (Repromer H, Dr. Maisch GmbH, Germany), following the method described by Kim *et al*. (2009). After quantifying OA production, the fungal OA-enriched solution was concentrated using a rotary vacuum evaporator at 40 °C before application, and was used as an indirect bioleaching agent without fungal biomass.

Extraction of arsenic

The bioleaching of As from abandoned mine soil was conducted using a fungal OA-enriched solution under the optimal conditions established in this study. Each soil sample was placed in a separate flask containing 100 mL of the fungal OA-enriched solution. The procedures that followed were performed as described in Kim *et al*. (2009).

Heavy Metal Extraction Capability of Fungal OA

In the BS and DY abandoned mine soils, six other heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) were detected using ICP-OES. Therefore, the heavy metal extraction capability of fungal-derived OA was investigated and compared with that of commercial OA.

Statistical Analysis

Statistical analyses were conducted to generate three-dimensional response surface plots and two-dimensional contour plots using SAS version 9.1.3 (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

In this study, soils from two abandoned mines were collected to investigate heavy metal contamination. Seven heavy metals were detected at varying concentrations (Table 1). Among these metals, As was the most significant pollutant, with concentrations exceeding 10,000 mg/kg in both mine soils, surpassing the “Standards of Countermeasures against Soil Contamination” in Korea (Table 1). The As concentration was twice as high in the DY soil (*ca.* 21,600 mg/kg) compared to the BS soil (*ca.* 10,070 mg/kg). Although Pb was the most abundant heavy metal (*ca.* 34,100 mg/kg) in the BS mine soil, its concentration was relatively low in the DY soil (*ca.* 6,420 mg/kg). Therefore, this study focused on optimizing the As leaching process using OA, taking into account the concentration of OA and added soil weight through RSM-CCD.

Fitted Model for Arsenic Extraction by Commercial Oxalic Acid

Table 3 shows the observed and predicted As extraction rates for 11 experimental conditions using CCD. The highest As extraction percentages were detected in run no. 5 (actual input values: 22 g/L of OA and 3 g/100 mL of added soil) (Table 3). The observed As extraction percentages were 94.8 ± 5.0% for the BS soil and 97.8 ± 4.2% for the DY soil. RSM analysis revealed that As removal efficiencies had a positive relationship with OA concentration but a negative relationship with added soil weight (Figs. 1a, 1b, 2a, and 2b). Based on these results, two RSM models were developed for the BS soil (Eq. (2)) and DY soil (Eq. (3)).

$$\text{BS soil} = 49.176 + 2.088A - 6.125B \tag{2}$$

$$\text{DY soil} = 37.723 + 3.933A - 4.559B - 0.050A^2 \tag{3}$$

Where A is the OA concentration and B is the added soil weight. The appropriateness of these models was examined using a lack-of-fit test. Both models showed insignificant values ($p = 0.058$ and 0.099 for the BS and DY soils, respectively) for lack of fit, indicating that the models were statistically significant for further analysis. The fitted RSM models showed high coefficients of determination ($R^2 = 0.967$–0.97), indicating strong correlations between variables and responses. The observed and predicted As extraction rates exhibited a significant linear relationship (Figs. 1c and 2c). These results indicate that the models predicted the rate of As extraction with high accuracy.

OA concentration (A) was the most significant parameter, accounting for 49.0% and 52.6% of the total variance for BS and DY soils, respectively. The amount of added soil (B) explained 46.9% and 40.0% of the total variance, making it the second most important factor. In the DY soil model, A^2 contributed 5.5% of the overall variance. However, there was no significant effect in the BS soil model from quadratics (A^2 and B^2) or interaction ($A \times B$).

Based on the equations, the optimal condition for BS soil was determined when the OA concentration ranged from 33.1 to 40.0 g/L and the added soil weight ranged from 3.0 to 5.3 g/100 mL (Fig. 1). Similarly, for the DY soil, an OA concentration of 34.1 to 40.0 g/L and an added weight of 3.0 to 3.3 g/100 mL of soil were appropriate for completely extracting the As from the soil (Fig. 2). These results suggest that 53,370 to 71,250 mg/kg of As in 3 g of abandoned mine soil can be completely released within 24 hours using 100 mL of a 33.1 to 34.1 g/L commercial OA solution.

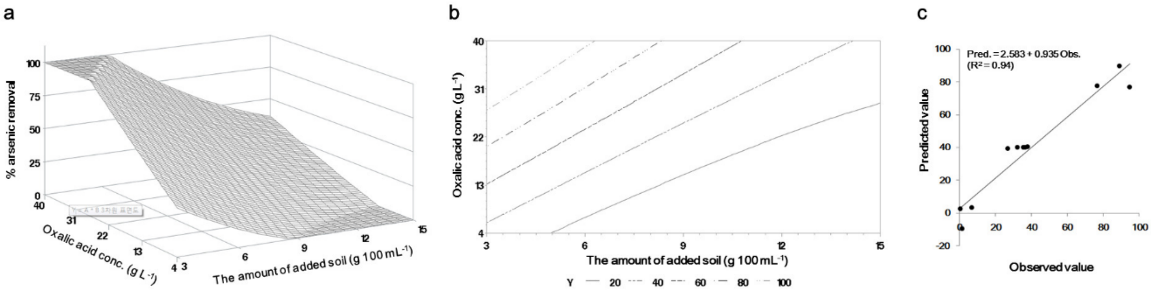

Fig. 1. Three-dimensional response surface plot (a) and two-dimensional contour plots (b) expression percent As removal from soil collected from the Bok Su abandoned mine and the relationship between observed and predicted yields of arsenic removal (c)

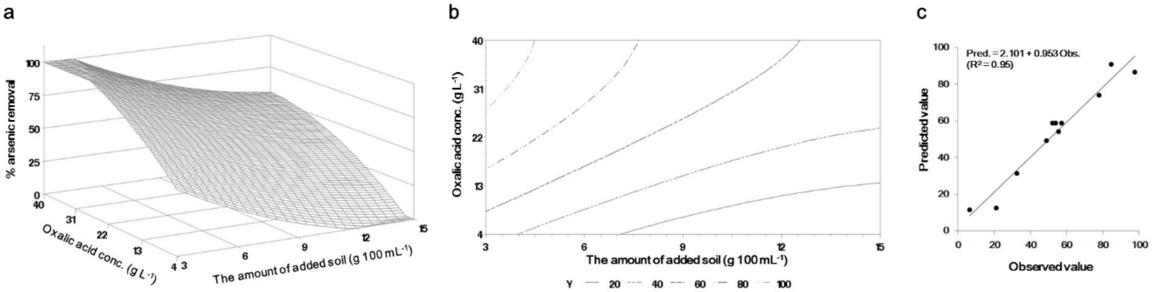

Fig. 2. Three-dimensional response surface plot (a) and two-dimensional contour plots (b) expression percent As removal from soil collected from the Dae Yang abandoned mine and the relationship between observed and predicted yields of arsenic removal (c)

According to previous studies, As mobility in soil is influenced by pH (Signes-Pastor *et al*. 2007; Zhang *et al*. 2024), redox potential (Masscheleyn *et al*. 1991), and soil amendments, such as P fertilization (Davenport and Peryea 1991). Moreover, soil texture, such as clay content, also influences As mobility (Dousova *et al*. 2016). Zhang *et al*. (2024) demonstrated the correlation between the As leaching and pH of leachate through the chemical speciation and valence of As. Additionally, the encapsulation effect of the cementitious matrix was significantly influenced by pH. This study revealed that the leached As concentration increased in strongly acidic leachate (pH 3), which induced the formation of pores and cracks. Conversely, the As concentration was lowest in the weakly alkaline leachate (pH 9) because of the facilitated cement hydration process, which enhanced As attachment to the surface of soil particles, promoting As encapsulation.

This study revealed that the arsenic extraction efficiency (as a percentage) was much higher in the DY mine soil than in the BS mine soil under insufficient OA concentration or excessive amounts of contaminated soil, according to RSM-CCD analysis (Table 3). These differences may indicate that the amount of treated soil influences leaching efficiency, the initial As concentration in the soil, soil pH, and the treated OA concentration (Tables 1 and 3). However, the clay fraction was not considered here because the result contradicted a previous study (Davenport and Peryea 1991). In the previous study, the As leaching efficiency was lower in high clay soils (Davenport and Peryea 1991). In contrast, this study found that the As leaching efficiency was higher in the DY soil, which had a higher clay fraction (BS, 7.9%; DY, 14.3%).

The As extraction using OA was investigated under varying conditions, including OA concentrations (1, 10, and 100 mM), initial As concentrations in aquifer sediments (2–10 mg/kg and 100 to 400 mg/kg), and pH levels (3.1, 2.2, and 1.4) in a previous study (Sun *et al*. 2016). The study revealed that the As extraction efficiency depended on the OA concentration and the initial As concentration in the sediment but was also affected by the pH range of 1 to 3. In that study, higher As extraction was observed in Vineland sediments, which had significantly higher As concentrations (101 to 374 mg/kg), compared to sediments from Dover, which had much lower As concentrations (2.4 to 12.7 mg/kg). The low initial As concentrations in the sediment resulted in a low leaching efficiency, which was likely due to the lower surface coverage of As. This could have made it less prone to interaction with OA and, consequently, limited the extraction efficiency (Sun *et al*. 2016). However, since both abandoned mine soils employed in this study contained very high As concentrations, determining whether As extraction can be attributed to low surface coverage in soils with lower initial As concentrations is difficult.

Table 3. Observed and Predicted Yields of Arsenic Removal from Soils in Two Abandoned Mines

Experimental point	Run no.	Coded value[a]		Actual value		Responses[c]			
						BS soil		DY soil	
		A	B	A	B	Observed (%)[b]	Predicted (%)	Observed (%)	Predicted (%)
Cube point	1	-1	-1	10	5	26.8 ± 2.4	39.4	49.0 ± 2.2	49.1
	2	-1	1	10	13	1.7 ± 0.1	-9.6	21.1 ± 1.0	12.3
	3	1	1	34	13	38.0 ± 1.1	40.5	55.6 ± 2.5	53.9
	4	1	-1	34	5	89.3 ± 3.6	89.5	84.9 ± 1.7	90.7
Star point	5	0	-1.5	22	3	94.8 ± 5.0	76.7	97.8 ± 4.2	86.3
	6	-1.5	0	4	9	0.7 ± 0.2	2.4	6.6 ± 0.6	11.3
	7	0	1.5	22	15	6.9 ± 0.5	3.2	32.6 ± 2.8	31.1
	8	1.5	0	40	9	75.8 ± 3.0	77.6	78.1 ± 2.5	73.7
Central point	9	0	0	22	9	35.7 ± 0.8	40.0	53.9 ± 9.9	58.7
	10	0	0	22	9	32.3 ± 0.3	40.0	57.5 ± 2.0	58.7
	11	0	0	22	9	36.8 ± 2.6	40.0	52.2 ± 4.2	58.7

[a] A, Oxalic acid concentration; B, The amount of added soil.
[b] Values represent the average of 3 replicates.
[c] Responses represents the experimentally measured dependent extraction efficiency used for model prediction

Here, it was demonstrated that by adjusting the high OA concentration and the proper weight of the contaminated soil, As extraction appeared to be relatively unaffected by these factors. These findings emphasize the critical need to optimize both the OA concentration and the amount of contaminated soil for efficient As leaching.

Fungal OA Production

Fungal LMWOAs have been studied not only for the various roles of fungi in wood decay, as saprobes or pathogens infecting plants, but also for their ability to detoxify metal compounds and solubilize heavy metals in soils (Dutton and Evans 1996; Gadd 1999). Among these, OA has recently garnered attention for its ability to solubilize and release metal ions from heavy metal-contaminated soils (Sayer and Gadd 1997; Gadd 1999; Mendes *et al*. 2020). The biosynthesis of OA by fungi is influenced by factors such as the availability of carbon or nitrogen, environmental pH, and the cultivation stage (Plassard and Fransson 2009). For instance, OA production by *Sclerotinia sclerotiorum* decreased when the pH was below 4 (Culbertson *et al*. 2007). Similarly, a well-known OA producer, *Aspergillus niger*, suppresses OA synthesis at low pH levels (Dixit and Hering 2003). These organic acids, once produced by fungi, contribute to a decrease in environmental pH. Although low-pH conditions contribute to the dissolution of As in soil, they negatively affect OA production by fungi. Therefore, the ability to produce OA under low pH conditions is crucial for sustainable OA production in low pH environments.

In this study, fungal OA production and the corresponding pH changes were monitored during the incubation period (Fig. 3). A sharp increase in OA production was observed within the first 4 days, reaching 1.087 g/L (1.21 mM), accompanied by a significant decrease in pH from 5.0 to 2.39. After the fourth day, the pH stabilized at approximately 2.0, while OA production continued to rise steadily, peaking at 1.627 g/L (1.81 mM) after 8 days. Subsequently, OA production fluctuated, with the highest level recorded after 20 days (1.666 g/L, 1.85 mM), comparable to the production observed on the eighth day. Based on these results, the eighth day was selected as the optimal incubation period to fungal OA production.

These findings demonstrate that *A. albida* KUC8802 can produce OA while sustaining production in low-pH environments—an exceptional trait that highlights its potential among fungi.

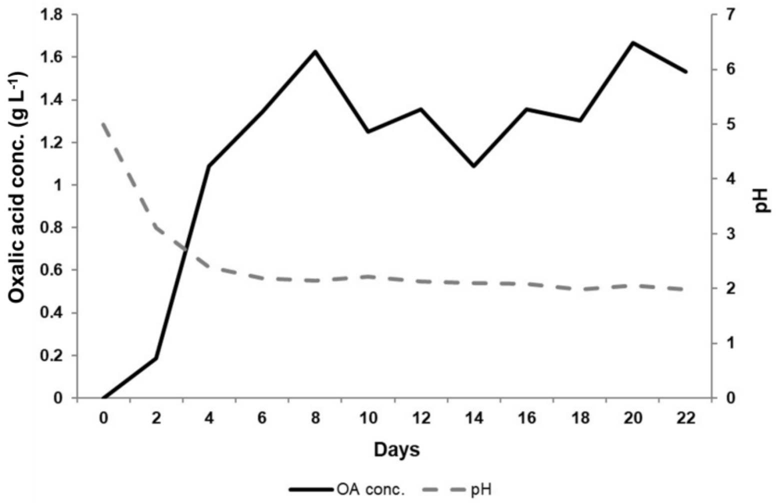

Fig. 3. Oxalic acid production and pH values from the fermentation broth of *Antrodia albida* KUC8802

Heavy Metal Extraction Capability of Fungal OA Compared to that of the Commercial OA

The fungal OA-enriched solutions were concentrated to 33.1 g/L (36.76 mM) and 34.1 g/L (37.88 mM) according to the fitted model and were applied to 3 g of BS and DY soils, respectively. The As extraction percentages of the fungal solutions exceeded 95% in both soil types, with no significant differences (Table 4). Furthermore, the As extraction efficiency of fungal-derived OA was similar to that of commercial OA (Table 4).

Table 4. Percentage Removal of Arsenic Using the Evaluated Model

Soil[a]	As removal efficiency (%)[b]	
	Oxalic acid produced by the fungus	Commercial oxalic acid
BS	98.7 ± 3.9	99.0 ± 2.1
DY	95.5 ± 4.8	96.6 ± 6.1

[a] BS, soil collected from the Bok Su abandoned mine; DY, Soil collected from the Dae Yang abandoned mine.
[b] Values represent the average of 3 replicates.

A previous bioleaching study investigated the extraction of As from CCA-treated sawdust using *A. albida* KUC8802 (Kim *et al*. 2009). In the study, OA was pre-produced by culturing the fungus for 10 days, yielding 2.4 g/L of OA. Subsequently, 100 mL of unfiltered fungal fermentation broth was applied to 3 g of CCA-treated sawdust over 10 days, resulting in a 94% As extraction efficiency (Kim *et al*. 2009). Notably, oxalic acid was the only organic acid detected in the fungal broth. Another study focused on bioremediation *via* bioaugmentation, where fungal inoculation at the contamination site promotes *in situ* OA production and subsequent contaminant removal (Seh-Bardan *et al*. 2012). For example, *Aspergillus fumigatus* has been used to evaluate the efficiency of heavy metal extraction from mine soils. The study employed both the distribution and surface application methods for fungal OA production (Seh-Bardan *et al*. 2012). However, the extraction process requires a long duration and exhibits limited efficiency. In the present study, the fungal culture broth was filtered to remove mycelia, and the OA-containing culture broth was directly applied to the contaminated soil. Additionally, ultrasound treatment was applied during the extraction process. This combined approach was likely responsible for the high As extraction efficiency observed within 24 hours, outperforming previous studies.

The extraction efficiencies of the OA solutions for the other six heavy metals from both abandoned mine soils were analyzed (Fig. 4). The extraction tendencies for the other heavy metals varied depending on the type of OA solution used. Commercial OA demonstrated higher extraction efficiencies for Cr, Cu, and Zn than fungal OA. Conversely, fungal OA solution exhibited higher extraction efficiencies for Cd, Ni, and Pb (Fig. 4). The extraction tendencies of fungal OA also differed between the abandoned mine soils. In the BS mine soil, the bioleaching efficiency was highest for Cd, followed by Cu, Zn, Ni, Cr, and Pb (Fig. 4). However, in the DY mine soil, the bioleaching efficiency was highest for Cd, followed by Cu, Pb, Cr, Zn, and Ni (Fig. 4). The leaching efficiency of heavy metals also differed between commercial OA in the BS and DY mine soils. In the DY mine soil, the leaching efficiency was highest for Cd, followed by Cu, Cr, Zn, Pb, and Ni. However, in the BS mine soil, the leaching efficiency was highest for Cu, followed by Cd, Zn, Cr, Ni, and Pb.

The initial concentrations of the six heavy metals in the soil may have influenced their extraction efficiencies. For three metals (Cd, Ni, and Pb), a high initial concentration negatively affected the extraction. However, Cu and Zn were more efficiently extracted by OA at high initial concentrations. This was likely due to variations in OA solubility depending on the heavy metal compound. For Pb, a single oxalate ion can bind to a single Pb ion in a monodentate complex (Ash *et al*. 2016). Additionally, lead oxalate is slightly soluble in water. Therefore, the Pb-leaching capability of the OA solution was low at sites with high Pb concentrations. The higher Pb extraction observed in the fungal OA-enriched broth suggests the possible involvement of additional factors. Similarly, Cd and Ni also showed increased extraction, indicating a broader effect of the fungal OA-enriched broth on metal solubilization. However, further investigation is needed to identify the substances in the broth that may influence Pb extraction. Experiments using isolated fungal organic acids could provide more precise insights into their specific contributions.

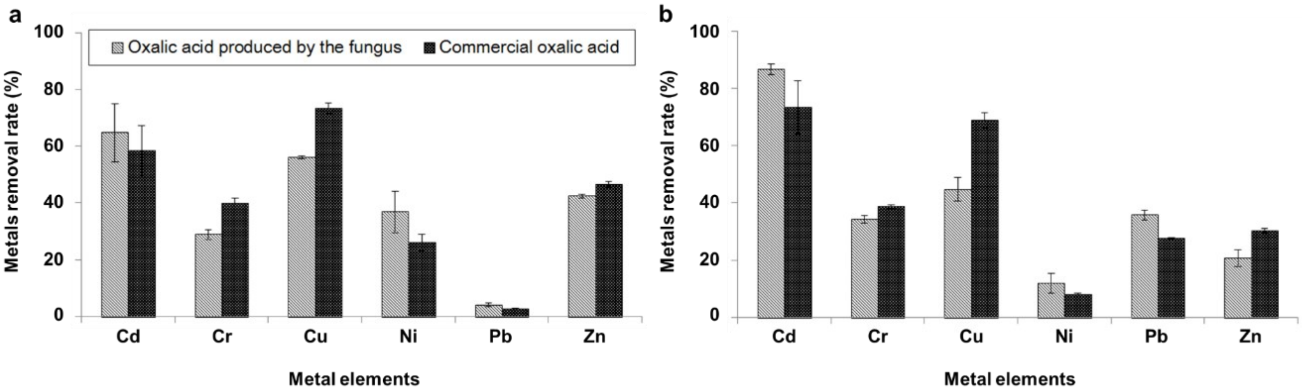

Fig. 4. Extraction rates of other heavy metals from soils collected from the Bok Su (a) and Dae Yang (b) abandoned mines

The present study primarily focused on optimizing the efficiency of arsenic extraction. However, it did not analyze arsenic speciation or the transformations of solid-phase minerals in the residual samples. Since arsenic can exist in multiple oxidation states, particularly As(III) and As(V), and may form secondary biogenic minerals in the presence of iron and microbial activity, it is essential for future studies to include analyses of arsenic speciation and mineralogical characterization. This will enhance our understanding of the transformation pathways and stabilization mechanisms involved in the bioleaching process.

Considerations for Rinsate Management and Environmental Safety

Although this study focused on laboratory-scale optimization, practical implementation of OA-based bioleaching must also address the safe and responsible management of arsenic- and metal-contaminated rinsate. If untreated, the release of metal-rich effluent into soil or water bodies could result in serious secondary contamination risks (Madjar *et al*. 2025). Therefore, field-scale applications should incorporate appropriate post-treatment solutions to mitigate these risks. Technologies such as chemical precipitation, membrane filtration, and adsorption using low-cost sorbents have been widely studied for removing heavy metals from contaminated water (Babel and Kurniawan 2003; Fu and Wang 2011). In addition, metal recovery and reuse strategies—such as bioelectrochemical systems—could provide sustainable endpoints (Pines *et al*. 2025).

Employing an integrated approach has potential not only to enhance the environmental safety of the remediation process but also to promote long-term feasibility and sustainability for field-scale applications.

CONCLUSIONS

1. The findings of this study highlight the potential of fungal-derived oxalic acid (OA) as an effective bioleaching agent for heavy metal-contaminated soils, particularly at abandoned mine sites.
2. Using response surface methodology with central composite design (RSM-CCD), the optimal conditions for As extraction were identified, demonstrating the importance of OA concentration and the added weight of contaminated soil in improving leaching efficiency. Under these conditions, extraction efficiencies exceeded 95% for both commercial and fungal-derived OA solutions.
3. Fungal OA demonstrated unique extraction patterns for other heavy metals compared to commercial OA, which was likely due to differences in metal solubility. Notably, fungal OA exhibited a higher extraction capability for Cd, Ni, and Pb, suggesting that additional factors in fungal solutions may enhance leaching capability.
4. The fungus *Antrodia albida* KUC8802 produced OA even under low pH conditions, supporting its potential for bioleaching applications. This method, combined with ultrasound, also achieves faster and more efficient metal extraction than traditional techniques, making it a promising solution for soil remediation.
5. Further studies are needed to expand the applicability of fungal OA across different heavy metal types and to enhance its remediation performance by integrating OA with lignocellulosic materials or other complementary biosorbents, thereby promoting more rapid and accessible adsorption and extraction. Exploring such synergistic systems represents a promising direction for future soil remediation research.
6. To further validate the bioleaching mechanism, future work may include X-ray diffraction, Fourier-transform infrared spectroscopy, redox potential measurements, and Scanning electron microscopy analyses to examine changes in mineral structure and surface properties.

ACKNOWLEDGMENTS

We would like to extend our sincere gratitude to Syaidatul Shahomlail for his invaluable contribution to this study, particularly in performing the experiments. We also thank Editage (www.editage.co.kr) for editing and reviewing this manuscript for English language.

REFERENCES CITED

Aghaie, E., Pazouki, M., Hosseini, M., Ranjbar, M., and Ghavipanjeh, F. (2009). "Response surface methodology (RSM) analysis of organic acid production for kaolin beneficiation by *Aspergillus niger*," *Chemical Engineering Journal* 147, 245-251. https://doi.org/10.1016/j.cej.2008.07.008

Arantes, V., Qian, Y., Milagres, A. M., Jellison, J., and Goodell, B. (2009). "Effect of pH and oxalic acid on the reduction of Fe^{3+} by a biomimetic chelator and on Fe^{3+}

desorption/adsorption onto wood: Implications for brown-rot decay," *International Biodeterioration & Biodegradation* 63, 478-483. https://doi.org/10.1016/j.ibiod.2009.01.004

Ash, C., Tejnecký, V., Borůvka, L., and Drábek, O. (2016). "Different low-molecular-mass organic acids specifically control leaching of arsenic and lead from contaminated soil," *Journal of Contaminant Hydrology* 187, 18-30. https://doi.org/10.1016/j.jconhyd.2016.01.009

Babel, S., and Kurniawan, T. A. (2003). "Low-cost adsorbents for heavy metals uptake from contaminated water: A review," *Journal of Hazardous Materials* 97(1-3), 219-243. https://doi.org/10.1016/S0304-3894(02)00263-7

Chaerun, S. K., Sulistyo, R. S., Minwal, W. P., and Mubarok, M. Z. (2017). "Indirect bioleaching of low-grade nickel limonite and saprolite ores using fungal metabolic organic acids generated by *Aspergillus niger*," *Hydrometallurgy* 174, 29-37. https://doi.org/10.1016/j.hydromet.2017.08.006

Cruz-Rodríguez, I. A., Rojas-Avelizapa, N. G., and Rivas-Castillo, A. M. (2022). "Microbially-produced organic acids as leaching agents for metal recovery processes," *Advancements of Microbiology* 61, 179-190. https://doi.org/10.2478/am-2022-019

Culbertson, B. J., Furumo, N. C., and Daniel, S. L. (2007). "Impact of nutritional supplements and monosaccharides on growth, oxalate accumulation, and culture pH by *Sclerotinia sclerotiorum*," *FEMS Microbiology Letters* 270, 132-138. https://doi.org/10.1111/j.1574-6968.2007.00678.x

Davenport, J., and Peryea, F. J. (1991). "Phosphate fertilizers influence leaching of lead and arsenic in a soil contaminated with lead arsenate," *Water, Air, and Soil Pollution* 57, 101-110. https://doi.org/10.1007/BF00282873

Din, G., Hassan, A., Rafiq, M., Hasan, F., Badshah, M., Khan, S., *et al.* (2020). "Characterization of organic acid producing *Aspergillus tubingensis* FMS1 and its role in metals leaching from soil," *Geomicrobiology Journal* 37, 336-344. DOI: 10.1080/01490451.2019.1701585

Dixit, S., and Hering J. G. (2003). "Comparison of arsenic (V) and arsenic (III) sorption onto iron oxide minerals: implications for arsenic mobility," *Environmental Science & Technology* 37, 4182-4189. https://doi.org/10.1021/es030309t

Dousova, B., Buzek, F., Lhotka, M., Krejcova, S., and Boubinova, R. (2016). "Leaching effect on arsenic mobility in agricultural soils," *Journal of Hazardous Materials* 307, 231-239. https://doi.org/10.1016/j.jhazmat.2015.12.030

Dutton, M. V., and Evans, C. S. (1996). "Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment," *Canadian Journal of Microbiology* 42, 881-895. https://doi.org/10.1139/m96-114

Fu, F., and Wang, Q. (2011). "Removal of heavy metal ions from wastewaters: A review," *Journal of Environmental Management* 92(3), 407-418. https://doi.org/10.1016/j.jenvman.2010.11.011

Gadd, G. M. (1999). "Fungal production of citric and oxalic acid: Importance in metal speciation, physiology and biogeochemical processes," *Advances in Microbial Physiology* 41, 47-92. https://doi.org/10.1016/S0065-2911(08)60165-4

Geng, H., Wang, F., Yan, C., Tian, Z., Chen, H., Zhou, B., *et al.* (2020). "Leaching behavior of metals from iron tailings under varying pH and low-molecular-weight organic acids," *Journal of Hazardous Materials* 383, article 121136.

https://doi.org/10.1016/j.jhazmat.2019.121136

Gharieb, M. M., and Gadd, G. M. (1999). “Influence of nitrogen source on the solubilization of natural gypsum ($CaSO_4$. $2H_2O$) and the formation of calcium oxalate by different oxalic and citric acid-producing fungi,” *Mycological Research* 103, 473-481. https://doi.org/10.1017/S0953756298007382

Islam, M. M. (2008). *Bioleaching of Refinery Hydroprocessing Ni-Mo Catalyst by Heterotrophic Fungi*, Master’s Thesis, Stanford University, USA.

Jomova, K., Alomar, S. Y., Nepovimova, E., Kuca, K., and Valko, M. (2025). “Heavy metals: Toxicity and human health effects,” *Archives of Toxicology* 99(1), 153-209. https://doi.org/10.1007/s00204-024-03903-2

Khalil, S., and Hassan, N. E. (2024). “Review of heavy metal removal from soil: Methods and technologies,” *Global Academic Journal of Agriculture and Biosciences* 6, article 3. https://doi.org/10.36348/gajab.2024.v06i06.003

Kim, G. H., Choi, Y. S., and Kim, J. J. (2009). “Improving the efficiency of metal removal from CCA-treated wood using brown rot fungi,” *Environmental Technology* 30, 673-679. https://doi.org/10.1080/09593330902858906

Krishnamurty, K. V., and Harris, G. M. (1961). “The chemistry of the metal oxalato complexes,” *Chemical Reviews* 61, 213-246. https://doi.org/10.1021/cr60211a001

Lapeyrie, F., Chilvers, G., and Bhem, C. (1987). “Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr.,” *New Phytologist*. 106, 139-146. https://doi.org/10.1111/j.1469-8137.1987.tb04797.x

Madjar, R. M., and Vasile Scăețeanu, G. (2025). “An overview of heavy metal contamination in water from agriculture: origins, monitoring, risks, and control measures,” *Sustainability* 17(16), article 7368. https://doi.org/10.3390/su17167368

Mandal, S., and Banerjee, P. C. (2004). “Iron leaching from China clay with oxalic acid: Effect of different physico-chemical parameters,” *International Journal of Mineral Processing* 74, 263-270. https://doi.org/10.1016/j.minpro.2004.01.004

Masscheleyn, P. H., Delaune, R. D., and Patrick Jr, W. H. (1991). “Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil,” *Environmental Science & Technology* 25, 1414-1419. https://doi.org/10.1021/es00020a008

Mendes, O. G., Murta, H. M., Valadares, R. V., da Silveira, W. B., da Silva, I. R., and Costa, M. D. (2020). “Oxalic acid is more efficient than sulfuric acid for rock phosphate solubilization,” *Minerals Engineering* 155, article 106458. https://doi.org/10.1016/j.mineng.2020.106458

Nasab, M. H., Noaparast, M., Abdollahi, H., and Amoozegar, M. A. (2020). “Indirect bioleaching of Co and Ni from iron rich laterite ore, using metabolic carboxylic acids generated by *P. putida*, *P. koreensis*, *P. bilaji* and *A. niger*,” *Hydrometallurgy* 193, article 105309. https://doi.org/10.1016/j.hydromet.2020.105309

Onireti, O. O., and Lin, C. (2016). “Mobilization of soil-borne arsenic by three common organic acids: Dosage and time effects,” *Chemosphere*. 147, 352-360. https://doi.org/10.1016/j.chemosphere.2015.12.129

Peters, R. W. (1999). “Chelant extraction of heavy metals from contaminated soils,” *Journal of Hazardous Materials* 66, 151-210. https://doi.org/10.1016/S0304-3894(99)00010-2

Pines Pozo, M. T., Lopez Fernandez, E., Villasenor, J., Leon-Fernandez, L. F., and Fernandez-Morales, F. J. (2025). “Metal recovery from wastes: A review of recent advances in the use of bioelectrochemical systems," *Applied Sciences* 15(3), article 1456. https://doi.org/10.3390/app15031456

Plassard, C., and Fransson, P. (2009). "Regulation of low-molecular weight organic acid production in fungi," *Fungal Biology Reviews* 23, 30-39. https://doi.org/10.1016/j.fbr.2009.08.002

Renella, G., Landi, L., and Nannipieri, P. (2004). "Degradation of low molecular weight organic acids complexed with heavy metals in soil," *Geoderma* 122, 311-315. https://doi.org/10.1016/j.geoderma.2004.01.018

Ren, W.-X., Li, P.-J., Geng, Y., and Li, X.-J. (2009). "Biological leaching of heavy metals from a contaminated soil by *Aspergillus niger*," *Journal of Hazardous Materials* 167, 164-169. https://doi.org/10.1016/j.jhazmat.2008.12.104

Salman, M., Athar, M., and Farooq, U. (2015). "Biosorption of heavy metals from aqueous solutions using indigenous and modified lignocellulosic materials," *Reviews in Environmental Science and Bio/Technology* 14, 211-228. https://doi.org/10.1007/s11157-015-9362-x

Sayer, J. A., Gadd, G. M. (1997). "Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*," *Mycological Research* 101, 653-661. https://doi.org/10.1017/S0953756296003140

Schwab, A., He, Y., and Banks, M. (2005). "The influence of organic ligands on the retention of lead in soil," *Chemosphere* 61, 856-866. https://doi.org/10.1016/j.chemosphere.2005.04.098

Schwab, A., Zhu, D., and Banks M. (2008). "Influence of organic acids on the transport of heavy metals in soil," *Chemosphere* 72, 986-994. https://doi.org/10.1016/j.chemosphere.2008.02.047

Seh-Bardan, B. J., Othman, R., Ab Wahid, S., Husin, A., and Sadegh-Zadeh, F. (2012). "Column bioleaching of arsenic and heavy metals from gold mine tailings by *Aspergillus fumigatus*," *CLEAN–Soil, Air, Water* 40, 607-614. https://doi.org/10.1002/clen.201000604

Seregin, I. V., and Kozhevnikova, A. D. (2024). "The role of low-molecular-weight organic acids in metal homeostasis in plants," *International Journal of Molecular Sciences* 25, article 9542. https://doi.org/ 10.3390/ijms25179542

Signes-Pastor, A., Burló, F., Mitra, K., and Carbonell-Barrachina, A. (2007). "Arsenic biogeochemistry as affected by phosphorus fertilizer addition, redox potential and pH in a west Bengal (India) soil," *Geoderma* 137, 504-510. https://doi.org/10.1016/j.geoderma.2006.10.012

Sun, J., Bostick, B. C., Mailloux, B. J., Ross, J. M., and Chillrud, S. N. (2016). "Effect of oxalic acid treatment on sediment arsenic concentrations and lability under reducing conditions," *Journal of Hazardous Materials* 311, 125-133. https://doi.org/10.1016/j.jhazmat.2016.02.060

Sun, Y., Luo, T., Zhong, S., Zhou, F., Zhang, Y., Ma, Y., *et al.* (2021). "Long-term effects of low-molecular-weight organic acids on remobilization of Cd, Cr, Pb, and As in alkaline coastal wetland soil," *Environmental Pollutants and Bioavailability* 33, 266-77. https://doi.org/10.1080/26395940.2021.1982406

Syed, Z., Sogani, M., Rajvanshi, J., and Sonu, K. (2023). "Microbial biofilms for environmental bioremediation of heavy metals: A review," *Applied Biochemistry and Biotechnology* 195(9), 5693-5711. https://doi.org/10.1007/s12010-022-04276-x

Wang, S., and Mulligan, C. N. (2013). "Effects of three low-molecular-weight organic acids (LMWOAs) and pH on the mobilization of arsenic and heavy metals (Cu, Pb, and Zn) from mine tailings," *Environmental Geochemistry and Health* 35, 111-118. https://doi.org/10.1007/s10653-012-9461-3

Wuana, R. A., and Okieimen, F. E. (2011). "Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation," *International Scholarly Research Notices* 1, article 402647. https://doi.org/10.5402/2011/402647

Zhang, Y., Xue, R., He, X., Cheng, Q., Hartley, W., and Xue S. (2020). "Effect of acid production by *Penicillium oxalicum* on physicochemical properties of bauxite residue," *Geomicrobiology Journal* 37, 929-936. https://doi.org/10.1080/01490451.2020.1801907

Zhang, W., Yu, H., Huang, J., and Jiao W. (2024). "Influence of pH on the leaching behavior of a solidified arsenic contaminated soil," *Environmental Technology* 45, 4169-4180. https://doi.org/10.1080/09593330.2023.2243392

Article submitted: July 3, 2025; Peer review completed: August 16, 2025; Revised verson received: November 7, 2025; Accepted: January 9, 2026; Published: January 22, 2026.
DOI: 10.15376/biores.21.1.2186-2201