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Experimental Observations and Assessment of Landfill Leachate Bioremediation by Autochthonous Fungi Species and their Effective Geoactivities

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ABSTRACT

Autochthonous fungi are potential successful agents in the process of bioremediation through their efficient adaptation to pollutant toxicity and competition with other microorganisms that are present in the leachate treatment plant. The leachate from municipal waste landfills was an important source of fungi. Even though some of these fungi have the potential to be human pathogens, such strains when used in biological treatment approaches may serve as a possible tool for integrating the leachate bioremediation process because of the extracellular enzymes produced by fungal strains. In this study, the leachate sample was tested with regard to four parameters, including total dissolved solids (TDS), electrical conductivity (EC), pH, and the decolorization of the leachate sample by a number of indigenous species of fungi, which were observed by use of the culture techniques. The scanning electron microscope coupled with energy dispersive x-ray analysis, UV-spectrum, and reflected and transmitted polarizing light microscope were used in the research project to observe and assess the obtained data from the experimental work. The results indicated the various levels of efficiency of the isolated fungal strains in eliminating color, lowering TDS, EC, and pH through their geoactivities potential in metal biosorption and immobilization by biomineralization of new minerals in the growth environment and eventually reducing the metal bioavailability and toxicity.

Keywords: autochthonous fungi, pathogenic fungi, biosorption, biomineralization, decolorization.

INTRODUCTION

Over recent years, it has grown increasingly clear that leachate from landfills poses significant threats, not only to the health of humans but also to the health of the surrounding ecosystem (Matejczyk et al., 2011; Tigini et al., 2014; Alimba et al., 2016). The term "landfill leachate" refers to the contaminated aqueous effluent that is produced by a landfill as a result of rainwater seeping through the landfill, the presence of water in the garbage that has been placed, surface water runoff, and biological decomposition (Bodzek et al., 2006; Di Maria and Sisani, 2017).

Landfill leachates are defined by extreme conditions, such as a high concentration of ammonia and refractory xenobiotics as well as a low BOD/COD ratio. These characteristics are the basis of the failure of their treatment in traditional plants. There is a need for alternative treatment methods. Because of the inefficiency of the treatments, the effluents that are discharged from wastewater treatment facilities continue to have a dark appearance and a high level of toxicity (Vedrenne et al., 2012; Kurniawan et al., 2010; Schiopu and Gavrilescu, 2010; Razarinah et al., 2015).

Nowadays, the decomposition of pollutants by fungi is one of the most inexpensive and environmentally favorable methods among biological processes such as xenobiotic degradation due to their complexity to degradation. The removal or reduction of xenobiotics by the use of the metabolic capabilities of fungi is a promising area of research known as fungal bioremediation. Fungi are a special kind of microbe that is capable of breaking down a broad variety of harmful xenobiotics in a very efficient manner. They exert their effects via extracellular ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, among others. Because of their ability to degrade xenobiotic compounds and generate polymeric byproducts, they are a valuable tool for the process of bioremediation. During the process of fungal remediation, the fungi use harmful substances, even insoluble ones, as a source of nutrients and convert them to more straightforward fragmented forms (Ellouze and Sayadi, 2016).

Moreover, the black color of the leachate is one of the significant issues, as it is difficult to remove and contributes to the formation of stratification problems in bodies of water by their extreme conditions. It is possible to use bioremediation processes for the treatment of the leachate, which are not only more effective but also more cost- and environmentally friendly (Saetang and Babel, 2010; Sudiana et al., 2022).

In the early years, several wastewater treatment solutions depending on multiple physiochemical processes were developed. However, these procedures are less successful and more costly (Lata et al., 2007; Hosseini et al., 2010). The many studies that have been conducted on biological approaches for dye removal have recently acquired prominence as a result of the low cost and environmentally acceptable character of these techniques. As a result of their ability to produce a wide range of extracellular proteins, organic acids, and other metabolites, fungi are regarded as superior biological agents for use in dye removal processes. This is mostly due to the fact that fungi have a greater capacity to adjust their metabolic processes in response to changes in their surrounding environment (Banat et al., 1996; Doğar et al., 2010; Coulibaly et al., 2003). In recent research (Kaushik and Malik, 2010), many distinct strains of the fungus Aspergillus sp. have been used for the elimination of a wide variety of synthetic dyes from a variety of different substrates.

The region of Iraqi Kurdistan, like other parts of the world, faces problems with the collection of waste materials and the management of landfills due to improper methods of waste disposal. The landfills in Kurdistan, particularly those in the province of Erbil, such as the one in Soran, are categorized as level II due to the lack of gas and leachate collecting systems. Because it does not have an environmentally engineered solution for these issues, the Soran City landfill has the potential to always cause serious pollution to the environment. This is accomplished by releasing leachate and gases into the environment's water, soil, and air in significant quantities. Biologically hazardous items, such as waste from households and slaughterhouses, are routinely dumped at this landfill (Aziz and Mustafa, 2018; Gardi, 2017).

The landfill in Soran City is responsible for all aspects that are linked to ecologically hazardous areas. Because of this, it was absolutely necessary to conduct an investigation into the levels of treatment of leachate which could pose a health risk to people working at the landfill, residents of the surrounding areas (less than 700–800 meters away), and domestic animals that feed and drink water within a range of meters of the dump. The contamination of both surface and subsurface water is the most obvious cause for worry due to the fact that the leachates are promptly dropped into the river that is nearby.

The aim of this study is to the evaluation of the airborne fungal spores and the capacity of the autochthonous fungi for removing leachate color and lowering TDS, EC, and PH which leak from the Soran city landfill into the Kawlokan river. This work is interesting because it utilizes a biological source for the decolorization of dyes, which is an eco-friendly technique to overcome the hazardous consequences of textile pollution. As a result of this, the study has the potential to make significant contributions.

METHOD AND MATERIALS

Study area

The study area is Soran City Landfill (SCL). Soran City is the boundary city of the capital of the Kurdistan region of Iraq. SCL is located between Soran City and Rwanduz City, about 700-800 m away from the Kawlokan river. The area of the landfill is about 61702 m² (Figure 1). The average age of SCL is 12 years, and the average daily dumping of waste is more than 125 tons. The type of the waste dumped at this area include home wastes, marketing waste, hospitals waste, electronic wastes, and waste water from septic tanks of home. The term "landfill leachate" refers to the contaminated aqueous effluent that is produced by the landfill as a result of rainwater seeping through the landfill, the presence of water in

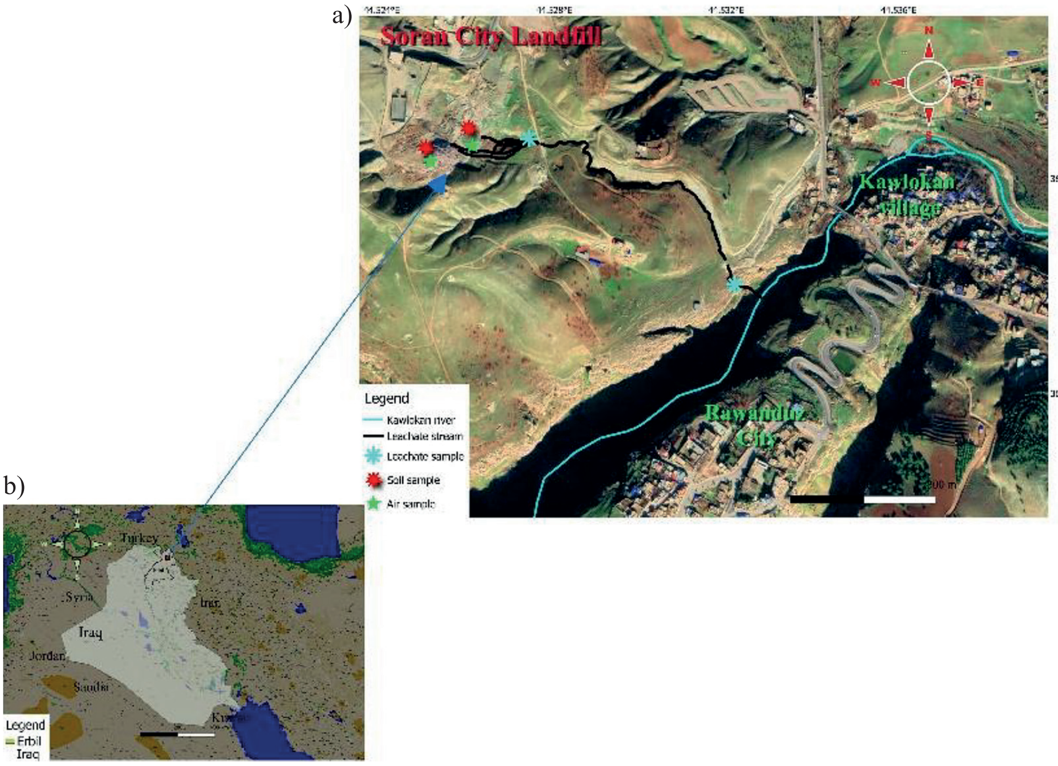

Figure 1. Location map of the study area sites and layout of the sampling points in Soran City Landfill, Erbil, Iraqi Kurdistan region. a) Study area detailing the sampling points in the landfill; b) Map of Iraq and Erbil Province. The black meandering line is actually the leachate stream as its flows from landfill sites into the Kawlokan river

the garbage that has been placed, surface water runoff, and biological decomposition. The SCL leachate is leakage from the landfill and made into a stream till enters the Kawlokan river.

Sample collection

For the purpose of isolating different types of fungi, the two soil samples were collected at Soran city landfill and one sample from Zozik mountain. The leachate samples were collected from the base of the landfill and from the stream of leachate that is 700–800 m long near the Kawlokan river. The fungi from the air of the landfill were isolated by putting 4 plates with 20 ml PDA medium on some place higher than the landfill surface by 30 cm and left them open for 20–30 minutes at two different points. The same procedure was done to isolate fungi from Zozik mountain. Table 1 was shown the details of the sample locations. Clean zip plastic bags were used for soil samples and clean plastic bottles were used for leachate collection. All collected samples were stored under 4 °C at the biogeoscience department-scientific research center from Soran university for further analysis, and the cultured plates for the isolation of fungi from air were directly incubated at 30 °C for 5–7 days. Figure 2 shown the experimental design of the study.

Table 1. Sample details

Types of samples	Location of samples	Altitude
Soil	On the surface of Soran city landfill site	696 m
Soil	At the bottom of Soran city landfill from surface soil	678 m
Soil	From Zosk mountain, away from urbanization area as a soil control sample	1226 m
Air	On the surface of Soran landfill site	696 m
Air	At the bottom of Soran landfill site	678 m
Air	From the Zosk mountain, away from urbanization area as an Air control sample	1226 m
Leachate	From the base of the landfill	664 m
Leachate	From the stream at 700-750m away from the landfill	613 m

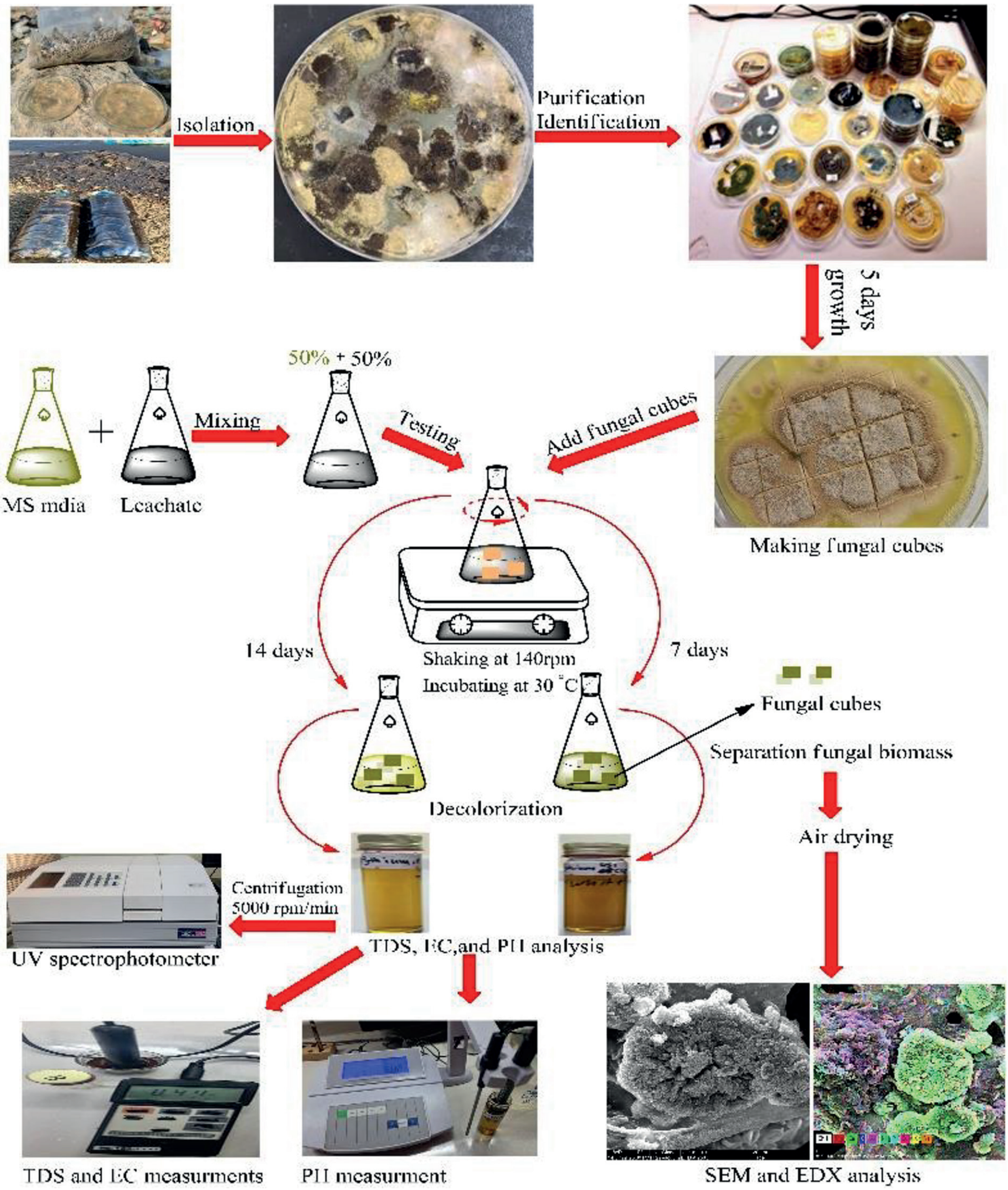

Figure 2. Graphical experimental design of the study

Isolation of fungi

The dilution method was used for the isolation of fungal species from soil and leachate. Four dilution factors (1:10) were applied and then transferred 100 µl from stock and all dilution factors onto the PDA medium which was supplemented with two types of antibiotics (chloramphenicol (50 mg/L) and streptomycin (30 mg/L)) for inhibition of the growth of the bacteria. The cultures were incubated at 30 °C for 5–7 days. After the colonies were formed, each colony was cultured on the new PDA to get a pure culture.

Identification of isolated fungi

The isolated fungi were identified according to the morphological characterization of the growth colonies and microscopic characterization of the fungal structures. Microscopic characterization of the isolated fungi was detected by using methylene blue to stain the fungal slides under the reflected and transmitted polarizing light microscope (model; Leica DM2700 P, Germany).

Characterization of the leachate sample

Chemical and physical characterizations of the leachate sample were done by measuring the pH, TDS, and EC parameters, and K, P, Fe, Na, Nb, Sn, Zr, S, Al, Rb, Ca, and Mg elemental content. The pH of the leachate samples was determined using a PHS-3BW Microprocessor PH/mV/temperature meter. TDS and electrical conductivity (EC) were measured by a conductivity meter (model; RS180-7127). The samples were air dried and homogenized, then the dried samples were crushed with a clean mortar and pestle and then sieved using a 0.65-micron sieve and analyzed for elements by inductively coupled plasma mass spectrometer (ICP-MS).

Assessing fungal interactions with the leachate

Fungal species have the ability to remove color from wastewater. Leachate is very dark blackish water that comes out of a landfill as the result of the biodegradation of solid waste material by microbes inside the landfill (Saetang and Babel, 2010; Bodzek et al., 2006; Di Maria and Sisani, 2017). In this study, different fungi species were tested for decolorization of the leachate. The experiment was done inside the 250 ml conical flask, which contained 200 ml of 50% of the MSM and 50% of the sterilized leachate. The composition of the MSM was NaCl (0.1g/l), KNO_3 (0.5 g/l), NH_4Cl (0.1 g/l), KH_2PO_4 (0.5 g/l), K_2HPO_4, (1.5 g/l), $MgSO_4$ (0.2 g/l), $FeSO_4.7H_2O$ (0.02 g/l), $CaCl_2.2H_2O$ (0.05 g/l), $CuSO_4$ (0.02 g/l), Glucose (0.4 g/l), Peptone (0.4 g/l), Yeast extract (0.4 g/l), Chloramphenicol (0.05 g/l), and Streptomycin (0.03 g/l) after modified from (Mishra and Malik, 2012). 18 isolated fungal species were tested. The flasks were inoculated with the three cubes discs (1–2 cm^2) of the 5 days cultured fungi. One flask was prepared as a control without inoculation with fungi. The flasks were held on the shaker at 140 rpm for 14 days at 30 °C. After 7 days of incubation, 60 ml from each flask was taken for further analysis, and it was repeated after 14 days of incubation (Figure 2).

Alteration of pH, TDS, and EC parameters

The pH, TDS, and EC of the treatment leachate sample were determined at 0 days, 7 days, and 14 days of the incubation to know the effect of fungi on them. The pH of the leachate samples was determined using a PHS-3BW Microprocessor PH/mV/temperature meter. TDS and electrical conductivity (EC) were measured by a conductivity meter (model; RS180-7127).

Leachate color removal efficiency

In order to determine the percentage of the removed color from the MSM contained leachate, the absorbance was read from each flask at 0 days, 7 days, and 14 days of incubation. The UV spectrophotometer (model; CE9500, Cecil Instruments) was used to determine the absorbances of the samples at the maximum absorbance was got at all the samples, and the control was used as blank. For detection of the absorbance, 2 ml of the media were centrifuged at 5000 rpm for 1 minute. Equation 1 was used to find the percentage of the decolorization according to the previous studies (Mathur et al., 2015).

$$D\,(\%) = (Ai - Af)/Af \times 100\% \quad (1)$$

where: D (%) – decolorization percentage;
Ai – the initial absorbance of the sample;
Af – the final absorbance of the sample.

Metal biosorption and biomineralization

A scanning electron microscope (FEI Model QUANTA 450) was used for imaging air-dried fungal biomass after it was separated from leachate, silver coated, and examined under the SEM for observation of the biominerals, their shapes, and the encrustation of fungal hyphae with metals and nanocrystals. The Energy Dispersive X-Ray Analysis (EDX) detector, Esprit (Bruker Nano Berlin, Germany) fitted to the microscope at an accelerating voltage of 25 kV, was used for elemental analysis and composition of biominerals.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software was used to analyze the frequency and percentage of each isolated species from the samples in this study.

RESULTS

Isolation and identification of fungi

The total number of isolated fungi in this study was 174. 153 isolated fungi were related to the samples collected from landfills (soil, air, and leachate). 21 isolates were observed from samples collected from Zozik mountain (soil and air) (Table 2). The isolated fungi were identified following the atlases of mycology and previous

Table 2. Isolation of fungi from samples of landfill

Name of fungi	Isolation of fungi from samples of landfill								Isolation of fungi from samples of Zozik mountain			
	Soil		Air		Leachate		Total number of fungi		Control samples		Total number of fungi	
	SS1	SS2	AS1	AS2	LS1	LS2	Isolated species	Total isolates	SCS	ACS	Isolated species	Total isolates
Aspergillus niger	7	6	3	4	1	1	22		2	1	3	
Aspergillus fumigatus	5	4	6	3	3	2	23		1	1	2	
Aspergillus terreus	2	1	1	0	1	0	5		0	0	0	
Aspergillus flavus	4	5	3	1	3	1	17		1	0	1	
Aspergillus pseudoelegans	2	0	0	0	0	0	2		0	0	0	
Aspergillus elegans	0	3	0	0	0	0	3		0	0	0	
*Penicillium sp.*1	3	2	3	2	2	1	13		3	2	5	
*Penicillium sp.*2	4	1	0	1	2	1	9		2	2	4	
*Penicillium sp.*3	1	2	0	0	0	0	3		0	0	0	
Rhizopus sp.	3	5	2	4	2	3	19	153	3	2	5	21
Alternaria sp.	2	0	1	3	0	0	6		0	0	0	
Mortierella sp.	3	3	2	1	0	0	9		1	0	1	
Trichoderma harzianum	2	0	0	0	0	0	2		0	0	0	
Ulocladium sp.	0	1	0	0	0	0	1		0	0	0	
Scedosporium apiospermum	2	3	1	2	0	2	10		0	0	0	
Geotrichum sp.	5	1	0	0	0	0	6		0	0	0	
Unknown fungi 1	2	0	0	0	0	0	2		0	0	0	
Unknown fungi 2	0	1	0	0	0	0	1		0	0	0	

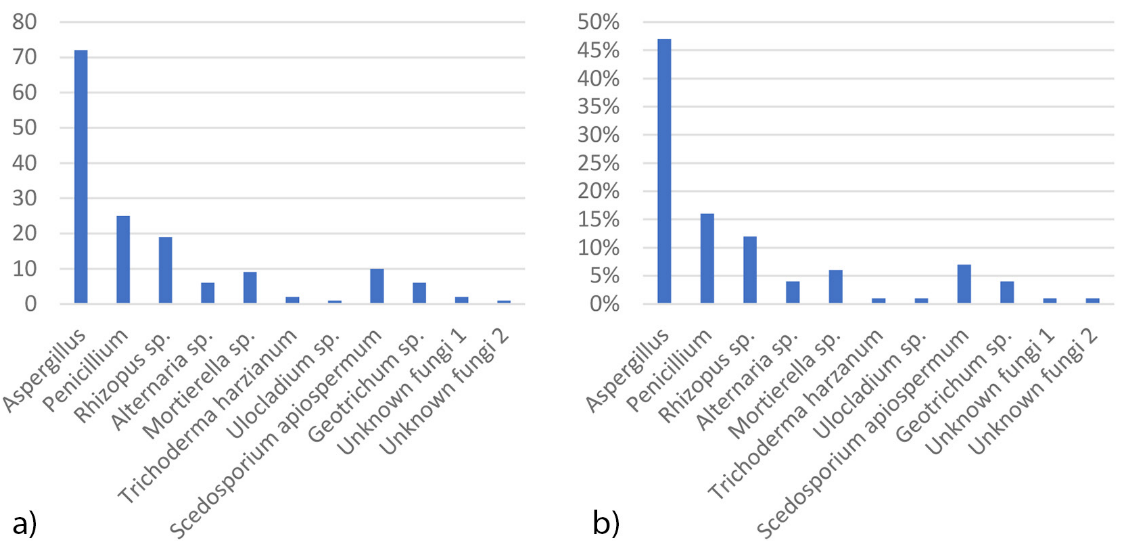

Figure 3. (a) Frequency and (b) percentage of fungal species observed from landfill samples

studies based on the colony morphologies and microscopic characterizations (Ahmed et al., 2022; Frisvad et al., 2004; Sciortino Jr, 2017; Alsohaili and Bani-Hasan, 2018; Eltariki et al., 2021; Nguyen et al., 2019; Visagie et al., 2013; Watanabe, 2002; Chiang et al., 2014; Di Teodoro et al., 2020; Campbell and Johnson, 2013; Grano-Maldonado et al., 2021).

The statistical analysis, Figure 3, shows 47% of the isolates from Soran City landfill sites belong to the genus of Aspergillus whose frequency was 72 and contained different species such as *Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus, Aspergillus pseudoelegans,* and *Aspergillus elegans,* and 16% and 12% were species of Penicillium and Rhizopus whose frequencies were 25 and 19, respectively. Other studies have shown that aspergillus and penicillium were the dominating species in landfills (Zegzouti et al., 2020). The species of penicillium were divided into three different species. Other isolates were classified under different genera and species, *Alternaria sp., Mortierella sp., Trichoderma harzianum, Ulocladium* sp.*, Scedosporium apiospermum,* and *Geotrichum* sp. (Table 2). Two isolates that were unknown were mold and both had conidia (Figure 5).

In samples of Zozik mountain, the frequency of the species was different from the samples of the landfill site (Figure 4). 43% of the isolates from Zozik mountain belong to the genus of Penicillium, whose frequency was 9 and contained different species as mentioned by numbers as shown in Table 2. 29% and 24% were species of Aspergillus and Rhizopus, whose frequencies were 6 and 5, respectively. Another isolate was classified under the genus of *Mortierella* sp. (Table 2).

Characterization of leachate sample

The pH value of the leachate sample was 8.93, meaning the leachate sample of the Soran city landfill was alkaline, which was considered as an indicator of the mature state of the landfill. EC and TDS are important parameters that reflect the degree of salinity and minerals of the leachate sample. EC and TDS values exhibited obvious values for the leachate samples were >20000 µS and >12800 mg/l, respectively. The rise in the EC value is related to the increase of the anion and

Table 3. Measured parameters of the leachate sample used in the experiment

Parameters	Values
pH	8.93
EC	>20000 µS
TDS	>12800 mg/l
Na	>10% (1% = 10000 ppm)
Ca	27829 ppm
K	23479 ppm
Mg	10896 ppm
Al	2523 ppm
Fe	3162 ppm
Nb	2.5 ppm
P	843 ppm
Rb	2 ppm
S	7421 ppm
Sn	6.8 ppm
Zr	8 ppm

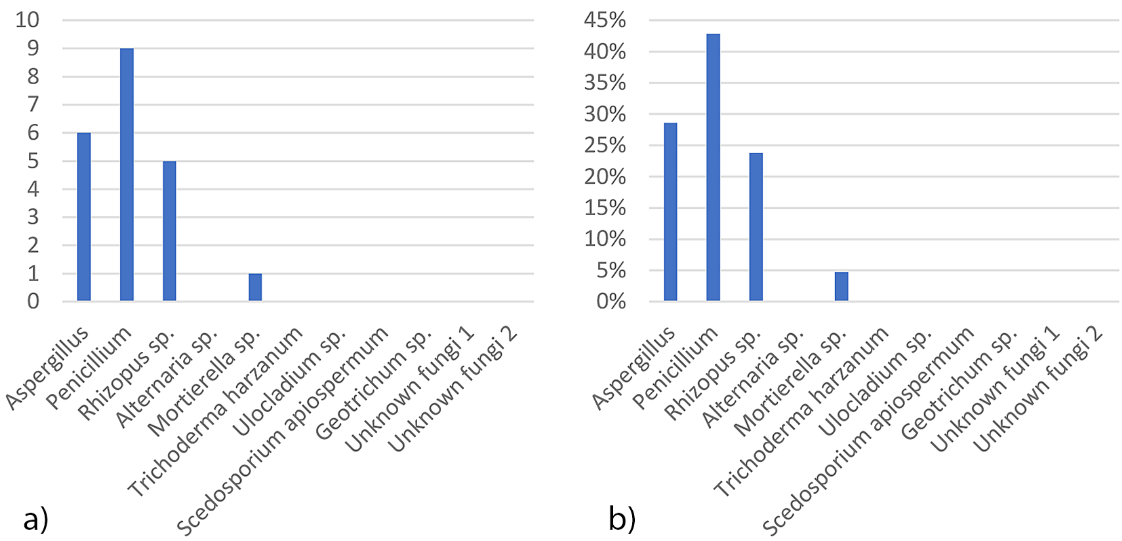

Figure 4. (a) Frequency and (b) percentage of fungal species observed from Zozik mountain samples

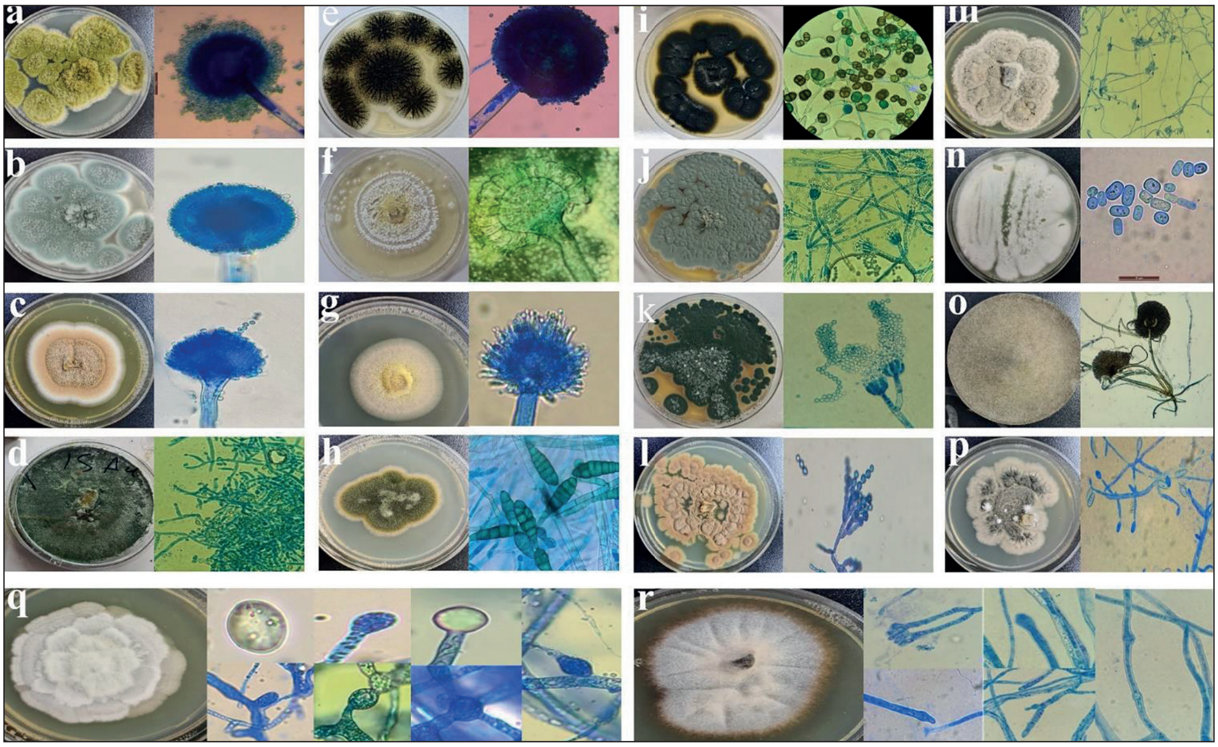

Figure 5. Identification of the observed fungi. Right images show morphology of the fungal culture after 4–5 days growth. Left images show microscopic characterization of fungal structures. a-*A. flavus*, b-*A. fumigatus*, c-*A. terreus*, d-*Trichoderma harzianum*, e - *A. niger*, f-*A. pseudoelegans*, g-*A. elegans*, h-*Alternaria* sp., i-Ulocladium sp., j-*Penicillium* sp.1, k-*Penicillium* sp.2, l-*Penicillium* sp.3, m-*Scedosporium apiospermum*, n-*Geotrichum* sp., o-*Rhizopus* sp., p-Unknown fungi 2, q-*Mortierella* sp., r-Unknown fungi1

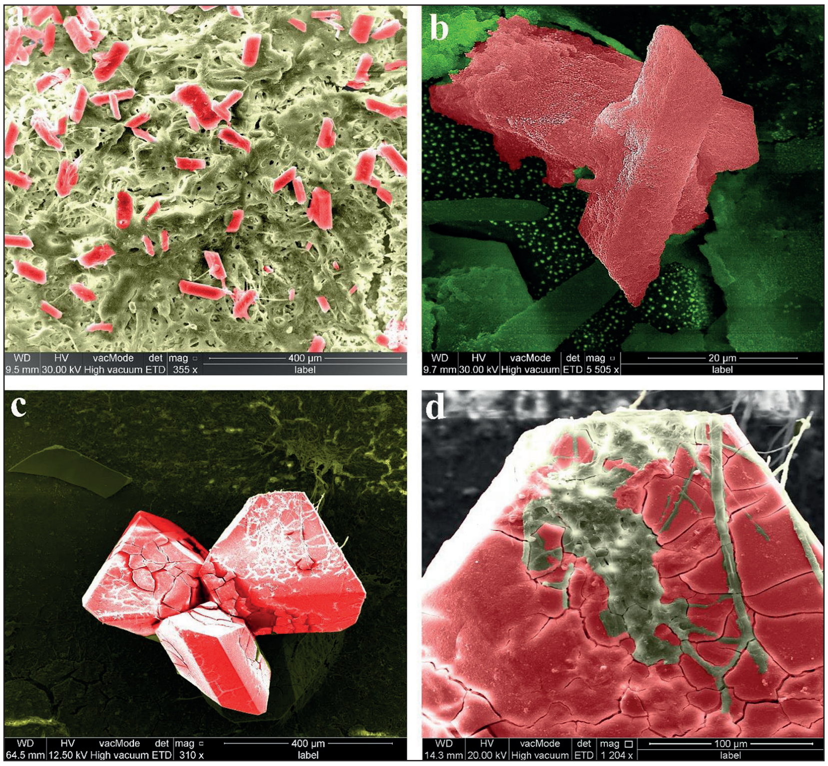

Figure 6. SEM micrograph images of fungal biomass showing biominerals (Figs. a-d) formed during the experiment. Both images are from the same sample. The crystals of fungal induced biominerals are shown in pseudo red color and are composed of mainly magnesium (Mg) and phosphate (P) based on EDX analysis. In Fig. 6d is clearly seen fungal hyphae colonizing the surface of these newly formed biominerals

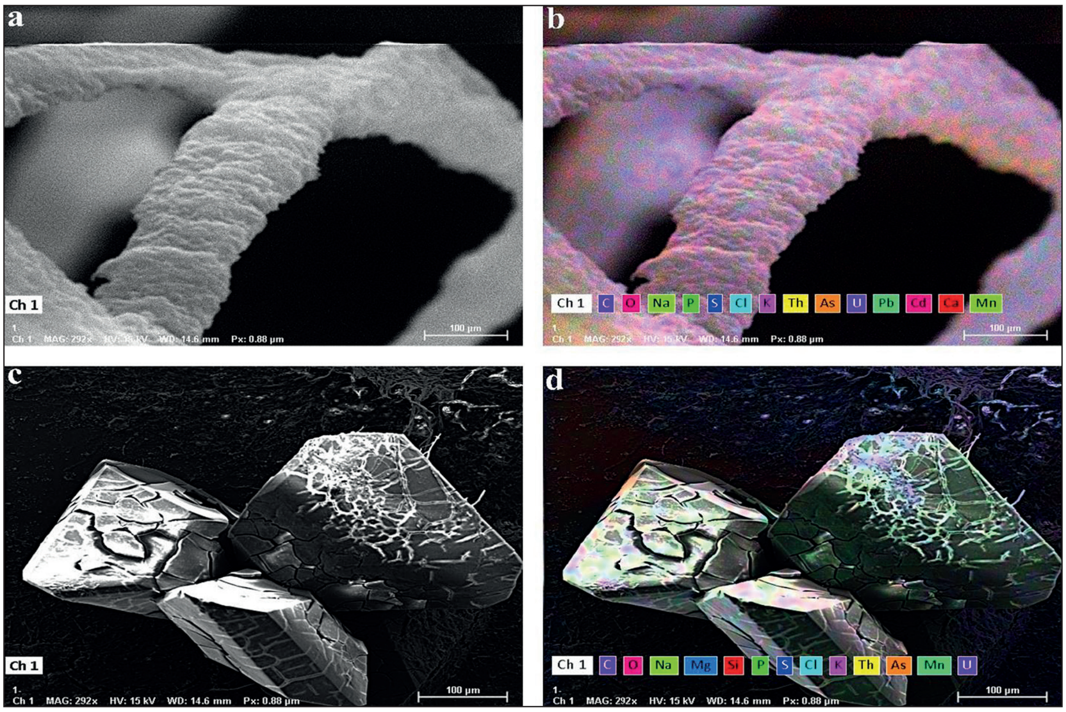

Figure 7. SEM-EDX images of: (a, b) densely encrusted fungal hyphae with biominerals formed due to interaction of the fungal mass with the various metals within the leachate. The nano-size encrusting minerals are totally covering the hyphae. This process involves immobilization and sequestration of metals from the leachate and thus environmental bioremediation. Figs. c, d are non-encrusting and freely formed biominerals in treated leachate. Fig. 7b and Fig. 7d are showing the elemental mapping of the immobilized elements within those biominerals

cation. The chemical composition of the leachate from the landfill was determined by ICP mass. The compounds with high concentrations are Na, Ca, K, Mg, S, Fe, and Al, whose concentrations are >100000 ppm, 27829 ppm, 23479 ppm, 10896 ppm, 7421 ppm, 3162 ppm, and 2523 ppm, respectively. Other elements shown in Table 3 were classified as minor elements whose concentrations were less than 1000 ppm (Xaypanya et al., 2018; Baettker et al., 2020; Siddiqi et al., 2022).

Geoactivities of autochthonous fungi

The result showed that there are a large number of biominerals were formed during the experimental period. Because there is a high concentration of elements in the leachate sample, as shown in Table 3, the mineral nucleation was grown to a large mineral. The biomineralization was induced by fungal surface and excretions (organic acids) (Kolo and Claeys, 2005). The SEM showed a very detailed images of the biominerals and the hyphal encrustation phenomena (Figure 6). EDX analysis was used to detect the composition of the minerals that formed in the treatment leachate. The EDX was shown the types of those elements were bound to the ligands of the hyphal surface, as shown in Figure 7 as an example for biosorption of toxic elements such as cadmium and lead, and some other elements such as sulfur, phosphor, zirconium, tin, magnesium, and chlore (Zahmatkesh et al., 2016).

Effect of fungi on EC, TDS, and pH

Table 4 show that the EC and TDS decreased after fungal treatment. Following a 7-day incubation, EC and TDS were 13120 µS/cm and 8397 in the control flask, while the EC and TDS values were decreasing inside all treatment flasks with fungi at different levels. The decreasing range of the fungal treatment was between 9230 µS/cm and 5907 mg/l with *A. fumigatus* to 7110 µS/cm and 4550 mg/l with unknown fungi 2. The EC and TDS values were increased in all flasks after 14 days of incubation compared to the 7 days of incubation except the flask with *A. pseudoelegans* and *A. elegans* were decreased the EC and TDS values. The range of EC value after 14 days incubation was between 9660 µS/cm and 6182 mg/l with *A. fumigatus* to 7440 µS/cm and 4762 mg/l with *S. apiospermum.* While the EC and TDS

Table 4. Changes in the leachate parameters after treatment with fungi

Fungi	7 days of incubation			14 days of incubation		
	EC (µS)	TDS (mg/l)	pH	EC (µS)	TDS (mg/l)	pH
Control	13120	8397	8.5	13170	8429	8.48
Penicillium sp.1	8870	5676.8	7.5	9550	6112	7.89
Penicillium sp.2	8040	5145.6	7.28	8440	5401.6	7.55
Geotrichum sp.	9180	5875.2	5.8	9450	6048	6.32
Trichoderma harzianum	8850	5664	6.9	9370	5996.8	7.48
Unkmown fungi 1	8370	5356.8	7.25	9020	5772.8	7.85
Mortierella sp.	8810	5638.4	7.21	8920	5708.8	7.69
Alternaria sp.	8450	5408	7.04	8850	5664	7.86
Rhizopus sp.	7540	4825.6	7.19	8110	5190.4	8.3
Ulocladium sp	8170	5228.8	7.03	8700	5568	7.42
Scedosporium apiospermum	7510	4806.4	7.15	7440	4761.6	7.36
Penicillium sp. 3	7430	4755.2	7.14	7880	5043.2	7.37
Unknown fungi 2	7110	4550.4	7.25	8150	5216	7.3
A.fumigatus	9230	5907.2	6.66	9660	6182.4	7.15
A.niger	8230	5267.2	7.24	8840	5657.6	7.41
A.terreus	8700	5568	6.82	9360	5990.4	7.17
A.flavus	8100	5184	7.48	8700	5568	7.76
A.pseudoelegans	8740	5593.6	6.14	8080	5171.2	7.96
A.elegans	8770	5612.8	7.76	5590	3577.6	7.93

values with *A. elegans* were less than the range, which was 5590 µS/cm and 3578 mg/l.

The pH value during the period of experiment was alkaline and it was between 8.48–8.5. The pH values for treatment samples after 7 days of incubation became acid to neutral and were between 5.8 with *Geotrichum* sp. and 7.76 with *A. elegans.* While the pH of the samples after 14 days of incubation was increased to become neutral and the range of pH value was between 6.32 for *Geotrichum* sp. and 8.3 for *Rhizopus* sp. (Table 4).

Decolorization of leachate with fungi

Decolorization of *Aspergillus* species, *Penicillium* species, and the other native fungi were presented in Figure 8. The color of the leachate is black before treatment, but after treatment using autochthonous fungi, there is a change in color from black to almost yellow color or the colorless nature of the wastewater. *Aspergillus* species were found to be the best decolorizer fungi in this study, as it also found in previous study (Mathur et al., 2015). The range of the decolorization percentage for *Aspergillus* species was between 15.3% and 79.3% after 7 days of incubation and it was increased to 59.5–92.9% after 14 days of incubation. In *Aspergillus* species, *A. flavus* was the best decolorizer fungus, which was removed color by 92.9%. The percentage of decolorization of the *Penicillium* group was between 4.65% and 23.4% after 7 days of incubation and it was increased to 30.8–58.9% after 14 days of incubation. Within penicillium species, *Penicillium* sp.1 has the highest level of decolorization by 58.9%. Other isolated fungi that have a high percentage of decolorization after 14 days of incubation were *Geotrichum* sp., *Unknown fungi* 1, *T. harzianum, Mortierella* sp., and *Alternaria* sp.; that they removed color by 82.2%, 74.3%, 73.9%, 66.6%, and 55.4%.

DISCUSSION

Air contamination with fungal spores

Municipal wastes are suitable substrates for the growth of different fungi that can release their conidia and mycelium particles into the air, which can lead to serious health threats to humans, especially to landfill workers and local residents (Frączek et al., 2017; Gołofit-Szymczak et al., 2019). In this study, 12 fungal species were isolated and identified from air samples. The isolated fungi were classified under the phylum of Ascomycota and Zygomycota. The species of fungi

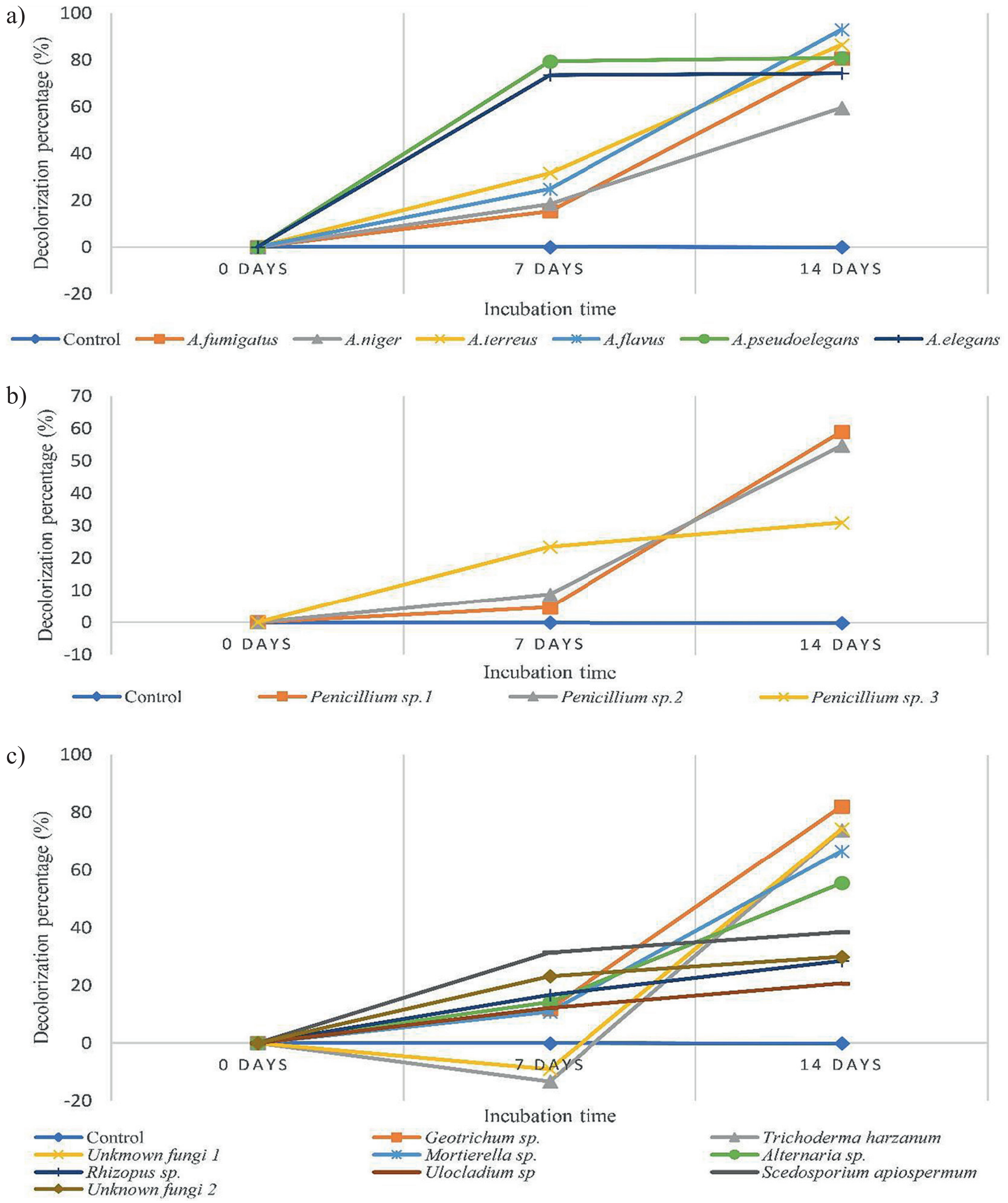

Figure 8. Decolorization percentage of treatment culture by fungi. a) Decolorization percentage of *Aspergillus* species, *A. flavus* has the highest percentage of color removal from culture treatment; b) Decolorization of *Penicillium* species, *Penicillium* sp.1 has the highest percentage of color removal among *Penicillium* species from culture treatment; c) Decolorization percentage of other isolated fungi, *Geotrichum* sp. has the third highest percentage of color removal from culture treatment after *A. flavus* and *A. terreus*

isolated from the landfill air samples were *Rhizopus* sp., *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Scedosporium apiospermum, Mortierella* sp., *Penicillium* sp.1*, Penicillium* sp.2*, Penicillium* sp.3*, and Alternaria* sp. The species identified from the Zozik mountain air sample were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mortierella* sp.*, Penicillium* sp.1*, and Penicillium* sp.2. The previous studies were observed those species of fungi from the landfill air are (Odonkor and Mahami, 2020; Schlosser et al., 2016; Madsen et al., 2016; Breza-Boruta, 2012; Li et al., 2021; Kalwasińska et al., 2014). This difference in the frequency of the species from both locations indicated the contamination of the air at the Soran city landfill because of the dumping of waste materials at the site that becomes a health risk for those people who live near the dumping site. Most of the isolated fungi are strong pathogens. *Aspergillus* *flavus* and *Aspergillus fumigatus* were observed with high frequency in the landfill air samples, which are the most dangerous species of aspergillus that cause severe infections related to aspergillosis infections. The spores of these species can cause bronchopulmonary hypersensitivity, which manifests itself with asthmatic spasms, fever, and general malaise, especially in Asia, the Middle east, and Africa due to the ability of these two species of fungi to survive in hot and climatic conditions compared to other species (Li et al., 2022).

Fungi as geoactive agents

Alteration of leachate pH, EC, and TDS parameters

Nutrients and heavy metals, among other chemical components, have varying degrees of solubility and biological availability depending on the water's PH. Metals may oxidize in water with a low pH. High levels of alkalinity in the water make it taste bad and leave deposits on plumbing and other water-using equipment. Toxic metals are more soluble under acidic conditions (Islam et al., 2017). The Environmental Protection Agency recommends against drinking water that is too acidic or alkaline (EPA). The EPA specifies that the pH range of safe drinking water is 6.5 to 8.5 (Hendrickson, 2017). In this study, the pH of the leachate was 8.48–8.5, which indicated the alkalinity of the environment and the mature phase of the landfill. Biological methods for the treatment of wastewater are known to the main methods for cleaning the water. Fungal geoactivities were considered in the bioremediation applications (Banat et al., 1996; Doğar et al., 2010; Coulibaly et al., 2003). Even so, the medium contained two strong buffers (KH_2PO_4 and K_2HPO_4) which strongly prevented the decrease of the pH. The results showed that the fungi were able to decrease the high pH to a neutral range due to the production of organic acid by the fungi as shown in Table 4. The best fungi to decrease the pH was *Geotrichum sp.* which was at 5.8 after 7 days of incubation. However, the pH was increased slightly at 14 days of incubation, as shown in Table 4. Several processes contribute to increase pH, including the formation of ammonia from decaying organic matter, the dissolving of solid Mn and Fe oxides under reducing circumstances, and the ligand exchange that occurs when organic anions replace the terminal OH^- of hydroxy oxides (Noble et al., 1996).

In metal-polluted environments, a variety of fungi belonging to all of the genera can still be observed, as well as the capacity to persist and grow in the face of extremely dangerous quantities of toxic metals is regularly seen. The existence of impermeable pigmented cell walls, extracellular polymers of chains of mono and di-saccharides, and organic excretion are examples of advantageous characteristics in which they can determine success. This is especially true in situations where they lead to detoxification of the metal species, such as by binding or precipitation. However, due to the inclusion of several direct and indirect physicochemical and biological factors in the process of survival, separation can be challenging in many instances. In contrast to the environmental modification of toxicity, geoactivities of fungi that may be involved in fungal survival include extracellular precipitation, complexation, and crystallization; transformation of metal species redox reactions, methylation, biosorption to cell walls, pigments, as well as extracellular polysaccharide; decreased transport or impermeability; efflux; intracellular compartmentation; precipitation; and/or sequestration (Ross, 1975; Gadd and Griffiths, 1977; Mehra and Winge, 1991).

In this study, the EC and TDS values decreased after 7 days of incubation (Table 4). The decreasing of EC and TDS is related to the biological mechanism of fungi to modify the toxic elements in the leachate by a biomineralization process. The biomineral was formed by inducing the environment by fungal excretion as organic acids such as oxalate acid. The result showed biominerals were formed inside most of the flask containing fungal cubes and the control did not contain minerals. The shape of the biomineral is shown in Figure 6, and the composition of the mineral was detected by EDX analysis as shown in Figure 7. Another reason for decreasing EC and TDS values from treatment flasks was related to the biosorption process, which was done on surface of fungal hyphae as shown in Figure 7, and some types of metals, including toxic heavy metals, were detected by EDX analysis on the surface of fungal hyphae, leading to their becoming encrusted with metals. An example of the highly toxic heavy metal was remediated in this study was cadmium that attached to the hyphal surface (Figure 7). Both mechanisms are areas of rising biotechnological interest due to the fact that the removal of potentially hazardous and/or valuable metals and radionuclides from polluted effluents can lead to detoxifying prior to their release to the environment. This can be environmentally beneficial (Gadd, 1988; McEldowney, 1990). Due to a rise in biosorption capabilities with rising pH up to the optimal pH value and thereafter a drop at higher pH values, the capacity for biomineral formation improved over the incubation period. A pH around 4.5–5.0 was ideal for the removal efficiency of metals that Metal-binding occurs at this optimal pH value as a result of electrostatic interactions between cationic species and the negatively charged biomass functional groups (Kuyucak and Volesky, 1988). When the pH was low, cell wall ligands strongly bonded to H_3O+ hydronium ions, creating a repulsive force that prevented metal cations from reaching the cell. A rise in pH would cause an increase in the number of exposed ligands, all of which would be negatively charged, attracting positively charged metallic ions, which would then bind onto the cell surface (Brady and Duncan, 1994). The quantities of TDS and EC were raised to a little higher level since it is possible that metal precipitation is responsible for the reduction in absorption that occurs at significantly higher pH (Brady and Duncan, 1994; Beveridge and Murray, 1980; Beveridge, 1989).

Leachate color removal (Decolorization)

The leachate contained a large amount of the organic substance as a result of biodegradation inside the landfill. The organic substances such as humic acids and fluvic acid are responsible for the dark color of the leachate (Bhalla et al., 2013; Aziz et al., 2007). In this study, the fungi were able to remove color from the leachate. After 7 days of incubation, the percentage of decolorization occurred in a range of 4.6–79%. This occurred due to the organic acids secreted by the fungi. While the percentage of decolorization after 14 days of incubation was found to increase to a range of 20.5–92.9%. The significant increase in removal color may be due to the attack of humic and fluvic substances by fungi as a source for uptaking nutrients. This causes the modification in the chemical structures of both of them (Ramli et al., 2021).

CONCLUSIONS

The Soran City Landfill is a possible source of fungal spores, and some of them have the potential to be harmful to humans. Even though many of the native fungi in garbage are pathogens, the vast majority of them are useful in bioremediation operations. Leachate coming from the landfill is a significant risk for environmental pollution. The remediation of this blackish liquid is a difficult task because of its harsh conditions, such as high TDS and EC, high concentrations of toxic elements, and high pH. This leads to the fact that landfill leachate poses a significant alarm to the development of environmentally friendly practices that are sustainable for treatment of the leachate. In light of this fact, the purpose of the research is to examine the isolating and identifying of autochthonous fungi as well as the use of these fungi for the treatment of leachate. We have shown that the geoactivities of the indigenous fungus have the ability to decolorize the leachate, reduce the level of total dissolved solids and electrical conductivity, and bring the PH of the leachate closer to neutral. Based on the findings of the research, it was determined that fungi are a useful tool for the remediation of hazardous components and possess the potential to cause biomineralization in their environments.

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