Phylogenetic Perspectives on Heavy Metal Hyperaccumulation in Fungal Lineages

Heavy Metal Hyperaccumulation in Fungal Lineages

Martinez C[1,2], Thompson JB[2], and Hawkins JA[2]

[1]*Department of Geography and Environmental Science, University of Reading, Reading, RG6 6AB, UK*

[2]*School of Biological Sciences, University of Reading, Reading, RG6 6UR, UK*

Corresponding Authors:

Martinez C: c.martinez@pgr.reading.ac.uk

Hawkins J.A.: j.a.hawkins@reading.ac.uk

Abstract

Across the fungal kingdom, the ability to hyperaccumulate and sequester toxic heavy metals from the environment appears to have evolved multiple times. Although in plants, animals, and bacteria the evolution of heavy metal hyperaccumulation is well studied, and despite potential applications of hyperaccumulation in mycoremediation, fungi are under-investigated. Here, we compile a novel dataset from 109 published sources that record hyperaccumulation from 204 species of Fungi, expanding previous compilations more than sixfold. In order to test whether melanisation is predictive of ability to hyperaccumulate, as has been suggested by melanin's role in reducing oxidative stress, a novel dataset describing the distribution of melanism was also constructed. These data are analysed in a phylogenetic framework, to explore the evolutionary history of heavy metal accumulation in fungi. We identify understudied groups and test whether melanism is over-represented in hyperaccumulating lineages. These analyses reveal potential 'hot nodes' for heavy metal hyperaccumulation in Fungi and tentatively support a relationship between hyperaccumulation and melanism. Our dataset and analyses highlight the necessity for further research into fungi and fungal hyperaccumulation, potentially opening new horizons for mycoremediation.

Main Text

1 Introduction

Hyperaccumulation is the ability to accumulate and sequester high concentrations of contaminants [1]. Heavy metals (HMs) in the soil are hyperaccumulated by some plants, fungi and other organisms and enter the food chain where they have an array of negative impacts from reduced soil fertility to impacts on human health with the HM acting as carcinogens and mutagens, and increasing the risk for conditions such as Parkinson's Disease [2,3,4,5]. Further, HMs enter the water systems and accumulate in agricultural crops, accumulating up the food chain and entering human livestock food supplies [6].

Whilst hyperaccumulation of HMs can present health risks, it can also be exploited to remediate contaminated sites, such as polluted rivers [7]. Although the threshold for recognising species as "hyperaccumulators" varies within the literature, there are some fungi that are consistently found to be able to take up high levels of potentially toxic HMs without experiencing serious toxic effects [8]. While some HMs, such as iron, are essential for normal fungal function at low levels but toxic at higher levels, other metals, such as mercury, are non-essential or have an unknown function but adversely impact normal fungal function at high concentrations [9]. An important ecological example is that hyperaccumulation of HMs can affect the development of mycorrhizal fungi and spore production, in certain fungi at higher HM concentrations [9,10].

In Glomeromycota this has implications for the farming industry given that most crop plants form, and rely on, mycorrhizal relationships [11]. However, not all fungi are negatively impacted by the presence of HMs and those that hyperaccumulate HMs can be used to improve soil quality, drawing HMs out of the soil and sequestering them, either by binding them extracellularly or elsewhere biochemically inaccessible, or through chelation and inactivation in the cytosol [10,11,12]. This protects the fungus from metabolic and oxidative damage from free radicals [10,11,12].

Mycoremediation, the removal or degradation of contaminants in the environment using Fungi that hyperaccumulate and sequester HMs and other contaminants, is an emerging area of interest for mycologists and remediators as a novel solution to environmental pollution [13,14]. While the current state of industrial mycoremediation is mostly small-scale and using a small number of species, the success of these interventions suggests that there is scope for mycoremediation on a larger-scale. Development of mycoremediation depends on the identification of effective HM hyperaccumulators, but this is difficult in the field or laboratory, because many fungi are difficult to culture or do not produce fruiting bodies [15].

Much of the work in identifying HM hyperaccumulating fungi consists of disparate projects, which tend to be highly situational and opportunistic. However, a phylogenetic approach towards identifying HM accumulators could streamline the discovery of mycoremediative agents [3]. This approach is not unprecedented, with similar work having been conducted in earthworms, bacteria, and plants [16,17,18]. Plant hyperaccumulators often form symbiotic relationships with mycorrhizal fungi, a field that has been well studied, however, the study of the fungal metal hyperaccumulators and their evolution is very limited [19]. Mycoremediation research would benefit from understanding predictors of HM hyperaccumulation and a potepotential predictors is melanisation. Melanisation is a common

adaptation in Fungi to extreme conditions that cause oxidising stress [20]. Suthar et al. (2023) suggested fungi growing in habitats with high HM concentrations - sometimes contaminated with radioisotopes - would likely be melanised [21]. Fungal melanins are effective biosorbents of HMs, reducing oxidative stress, allowing fungi to uptake and sequester HMs such as $Pb^{2+,}$ Cd^{2+}, and other HMs, especially in aquatic environments [22]. Fungal melanins have been shown to assist in HM chelation with melanised fungi often observed growing in environments with industrial contamination or at roadsides [23]. It has also been found that melanin allows fungi to uptake and chelate metals taken up from the soil that would otherwise be toxic to them, such as silver nitrate [24,25,26]. This testifies to the adaptability and remediative capabilities of fungi, prompting the question whether melanin production is correlated with the ability to hyperaccumulate HMs in the environment.

Here, we present a database of 204 species of HM accumulating fungi and 244 melanising fungi from 127 sources, and test whether melanisation predicts HM hyperaccumulation in a phylogenetic framework. We find that there is a significant phylogenetic signal for HM hyperaccumulation in fungi, we identify 315 hot nodes with higher-than-expected HM hyperaccumulating and melanising fungi, and find a modest link between the two traits; melanistic fungi are over-represented in the hot nodes. Our database and results provide a solid foundation for future data collection and comparative research in fungi.

2 Materials and methods

2.1 Data collection

A literature review was performed using publicly available data. Literature searches were conducted in Google Scholar, ScienceDirect, JSTOR, PubMed and the book *Mycelium Running* [27]. This data was collated to construct a dataset of known fungal HM hyperaccumulators (Appendix A). A search term strategy was established to retrieve data for analysis, combining search terms which were categorised into two groups. The first related to HMs, and hyperaccumulation and consisted of: 'heavy metal*', 'hyperaccumulation', 'remediation'. 'uptake', 'HM uptake', 'HM hyperaccumulation', 'mycoremediation'. 'remediation', 'metals'. The second related to fungi and consisted of: 'fungal', 'fungi', 'fungus', 'mycorrhizal', and 'mushroom'. For the melanisation data collection, a similar method was employed using the same fungal terms and search terms relating to melanisation consisting of: 'melanin', 'melanisation', 'melanised', and 'melanin production' (Appendix C). For both HM hyperaccumulation and melanisation, the literature comprises of multiple methods of identification of those traits, often based on observation and opportunistic

sampling as opposed to systematic testing. Where possible, the data was corroborated through multiple sources to justify its inclusion in the dataset.

Species names recorded in the literature review were corrected to match the taxonomy used in a fungal phylogeny produced by Li et al (2021) [28]. This was the most recent comprehensive genome scale fungal phylogeny available. The phylogeny comprises of 1644 fungal taxa from 247 genera and 90 families, and with 28 outgroup taxa, and approximately 85% of the relationships inferred were considered well supported and resolved [28]. The species in the phylogeny were coded for presence (1) or absence (0) of evidence of the ability to hyperaccumulate HMs (Appendix C), and the same was done for the presence/absence of evidence of melanin production (Appendix D).

2.2 Statistical analysis

2.2.1 Phylogenetic signal

Two tests for phylogenetic signal were conducted using the phylo.d function implemented in the R package "caper" [29]. The first, tested for the phylogenetic signal of HM hyperaccumulation in Fungi as a binary trait. The fungal phylogeny [28] and hyperaccumulation data, coded 1/0 respectively, (Appendix C). If the "Estimated D" value is below 0, there is good evidence for the clustering of the trait. A highly conserved trait will give a value close to 1. Two p-values are generated: Pval1 (the probability of E(D) is a result of no (random) phylogenetic structure) and Pval0 (the probability of E(D) resulting from no (random) phylogenetic structure [29]. The D value is calculated from the sum of the changes observed in the estimated nodal values of the trait along the edges of the phylogeny.

2.2.2 Hot node identification

To identify nodes with more HM hyperaccumulating species than expected, we used the "hot nodes" approach [30,31,32,33,34]. This is based on the principle that certain phylogenetic lineages, in traits which show phylogenetic signal, harbour more species with the trait than expected under random distributions. We computed a non-parametric p-value for each internal node, comparing the number of hyperaccumulating descendants against a null distribution of 999 random shuffles of the trait across the phylogeny. Nodes were considered significant if they had a p-value below 0.05 (5% nominal alpha, one-tailed test). The R code provided by Atienza-Barthelemy et al. (2024) [34] was used and the full script, for all comparative analyses, is available at: https://github.com/CMartinezData/Fungi-Heavy-Metal-Hyperaccumulation. We then compared the overlaps of melanising species with those in the HM hyperaccumulating hot nodes.

3 Results

3.1 Data coverage

We found 109 sources that recorded at least one fungal taxon as HM hyperaccumulating; in total there were 72 genera and 157 species recorded in our dataset (Appendix A). Similarly, we record 244 species which have melanin from 18 sources (Appendix B). The number of species that were both hyperaccumulating and had melanin was 20, representing 9.8% of hyperaccumulators and 8.2% of the species with melanin.

After filtering for species also present in the phylogeny [28], 204 HM hyperaccumulating and 48 melanin-producing species were retained for subsequent phylogenetic analyses and were scored binarily. These comprise 12.2% and % of the species in the phylogeny, respectively. In the cases where a source reported only at genus and not species level, we scored all species in the genus as the same state. The filtered dataset is available in Appendix B.

3.2 Phylogenetic signal

We found strong phylogenetic signal for HM hyperaccumulation in the fungal kingdom (D = -0.1794588) (Figure 1). The D statistic calculates two p values under different null models, and we found that he probability of E(D) resulting from no (random) phylogenetic structure was 0, while the probability of E(D) resulting from Brownian phylogenetic structure was 0.926. Together, these reveal that HM hyperaccumulation is likely to be not randomly distributed, and evolve similarly to a Brownian motion model of evolution.

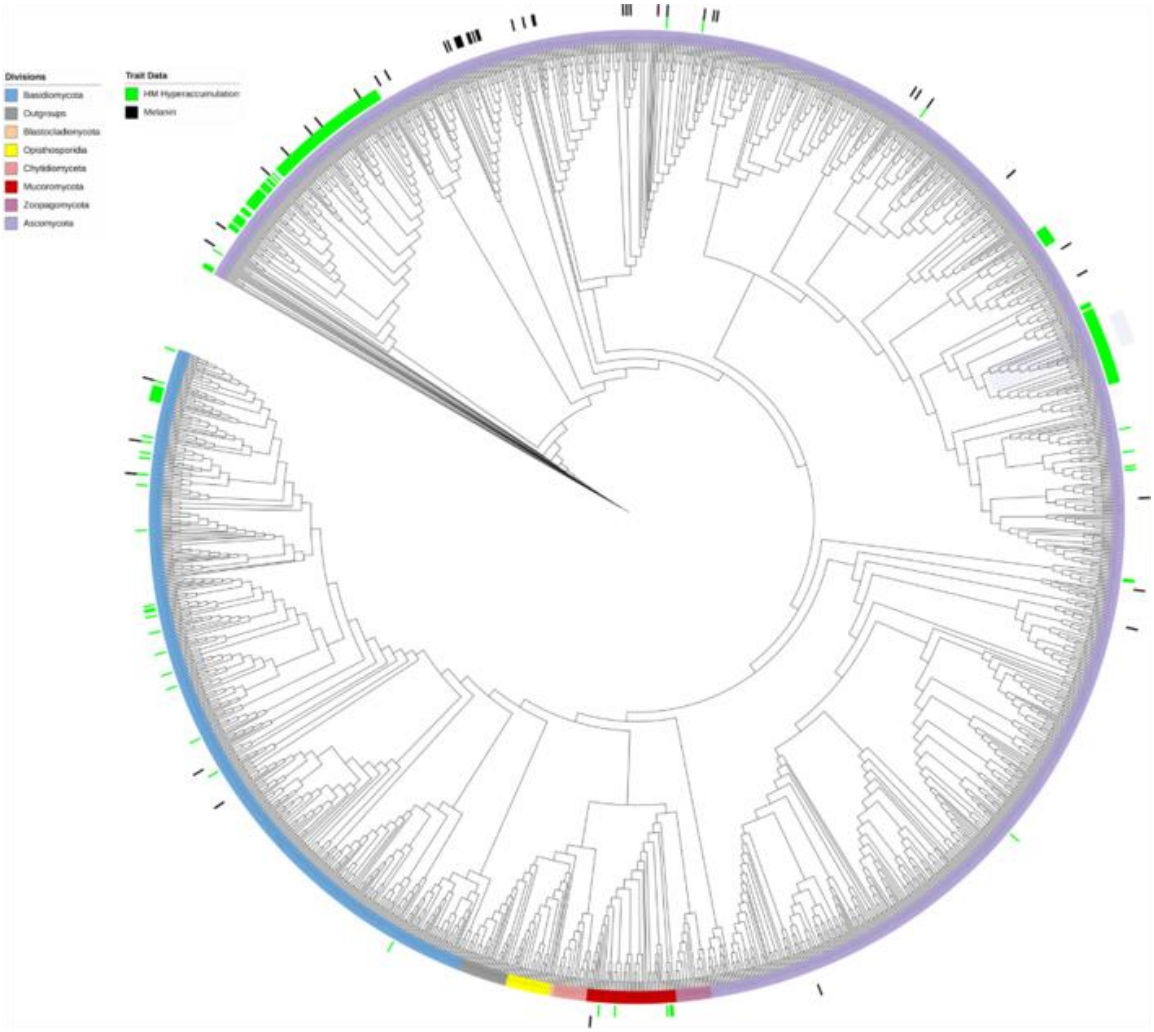

Figure 1: *Phylogenetic distribution of heavy metal hyperaccumulation and melanin across the fungal kingdom. lack outer ring: melanin, green middle ring: HM hyperaccumulation, coloured innermost ring: fungal divisions. Figure prepared using iTOL [35] and phylogeny is from Li et al., (2021)[28].*

3.3 Hot nodes

We identified 315 HM hyperaccumulation hot nodes, comprising 18.8% of the species in the phylogeny. While melanised fungi make up 2.9% of the dataset, 10 species (3.3%) were found to intersect with species in the HM hyperaccumulation hot nodes. This is a very modest increase in the proportion of melanistic species in the HM hyperaccumulation hot nodes as compared to the phylogeny as a whole.

4 Discussion

Despite the poor data availability previously, we found 204 species to be recorded HM hyperaccumulators, a sixfold improvement. Prior to our study, Stamets (2005) reported that 36 species were known hyperaccumulators [14]. Our study represents the first systematic and the most comprehensive survey of fungal HM hyperaccumulation reported in the literature. Nevertheless, the proportion of fungal species reported with this trait is very small, limiting the kinds of analyses possible and the conclusions that can be drawn from the data. Despite this, we show the distribution of ability to accumulate HMs is phylogenetically structured and may be related to melanin production and we used the hot node approach to very tentatively point to a relationship between HM hyperaccumulation and melanin production. More research into HM hyperaccumulation and melanin in fungi, constructing larger datasets, would be invaluable for producing more robust analyses and highlighting potential hot nodes for fungal HM hyperaccumulators.

We find that HM hyperaccumulation shows phylogenetic signal across fungi. This indicates that there are evolutionary patterns in the distribution of the ability to hyperaccumulate heavy metals within the kingdom Fungi and the distribution is not random. The ability to hyperaccumulate heavy metals appears to have evolved in certain groups of fungi, perhaps conveying an adaptive advantage in contaminated niches. This provides a jumping off point for more structured investigation into mycoremediative potential across the fungal Kingdom. Current practice in mycoremediation involves the identification of fungi living within contaminated habitats followed by the culturing and 'training' of specific strains within those species for the purposes of remediating specific contaminants [36]. This opportunistic approach has highlighted the abundance of HM hyperaccumulators within phyla such as the Basidiomycota and Ascomycota [37,38]. Filamentous and fruiting body fungi have an advantage in hyperaccumulation over yeasts (and other single celled organisms) as the densely packed hyphae collect the HMs in a solid and stable form sequestered away, however, they are also most likely to be identified and tested within contaminated environments given their larger and more prominent body size and structure [37,39,14]. Fungi present in a large range of habitats are more likely to have strains capable of hyperaccumulation and are more likely to be manipulated to be more effective mycoremediators, given that they are more likely to encounter contaminated environments as well as being more likely to be detected [14]. Fungi that grow in soil or aquatic environments, as opposed to those in host organisms, would likely have a higher HM tolerance given the normal levels of heavy metal exposure from their environment. Further, it follows that saprophytic and mycorrhizal fungi that live in the soil, and fungi in other environments where

significant amounts of HMs are present within their habitat and nutrient supply have evolved to be effective HM hyperaccumulators [9]. This pattern has been noticed with white rot fungi due to their specific lignin degrading enzymes [40,41]. As evidenced here, there are many factors that may lead to the clustering of HM tolerance as a trait within the kingdom Fungi.

Sourcing data from publications, as we do here, may introduce sampling biases. In their construction of the phylogeny, Li et al. used 11 data matrices generated through subsampling genes from the full data matrix to sensitivity test for biases including the removal of taxa or genes to facilitate improved phylogenomic inference using a concatenation-based model [28]. It was found that sampling was biased towards Ascomycota and Basidiomycota with the Saccharomycotina, Peziomycotina, and Agaricomycotina dominating the data [28]. This is similar to the sampling bias found during the literature review as these taxa are highly studied and reported on compared to other fungi, especially those that do not form fruiting bodies. The bias in research to focus study on fungi that form fruiting bodies is a known issue within many aspects of mycology but efforts are being made to reduce the biases. This includes ecological surveying, in which recent advances in eDNA analysis aim to reduce the sampling bias by maximizing sequencing of fungal diversity in a sample [42]. However, the eDNA approach is still in development and, as such, data collected on HM hyperaccumulation and melanisation in fungi is subject to the same biases.

Scoring melanin as present or absent introduces a different challenge. Fungal melanisation is caused, not by a single mechanism and application, but through several melanin precursors and synthesis pathways [28]. These melanins are produced by a variety of genes across different fungi and expressed differently under different environmental conditions [43,26]. This makes identification of melanised fungi problematic, relying on either observation of fruiting bodies or culturing and genetic analysis, subject to the environmental conditions that may promote or repress melanin production and expression [26].

5 Conclusions

We have presented the data and analyses here as moving towards an approach for rational discovery of fungal HM accumulators for mycoremediation. Until now, there has been no such systematic search for candidate species. One benefit of the current, opportunistic approach to identification of candidate HM hyperaccumulator species is that it relies on local uses for local species. The limited introduction of non-native species into new areas may be an advantage, since the spread of fungal pathogens through spores and mycorhizal associations can pose dangers to human and environmental health [44]. While non-native fungi may provide novel remediative solutions for contaminated soils, a case-by-case

ecological impact review should be undertaken before the culturing and introduction of mycoremediators in non-native environments.

Here we focus on HM hyperaccumulation. However, Fungi have been noted to be tolerant of other extreme conditions, including radiative environments [45,46]. In this case, melanin may also be indicative of the ability to tolerate extreme conditions. The approach we take here could be applied to fungi that may provide novel remediative solutions to these very specific conditions, but in this case there is an even greater need for baseline documentation.

This research demonstrates the viability of a phylogenetics-based approach towards more effective HM hyperaccumulation using fungi. Through the identification of potential 'hot nodes' and discussion of factors that may indicate a greater ability to hyperaccumulate HMs, we establish a groundwork for future systematic sampling and testing within the fungal kingdom. However, it is also evident that the limited information available on fungal HM hyperaccumulation and the sampling biases present in mycological research greatly impacts the reliability of conclusions drawn from phylogenetics based approaches at this time. With improved sampling, systematic testing, and larger, more comprehensive, datasets, the biases limiting this research could be overcome, opening up new horizons for mycoremediation.

6 Electronic supplementary information

Appendices A-D are available as Electronic Supplementary Information.

7 Author contributions

Study conception and design: Martinez, C., Hawkins, J.A.; data collection: Martinez, C.; analysis and interpretation of results: Martinez, C., Hawkins, J.A., Thompson, J.; draft manuscript preparation: Martinez, C., Hawkins, J.A., Thompson, J.B. All authors reviewed the results and approved the final version of the manuscript.

8 Acknowledgments

Many thanks to Dr. Louise Johnson and Dr. Andrew Meade at the University of Reading for support during the development of this paper. Thanks also to: Ester Gaya from Kew Gardens, and the Rokas Laboratory for providing the phylogeny from their 2021 paper Li et al., (2021).

9 Ethical Statement (Optional.)

"Not applicable."

10 Conflict of interest

The authors declare that they have no conflict of interest.

11 Data availability

The fungal phylogeny is available from:	Li Y, Steenwyk JL, Chang Y, Wang Y, James TY, Stajich JE, Spatafora JW, Groenewald M, Dunn CW, Hittinger CT, Shen XX, Rokas A. A genome-scale phylogeny of the kingdom Fungi. *Curr Biol*. 2021 Apr 26;31(8):1653-1665.e5. doi: 10.1016/j.cub.2021.01.074. Epub 2021 Feb 18. PMID: 33607033; PMCID: PMC8347878.
Data available within the article or its supplementary materials.	All data generated or analysed during this study are included in this published article [and its supplementary information files]: https://github.com/CMartinezData/Fungi-Heavy-Metal-Hyperaccumulation/tree/main
Code used for phylogenetic analyses.	All code used for the phylogenetic analysis during this study is available via GitHub at: https://github.com/CMartinezData/Fungi-Heavy-Metal-Hyperaccumulation

12 References

1. Rascio, N. and Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, 180(2), pp.169–181. doi:https://doi.org/10.1016/j.plantsci.2010.08.016.
2. Khan, I., Ali, M., Aftab, M., Shakir, S., et al. (2019). Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metallotolerant fungi. *Environmental Monitoring and Assessment*, 191(10). doi:https://doi.org/10.1007/s10661-019-7781-9.
3. Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. and Wang, M.-Q. (2021). Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics*, [online] 9(3), p.42. doi:https://doi.org/10.3390/toxics9030042.
4. Zhuang, P., Lu, H., Li, Z., Zou, B. and McBride, M.B. (2014). Multiple Exposure and Effects Assessment of Heavy Metals in the Population near Mining Area in South China. *PLoS ONE*, 9(4), p.e94484. doi:https://doi.org/10.1371/journal.pone.0094484.
5. Pyatha, S., Kim, H., Lee, D. and Kim, K. (2022). Association between Heavy Metal Exposure and Parkinson's Disease: a Review of the Mechanisms Related to Oxidative Stress. *Antioxidants*, [online] 11(12), p.2467. doi:https://doi.org/10.3390/antiox11122467.
6. Placek, A., Grobelak, A. and Kacprzak, M. (2015). Improving the phytoremediation of heavy metals contaminated soil by use of sewage sludge. *International Journal of Phytoremediation*, 18(6), pp.605–618. doi:https://doi.org/10.1080/15226514.2015.1086308.
7. Pini, A.K. and Geddes, P. (2020). Fungi Are Capable of Mycoremediation of River Water Contaminated by E. coli. *Water, Air, & Soil Pollution*, 231(2). doi:https://doi.org/10.1007/s11270-020-4464-7.
8. Priyanka and Dwivedi, S.K. (2023). Fungi mediated detoxification of heavy metals: Insights on mechanisms, influencing factors and recent developments. *Journal of Water Process Engineering,* [online] 53, p.103800. doi:https://doi.org/10.1016/j.jwpe.2023.103800.
9. Gajewska, J., Floryszak-Wieczorek, J., Sobieszczuk-Nowicka, E., Mattoo, A. and Arasimowicz-Jelonek, M. (2022). Fungal and oomycete pathogens and heavy metals: an inglorious couple in the environment. *IMA Fungus*, 13(1). doi:https://doi.org/10.1186/s43008-022-00092-4.
10. Pawlowska, T.E. and Charvat, I. (2004). Heavy-Metal Stress and Developmental Patterns of Arbuscular Mycorrhizal Fungi. Applied and Environmental *Microbiology,*

70(11), pp.6643–6649. doi:https://doi.org/10.1128/aem.70.11.6643-6649.2004.
11. Chang, Q., Diao, F., Wang, Q., Pan, L., et al. (2018). Effects of arbuscular mycorrhizal symbiosis on growth, nutrient and metal uptake by maize seedlings (Zea mays L.) grown in soils spiked with Lanthanum and Cadmium. Environmental *Pollution,* 241, pp.607–615. doi:https://doi.org/10.1016/j.envpol.2018.06.003.
12. Chen, J., Guo, J., Li, Z., Liang, X., et al. (2022). Effects of an Arbuscular Mycorrhizal Fungus on the Growth of and Cadmium Uptake in Maize Grown on Polluted Wasteland, Farmland and Slopeland Soils in a Lead-Zinc Mining Area. *Toxics*, [online] 10(7), p.359. doi:https://doi.org/10.3390/toxics10070359.
13. Kulshreshtha, S., Mathur, N. and Bhatnagar, P. (2014). Mushroom as a product and their role in mycoremediation. *AMB Express,* [online] 4(1). doi:https://doi.org/10.1186/s13568-014-0029-8.
14. Stamets, P., 2005. *Mycelium running: How mushrooms can help save the world*. Berkeley, CA: Ten Speed Press.
15. Gautam, A.K., Verma, R.K., Avasthi, S., Sushma, et al., (2022). Current Insight into Traditional and Modern Methods in Fungal Diversity Estimates. *Journal of Fungi,* 8(3), p.226. doi:https://doi.org/10.3390/jof8030226.
16. Hobbelen, P.H.F., Koolhaas, J.E. and van Gestel, C.A.M. (2006). Bioaccumulation of heavy metals in the earthworms Lumbricus rubellus and Aporrectodea caliginosa in relation to total and available metal concentrations in field soils. *Environmental Pollution,* 144(2), pp.639–646. doi:https://doi.org/10.1016/j.envpol.2006.01.019.
17. Nnaji, N.D., Anyanwu, C.U., Miri, T. and Onyeaka, H. (2024). Mechanisms of Heavy Metal Tolerance in Bacteria: A Review. *Sustainability,* 16(24), pp.11124–11124. doi:https://doi.org/10.3390/su162411124.
18. Reeves, R.D., Baker, A.J.M., Jaffré, T., Erskine, P.D., et al. (2017). A global database for plants that hyperaccumulate metal and metalloid trace elements. *New Phytologist,* 218(2), pp.407–411. doi:https://doi.org/10.1111/nph.14907.
19. Boyd, R.S. and Martens, S.N. (1998). The significance of metal hyperaccumulation for biotic interactions. *Chemoecology*, 8(1), pp.1–7. doi:https://doi.org/10.1007/s000490050002.
20. Grishkan, I. (2011). Ecological Stress: Melanization as a Response in Fungi to Radiation. *Extremophiles Handbook,* pp.1135–1145. doi:https://doi.org/10.1007/978-4-431-53898-1_54.
21. Suthar, M., Dufossé, L. and Singh, S.K. (2023). The Enigmatic World of Fungal Melanin: A Comprehensive Review. *Journal of Fungi,* 9(9), pp.891–891. doi:https://doi.org/10.3390/jof9090891.
22. Tran-Ly, A.N., Ribera, J., Schwarze, F.W.M.R., Brunelli, M. and Fortunato, G. (2020). Fungal melanin-based electrospun membranes for heavy metal detoxification of water. *Sustainable Materials and Technologies,* 23(e00146), p.e00146.

doi:https://doi.org/10.1016/j.susmat.2019.e00146.

23. Dadachova, E., Bryan, R.A., Howell, R.C., Schweitzer, A.Det al. (2007). The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. *Pigment Cell & Melanoma Research*, 21(2), pp.192–199. doi:https://doi.org/10.1111/j.1755-148x.2007.00430.x.
24. Ban, Y., Tang, M., Chen, H., Xu, Z. et al. (2012). The Response of Dark Septate Endophytes (DSE) to Heavy Metals in Pure Culture. *PLoS ONE*, 7(10), p.e47968. doi:https://doi.org/10.1371/journal.pone.0047968.
25. García-Rivera, J. and Casadevall, A. (2001). Melanization of Cryptococcus neoformans reduces its susceptibility to the antimicrobial effects of silver nitrate. *Medical Mycology,* 39(4), pp.353–357. doi:https://doi.org/10.1080/714031043.
26. Liu, R., Meng, X., Mo, C., Wei, X. and Ma, A. (2022). Melanin of fungi: from classification to application. *World Journal of Microbiology and Biotechnology,* 38(12). doi:https://doi.org/10.1007/s11274-022-03415-0.
27. Stamets, P. and Zwickey, H. (2014). Medicinal Mushrooms: Ancient Remedies Meet Modern Science. *Integrative medicine (Encinitas, Calif.),* [online] 13(1), pp.46–7. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4684114/.
28. Li, Y., Steenwyk, J.L., Chang, Y., Wang, Y., James, T.Y., Stajich, J.E., Spatafora, J.W., Groenewald, M., Dunn, C.W., Hittinger, C.T., Shen, X.-X. and Rokas, A. (2021). A genome-scale phylogeny of the kingdom Fungi. *Current Biology,* 31(8), pp.1653-1665.e5. doi:https://doi.org/10.1016/j.cub.2021.01.074.
29. Fritz, S.A. and Purvis, A. (2010). Selectivity in Mammalian Extinction Risk and Threat Types: a New Measure of Phylogenetic Signal Strength in Binary Traits. Conservation Biology, 24(4), pp.1042–1051. doi:https://doi.org/10.1111/j.1523-1739.2010.01455.x.
30. Saslis-Lagoudakis, C.H., Savolainen, V., WIlliamson, E.M., Forest, F., Wagstaff, S.J., Baral, S.R., Watson, M.F., Pendry, C.A. and Hawkins, J.A. (2012). Phylogenies reveal predictive power of traditional medicine in bioprospecting. *Proceedings of the National Academy of Sciences USA*. , 109(39), pp.15835–40. doi:https://doi.org/10.1073/pnas.1202242109.
31. De Souza, E.N.F. and Hawkins, J.A. (2017). Comparison of Herbarium Label Data and Published Medicinal Use: Herbaria as an Underutilized Source of Ethnobotanical Information. *Economic Botany,* 71(1), pp.1–12. doi:https://doi.org/10.1007/s12231-017-9367-1.
32. Lei, D., Al Jabri, T., Teixidor-Toneu, I., Saslis-Lagoudakis, C.H., et al. (2020).

Comparative analysis of four medicinal floras: Phylogenetic methods to identify cross-cultural patterns. *PLANTS, PEOPLE, PLANET,* 2(6), pp.614–626. doi:https://doi.org/10.1002/ppp3.10131.

33. Thompson, J.B. and Hawkins, J.A. (2024). Phylogeny and bioprospecting: The diversity of medicinal plants used in cancer management. *PLANTS, PEOPLE, PLANET,* 7(1). doi:https://doi.org/10.1002/ppp3.10566.
34. Atienza-Barthelemy, D., Macía, M.J. and Molina-Venegas, R. (2024). Hot node limitations and impact of taxonomic resolution on phylogenetic divergence patterns: A case study on Ecuadorian ethnomedicinal flora. *PLANTS, PEOPLE, PLANET*, 7(3), pp.644–653. doi:https://doi.org/10.1002/ppp3.10594.
35. Letunic, I. and Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research,* 49(W1), pp.W293–W296. doi:https://doi.org/10.1093/nar/gkab301.
36. Kulshreshtha, S., Mathur, N., Bhatnagar, P. and Kulshreshtha, S. (2013). Cultivation of Pleurotus citrinopileatus on handmade paper and cardboard industrial wastes. *Industrial Crops and Products,* 41, pp.340–346. doi:https://doi.org/10.1016/j.indcrop.2012.04.053.
37. Ghosh, S., Rusyn, I., Dmytruk, O.V., Dmytruk, K.V., et al. (2023). Filamentous fungi for sustainable remediation of pharmaceutical compounds, heavy metal and oil hydrocarbons. *Frontiers in Bioengineering and Biotechnology,* 11. doi:https://doi.org/10.3389/fbioe.2023.1106973.
38. Shourie, A. and Vijayalakshmi, U. (2022). Fungal Diversity and Its Role in Mycoremediation. *Geomicrobiology Journal,* 39(3-5), pp.426–444. doi:https://doi.org/10.1080/01490451.2022.2032883.
39. Kumar, V. and Dwivedi, S.K. (2021). Mycoremediation of heavy metals: processes, mechanisms, and affecting factors. *Environmental Science and Pollution Research,* 28(9), pp.10375–10412. doi:https://doi.org/10.1007/s11356-020-11491-8.
40. Baldrian, P. (2003). Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology,* 32(1), pp.78–91. doi:https://doi.org/10.1016/s0141-0229(02)00245-4.
41. Guilherme, A., Reyes, C., Maurício César Palmieri, Bevilaqua, D. and Regina, S. (2021). Innovative method for encapsulating highly pigmented biomass from Aspergillus nidulans mutant for copper ions removal and recovery. *PLOS ONE*, 16(11), pp.e0259315–e0259315. doi:https://doi.org/10.1371/journal.pone.0259315.
42. Runnel, K., Lõhmus, P., Küngas, K., Marmor-Ohtla, L., Palo, A., Pütsepp, G., Tamm, H., Tammekänd, I. and Lõhmus, A. (2024). Aerial eDNA contributes vital

information for fungal biodiversity assessment. *Journal of Applied Ecology*, 61(10), pp.2418–2429. doi:https://doi.org/10.1111/1365-2664.14691.

43. De Souza, R.A., Kamat, N.M. and Nadkarni, V.S. (2018). Purification and characterisation of a sulphur rich melanin from edible mushroom Termitomyces albuminosus Heim. *Mycology,* 9(4), pp.296–306. doi:https://doi.org/10.1080/21501203.2018.1494060.
44. Kainat, N., Mujtaba, M., Wang, Y. and Zhou, B. (2025). Effectors of plants pathogenic fungi and fungal like microbes: a comprehensive review on mechanisms, roles, and host interactions. *Frontiers in Plant Science*, 16. doi:https://doi.org/10.3389/fpls.2025.1626960.
45. Dighton, J. and Horrill, A.D. (1988). Radiocaesium accumulation in the mycorrhizal fungi Lactarius rufus and Inocybe longicystis, in upland Britain, following the Chernobyl accident. *Transactions of the British Mycological Society,* 91(2), pp.335–337. doi:https://doi.org/10.1016/s0007-1536(88)80223-7.
46. Zhdanova, N.N., Tugay, T., Dighton, J., Zheltonozhsky, V. and Mcdermott, P. (2004). Ionizing radiation attracts soil fungi. *Mycological Research,* 108(9), pp.1089–1096. doi:https://doi.org/10.1017/s0953756204000966.