Proceedings of the ICBBG2025
2025 International conference on Bio-mediated and Bio-inspired Geotechnics
18-20 May, 2025 | Tempe, Arizona, USA
https://doi.org/10.53243/ICBBG2025-91

A Preliminary Study of Growth Response and Copper Tolerance in the Desert Fungus *Podaxis pistillaris*

Ryan Cotter,[1] Emmanuel Salifu, PhD,[2] and Caitlyn A. Hall, PhD[3]

[1]Department of Chemical and Environmental Engineering, The University of Arizona, 1200 E University Blvd, Tucson, AZ 85721, USA; Email: ryanc2@arizona.edu
[2]Center for Bio-mediated and Bio-inspired Geotechnics, School of Sustainable Engineering and the Built Environment, Arizona State University, 1151 S Forest Ave, Tempe, AZ 85287, USA; E-mail: emmanuel.salifu@asu.edu
[3]Department of Biosystems Engineering, The University of Arizona, 1200 E University Blvd, Tucson, AZ 85721, USA; E-mail: cahall@arizona.edu

ABSTRACT

This study evaluates the copper tolerance of *Podaxis pistillaris*, a desert fungus isolated from urban areas in Tucson, Arizona, as a potential sustainable approach for remediating soil contamination. The tolerance of two strains, PP1-5 and PP1-6, was investigated in growth media spiked with copper at concentrations similar to those found in Arizona's copper mine tailings. The growth area of the cultures was monitored daily over 21 days. PP1-5 exhibited greater tolerance, with minimal growth area reductions at copper concentrations below 1 mM. In contrast, PP1-6 was more sensitive, showing significant growth area reductions at concentrations above 0.25 mM. These findings enhance our understanding of *P. pistillaris*'s interactions with copper and its ability to survive in arid and semi-arid soils with elevated copper levels. This study lays the groundwork for future research into the biotechnological applications of *P. pistillaris* for mycoremediation of heavy metal-contaminated soils.

INTRODUCTION

Tailings ponds hold the waste rock slurry from mining operations and contain high concentrations of residual heavy metals, presenting significant health and environmental hazards (Chen et al. 2024). The mining sector in the southwestern United States is a historically expansive and growing industry, making the development of efficient, inexpensive, and more sustainable remediation technologies important in this region (Parades-Aguilar et al. 2024). Copper, one of the most vital resources mined in the southwestern United States (Brooks et al. 2024), has become the focal point of remediation efforts and is the focus of the present study.

In recent years, bio-mediated technologies for remediating tailings have gained increasing attention due to their potential for sustainability and efficiency (Zhang et al. 2024). One notable bio-mediated technology is mycoremediation, which uses fungi as the primary organism for remediation. Fungi exhibit several mechanisms to tolerate and sequester heavy metals, including the adsorption of metal ions to their cell wall, the production of chelating metabolites, and the utilization of transporter proteins to store heavy metals internally. Mycoremediation has been successfully applied to the remediation of mine tailings (Palanivel et al. 2023). However, arid

climates such as the Southwestern United States present unique challenges for *in-situ* mycoremediation applications. Environmental factors, including high temperatures, high soil salinity, high evapotranspiration rates, low soil moisture, low soil nutrients, and infrequent rainfall, are stressful conditions for living organisms (Rani & Paul 2023). To address this, many studies focus on isolating fungi native to these arid environments that are adapted to the aforementioned environmental conditions (Obuekwe et al. 2005).

One fungus capable of thriving in arid and semi-arid environments is *Podaxis pistillaris*. This ubiquitous species is adapted for survival in hot deserts, growing optimally at temperatures between 35-40 ℃ and producing thick-walled spores resistant to desiccation (Conlon et al. 2019). Strains of *P. pistillaris* isolated from Saudi Arabia have shown the ability to tolerate and accumulate lead and cadmium (Hashem & Al-Rahmah 1993). As a mushroom-forming fungus, *P. pistillaris* also has the advantage of accumulating heavy metals in its fruiting bodies, providing a convenient route to isolate and remove heavy metals from contaminated soils (Damodaran et al. 2013). Together, these qualities make the species a potential candidate for *in-situ* mine tailing remediation in the southwestern United States.

Testing the tolerance of *P. pistillaris* at copper concentrations similar to those found in mine tailings is a crucial first step toward ascertaining its remediation efficacy. Although tolerance does not directly indicate remediation capability, it provides vital insights into *P. pistillaris*'s interactions with copper and the potential implications for remediation. Therefore, this study aims to evaluate the copper (Cu) tolerance of two strains of *P. pistillaris* isolated from Tucson, Arizona. By comparing the growth response of each strain under varying Cu concentrations, we seek to understand how strain variability affects Cu tolerance. Ultimately, this research will provide a preliminary understanding of the role that fungal strain differences may play in enhancing or limiting the remediation capabilities of *P. pistillaris* in copper-contaminated environments.

METHODS AND MATERIALS

Fungal strain isolation. Mature fruiting bodies of *P. pistillaris* were collected in Tucson, Arizona. Spores of the fruiting bodies were germinated on potato dextrose broth agar (PDB-A; 25 g PDB:20 g nutrient agar:1 L deionized (DI) water) with PDB composed of 20 g/L potato extract and 4 g/L dextrose. Germination occurred over 14 days at 25 ℃. Successful cultures were transferred to PDB-A plates. Genetic identification via Sanger sequencing on an Applied Biosystems 3730 DNA Analyzer (ThermoFisher Scientific), confirmed the isolation of two strains of *P. pistillaris* labeled *PP1-5* and *PP1-6*, respectively.

Sample preparation. Potato dextrose agar (PDA) was prepared by dissolving 39 g PDA (20 g potato extract: 4 g dextrose: 15 g nutrient agar) in 1 L DI water to culture the two strains of *P. pistillaris*. All materials used to prepare the media were sterilized by autoclaving for 15 minutes at 121 ℃. Copper, in the form of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (GFS Chemicals) was added to the PDA solutions after autoclaving. The PDA was prepared in batches through sequential dilution from the highest to lowest copper concentration, accounting for a volume of 13 mL in each PDA plate. Two experimental sets were created: 1) trace concentrations of 1 mM, 0.75 mM, 0.5 mM, and 0.25 mM; and 2) elevated concentrations of 2 mM, 1.75 mM, 1.5 mM, and 1.18

mM. Standard PDA plates with 0 mM of added copper were prepared as controls. All experimental and control specimens were prepared in triplicates for each fungal strain.

Each PDA plate was inoculated with a 7 mm diameter disk of *PP1-5* or *PP1-6* biomass from their respective PDB-A cultures. The *PP1-5* and *PP1-6* plates were prepared in separate batches to prevent cross-contamination. After inoculation, the plates were wrapped with parafilm, placed into labeled gallon Ziploc bags and sealed. The bags were then placed in an incubator at 35 °C.

Cultures were allowed to grow for three days before initial imaging. Subsequently, images were taken daily for up to 21 days. Plates that showed no mycelial growth after 14 days or exhibited contamination were discarded. On Day 22, the lids of the plates were removed, and final overhead images of the cultures were taken.

Mycelial growth measurement via image analysis. Imaging was conducted in a Smith-Victor 25" LED Desktop Studio/shooting tent fitted with white LEDs and a reflective silver interior. A matte black background sweep was installed in the interior of the box. An iPhone 13 with dual 12-megapixel cameras (Apple Inc.) was mounted at the top of the box for imaging. The iPhone 13 camera's settings were adjusted to a magnification of 2.5, a tone of -100, a warmth of 50, and an exposure of -1.3. A small adjustable vertical platform was used to elevate the plates closer to the camera. Due to high levels of condensation on the lids of some of the plates, plates were flipped upside down and imaged from the bottom. Plates were labeled with relevant information on their bottoms to maintain organization. A line was also drawn perpendicular to the edge of the bottom of the plate to act as a reference point for the consistent positioning of the plates in each image.

After the growth period, the images were downloaded from the iPhone 13 to a computer and analyzed using Fiji ImageJ to measure the growth area of each culture. The images were first set to 8-bit grayscale. Then, the line tool was used to measure 1 cm on the ruler within the image, setting the cm/pixel scale for all subsequent measurements. The freehand drawing tool was used to trace the growth area of the culture, and the built-in measurement tool provided the growth area in cm^2. This process was repeated for all images.

RESULTS AND DISCUSSION

Figure 1A and Figure 1B show the daily growth areas monitored over 21 days for both strains of *P. pistillaris*. The results indicate a reduction in growth with increased Cu concentration for both *P. pistillaris* strains. The final average growth area for the *PP1-5* strain under the 0 mM Cu control treatment was 46.1 cm^2, followed by 36.3 cm^2 at 0.25 mM, 28.9 cm^2 at 0.5 mM, 24.7 cm^2 at 0.75 mM, 23.0 cm^2 at 1 mM, 19.7 cm^2 at 1.18 mM, and 11.0 cm^2 at 1.5 mM. While, the final average growth area for the *PP1-6* strain under the 0 mM Cu control treatment was 34.6 cm^2, followed by 16.6 cm^2 at 0.25 mM, 9.2 cm^2 at 0.5 mM, 7.4 cm^2 at 0.75 mM, 8.2 cm^2 at 1 mM, 6.3 cm^2 at 1.18 mM, and 5.2 cm^2 at 1.5 mM. No growth was observed at 2 mM Cu for *PP1-5* or *PP1-6*, indicating that the toxicity threshold for *P. pistillaris* growth is between 1.5 mM and 2 mM Cu. In the field, copper mine tailings in Arizona have been measured to have an average copper concentration of 526.4 mg/kg (Haque et al. 2008). Assuming a copper speciation of purely copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) and an average soil bulk density of 1.43 g/mL (Gitari et al. 2018), this corresponds to a copper concentration of approximately 3 mM. Therefore, some prior

treatments or additional amendments would be necessary for *PP1-5* and *PP1-6* to be applied *in-situ* in this particular scenario.

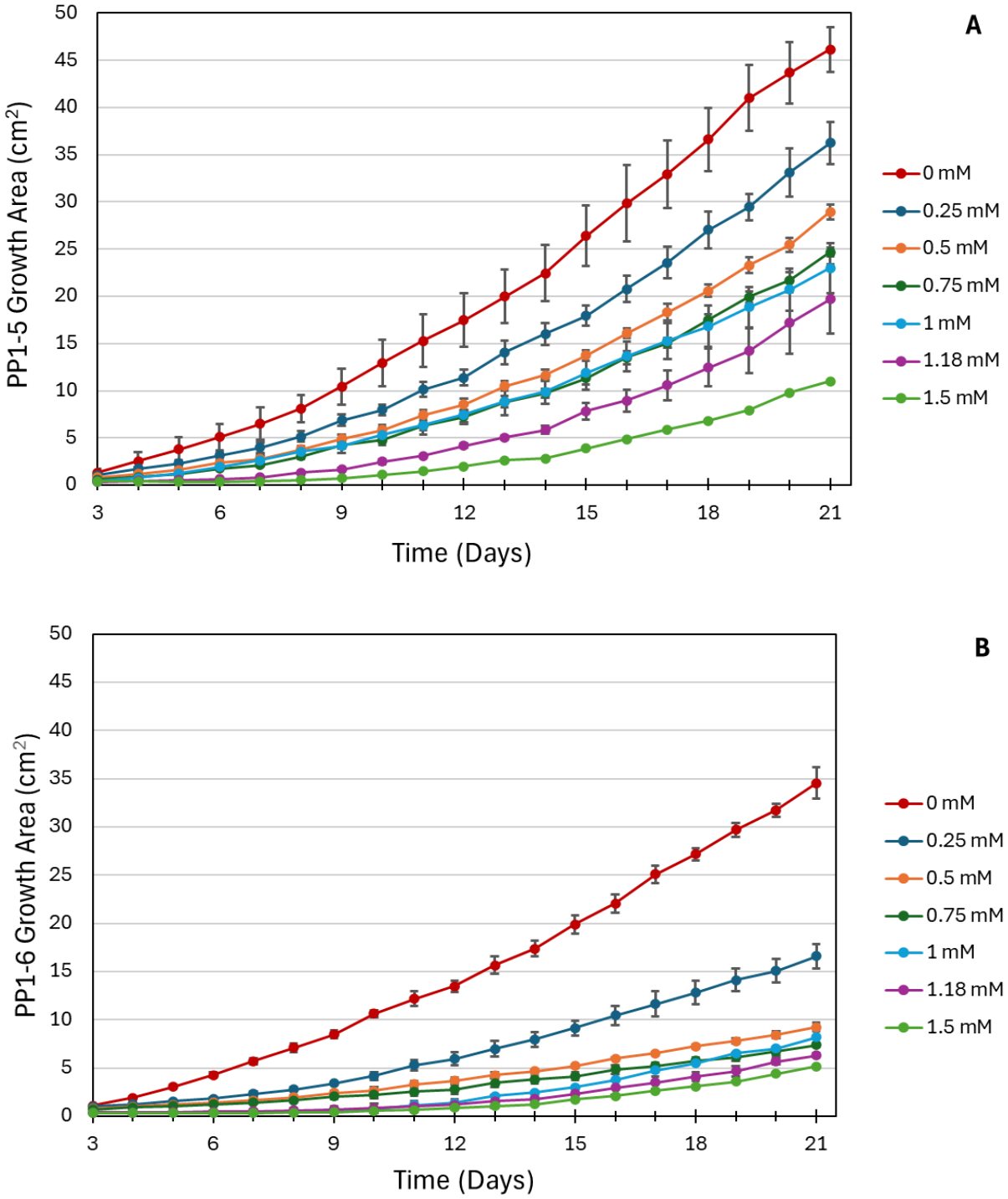

Figure 1. Average mycelial growth area of *PP1-5* (A) and *PP1-6* (B) over the 21-day growth period at 0 mM, 0.25 mM, 0.5 mM, 0.75 mM, 1 mM, 1.18 mM, and 1.5 mM Cu concentrations. The standard deviation of each triplicate set at each time point was calculated and incorporated as error bars.

As shown in Figure 1, *PP1-5* and *PP1-6* exhibited limited deviations in growth area between replicates at elevated copper concentrations (1.5 mM for *PP1-5* and concentrations greater than 0.25 mM for *PP1-6*) compared to the visible deviations between replicates at lower copper concentrations. Furthermore, many replicates failed to grow at these elevated copper concentrations. Growth was observed for 1 out of 3 total replicates for *PP1-5* at 1.5 mM and 5 out of 12 total replicates for *PP1-6* at concentrations greater than 0.25 mM. These observations could be explained by phenotypic heterogeneity in the genetically identical replicates of *PP1-5* and *PP1-6*. Heterogeneity can contribute to the selection of advantageous phenotypes in the face of environmental stress, such as industrial pollutants. In many cases, these phenotypes make trade-offs between biological capabilities to survive (Hewitt et al. 2016). In the case of *PP1-5* and *PP1-6*, individuals exhibiting limited growth rates in return for more effective copper tolerance mechanisms likely dominated the successful replicates at elevated copper concentrations.

The similarity in toxicity thresholds suggests a common tolerance level among the strains; however, the overall growth and response to Cu dosing indicates that strain type may still influence *P. pistillaris*'s reaction to Cu presence. Figure 2 illustrates the differences in growth between PP1-5 and PP1-6 over the 22-day experiment under 0 mM, 0.25 mM, and 1.5 mM Cu conditions. Growth reduction in both strains was non-linear, suggesting that higher concentrations of Cu lead to increasingly pronounced inhibition of growth. Biomass growth reduction in *PP1-5* stayed below 50% for treatments up to 1 mM Cu and reached a maximum reduction of 76.1% at 1.5 mM Cu. In contrast, *PP1-6* showed more pronounced reductions, with growth decreasing by over 50% at every Cu concentration, peaking at 85.0% at 1.5 mM. When comparing copper tolerance, *PP1-5* demonstrated higher tolerance across all Cu concentrations, while *PP1-6* showed greater sensitivity, especially at concentrations above 0.25 mM. This increased sensitivity in *PP1-6* is likely due to a higher bioaccumulation rate, where fungi actively produce metal-tolerant proteins to transport heavy metals into their cells (Chen et al. 2018). However, elevated and unregulated concentrations of heavy metals in fungal cells can lead to toxic effects, as seen in the reduced growth area of both *PP1-5* and *PP1-6* at higher copper concentrations (Szada-Borzyszkowska et al. 2024).

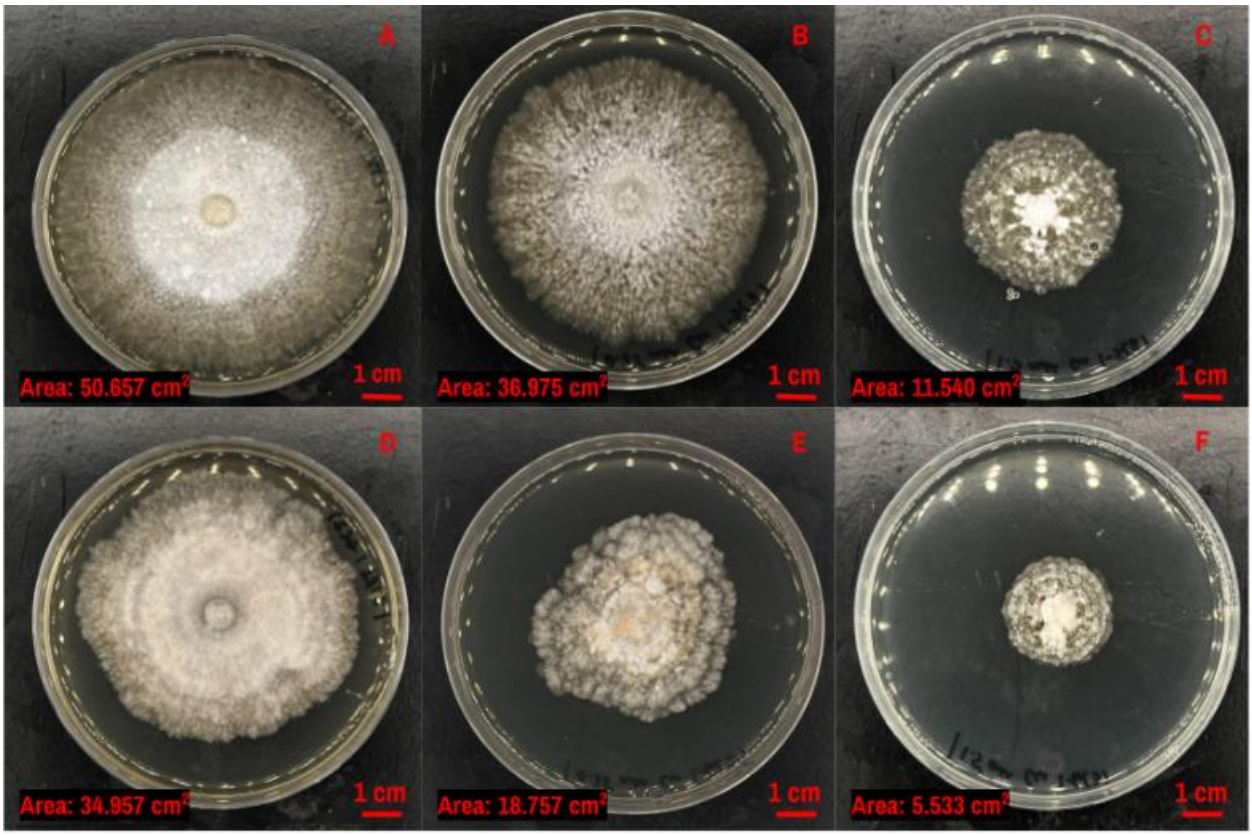

Figure 2. Radial growth of *PP1-5* at 22 days on (A) 0 mM Cu PDA, (B) 0.25 mM Cu PDA, and (C) 1.5 mM Cu PDA; and *PP1-6* at 22 days on (D) 0 mM Cu PDA, (E) 0.25 mM Cu PDA, and (F) 1.5 mM Cu PDA.

While bioaccumulation offers some explanation for the differences between the two strains, the current growth data does not fully account for other potential interactions with copper. For example, *PP1-5* and *PP1-6* might differ in their ability to produce metal-chelating compounds, which can neutralize copper or increase its bioavailability (Xie et al., 2019). Future work should quantify these additional heavy metal interactions to develop a more complete remediation profile for these strains.

When selecting fungi for mycoremediation, species and strain specificity are critical considerations due to the variations in remediation efficacy, degradation pathways, and contaminant interactions (Kózka et al. 2023). Despite these differences, clustering growth areas across treatments indicates a comparable overall response between the strains at each Cu concentration tested. It is important to note that the response to elevated copper concentrations by *PP1-5* and *PP1-6* may not represent all *P. pistillaris* strains, as other strains might tolerate higher Cu levels. Significant variations can exist among fungal communities, even those collected from the same local region, underscoring the importance of testing a variety of species and strains for their remediation capabilities (Nascimento et al. 2011). Isolating and testing other *P. pistillaris* communities for their heavy metal tolerance and sequestration properties is crucial to identifying a group of strains suitable for mine tailing remediation in arid and semi-arid environments.

Throughout the growth period, *PP1-5* consistently outperformed *PP1-6* at every Cu concentration. The maximum average growth areas of the two strains were only comparable on the control plates, with increasing copper concentrations leading to notable deviations in growth between them. Co-culturing may offer a solution to unite and complement the distinct heavy metal tolerance and sequestration properties of *PP1-5* and *PP1-6*. This method leverages interactions between different organisms to withstand stressful environmental conditions, as demonstrated in phytoremediation. However, factors such as physical size, biochemical characteristics, and associated microbial communities can influence compatibility between strains (Zhang et al. 2023). Although *P. pistillaris* does not form known symbiotic relationships with plants, co-culturing *PP1-5* and *PP1-6* could be a valuable step toward combining their varied copper tolerance and sequestration capacities for enhanced remediation (Adeyemi et al. 2021).

CONCLUSION

Two strains of *P. pistillaris* were evaluated for their ability to tolerate elevated concentrations of copper. When the growth response between PP1-5 and PP1-6 was compared, PP1-5 exhibited a notable tolerance to elevated copper concentrations, maintaining relatively minimal growth area reductions at concentrations greater than 1 mM. Comparatively, strain PP1-6 was more sensitive to elevated copper concentrations, and major reductions in growth area were observed at concentrations greater than 0.25 mM. These differences in copper tolerance are likely due to varying rates of bioaccumulation between the two fungal strains. However, little can be extrapolated about the other ways these strains interact with heavy metals. Future work will consider how other mechanisms, such as biosorption or chelation, differ between PP1-5 and PP1-6 and how these factors dictate their remediation efficacies. Quantifying levels of copper removal by each fungus will be a promising next step. Future experiments will also isolate and explore different strains of *P. pistillaris* and consider the complementary benefits of co-culturing for copper tolerance and remediation. These next steps are integral to creating a fully developed concept for a mycoremediation strategy to apply to mine tailings in arid and semi-arid environments. Given the promising copper tolerance observed in PP1-5, *P. pistillaris* shows potential as a candidate for bioremediation in heavy metal-contaminated sites.

REFERENCES

Adeyemi, N. O., Atayese, M. O., Sakariyawo, O. S., Azeez, J. O., Abayomi Sobowale, S. P., Olubode, A., Mudathir, R., Adebayo, R., & Adeoye, S. (2021). Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead and zinc contaminated soils. *Rhizosphere*, *18*, 100325. https://doi.org/10.1016/j.rhisph.2021.100325

Brooks, C. F., Juanals, B., & Minel, J.-L. (2024). Environmental Debates in the Media: The Case of Hardrock Mining in Arizona and Energy Transition Dilemmas. *Sage Open*, *14*(3), 21582440241267847. https://doi.org/10.1177/21582440241267847

Chen, J., Liu, J., Hu, M., Liu, J., Yu, Y., Zhou, Y., Bao, N., Han, X., Zhao, X., & Guo, F. (2024). Potential and characteristics of heavy metals electrokinetic removal from the copper-zinc mine tailings: Study on the simulated and actual tailings. *Chemical Engineering Journal*, *496*, 154245. https://doi.org/10.1016/j.cej.2024.154245

Chen, M., Wang, L., Hou, J., Yang, S., Zheng, X., Chen, L., & Li, X. (2018). Mycoextraction: Rapid Cadmium Removal by Macrofungi-Based Technology from Alkaline Soil. *Minerals*, *8*(12). https://doi.org/10.3390/min8120589

Conlon, B. H., Aanen, D. K., Beemelmanns, C., de Beer, Z. W., De Fine Licht, H. H., Gunde-Cimerman, N., Schiøtt, M., & Poulsen, M. (2019). Reviewing the taxonomy of *Podaxis*: Opportunities for understanding extreme fungal lifestyles. *Fungal Biology*, *123*(3), 183–187. https://doi.org/10.1016/j.funbio.2019.01.001

Damodaran, D., Balakrishnan, R. M., & Shetty, V. K. (2013). The Uptake Mechanism of Cd(II), Cr(VI), Cu(II), Pb(II), and Zn(II) by Mycelia and Fruiting Bodies of Galerina vittiformis. *BioMed Research International*, *2013*(1), 149120. https://doi.org/10.1155/2013/149120

Gitari, W., Thobakgale, R., & Akinyemi, S. A. (2018). Mobility and Attenuation Dynamics of Potentially Toxic Chemical Species at an Abandoned Copper Mine Tailings Dump. *Minerals*, *8*, 64. https://doi.org/10.3390/min8020064

Haque, N., Peralta-Videa, J. R., Jones, G. L., Gill, T. E., & Gardea-Torresdey, J. L. (2008). Screening the phytoremediation potential of desert broom (*Baccharis sarothroides* Gray) growing on mine tailings in Arizona, USA. *Environmental Pollution*, *153*(2), 362–368. https://doi.org/10.1016/j.envpol.2007.08.024

Hashem, A. R., & Al-Rahmah, A. N. (1993). Growth of Podaxis pistillaris Collected from Saudi Arabia at Different Concentrations of Cadmium and Lead. *Journal of King Saud University*, *5*(2), 127–135.

Hewitt, S. K., Foster, D. S., Dyer, P. S., & Avery, S. V. (2016). Phenotypic heterogeneity in fungi: Importance and methodology. *Fungal Biology Reviews*, *30*(4), 176–184. https://doi.org/10.1016/j.fbr.2016.09.002

Kózka, B., Sośnicka, A., Nałęcz-Jawecki, G., Drobniewska, A., Turło, J., & Giebułtowicz, J. (2023). Various species of *Basidiomycota* fungi reveal different abilities to degrade pharmaceuticals and also different pathways of degradation. *Chemosphere*, *338*, 139481. https://doi.org/10.1016/j.chemosphere.2023.139481

Obuekwe, C. O., Badrudeen, A. M., Al-Saleh, E., & Mulder, J. L. (2005). Growth and hydrocarbon degradation by three desert fungi under conditions of simultaneous temperature and salt stress. *International Biodeterioration & Biodegradation*, *56*(4), 197–205. https://doi.org/10.1016/j.ibiod.2005.05.005

Palanivel, T. M., Pracejus, B., & Novo, L. A. B. (2023). Bioremediation of copper using indigenous fungi *Aspergillus* species isolated from an abandoned copper mine soil. *Chemosphere*, *314*, 137688. https://doi.org/10.1016/j.chemosphere.2022.137688

Parades-Aguilar, J., Calderon, K., Agustin-Salazar, S., Cerruti, P., Ambrogi, V., Gamez-Meza, N., & Medina-Juarez, L. A. (2024). Isolation and identification of metallotolerant bacteria with a potential biotechnological application. *Scientific Reports*, *14*(1), 3663. https://doi.org/10.1038/s41598-024-54090-0

Rani, J., & Paul, B. (2023). Challenges in arid region reclamation with special reference to Indian Thar Desert—Its conservation and remediation techniques. *International Journal of Environmental Science and Technology*. https://doi.org/10.1007/s13762-022-04746-z

Szada-Borzyszkowska, A., Krzyżak, J., Rusinowski, S., Sitko, K., & Pogrzeba, M. (2024). Toxic effect of mercury on arbuscular mycorrhizal fungi colonisation and physiological status of three seed-based *Miscanthus* hybrids. *Journal of Trace Elements in Medicine and Biology*, *83*, 127391. https://doi.org/10.1016/j.jtemb.2024.127391

Xie, Y., Li, X., Huang, X., Han, S., Amombo, E., Wassie, M., Chen, L., & Fu, J. (2019). Characterization of the Cd-resistant fungus *Aspergillus aculeatus* and its potential for increasing the antioxidant activity and photosynthetic efficiency of rice. *Ecotoxicology and Environmental Safety*, *171*, 373–381. https://doi.org/10.1016/j.ecoenv.2018.11.123

Zhang, Q., Ma, L., Peng, Y., & Yan, X. (2024). Sustainable bioleaching of heavy metals from coal tailings using *Bacillus inaquosorum B.4*: Mechanistic insights and environmental implications. *Journal of Environmental Chemical Engineering*, *12*(5), 113400. https://doi.org/10.1016/j.jece.2024.113400

Zhang, Y., Li, M., Su, A., Lv, X., Qiu, Y., & Xu, Y. (2023). Co-planting improves the phytoremediation efficiency of combined phenanthrene and copper co-contaminated soils. *Journal of Cleaner Production*, *382*, 135380. https://doi.org/10.1016/j.jclepro.2022.135380