

Chromium, Cadmium, and Copper-Resistance and Removal Potential of Bacteria and Fungi Isolated from Two Bryophytes' Surfaces and Substratum

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Abstract

Bryophytes are known to accumulate heavy metals on their surfaces by biosorption; hence, it is probable that microorganisms inhabiting their surfaces may be metal-tolerant. Bacteria and fungi were therefore isolated from the surface and substratum of two mosses, *Polytrichum commune* and *Tortula muralis*, used as test bryophytes, using nutrient agar (NA) and malt extract agar (MEA). The isolates were initially identified by standard morphological and biochemical methods and subsequently screened for metal-resistance in NA and MEA containing 100 mg/L Cr and Cd, and Cu 600 mg/L. Metal-tolerance index (MTI) was determined in nutrient broth containing 300 mg/L Cr and Cd, and MEA containing 800mg/L for Cu. Isolates with MTI >0.50 for the three metals were selected, identified by molecular methods, and tested for minimum inhibitory concentration (MIC) and removal of Cr, Cd, and Cu from aqueous media. The selected isolates were tested for MTI using combined concentrations of Cr, Cd, and Cu. The isolates' identities were confirmed as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus mirabilis*, and *Aspergillus oryzae*, and their MICs (mg/L) were: Cr, 350-550; Cd, 300-350; and Cu, 650-750. Metal removal from the aqueous media by the four organisms was 14.06-77.28% with significant differences by metal ($F=4.40-631.40$; $P=0.037-0.000$) and by organism ($F=122.90-541.80$; $P=0.000$). The MTI of the combined metals was low (<0.50), and it declined with increasing concentrations. *Pseudomonas aeruginosa* tolerated the highest combined concentrations, albeit with 0.10 MTI. Thus, the MIC levels and metal-removal capabilities indicate these organisms as potentially useful for bioremediation of metal-polluted sites.

Keywords: *Polytrichum commune*; *Tortula muralis*, microbial metal-tolerance; chromium; cadmium; copper.

Резюме

Известно е, че бриофитите натрупват тежки метали по повърхностите си чрез биосорбция, следователно е вероятно микроорганизмите, обитаващи техните повърхности, да са металоустойчиви. Поради това изолирахме бактериите и гъбите от повърхността и субстрата на два вида мъхове, *Polytrichum commune* и *Tortula muralis*, използвани като тестови бриофити върху хранителен агар (NA) и агар с малцов екстракт (MEA). Изолатите първоначално бяха идентифицирани чрез стандартни морфологични и биохимични методи и впоследствие скринирани за металоустойчивост в NA и MEA, съдържащи 100 mg/L Cr и Cd, и 600 mg/L Cu. Индексът на металоустойчивост (MTI) беше определен в хранителен бульон, съдържащ 300 mg/L Cr и Cd, и MEA, съдържащ 800 mg/L за Cu. Изолати с MTI > 0.50 за трите метала бяха избрани, идентифицирани чрез молекулярни методи и тествани за минимална инхибираща концентрация (MIC) и за отстраняване на Cr, Cd и Cu от водна среда. Избраните изолати бяха тествани за MTI, използвайки комбинирани концентрации на Cr, Cd и Cu. Идентичността на изолатите беше потвърдена като *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus mirabilis* и *Aspergillus oryzae*, а техните MIC (mg/L) бяха съответно: Cr, 350-550; Cd, 300-350; и Cu, 650-750. Отстраняването на метал от водната среда от четирите организма беше 14.06-77.28% със значителни разлики по метал ($F=4.40-631.40$; $P=0.037-0.000$) и по организъм ($F=122.90-541.80$; $P=0.000$). MTI на комбинираните метали беше нисък (<0.50) и намаляваше с увеличаване на концентрациите. *Pseudomonas aeruginosa* понасяше най-високите комбинирани концентрации, макар и с 0.10 MTI. В заключение, нивата на минимална инхибираща концентрация (МИК) и способността за отстраняване на метали показват, че тези организми са потенциално полезни за биоремедиация на замърсени с метали места.

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Introduction

Naturally, heavy metals are present in the environment, but anthropogenic activities have increased their concentrations above acceptable limits, thereby causing harm to man and his environment (Muhammad *et al.*, 2011; Ali *et al.*, 2019). The anthropogenic activities that increase heavy metal concentrations include mining, untreated industrial waste waters, the paint industry, agricultural pesticides, fertilizers, and municipal wastes (Liu *et al.*, 2018). Municipal dumpsite wastes such as textiles, automobile brake pads, batteries, electronic waste, and metal scrapings are also sources of metal pollution in the environment (Mokogwu and Ejechi, 2022).

Conventional methods such as chemical precipitation, flocculation, coagulation, solvent extraction, adsorption, complexation, electro-kinetics, membrane filtration, reverse osmosis, electrochemical treatment, activated carbon, ion exchange, and electrodialysis have been employed at an industrial scale for heavy metal removal from effluents (Srivastava *et al.*, 2008; Mohammed *et al.*, 2011; Razzak *et al.*, 2022). However, these conventional methods have setbacks like high cost, high energy consumption rate, and inability to completely remove the heavy metals, thereby generating toxic by-products (Yan and Viraraghavan, 2000; Zafor *et al.*, 2007; Li *et al.*, 2008; Srivastava *et al.*, 2008). This has led to the investigation of the use of agricultural wastes, plants, and microorganisms as alternative eco-friendly approaches for the removal of metals from wastes and the environment (Srivastava *et al.*, 2008; Akpomie and Ejechi, 2016; Crini *et al.*, 2019).

Microorganisms have been extensively investigated for their capacity to remove metals from the environment (e.g. Oyewole *et al.*, 2019; Vacar *et al.*, 2021; Wróbel *et al.*, 2023; Firinca *et al.*, 2024; Tang *et al.*, 2024). Microbes have various mechanisms by which they remove these metals, such as biosorption, extracellular precipitation, uptake, mineralization, enzymatic oxidation, sequestration, production of chelating agents, chemotrophic and autotrophic leaching, methylation, and redox transformations (Njoku *et al.*, 2020; Nnaji *et al.*, 2024). Metal-tolerant microorganisms have often been isolated from various polluted sites as listed earlier for bioremediation and biotechnological applications (Chellaiah *et al.*, 2009; Dahnoun *et al.*, 2024). However, there may be other sources of metal-resistant bacteria that are yet to be explored.

Plant microbiome, especially in the rhizosphere, is known to promote plant growth under a

symbiotic association, and this may be extended to detoxification of heavy metal pollutants according to the reviews by González and Ghneim-Herrera (2021). Thus, it will be worthwhile investigating microorganisms in the plant microbiome for metal-tolerance potential and ultimate use in bioremediation strategies. Bryophytes are a group of plants that are early colonizers of soil and are associated with diversity of bacteria and fungi (Cao *et al.*, 2020). They lack roots but are anchored to the soil by rhizoids, where the rhizosphere-inhabiting microorganisms assist in nutrient cycling and protection of the bryophyte, like the rhizosphere of higher plants (Chen and Nelson, 2022). The organisms involved in nutrient cycling may be metal-tolerant, especially as bryophytes are known for absorption and accumulation of metals from the environment (Tremper *et al.*, 2004; Fasani *et al.*, 2022). Thus, bryophytes are taken to be useful bio-indicators of a metal-polluted environment (Printarakul and Meeinkuirt, 2022). It is therefore not unlikely that microorganisms in the substratum and surfaces of bryophyte gametophytes, where biosorption of metals occurs, may exhibit metal tolerance. Such microorganisms can be potentially useful in biotechnological applications if found to be metal-resistant. Thus, the objectives of this study are to: 1. assess the metal resistance potential of microorganisms present in the substratum and surfaces of bryophytes using two widespread mosses, *Polytrichum commune* and *Tortula muralis*, with Cu, Cd, and Cr as test metals; and 2. ascertain the extent to which the isolates can remove these metals from aqueous medium.

Materials and Methods

Collection of bryophytes samples

Gametophytes and substratum materials of *P. commune* Hedw and *T. muralis* Hedw were collected from six locations in three towns (Abraka, Obiaruku and Warri) located in the Niger Delta region of Nigeria. The sources were: abandoned deteriorating building blocks on the soil, stone surfaces and roadside soil for *P. commune* (Fig. 1); and deteriorating painted and unpainted walls for *T. muralis* (Fig. 2.). The gametophytes were gently removed manually using sterile hand gloves and scalpel and kept in paper bags made of newspaper in accordance with the procedure of Buck and Thiers (1996). Their identities were confirmed in the Botany Department of Delta State University, Abraka, Nigeria.

Isolation and identification of microorganisms

The gametophytes and the adhering substratum materials were placed in deionised water

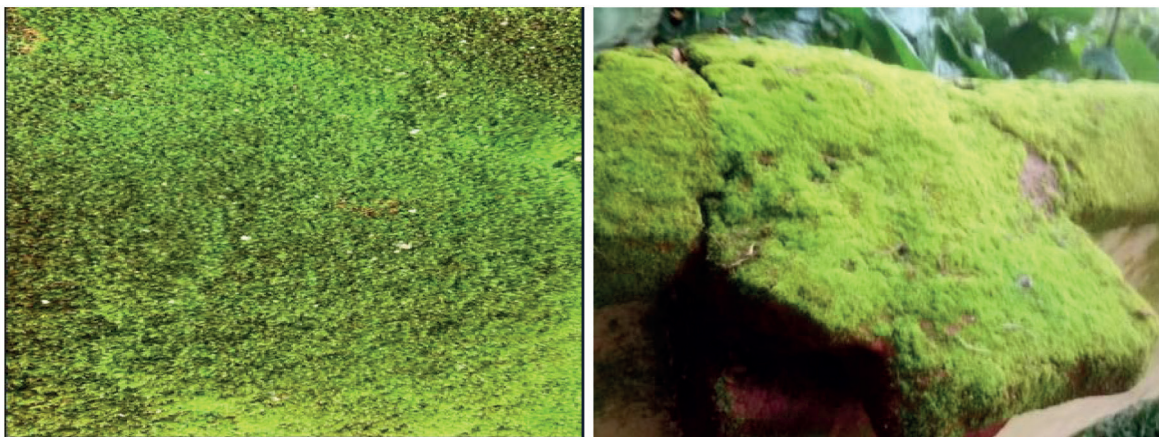


Fig. 1. Images of *P. commune* on roadside soil

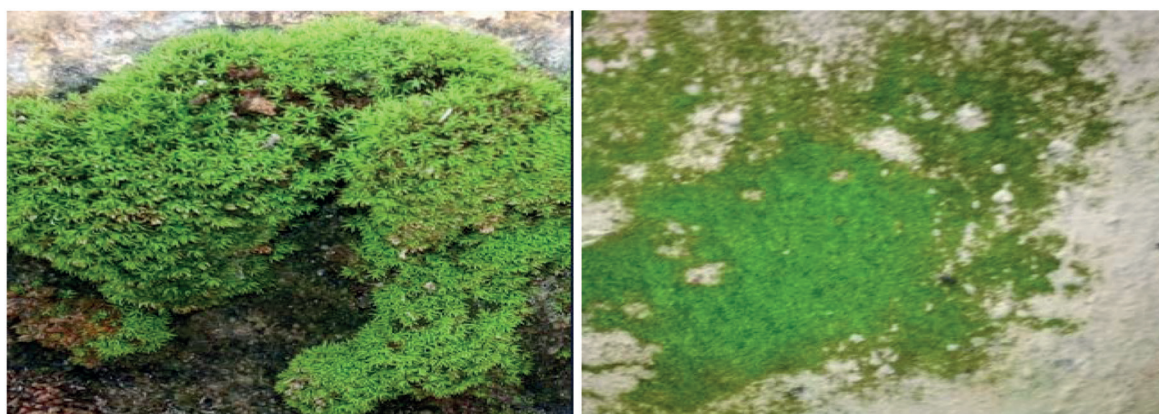


Fig. 2. Images of *T. muralis* on painted wall

and shaken vigorously to dislodge microorganisms. Thereafter, Malt extract (MEA) and Nutrient agar (NA) plates were inoculated with 1 ml of the water extract after appropriate serial dilution and incubated at room temperature ($30 \pm 2^\circ\text{C}$) for 24-48h. Distinct colonies were sub-cultured on fresh agar plates for purification and stored on agar slants till needed. The bacterial isolates were subsequently identified by morphology and biochemical characteristics (Gram staining, motility, oxidase, catalase, citrate, indole, glucose, lactose and triple sugar iron test) according to standard methods following Bergey's Manual of Determining Bacteriology (Holt *et al.*, 1994; Cheesbrough, 2006). The fungal isolates were identified by visual and microscopic methods after staining with lactophenol cotton blue. The fungal identification protocol was based on septate and non-septate hyphae, shape and colour of spores, and arrangement of spores on the hyphae and visual macroscopic appearance on agar plates using the keys of Dugan (2017).

Selection of metal-resistant isolates

Analytical grades of three salts: Chromium Chloride hexahydrate [$\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$], Cadmium Chloride monohydrate [$\text{CdCl}_2 \cdot \text{H}_2\text{O}$], and Copper (II) sulphate [CuSO_4] were obtained from the

Chemistry department of Delta State University, Abraka, and used in all the experiments. They were filter-sterilised using a $0.22 \mu\text{m}$ pore size Millipore membrane filter before use. NA and MEA plates were incorporated with the test metals using 100 mg/L for Cr^{6+} , and Cd^{2+} , and 600 mg/L for Cu^{2+} . The choice of these concentrations was based on the reports of Allen *et al.* (1997), Wei and Wee (2011), and Mokogwu and Ejechi (2022). Any isolate that grew at these concentrations is considered resistant. The concentrations were cross-checked with an Atomic Absorption Spectrometer (Perkin Elmer, Model 3100). An inoculum of 10^2 bacterial cells/fungal spores per ml in normal saline was developed based on plate counts, and the metal-incorporated NA and MEA plates were subsequently inoculated with the standardized inoculum. The plates were incubated at room temperature ($30 \pm 2^\circ\text{C}$) for 24-48h (bacteria) and 48-72h (fungi). The isolates that grew were sub-cultured and set aside for further tests.

Determination of the metal tolerance index (MTI) of selected metal-resistant microorganisms

The metal-resistant isolates were tested for metal tolerance with 300 mg/L of Cr and Cd and 800 mg/L for Cu in Nutrient Broth (NB). The broth

was inoculated with 10^2 bacteria cells/ml, and the absorbance was taken at 600 nm at the beginning and repeated after 48 h incubation at ambient temperature ($30 \pm 2^\circ\text{C}$). While the inoculated NB without metals served as the control. MTI was calculated by dividing the absorbance of the medium with test metals by the control absorbance after incubation (Oladipo *et al.*, 2018). With respect to fungal isolates, MEA was incorporated with the same concentration of metals and centrally inoculated with 5 mm diameter mycelium cut from the edge of a 5-day-old culture. The mycelia diameter was measured after incubation for 5 days at room temperature. Control plates without metals were also prepared. The MTI was calculated as before based on the mycelia extension dimensions. All experiments were conducted in five replicates.

Molecular identification of isolates with the MTI >0.5

The procedure of Sendolo *et al.* (2022) and Mishra *et al.* (2023) was adopted for the DNA extraction from isolates with MTI > 0.5. The bacterial and fungal cells with 50 mg wet weight were suspended in 200 μL of nuclease-free water and transferred to ZR Bashing Bead™ lysis tubes containing 750 μL of lysis solution that was supplemented with 0.5% (v/v) β -mercaptoethanol to enhance cell disruption. Homogenization in a bead beater at maximum speed for 5 minutes, followed by efficient lysis of the bacterial and fungal isolates. The lysates were centrifuged at $10,000 \times g$ for 1 minute, supernatants were then transferred to Zymo-Spin™ IV spin filters to remove debris. Thereafter, fungal/bacterial DNA binding buffer measuring 1,200 μL was transferred to the filtrate, and 800 μL aliquots of this mixture were loaded onto Zymo-Spin™ IIC columns and centrifuged at $10,000 \times g$ for 1 minute. DNA pre-wash buffer and DNA wash buffer were then used to wash the columns. DNA elution buffer measuring 100 μL was used to elute DNA and checked for quality by electrophoresis using 1% agarose gel stained with ethidium bromide. PCR amplification of the 16S rRNA using primers 1F (5'-GTGCCAGCAGCCGCGGTAA-3') and 2R (5'-AGACCCGGAACGTATTCAC-3') for bacteria; and [ITS-1F (5'-TCCGTAGGTGAACCTGCGG-3'); and ITS-4R (5'-TCCTCCGCTTATTGATATGC-3')] for fungi, was performed in 25 μL of 2 $\times$ PCR master mix (0.05 U/ μL Taq DNA polymerase, 4 mM MgCl_2 , 0.4 mM dNTPs), 10 μL genomic DNA template, 2.5 μL each of forward/reverse primers, and 10 μL nuclease-free water. Thermal cycling in a C1000 cycler (BioRad), initial dena-

turation at 94°C for 3 min, 37 cycles at 94°C (30 s), 50°C (30 s), and 72°C (1 min), and final extension at 72°C (7 mins). Amplicons were confirmed by electrophoresis on 1% agarose gel with ethidium bromide (10 $\mu\text{g}/\text{mL}$), visualized by a gel doc 2000 system (BioRad), and purified with a Zymoclean™ gel DNA recovery kit.

Sequencing was undertaken with the genetic analyzer 3130 xl (Applied Biosystems) using the procedure outlined in the manufacturer's manual. The chromatograms resulting from the sequencing reaction and the resulting nucleotide sequence were edited using Ridom Trace Edit. The 16S rRNA for bacteria and that of ITS for fungi sequences generated were compared with other referenced sequences available in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) database using the basic alignment search tool (BLASTn). Alignment of the nucleotide sequences, phylogenetic, and molecular evolution was ascertained with MEGA 11 version 6.0.

Assessment of minimum inhibitory concentrations (MIC)

The MIC with respect to the three test metals was determined for the organisms that were identified by molecular methods. For this purpose, MEA and NA plates containing various metal concentrations of 100-1000 mg/L were prepared, inoculated as before, and thereafter set aside at ambient temperature for 48h with respect to bacteria and 72 h for the fungus. The lowest concentration that inhibited growth was the MIC.

Metal removal assay

Isolates with the highest MIC for each of the metals were tested for the capacity to remove metals in aqueous solutions using 1000 mg/L in 100 ml of nutrient or potato dextrose broths. Flasks (250 ml) containing nutrient and potato dextrose broths were inoculated with 10^3 /ml bacterial or fungal spores, incubated at ambient temperature ($30 \pm 2^\circ\text{C}$), and agitated at 150 rpm for 24-48h. Thereafter, the flasks were centrifuged, and AAS was used to determine the residual metals' concentrations in the supernatant. Control sets were not inoculated with the test bacteria or fungus. The residual metal concentration was subtracted from the control and expressed as % removal. The experiments were conducted in five replicates.

Assessment of the tolerance of combined Cr, Cd, and Cu by the selected bacteria and fungus

The MTI determination procedure was repeated, but with NB and MEA incorporated with

combinations of Cr, Cd, and Cu. The tests were conducted in 6 steps, beginning with 50% of the concentrations (Cr, 50mg/L; Cd, 50 mg/L; Cu, 300 mg/L) used for the initial screening of isolates for metal resistance. These concentrations were increased at each step by 50 mg/L, as shown in Table 1.

Table 1. Combined concentration of Cr, Cd, and Cu series used for the tolerance index

Combination order	Concentration (mg/L) combination		
	Cr	Cd	Cu
1	50	50	300
2	100	100	350
3	150	150	400
4	200	200	450
5	250	250	500
6	300	300	550

Determination of Cr, Cd, and Cu concentrations on gametophyte surface deposits and substratum

The procedure of Jiang *et al.* (2020) was adopted for determining the three metals' concentrations on the bryophyte surface and the underlying substratum materials. The gametophytes were dried to constant weight in an oven at 50°C and milled, while pestle and mortar were used to grind the underlying materials after similarly drying to constant weight. Thereafter, 0.5 g samples were digested with a mixture of 37% hydrochloric and 65% nitric acid and set aside to stand overnight. This was followed by dissolution in deionized water and filtration with Whatman no. 1 filter paper to remove impurities. The filtrate was subsequently made up to 25 ml for gametophytes and 50ml for

the soil/materials with de-ionized water. Thereafter, the three metals' concentrations were determined with AAS.

Statistical Analysis

ANOVA was used to compare the differences between the levels of removal of the 3 test metals from aqueous media. The abilities of the fungus and bacteria to remove the metals from the media were also compared by ANOVA.

Result

The identities of the bacteria and fungi isolated from the bryophytes and the adhering substratum soil/materials are presented in Table 2. It was not considered necessary to separate the isolates based on the two bryophytes because *P. commune* and *T. muralis* were merely used as test grounds.

The table also presents the findings of the metal-resistant screening tests, where 5 bacteria (*Pseudomonas* sp. *P. aeruginosa*, *Bacillus* sp, *B. subtilis*, *Proteus mirabilis*, and a fungus (*Aspergillus* sp) were the isolates resistant to the three metals. The non-resistant isolates were subsequently dropped from further tests. Although there are a few exceptions, the results in Table 3 show that these metal-resistant isolates were generally moderately tolerant (>0.4). *P. aeruginosa*, *Bacillus* sp and *P. mirabilis* were the bacterial isolates that had MTI greater than 0.50 concerning the three metals (Table 3). The MTI of the only fungal isolate (*Aspergillus* sp.) was <0.50 as it concerns Cr and Cd, but >0.50 with respect to Cu (Table 3). While the MTI of *B. subtilis* was <0.50 for the three metals, that of *Pseudomonas* sp. was >0.5 with Cr and Cd (Table 3). The identities of the lone fungus and the

Table 2. Selection of Cr, Cd and Cu-resistant bacteria and fungi amongst isolates from the two moss bryophytes gametophyte surfaces and substratum (*P. commune* and *T. muralis*)

Isolates		Metals		
		Cr	Cd	Cu
Bacteria	<i>Pseudomonas</i> sp.	+	+	+
	<i>P. aeruginosa</i>	+	+	+
	<i>Bacillus</i> sp.	+	+	+
	<i>Bacillus</i> sp.	+	+	+
	<i>Proteus</i> sp.	+	-	-
	<i>Proteus mirabilis</i>	+	+	+
	<i>Enterobacter</i> sp.	-	+	-
	<i>E. coli</i>	+	+	-
	<i>Klebsiella</i> sp.	-	-	+
Fungi	<i>Aspergillus niger</i>	+	+	-
	<i>Aspergillus</i> sp.	+	+	+
	<i>Rhizopus</i> sp.	+	-	-

Table 3. Metal tolerance index (MTI) of metal-resistant isolates

Metal/concentration	Isolates	MTI	
		Mean	SD
Cr (300 mg/L)	<i>Pseudomonas sp.</i>	0.48	0.02
	<i>P. aeruginosa</i>	0.72	0.02
	<i>B. subtilis</i>	0.52	0.03
	<i>Bacillus sp.</i>	0.64	0.02
	<i>P. mirabilis</i>	0.51	0.02
	<i>Aspergillus sp.</i>	0.42	0.03
Cd (300 mg/L)	<i>Pseudomonas sp.</i>	0.36	0.01
	<i>P. aeruginosa</i>	0.65	0.01
	<i>B. subtilis</i>	0.36	0.02
	<i>Bacillus sp.</i>	0.50	0.02
	<i>Proteus mirabilis</i>	0.50	0.03
	<i>Aspergillus sp.</i>	0.43	0.02
Cu (800 mg/L)	<i>Pseudomonas sp.</i>	0.54	0.03
	<i>P. aeruginosa</i>	0.65	0.02
	<i>Bacillus subtilis</i>	0.29	0.01
	<i>Bacillus sp.</i>	0.55	0.01
	<i>P. mirabilis</i>	0.55	0.02
	<i>Aspergillus sp.</i>	0.53	0.02

three bacteria with MTI>0.50 were subsequently confirmed by molecular characterization as indicated in Figures 3-4 and shown in Table 4.

The MICs of the three metals were generally high (≥ 350 mg/L, with that of Cu being the highest (Table 5). There was marked variation in the reduction of the concentrations of the three metals in the aqueous media by the four organisms (Table 6). While *P. aeruginosa* removed more of Cr and Cd than Cu, *B. cereus* removed Cr and Cu better than Cd. Reduction of the levels of Cd and Cu by *P. mirabilis* was more than that of Cr. Reductions of metal concentrations by the bacterial species were generally greater than 50%, while it was lower with

the fungus *A. oryzae*. Cu was the least removed by *A. oryzae* (Table 6). The capacity of the organisms to reduce the metal concentrations in the aqueous medium can be seen in Figure 5. ANOVA showed that the bacteria and fungi differed significantly in their ability to remove the three metals (Fig. 5). While the reduction of Cr and Cd concentrations in the medium was best achieved with *P. aeruginosa*, the highest Cu removal was by *P. mirabilis* (Fig. 5). The fungus *A. oryzae* had the least ability to remove any of the three metals.

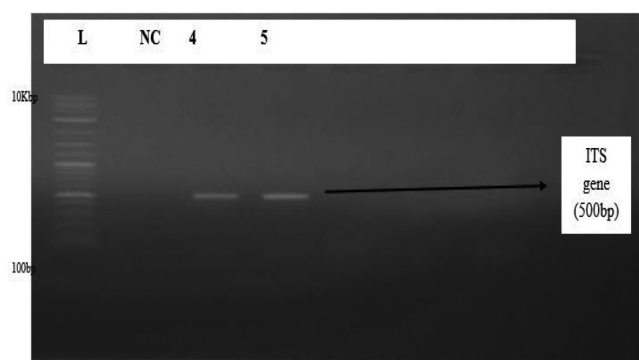


Fig. 3. The agarose gel electrophoresis of the amplified ITS region from the selected fungal isolates. The electrophoresis profile indicates that isolates 4 and 5 corresponds to the ITS bands at 500 bp, whereas lane L correspond to 1kb plus molecular ladder (100bp-10,000Kbp) while NC denotes no DNA templates

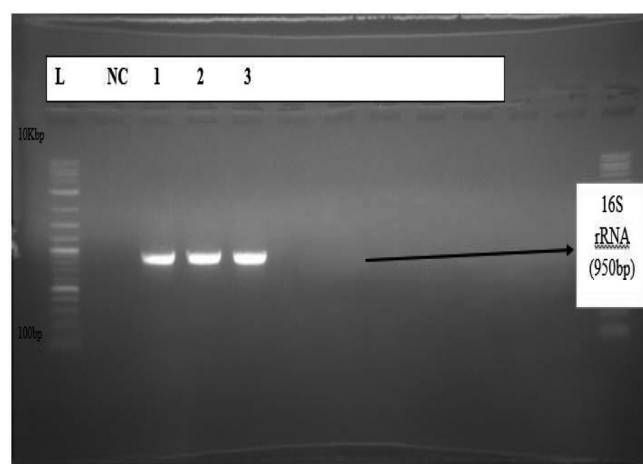


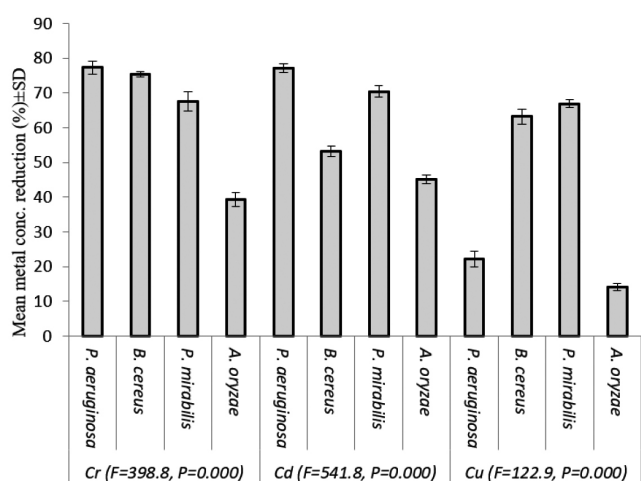
Fig. 4. Polymerase chain reaction results for bacteria isolates. L is 1kb plus ladder (100bp-10,000Kbp) DNA ladder (Molecular marker). Isolate 1, Isolate 2, and Isolate 3 corresponds to the 16S rRNA gene with bands at 900bp. NC: no DNA template.

Table 4. Molecular identification: NCBI Blast showing the sequence identity of the isolates

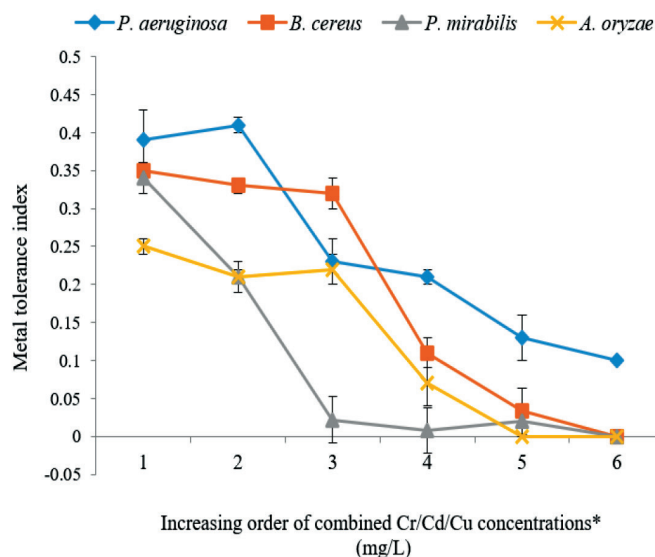
Sample ID of isolates	Query length (Bp)	Closest relative sequence	% Identity	E-value	Accession Number
1	842	<i>P. aeruginosa</i>	99.17	0.0	PX112461
2	720	<i>P. mirabilis</i>	97.29	0.0	PX112463
3	813	<i>B. cereus</i>	99.75	0.0	PX112464
4	553	<i>A. oryzae</i>	99.46	0.0	PX116176
5	567	<i>A. oryzae</i>	99.64	0.0	PX116730

Table 5. Minimum inhibitory concentration (MIC) of molecular identified isolates with high MTI

Isolates	MIC (mg/L)		
	Cr	Cd	Cu
<i>P. aeruginosa</i>	550	450	950
<i>P. mirabilis</i>	400	400	900
<i>B. cereus</i>	450	450	900
<i>A. oryzae</i>	350	350	850

**Fig. 5.** Comparison of the ability of bacteria and fungus isolates to remove Cr, Cd and Cu from aqueous media

The tolerance of combined Cr, Cd, and Cu by the four organisms was investigated, and the results, as indicated by the MTI, are presented in Fig. 6. The tolerance trends, which varied with the bacterial species and fungi, were generally low (<0.45), and they decreased with increasing levels of the metals (Fig. 6).

**Fig. 6.** Trends in the tolerance of combined Cr, Cd and Cu by bacteria and fungus isolates.

*See Table 1 in Materials and Methods for combination concentrations

Although the MTI was low, *P. aeruginosa* was the only organism that tolerated greater levels of the combined metals when compared to other isolates (Fig. 6). The concentrations of the three metals in the bryophytes' substratum materials were below the WHO (1996) recommended limits except for Cd in the deteriorating painted wall and roadside soil, where it was higher (Table 7). However, the concentrations of the three metals on the bodies of the two mosses were markedly higher than the levels in the substratum materials and the WHO permissible limits (Table 7).

Table 6. Removal of metals from aqueous solution by metal-resistant bacteria and fungus

Isolates	Mean reduction (%) ± SD			F(P)
	Cr	Cd	CU	
<i>P. aeruginosa</i>	^a 77.28±1.80	^b 77.088±1.32	^{ab} 22.20±2.28	148.19(0.000)
<i>B. cereus</i>	^c 75.54±0.8	^c 53.20±1.40	^c 63.20±2.16	248.40 (0.000)
<i>P. mirabilis</i>	64.54±2.79	^d 70.38±1.62	^d 66.90±1.06	4.40 (0.037)
<i>A. oryzae</i>	^e 39.34±1.92	^e 45.10±1.28	^e 14.06±1.05	631.40 (0.000)

Post hoc: values with similar superscripts across rows are significantly different (^aP=0.000; ^bP=0.000; ^cP=0.000; ^dP=0.037; ^eP=0.000)

Table 7. Concentration of chromium, cadmium and copper on bryophyte surfaces and substratum materials

Source	Mean conc. (mg/kg) $\pm$ SD		
	Chromium	Cadmium	Copper
Deteriorating building block	1.37 $\pm$ 0.30	0.07 $\pm$ 0.004	18.43 $\pm$ 3.06
Deteriorating painted wall	21.98 $\pm$ 3.08	0.83 $\pm$ 0.015	19.03 $\pm$ 3.27
Deteriorating unpainted wall	1.77 $\pm$ 0.10	0.71 $\pm$ 0.02	17.03 $\pm$ 3.60
Roadside soil	51.30 $\pm$ 5.40	1.72 $\pm$ 0.08	27.42 $\pm$ 3.23
<i>P. commune</i> gametophyte	122.24 $\pm$ 8.26	2.03 $\pm$ 0.37	38.54 $\pm$ 4.60
<i>T. muralis</i> gametophyte	105.65 $\pm$ 7.48	1.86 $\pm$ 0.29	29.42 $\pm$ 3.42
WHO (1996) permissible limits	100	0.8	36

Discussion

The microorganisms found on the bryophyte gametophytes and substratum were diverse and not unexpected because such a diversity is known to be associated with bryophytes (González and Ghneim-Herrera, 2021). Reports have shown that *P. commune* and *T. muralis* can accumulate nutrients and heavy metals from the atmosphere and soil (Nasrudi *et al.*, 2007; Zawadzki *et al.*, 2016; Ojiodu and Olumayede, 2018), and this can influence the occurrence of diverse microorganisms, including non-metal resistant species, as the results showed. The high concentrations of the three test metals on the moss gametophyte suggest that a substantial number of the metal-resistant isolates may have originated from there. The soil or substratum materials with lower concentrations of metals may not present a sufficient “hot spot” for the emergence of metal-resistant organisms of high MIC magnitudes, like the gametophytes. Jiang *et al.* (2020) found lower concentrations of metals, including Cr and Cd, in the substratum of moss bryophytes, as was observed in this study. This can be attributed to a reduced entry of atmospheric pollutants to the bryophyte substratum because the gametophyte acts like a shield.

The high concentration of metals on the moss gametophytes may be associated with traffic vehicle exhaust fumes and emissions from industries and household activities in the towns. These emissions contain heavy metals, which include Cr, Cd, and Cu (Ali *et al.*, 2017; Nwaedozie and Nyan, 2018). Rainfall, wind, and gravity may bring about the settlement of these heavy metals emanating from dust, emissions, and bioaerosols on the bryophytes. Some of the microorganisms on the bryophyte surfaces and substrata may subsequently develop metal resistance. Indeed, some members of the bryophyte microbiome may have come from bioaerosols. Bryophytes lack a conducting system; hence, the light cuticle and epidermis of the gametophyte

are used to accumulate metals and absorb nutrients with a capacity greater than their root-like rhizoids (Stanković *et al.*, 2018). However, some bryophyte substrates may contain high concentrations of metals, which makes the rhizosphere remain a source of metal-resistant organisms. For example, *T. muralis* was extracted from painted walls in this Nigerian-based study, whereas some paints used in Nigeria contain heavy metals. Heavy metals, which include Cr and Cd that are above permissible levels are present in some paints often used in Nigeria (Apanpa-Qasim *et al.*, 2016; Akindele and Joseph, 2024). Thus, bryophytes growing on a painted wall as substratum may enable the development of metal-resistant microorganisms in their rhizosphere. High levels of metals can be absorbed and accumulated via the rhizoids in the substratum (Printarakul and Meeinkuirt, 2022) in a symbiotic relationship with rhizosphere bacteria and fungi. For example, Das *et al.* (2017) and Simón-Solá *et al.* (2019) reported that plants with high heavy metal accumulation capacity do so with the support of microorganisms, which use their exudates as electron donors to enhance metal mobilization and detoxification. It can, therefore, be deduced that this process promotes metal-resistance amongst the rhizosphere microorganisms.

Pseudomonas aeruginosa, *Bacillus cereus*, *Proteus mirabilis*, and *Aspergillus oryzae* were the most Cr-, Cd-, and Cu-resistant isolates from the mosses, as shown by the MTI and MIC results. The MIC levels are substantial to make them potential “candidates” for bioremediation applications. As indicated by the results of the MIC and removal of metals from aqueous medium, *P. aeruginosa* stood out as an outstanding metal-resistant organism. This was not unexpected because several reports show that it is a versatile multi-metal resistance organism (Chellaiah, 2018; Abdelbary *et al.*, 2019; Feruke-Bello *et al.*, 2023). Generally, species of *Bacillus*, *Proteus*, and *Aspergillus* isolated from

metal-polluted sites are known to resist metals, although the level of resistance may vary (Girdhar *et al.*, 2022). A recent report (Mokrani *et al.*, 2026) also showed that strains of *Bacillus* and *Pseudomonas* demonstrated marked variability in their resistance to Cr, Cd, and Cu. A similar variation in resistance to Cr, Cd, and Cu by metal type and species was also encountered in this investigation. These variations suggest the absence or moderate operation of some specific metal-resistance mechanisms among the bacteria.

The mechanisms used by microorganisms to resist or adapt to metal toxicity vary, and they have been well documented by Mathivanan *et al.* (2021). They include: changes in the morphology of the microorganisms (e.g. reduced cell size) in response to toxic stressors; efflux pump mechanisms based on active transport; production of siderophores which chelate metals from the environment; production of metallothioneins, a cysteine-rich protein that sequester metals intracellularly in the process of detoxification; biotransformation to non-toxic forms; and biosorption or binding of metals with exopolysaccharides produced by some bacteria. Possession of some or all six mechanisms outlined above potentially qualifies any organism for application in a bioremediation strategy. The high MIC and level of removal of the three metals from aqueous solution by the strains of *P. aeruginosa*, *B. cereus*, *P. mirabilis*, and *A. oryzae* isolated from the bryophytes in this study suggest that they possess some of these resistance mechanisms, which are useful for bioremediation. *P. aeruginosa* uses almost all the mechanisms stated above for the removal of heavy metals, depending on concentrations and bioavailability (Abdelbary *et al.*, 2019; Hassen and Abbasi, 2025). *Bacillus* species have been reported to remove Cr, Cu, and Cd by transformation, efflux system, and efflux/chelation, respectively (Alotaibi *et al.*, 2021), while changes in morphology and biosorption with exopolysaccharides have been associated with heavy metal removal, especially Cd by *Proteus mirabilis* (Niu *et al.*, 2024). With respect to *Aspergillus* species, adsorption, chelation, and sequestration are known to be some of the metal removal mechanisms it uses (Badr, 2025).

Various concentrations of Cr, Cd, and Cu were encountered on bryophyte surfaces and substrata, thereby indicating that the bacterial isolates were already exposed to the three metals. It was therefore expected that they would tolerate the combined metals, which was not the case. The MTI of the combined metals was generally low and var-

ied. The variation was not unexpected because it was also encountered in the MTI and MIC values in the single metal tests. The low MTI of the combined metals can be attributed to the non-availability of some metal-specific tolerance mechanisms or the level of operation. It therefore follows that while an organism may tolerate some of the metals, it becomes vulnerable to others and growth ceases. Tolerance of multiple heavy metals is important for bioremediation because of the likely presence of more than one metal in polluted environments.

The paramount study objective was to demonstrate the likely presence of metal-resistant microorganisms on bryophyte surfaces and rhizosphere. This was achieved, and the capacity of the metal-resistant isolates to remove high concentrations of Cr, Cd, and Cu from aqueous media makes them potential “candidates” for bioremediation strategies, especially as they demonstrated multiple resistances to the three test metals. Although this multiple resistance failed to operate when the metals were combined, it indicated that a consortium of the isolates would be most useful in bioremediation. With a consortium, one species can make up for the limited resistance mechanism of the other, as indicated by the differences in the levels of resistance to each of the three metals among the organisms.

Conclusion

A diversity of microorganisms was isolated from the gametophyte and substratum of the test moss bryophytes, *P. commune* and *T. muralis*, but only *Pseudomonas* sp. *P. aeruginosa*, *Bacillus* sp, *B. subtilis*, *P. mirabilis*, and *Aspergillus* sp. resisted Cr, Cd, and Cu. However, only the species identified as *P. aeruginosa*, *B. cereus*, *P. mirabilis*, and *A. oryzae* by molecular methods had the highest MICs with regard to the three metals. The high concentrations of the three metals found on the moss gametophytes provided an environment for the emergence of metal-resistant microbial species on the gametophytes. The support for this inference comes from the knowledge that gametophyte surfaces are areas where emissions from industrial and household activities and dust containing heavy metals are adsorbed. The high MICs of the isolates were substantiated by the finding that over 50% of Cr, Cd, and Cu were removed from the aqueous medium. The results from this study, therefore, substantiate the widely held view that metal-resistant microorganisms are usually present where high concentrations of metals occur. Thus, it can be concluded

that metal-resistant bacteria and fungi can be found on bryophyte surfaces and substratum/rhizosphere, like in other waste-polluted environments. The study outcome also draws attention to bryophytes as potential sources of microorganisms for the remediation of metal-polluted environments, singularly or in consortia.

References

- Abdelbary, S., M. S. Elgamal, A. Farrag (2019). Trends in heavy metals tolerance and uptake by *Pseudomonas aeruginosa*. In: Sriramulu, D. (Ed). *Pseudomonas aeruginosa—An Armory within*, IntechOpen, London, pp.122. DOI: 10.5772/intechopen.85875.
- Akindele, A. F. I., A. Joseph (2024). Health risk assessment of lead, cadmium, heavy metals and metalloids in residential paint flakes from indoor wall surfaces. *Environ. Monit. Assess.* **196**: 1261. <https://doi.org/10.1007/s10661-024-133244>.
- Akpomie, O. O., B. O. Ejechi (2016). Removal of Cr⁶⁺ from tannery effluents with mixed cultures of bacteria. *Inter. J. Environ. Waste Man.* **17**: 60-70. <https://doi.org/10.15041/IJEW.2016.076431>.
- Allen, D. A., B. Austin, R. R. Colwell (1997). Antibiotic resistance patterns of metal-tolerant bacteria isolated from an estuary. *Antimicrob. Agents. Chemother.* **12**: 545-547. <https://doi.org/10.1128/AAC.12.4.545>.
- Ali, H., I. Khan, I. Ilahi (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *J. Chem.* **2019**: 6730305. <https://doi.org/10.1155/2019/6730305>.
- Ali, M. U., G. Liu, B. Yousaf, Q. Abbas, H. Ullah, M. A. M. Munir, B. Fu (2017). Pollution characteristics and human health risks of potentially ecotoxic elements (PTE) in road dusts from metropolitan area of Hefei, China. *Chemosphere* **181**: 111-121. doi: 10.1016/j.chemosphere.2017.04.061.
- Alotaibi, B. S., M. Khan, S. Shamim (2021). Unraveling the Underlying Heavy Metal Detoxification Mechanisms of Bacillus Species. *Microorganisms* **9**: 1628. <https://doi.org/10.3390/microorganisms9081628>.
- Apanpa-Qasim, A. F. I., A. A. Adeyin, S. N. Mudliar, K. Raghunathan, P. Thawale (2016). Examination of lead and cadmium in water-based paints marketed in Nigeria. *J. Health. Pollut.* **6**: 43-49. DOI:10.569612156-9614-6.12.43.
- Badr, S. Q. (2025). Genes responsible for heavy metal bioaccumulation in fungi *Aspergillus*. *J. Med. Genet. Clin. Biol.* **2**: 469-474. <https://doi.org/10.61796/jmgcb.v2i10.1417>.
- Buck, W. R., B. M. Thiers (1996). Guidelines for Collecting Bryophytes. Selected guidelines for ethnobotanical Research: A field manual. *Advances Econ. Botany* **10**: 143-146. <https://www.jstor.org/stable/43927616>.
- Cao, W., Y. Xiong, D. Zhao, H. Tan, J. Qu (2020). Bryophytes and the symbiotic microorganisms, the pioneers of vegetation restoration in karst rocky desertification Areas in southwestern China. *Appl. Microbiol. Biotech.* **104**: 873-891. <https://doi.org/10.1007/s00253-019-10235-0>.
- Cheesbrough, M. (2006). *District laboratory practice in tropical countries. Part 2*. Cambridge University Press, Cambridge. www.ambrig.org 9780521676311.
- Chen, K. H., J. Nelson (2022). A scoping review of bryophyte microbiota: diverse microbial communities in small plant packages. *J. Exp. Bot.* **73**: 4496-4513. doi: 10.1093/jxb/erac191.
- Chellaiah, E. R. (2018). Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: A mini-review. *Appl. Water Sci.* **8**: 154. doi:10.1007/s13201-018-0796-5.
- Crini, G., E. Lichtfouse (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environ. Chem. Lett.* **17**: 145-155. DOI:org/10.1007/s103311-018-07859.
- Dahnoun, K., F. Djadouni, B. Essghaier, C. Naccache, N. Zitouna (2024). Characterization and bioremediation potential of heavy-metal resistant bacteria isolated from agricultural soil. *Turkish J. Agric. For.* **48**: 607-617. doi:10.55730/1300-011X.3205.
- Das, S., M. L. Chou, J. S. Jean, H. J. Yang, K. P. Joo (2017). Arsenic-enrichment enhanced root exudates and altered rhizosphere microbial communities and activities in hyperaccumulator *Pteris vittata*. *J. Hazard. Mater.* **325**: 279-287. Doi:org/10.1016/j.hazmat.2016.12.006.
- Dugan, F. M. (2017). *The identification of fungi: An illustrated introduction with keys, glossary and guide to literature*. American Phytopathological Society, St Paul MN 55121 USA. <https://doi.org/10.1094/9780890545041>.
- Fasani, E., M. Li, C. Varotto, A. Furini, G. DalCorso (2022). Metal detoxification in land plants: From bryophytes to vascular plants, state of the art and opportunities. *Plants* **11**: 237. <https://doi.org/10.3390/plants11030237>.
- Fruke-Bello, Y. M., O. Odeyemi, G. O. Babalola (2023). Bioremediation potential of heavy metals multi-tolerant *Pseudomonas* spp. isolated from municipal waste dumpsite at Ileife, Osun Stat, Nigeria. *J. Pet. Environ. Biotechnol.* **14**: 543. <https://doi.org/10.5281/zenodo.159504>.
- Firinca, C., L. G. Zamfir, M. Constantin, I. Raut, L. Capra, D. Popa, M. L. Jinga, A. M. Baroi, R. Fierascu, N. O. Corneli, C. Postolache, M. Doni, A. Gurban, L. Jecu, T. E. Seasan (2024). Microbial removal of heavy metals from contaminated environments using metal-resistant indigenous strains. *J. Xenobiot.* **14**: 51-78. <https://doi.org/10.3390/jox14010004>.
- Girdhar, M., Z. Tabassum, K. Singh, A. Mohan (2022). A review on the resistance and accumulation of heavy metals by different microbial strains. In: Mendes, K. F., R. N. de Souza, K. C. Mielke (Eds). *Biodegradation technology of organic and inorganic pollutants*, IntechOpen, London, pp. 1-31. DOI: <http://dx.doi.org/10.5772/intechopen.101613>.
- González, S., T. Ghneim-Herrera (2021) Heavy Metals in soils and the remediation potential of bacteria associated with the plant microbiome. *Front. Environ. Sci.* **9**: 604216. doi: 10.3389/fenvs.2021.604216.
- Hassen, B., M. S. Abbassi (2025). Molecular mechanism of heavy metal resistance and cross-resistance to antibiotics in *Pseudomonas aeruginosa*. *Lett. Appl. Microbiol.* **78**: ovaf094. Doi:1093/lambio/ovaf094.
- Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, S. T. Williams (1994). *Bergey's manual of determinative bacteriology* 9th ed. Williams and Wilkins Publishers, Maryland.
- Jiang, Y., X. Zhang, R. Hu, J. Zhao, M. Fan, M. Shaaban, Y. Wu (2020). Urban atmospheric environment quality assessment by naturally growing bryophytes in Central China. *Int. J. Environ. Res. Public Health* **17**: 4537.

- doi:10.3390/ijerph17124537.
- Li, H., T. F. Xiao, Y. Shuang, L. B. He, Z. P. Ning, J. L. Sun, J. Q. Peng, D. H. Li, C. S. Zhu (2008). Hydrogeochemistry of cadmium in Jinding Pb-Zn mining district in Yunnan, China. *Geochimica* **27**: 21-27. Doi:10.1007/s11631-008-0021-6.
- Liu, L., W. Li, W. Song, M. Guo (2018). Remediation techniques for heavy metal contaminated soils: Principles and applicability. *Sci. Total Environ.* **633**: 206-219. <https://doi.org/10.1016/j.scitoenv.2018.03.161>.
- Mathivanan, K., J. U. Chandrika, A. Vinothkanna, H. Yin, X. Liu, D. Meng (2021). Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment – A review. *Ecotoxicol. Environ. Saf.* **226**: 112863. <https://doi.org/10.1016/j.ecoenv.2021.112863>.
- Mishra, G., R. Meena, R. Kant, S. Pandey, H. Ginwal, M. Bhandari (2023). An improved shorea robusta genomic DNA extraction protocol with high PCR fidelity. *Biol. Methods Protoc.* **8**: 1-6. <https://doi.org/10.1093/biomethods/bpad039>.
- Mokogwu, C. M., B. O. Ejechi (2022). Prevalence of antibiotics and Cr, Cd, Cu metals co-resistant coliforms in refuse dumpsites and air of surrounding buildings in a Nigerian urban setting. *Environ. Prot. Res.* **112**: 1-15. <https://doi.org/10.37256/epr.2220221826>.
- Mokrani, S., Z. Benouguef, K. Houali, L. Bensidhoum, A. Derguini, N. A. Ibrahim, N. S. Basher, E. Nabti (2026). Resistance mechanisms of rhizospheric *Bacillus* and *Pseudomonas* strains against heavy metal contamination (Cu, Cr and Cd) and their antifungal properties. *Microorganisms* **14**: 644. <https://doi.org/10.3390/microorganisms14030644>.
- Muhammad, S., M. T. Shah, S. Khan (2011). Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan region, Northern Pakistan. *Microchem. J.* **98**: 334-343. <https://doi.org/10.1016/j.microc.2011.03.003>.
- Nasrudi, T., A. Badacsonyi, I. Keresztényi, D. Podar, Z. Csintalan, Z. Tuba (2007). Comparison of two metal surveys by moss *Tortula muralis* in Budapest, Hungary. *Environ. Monit. Assess* **134**: 279–285. Doi: 10.1007/s10661-007-9617-2.
- Niu, S., H. Song, X. Zhang, X. Bao, T. Li, L. He, Yong Lia, Y. Li, D. Zhang, J. Bai, S. Liu J. Guo (2024). The Cd resistant mechanism of *Proteus mirabilis* Ch8 through immobilizing and detoxifying. *Ecotoxicol. Environ. Saf.* **278**: 116432. <https://doi.org/10.1016/j.ecoenv.2024.116432>.
- Njoku, K. I., O. R. Akinyede, O. F. Obidi (2020). Microbial remediation of heavy metals contaminated media by *Bacillus megaterium* and *Rhizopus stolonifer*. *Sci. Afr.* **10**: 1-8. <https://doi.org/10.1016/j.sciaf.2020.00545>.
- Nnaji, N. D., C. U. Anyanwu, T. Miri, H. Onyeaka (2024). Mechanisms of heavy metal tolerance in bacteria: A review. *Sustainability* **16**: 11124. <https://doi.org/10.3390/su162411124>.
- Nwaeozie, G., S. E. Nyan (2018). Determination of heavy metals in soot from petroleum vehicles exhaust tailpipes. *Inter. J. Environ. Agric. Biotech.* **3**: 2233-2243. <https://dx.doi.org/10.22161/ijeab/3.6.39>.
- Oladipo, O. G., O. O. Awotoye, A. Olayinka, C. C. Bezuidenhout, M. S. Maboeta (2018). Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. *Brazilian J. Microbiol.* **49**: 29-37. Doi: [10.1016/j.bjm.2017.06.003](https://doi.org/10.1016/j.bjm.2017.06.003).
- Ojiodu, C. C., E. G. Olumayede (2018). Biomonitoring of heavy metals using *Polytrichum commune* as a bioindicator in a macroenvironment, Lagos State, Southwestern – Nigeria. *FUW Trends Sci. Technol. J.* **3**: 287 – 291. <https://doi.org/10.21203/RS.3.RS-2926408/V1>.
- Oyewole, O. A., S. S. L. Zobeashia, E. O. Oladoja, R. O. Raji, E. E. Odiniya, A. M. Musa (2019). Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil. *SN Appl. Sci.* **1**: 857 <https://doi.org/10.1007/s42452-019-0879-4>.
- Printarakul, N., W. Meeinkuirt (2022). The bryophyte community as bioindicator of heavy metals in a waterfall outflow, *Sci. Rep.* **12**: 6942. <https://doi.org/10.1038/s41598-022-10980-9>.
- Razzak, S. A., M. A. Faruque, Z. Alsheikh, M. M. Hossain, R. A. Lucky, A. S. Bassi, H. De Lasa (2022). A comprehensive review on conventional and biological- driven heavy metals removal from industrial wastewater. *Environ. Adv.* **7**: 1-26. <https://doi.org/10.1016/j.envadv.2022.100168>.
- Sendolo, D., G. I. Baysah, C. O. Ezeamagu (2022). Detection of heavy metal-resistance gene in bacteria isolated from clinical and environmental sources using polymerase chain reaction (PCR). *Pan-African J. Health Environ. Sci.* **1**: 83–92. <https://doi.org/10.56893/ajhes.2022-vli2.239>.
- Simón-Solá, M. Z., N. Lovaisa, J. S. Dávila-Costa, C. S. Benimeli, M. A. Polti, A. Alvarez (2019). Multi-resistant plant growth-promoting actinobacteria and plant root exudates influence Cr (VI) and lindane dissipation. *Chemosphere* **222**: 679–687. <https://doi.org/10.1016/j.chemosphere.2019.01.197>.
- Srivastava, J., H. Chandra, K. Tripathi, R. Naraian, R. K. Sahu (2008). Removal of chromium (VI) through absorption by the *Pseudomonas* spp. isolated from tannery effluent. *J. Basic Microbiol.* **48**: 135-139. <https://doi.org/10.1002/jcbm.200700291>.
- Stanković, J. D., A. D. Sabovljević, M. S. Sabovljević (2018). Bryophytes and heavy metals: a review. *Acta Bot. Croat.* **77**: 109–118. DOI: 10.2478/botcro-2018-0014.
- Tang, H., G. Xiang, W. Xiao, Z. Yang, B. Zhao (2024) Microbial mediated remediation of heavy metals toxicity: mechanisms and future prospects. *Front. Plant Sci.* **15**: 1420408. doi: 10.3389/fpls.2024.1420408.
- Tremper, A. H., M. Agneta, S. Burton, D. E. Higgs (2004). Field and laboratory exposures of two moss species to low level metal pollution. *J. Atmos. Chem.* **49**: 111-120. <https://doi.org/10.1007/s/0874-004-1218-7>.
- Vacar, C. L., E. Covaci, S. Chakraborty, B. Li, D. C. Weindorf, T. Frentiu, M. Pârvu, D. Podar (2021). Heavy metal-resistant filamentous Fungi as potential mercury bioremediators. *J. Fungi* **7**: 386. <https://doi.org/10.3390/jof7050386>.
- Wei, L. S., W. Wee (2011). Antibigram and heavy metal resistance pattern of *Salmonella* spp. isolated from wild Asian sea bass (*Lates calcarifer*) from Tok Bali, Kelantan, Malaysia. *Jordan J. Biol. Sci.* **4**: 125-128. https://jjbs.hu.edu.jo/files/v4n3/Paper_number2_modified.pdf.
- WHO (1996). Permissible limits of heavy metals in soil and plants (Geneva: World Health Organization), Switzerland.
- Wróbel, M., W. Sliwakowski, P. Kowalczyk, K. Kramkowski, J. Dobrzynski (2023). Bioremediation of Heavy Metals by the Genus *Bacillus*. *Int. J. Environ. Res. Public Health* **20**:

4964. <https://doi.org/10.3390/ijerph20064964>.
- Yan, G., T. Viraraghavan (2000). Effect of pretreatment on the bioadsorption of heavy metal on *Mucor rouxii*. *Water SA*. **26**: 119-123.
- Yazdankhah, S., K. Rudi, A. Bernhoft (2014). Zinc and copper in animal feed-development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. *Microb. Ecol. Health Dis.* **25**: 25862-25865. <https://doi.org/10.3402/mehd.v25.25862>.
- Zafar, S., F. Aqil, I. Ahmad (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Biore-sour. Technol.* **98**: 2557-2561. <https://doi.org/10.1016/j.biortech.2006.09.051>.