# Effects of Heavy Metal Contamination on Fungal Diversity in *Pinus brutia* Shoots

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The effects of heavy metal pollution have become a significant global issue in recent years. The primary objective of the present study was to compare the heavy metal concentrations in *Pinus brutia* shoots grown in an organized industrial zone (OIZ) and a forested area (Adalar) and to examine how these heavy metals affect fungal microbiota. The results achieved here showed that Ni and V concentrations were lower than the detectable limits in both the Adalar and the OIZ region, whereas Se and Cu concentrations were lower than the detectable limits in the shoots collected from the Adalar. Concentrations determined in samples collected from the OIZ were approximately 6 times higher for Cr and 16 times higher for Zn in comparison to the samples collected from the Adalar. Metagenomic analysis revealed that the most common fungal genera were *Aureobasidium*, *Gibberella*, *Hazslinszkyomyces*, *Alternaria*, *Cladosporium*, *Buckleyzyma*, *Lasiodiplodia*, and *Hormonema* for the OIZ area and *Hormonema*, *Aureobasidium*, *Alternaria*, *Cladosporium*, *Arthrinium*, *Fonsecazyma*, and *Truncatella* for the Adalar region. In the future, this study may serve as a reference for the development of innovative strategies for the remediation of heavy metal pollution for a sustainable and clean environment using biological sources.

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## INTRODUCTION

Environmental pollution caused by heavy metals has become a significant threat to living organisms in many ecosystems. Most heavy metals are toxic and carcinogenic, even when in trace amounts, and they pose a serious threat to human life (Ghoma *et al.* 2023). The reduction of the accumulation of toxic metals in various ecosystems, such as soil, air, and water, is one of the most important concerns today (Key *et al.* 2021). In general, physical and chemical remediation approaches can neutralize many heavy metal pollutants. However, they also have disadvantages, including increased energy consumption, the need for additional chemicals, and the risk of secondary pollution. Biological remediation of heavy metal pollution draws significant interest, primarily due to its advantages such as high efficiency at low metal concentrations, cost-effectiveness, and environmental friendliness (Shourie and Vijayalakshmi 2022). Fungi, which are decomposers, have a unique ability to metabolize various organic and inorganic pollutants by utilizing them as sources of energy and carbon, and to reduce them to harmless concentrations and non-hazardous status. Given the versatility of fungi in terms of their ecology, nutritional habits, adaptations, morphologies, physiology, and metabolism, mycoremediation is considered an effective method for pollutant remediation (Chen *et al.* 2022). Therefore, the identification of mycoremediation agents, including fungi, is being comprehensively studied.

Endophytic fungi are present in almost every plant in their natural environment and contribute to the plant's growth and tolerance to environmental stress conditions. Some of these fungal endophytes can support plant growth in cases of high heavy metal accumulation (Li *et al.* 2012). These endophytes can enhance plant tolerance to heavy metal stress by immobilizing heavy metals and limiting their uptake and distribution by plants (Zheng *et al.* 2023). Certain endophytic fungi can affect the expression of stress-related genes by host plants, and thereby the stress resistance of the plant is triggered (Domka *et al.* 2019). Fungal endophytes are ideal organisms for the biological remediation of heavy metals because of their ability to form extensive mycelial networks, the non-selectivity of their catabolic enzymes, and their independence from pollutants as a growth substrate (Akpasi *et al.* 2023).

Differing from traditional culture-based methods, next-generation sequencing (NGS) and similar techniques can identify a broader range of fungal taxa associated with various plants when used in analyzing the endophytic flora (Deng and Cao 2017). The NGS technologies and systems biology allow for the simultaneous discovery of all members of microbial communities and their interactions with host plants. Scanning metagenomic libraries resulted in the identification of numerous new biomolecules and different fungal taxa from various environments (Datta *et al.* 2020). The rDNA-ITS gene region is used for the identification of organisms in the fungal microbiota using metagenomic NGS (Antil *et al.* 2023).

Fungi, which have the potential to reduce the accumulation of pollutants in nature, play a significant role in the detoxification of waste materials (Singh *et al.* 2020). Previous studies showed that endophytic fungi isolated from plants growing in areas contaminated with heavy metals are more tolerant to pollution, which makes them potentially more effective for microbial-assisted phytoremediation (Domka *et al.* 2019). To implement microorganism-assisted phytoremediation approaches in the field, it is necessary to better understand the diversity and ecology of plant-associated microorganisms. Therefore, it is necessary to identify fungal species, targeting specific pollutants and their associated plants to achieve effective results (Singh *et al.* 2020). In this study, the Samsun Organized Industrial Zone, characterized by high levels of heavy metal pollution, and a forested area (Adalar) with low levels of heavy metal contamination were selected as study areas. The present study aims to determine the accumulation of heavy metals in the shoot tissues of *Pinus brutia* grown in these areas and to identify endophytic fungi using metagenomic methods. This study can contribute to biological remediation approaches for heavy metals by determining the accumulation of heavy metals and fungal diversity in the study area.

## EXPERIMENTAL

### Metals Analysis in Shoot Tissues

In this study, shoot samples from *P. brutia* plants were collected from Samsun OIZ, where heavy metal contamination is high, and from the Adalar forest area, which represents the control area with lower levels of heavy metal pollution (Fig. 1). After removing the outer bark, the shoot samples were subjected to surface disinfection (Turkkan *et al.* 2020). The shoot samples were then placed in petri dishes and left at room temperature for 15 days, followed by drying in an oven at 45 °C for one week. A total of 0.5 g of dried samples were placed in a microwave oven with 6 mL of 65% $HNO_3$ and 2 mL of 30% $H_2O_2$. The solutions achieved were then transferred to flask bottles, and the total volume was adjusted to 50 mL with ultrapure water. The shoot samples prepared were analyzed using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) in triplicate. The values obtained from the analysis were multiplied by the dilution factor to calculate metal concentrations (Cebi Kilicoglu 2023; Cetin *et al*. 2023; Isinkaralar *et al.* 2023; Sulhan *et al.* 2023). Statistical analyses were performed using SPSS 22.0 software, and the data were interpreted using analysis of variance and Duncan's test (Cobanoglu *et al.* 2023; Kuzmina *et al.* 2023).

## Amplicon-based Metagenomic Analysis

Next-generation sequencing for amplicon-based metagenomic analysis was performed by Macrogen Inc., Company (Seoul, South Korea). The Qiagen DNeasy Power Soil Pro kit was used for DNA isolation. While establishing the metagenomic library, the amplification of the fungal rDNA-ITS target gene region was conducted using Forward ITS3: (5´ GCATCGATGAAGAACGCAGC 3´) and Rewerse ITS4: (5´ TCCTCCGCTT-ATTGATATGC 3´) primers (White *et al.* 1990), with bidirectional sequencing for each sample. Illumina MiSeq technology was used for sequence analysis of the final library. Statistical mycobiome bioinformatics, from phylum to genus level, was conducted using QIIME 2 (Bolyen *et al.* 2019) to determine fungal diversity. Raw sequence data were quality-filtered using DADA2 (Callahan *et al.* 2016). For each sample, paired-end reads were merged after filtering, and operational taxonomic units (OTUs) were generated. All amplicon sequence variants for fungi were aligned using the UNITE classifier reference database, and all taxa belonging to the mycobiome were identified.

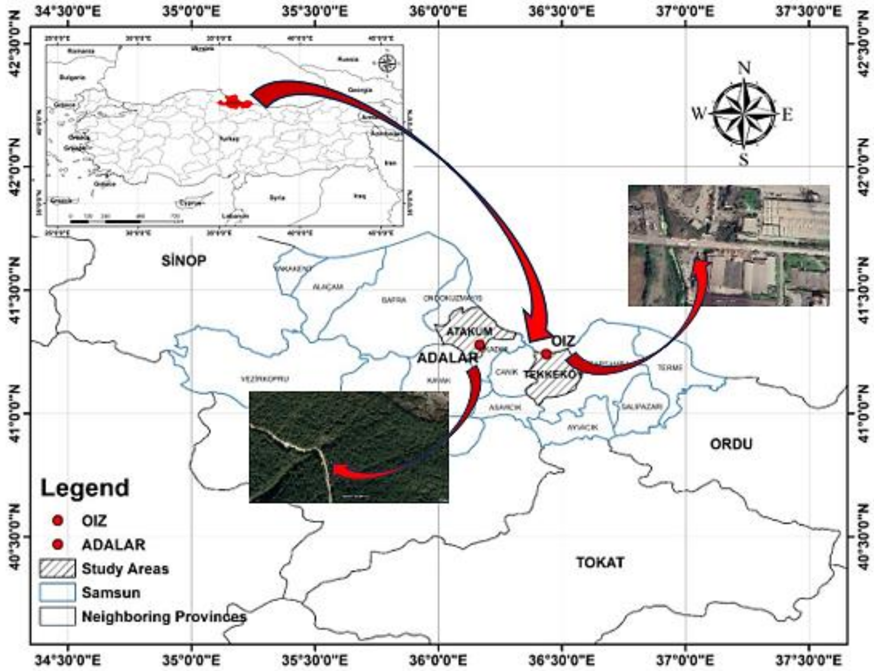


**Fig. 1.** Study area. Adalar and Organized Industrial Zone, Samsun Province, Türkiye

## RESULTS

Ni and V elements remained below detectable limits in the shoot samples of *P. brutia* collected from these research areas. The characterization of Cu, Pb, Cd, Se, Cr, and Zn pollutants is shown in Table 1.

**Table 1.** Heavy Metal Concentrations in *P. brutia* Shoot Samples

| Element | Adalar | OIZ | F |
|---|---|---|---|
| Pb (ppb) | 1394.5 a | 1793.8 b | 223.6*** |
| Cr (ppb) | 121.5 a | 771.2 b | 11204.7*** |
| Cu (ppb) | Under limit | 3795.0 | - |
| Zn (ppb) | 314.8 a | 5032.7 b | 2218.1*** |
| Se (ppb) | Under limit | 242.5 | - |
| Cd (ppb) | 346.5 a | 369.5 b | 27.3* |

***: $p < 0.001$; *: $p < 0.01$; a, b: Groups in which the values were clustered as a result of the Duncan’s test, ppb: Concentration value

Analyzing Table 1, it is evident that there were significant differences between element concentrations of *P. brutia* shoots collected from the OIZ (Organized Industrial Zone) and Adalar regions. Variance analysis revealed that there were statistically significant differences at a confidence level of at least 99% ($p < 0.01$) between the samples collected from the OIZ and Adalar regions for all elements. Among the elements studied, Cu and Se concentrations in samples collected from the Adalar region were below detectable limits. In the shoot samples collected from OIZ, concentrations of Cu, Se, Cd, Zn, Pb, and Cr, except for Ni and V, were higher when compared to those found in shoot samples collected from the Adalar. The concentrations determined in samples collected from the OIZ region were approximately 6 times higher in Cr and approximately 16 times higher in Zn when compared to the samples collected from the Adalar region. Furthermore, Se and Cu elements were found at higher concentrations in samples collected from the OIZ region, while they were below detectable limits in the samples from the Adalar region.

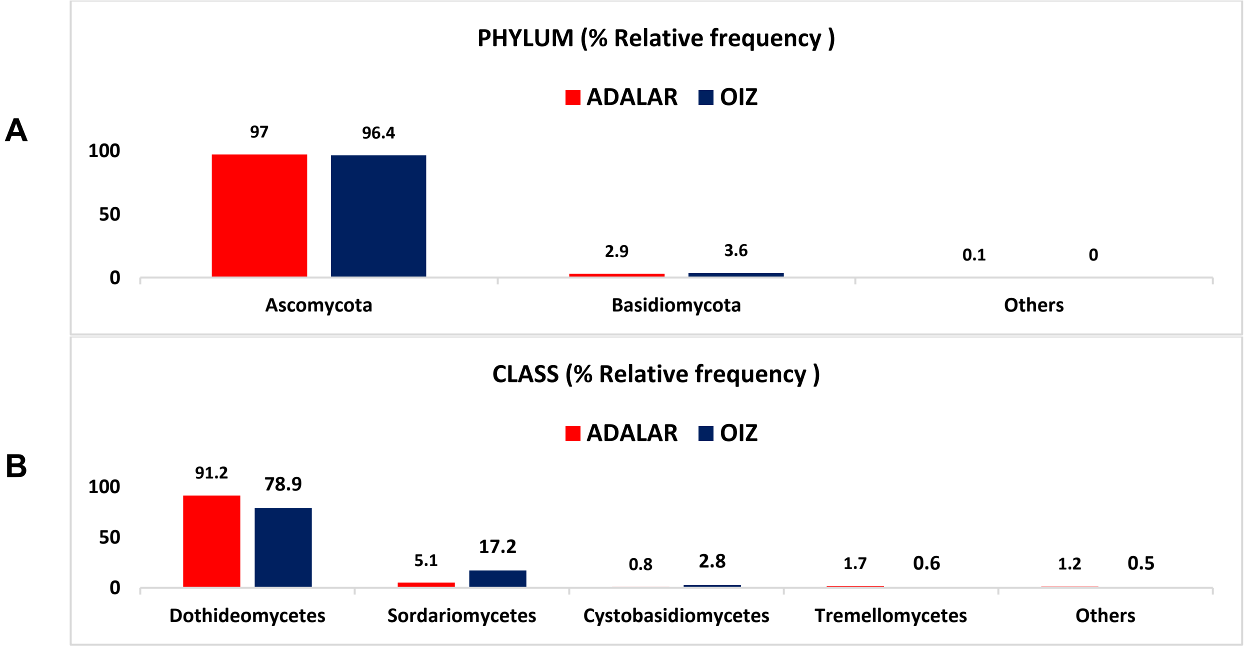
Given the rDNA-ITS microbiome analysis results of *P. brutia* shoots, fungal endophytes obtained from the industrial zone belonged to the Ascomycota phylum (relative frequency: 96.4%) and Basidiomycota (3.6%). Fungi belonging to the Ascomycota (97%) and Basidiomycota (2.9%) phyla were also detected in similar proportions in the shoot samples collected from the forested area (Fig. 2.A).

Examining the shoot samples obtained from these regions at the class level, Dothideomycetes were the most represented taxa, with a relative frequency of 78.9% in the OIZ mycobiota and 91.2% in the Adalar mycobiota. The Sordariomycetes class was present at 17.2% in OIZ samples and 5.1% in Adalar samples. Taxa belonging to the Cystobasidiomycetes class were found at a relative frequency of 2.8% in the shoot samples obtained from OIZ, whereas they were detected at less than 1% in samples obtained from the Adalar region. Members of the Tremellomycetes class were identified at 1.7% in the samples obtained from the Adalar region and less than 1% in the samples obtained from OIZ (Fig. 2.B).

When compared at the order level, the relative frequency of taxa represented at a level higher than 1% in the samples obtained from the OIZ zone were determined as Dothideales (44.9%), Pleosporales (26.1%), Hypocreales (15.6%), Capnodiales (6.7%), Sordariales (1.6%), and Botryosphaeriales (1.2%). In the Adalar region, the orders with a frequency above 1% were identified as Dothideales (68%), Pleosporales (15.3%), Capnodiales (7.7%), Xylariales (4.3%), and Tremellales (1.6%). Tremellales members were represented below 1% in the OSP region, and no Xylariales members were found in this region. In the Adalar shoot mycobiota, Sordariales and Botryosphaeriales orders were below 1%, and no Hypocreales order members were found (Fig. 2.C).

Examining the mycobiota of *P. brutia* shoots, 10 families with a relative frequency of over 1% were identified in the OIZ region, followed by Aureobasidiaceae (43.9%), Nectriaceae (15.5%), Coniothyriaceae (9.6%), Pleosporaceae (8.1%), Didymellaceae (7.2%), Cladosporiaceae (6.7%), Buckleyzymaceae (2.5%), Sordariaceae (1.6%), Botryosphaeriaceae (1.2%), and Dothioraceae (1%). Examining the shoot mycobiota obtained from the Adalar region, 7 family with a relative frequency above 1% were identified: Dothioraceae (46.4%), Aureobasidiaceae (21.6%), Pleosporaceae (7.9%), Cladosporiaceae (7.7%), Didymellaceae (6.5%), Apiosporaceae (2.3%), and Bulleraceae (1.4%) (Fig. 2.D).

When the mycobiome of *P. brutia* was analyzed at the genus level, 8 fungal taxa with a relative frequency above 1% were observed in the OSP region: *Aureobasidium* (43.9%), *Gibberella* (15.2%), *Hazslinszkyomyces* (9.4%), *Alternaria* (8.1%), *Cladosporium* (6.7%), *Buckleyzyma* (2.5%), *Lasiodiplodia* (1.2%), and *Hormonema* (1%). As a result of the meta-barcoding analysis of the Adalar mycobiome, 7 genera with a relative frequency above 1% were identified. These genera were *Hormonema* (46.4%), *Aureobasidium* (21.6%), *Alternaria* (7.9%), *Cladosporium* (7.6%), *Arthrinium* (2.3%), *Fonsecazyma* (1.4%), and *Truncatella* (1.1%). *Arthrinium*, *Fonsecazyma*, and *Truncatella* genera were not found in the shoot mycobiota obtained from the OIZ region, whereas *Gibberella*, *Hazslinszkyomyces*, and *Lasiodiplodia* genera were not detected in the shoot samples obtained from Adalar region, and *Buckleyzyma* was identified at a level lower than 1% (Fig. 2.E).



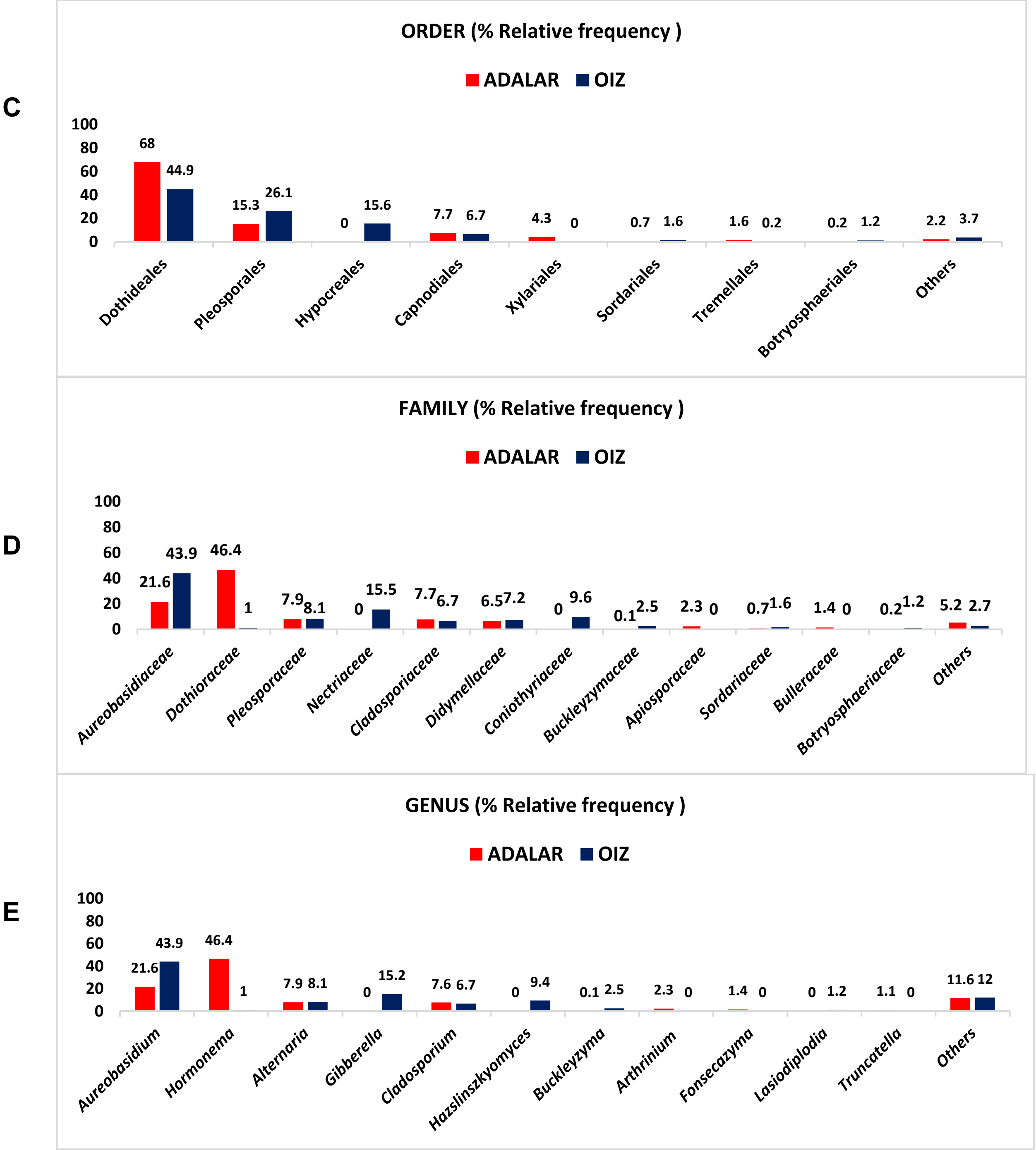


**Fig. 2.** Relative frequency of *P. brutia* shoot microbiota in study areas at different taxonomic levels: A. Phylum, B. Class, C. Order, D. Family, and E. Genus

## DISCUSSION

In this study, the concentrations of heavy metals, such as Cu, Pb, Cd, Se, Cr, Ni, Zn, and V, in the shoots of *P. brutia* grown in the OIZ and forest areas were determined, and how these heavy metals affected fungal endophyte diversity was investigated. While Cu and Se remained below detectable limits in samples collected from the forested area, Ni and V elements remained below detectable limits in both study areas. Cu, Se, Cr, and Zn concentrations in samples collected from the OIZ were significantly higher when compared to the Adalar region, indicating that the OIZ is an important source of heavy metals. These elements are among the most threatening to humans and other living beings, and they can be toxic, harmful, carcinogenic, and even lethal even at low concentrations (Badea *et al.* 2018; Cetin *et al.* 2022; Erdem *et al.* 2023). Due to their harmful effects, these four elements are listed both on the priority pollutant list of the Agency for Toxic Substances and Disease Registry (ATSDR) (Savas *et al.* 2021; Koc *et al.* 2023) and on the list of 13 priority metal pollutants created by the United States Environmental Protection Agency due to their acute and chronic toxicity (Hsieh *et al.* 2004). The high concentrations of heavy metals in samples collected from the OIZ region indicate that heavy metal pollution in this area is of serious concern. Previous studies also showed that heavy metal pollution in the industrial zone, where the research was conducted, is at very high levels (Karacocuk *et al.* 2022; Istanbullu *et al.* 2023). Furthermore, previous research emphasizes that industrial activities are one of the most significant sources of heavy metals (Ucun Ozel *et al.* 2019; Yayla *et al.* 2022).

The most abundant fungal genera in the OIZ *P. brutia* shoot mycobiota are *Aureobasidium* (43.89%), *Gibberella* (15.17%), *Hazslinszkyomyces* (9.38%), *Alternaria* (8.07%), *Cladosporium* (6.65%), *Buckleyzyma* (2.45%), *Lasiodiplodia* (1.16%), and *Hormonema* (1.03%). In the Adalar region shoot mycobiota, the predominant genera are *Hormonema* (46.41%), *Aureobasidium* (21.59%), *Alternaria* (7.94%), *Cladosporium* (7.64%), *Arthrinium* (2.28%), *Fonsecazyma* (1.39%), and *Truncatella* (1.05%). Previous studies suggested that fungi with a significant adaptation to a specific heavy metal are more dominant taxa in areas contaminated with these toxic metals (Deng Cao 2017). Furthermore, it is suggested that some fungal species can adapt to metal toxicity due to prolonged exposure to toxic metals (Khan *et al.* 2017). Among the most abundant genera found in the OIZ shoot mycobiota, previous studies have reported that *Aureobasidium* (Cu, Pb, Cd), *Gibberella* (Cu, Zn), *Alternaria* (Se, Cr, Cu, Cd, Ni, Pb, Zn), *Cladosporium* (Pb, Zn, Cu, Mn, Cd), and *Hormonema* (Cu, Ni) are microfungi that exhibit resistance to some heavy metals (Helander 1995; Kumar and Dwivedi 2021; Refaey *et al.* 2021; Zhang *et al.* 2022).

The *Aureobasidium* genus, which is highly detected in the shoots in both regions, can continue to thrive in environments with high levels of Pb and Cd by adsorbing Cu and performing the bioaccumulation of these metals (Mowll and Gadd 1984; Vaid *et al.* 2022). Cu, Pb, and Cd were significantly higher than the acceptable limits in the OIZ. Because of the tolerance to these toxic metals, *Aureobasidium* species have become the dominant species in the shoots of the plant, even in the presence of these high concentrations in the OIZ.

Fungi belonging to the *Hormonema* genus found in both the Adalar and OIZ shoots have also been isolated as pine endophytes in similar studies (Helander 1995). In this study, the tolerance of species isolated near the factory and those isolated from the control area 8 km away from Cu and Ni was examined. It was observed that the *Hormonema* strains isolated near the factory were more tolerant to Cu and Ni under *in vitro* conditions compared to those isolated from the control area 8 km away (Helander 1995). As shown in the results achieved in the present study, fungal taxa with tolerance to heavy metals can thrive in the environment, whereas some taxa without such tolerance can gain tolerance to heavy metals over time in response to pollution. It is evident that environmental conditions, such as heavy metals, can influence the mycobiota. Similarly, it was observed in the present study that some of the fungi identified in the OIZ shoots have resistance to toxic metals, and some taxa without tolerance can gain tolerance to toxic metals over time (Soanes and Richards 2014). To date, there has been no report on heavy metal detoxification by the *Hazslinszkyomyces*, *Buckleyzyma*, and *Lasiodiplodia* genera that were identified in the OIZ. However, the findings indicate that these genera represented in the mycobiota of OIZ shoots contaminated with heavy metals have tolerance to these metals. Detailed molecular studies are required to understand the resistance and detoxification metabolism of these fungi.

In the field of OIZ, despite the high levels of heavy metal pollution, the concentrations of Ni and V elements in *P. brutia* shoots were below the threshold values, contrary to expectations. However, among the dominant species in the shoot mycobiota of the region, fungi belonging to the *Alternaria* genus are known to be tolerant to Ni and have adapted to living in environments with high metal accumulations (Lindblom *et al.* 2018; Refaey *et al.* 2021). To the best of the author's knowledge, there is no study reporting any fungal genera identified in the OIZ being involved in V detoxification. Nevertheless, it should not be ignored that the phytoremediation ability of *P. brutia* in the OIZ may contribute to keeping V concentrations lower than the detectable limits. Furthermore, it is known that some fungal taxa without inherent tolerance can develop tolerance to toxic metals over time. Some microorganisms not only adapt but also act as cleansing organisms in the environment, beyond adaptation to heavy metal pollution (Bruins *et al.* 2000). It can be said that both the detoxification of Ni by fungi of the *Alternaria* genus and the phytoremediation of these elements by *P. brutia* in OIZ shoots contribute to keeping the accumulation of these toxic elements in the shoots below the threshold values.

In recent years, toxic metal pollution has become a serious global issue. Researchers primarily focused on developing more efficient and cost-effective methods for the remediation of pollutants. The technological and economic limitations of traditional methods resulted in the development of new technologies for pollutant remediation. The positive impact of microbial symbiotic relationships on plant growth and stress tolerance suggests that microbial-assisted phytoremediation could be used as an alternative solution for the detoxification of toxic metals.

## CONCLUSIONS

1. Isolation of endophytic fungi from plants growing in areas contaminated with toxic metals indicates that they may be more tolerant to pollution, making them potentially more attractive for use in microbial-assisted phytoremediation.
2. The biological cultivation of metal-absorbing fungi for on-site reclamation of heavy metal pollution can be employed as an effective region-specific method.
3. Identifying new fungi that are resistant to heavy metals will contribute to this technology for mitigating global environmental issues.
4. This study provides valuable information that can assist in detoxification processes by examining the relationship between metal accumulations and fungal diversity of *P. brutia* shoots in the Samsun industrial zone and the control area.

## Data Availability

All data included in this study are available upon request by contact with the corresponding author.

## Declaration

The authors declare no conflict of interest.

## REFERENCES CITED

Akpasi, S. O., Anekwe, I. M. S., Tetteh, E. K., Amune, U. O., Shoyiga, H. O., Mahlangu, T. P., and Kiambi, S. L. (2023). “Mycoremediation as a potentially promising technology: Current status and prospects – A review,” *Applied Sciences* 13(8), article 4978. DOI: 10.3390/app13084978

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R., *et al.* (2023). “DNA barcoding, an effective tool for species identification: A review,” *Molecular Biology Reports* 50(1), 761-775. DOI: 10.1007/s11033-022-08015-7

Badea, M., Luzardo, O. P., González-Antuña, A., Zumbado, M., Rogozea, L., Floroian, L., Alexandrescu, D., Moga, M., Gaman, L., Radoi, M., *et al.* (2018). “Body burden of toxic metals and rare earth elements in non-smokers, cigarette smokers and electronic cigarette users,” *Environmental Research* 166, 269-275. DOI: 10.1016/j.envres.2018.06.007

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., *et al* (2019). “Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2,” *Nature Biotechnology* 37(8), 852-857. DOI: 10.1038/s41587-019-0209-9

Bruins, M. R., Kapil, S., and Oehme, F. W. (2000). “Microbial resistance to metals in the environment,” *Ecotoxicology and Environmental Safety* 45(3), 198-207. DOI: 10.1006/eesa.1999.1860

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). “DADA2: High-resolution sample inference from Illumina amplicon data,” *Nature Methods* 13(7), 581-583. DOI: 10.1038/nmeth.3869

Cebi Kilicoglu, M. (2023). “Metagenomic analysis of the fungal microbiome in contaminated soil with heavy metal,” *Turkish Journal of Agriculture-Food Science and Technology* 11(9), 1671-1677. DOI: 10.24925/turjaf.v11i9.1671-1677.6261

Cetin, M., Aljama, A. M. O., Alrabiti, O. B. M., Adiguzel, F., Sevik, H., and Zeren Cetin, I. (2022). “Using topsoil analysis to determine and map changes in Ni Co pollution,” *Water, Air, and Soil Pollution* 233(8), article 293. DOI: 10.1007/s11270-022-05762-y

Cetin, M., Cebi Kilicoglu, M., and Kocan, N. (2023). “Usability of biomonitors in monitoring the change of tin concentration in the air,” *Environmental Science and Pollution Research* 30, 112357-112367. DOI: 10.1007/s11356-023-30277-2

Chen, L., Zhang, X., Zhang, M., Zhu, Y., and Zhuo, R. (2022). “Removal of heavy-metal pollutants by white rot fungi: Mechanisms, achievements, and perspectives,” *Journal of Cleaner Production* 354, article 131681. DOI: 10.1016/j.jclepro.2022.131681

Cobanoglu, H., Sevik, H., and Koç, I. (2023). “Do annual rings really reveal Cd, Ni, and Zn pollution in the air related to traffic density? An example of the cedar tree,” *Water, Air and Soil Pollution* 234(2), article 65. DOI: 10.1007/s11270-023-06086-1

Datta, S., Rajnish, K. N., Samuel, M. S., Pugazlendhi, A., and Selvarajan, E. (2020). “Metagenomic applications in microbial diversity, bioremediation, pollution monitoring, enzyme and drug discovery. A review,” *Environmental Chemistry Letters* 18, 1229-1241. DOI: 10.1007/s10311-020-01010-z

Deng, Z., and Cao, L. (2017). “Fungal endophytes and their interactions with plants in phytoremediation: A review,” *Chemosphere* 168, 1100-1106. DOI: 10.1016/j.chemosphere.2016.10.097

Domka, A. M., Rozpaądek, P., and Turnau, K. (2019). “Are fungal endophytes merely mycorrhizal copycats? The role of fungal endophytes in the adaptation of plants to metal toxicity,” *Frontiers in Microbiology* 10, article 371. DOI: 10.3389/fmicb.2019.00371

Erdem, R., Aricak, B., Cetin, M., and Sevik, H. (2023). “Change in some heavy metal concentrations in forest trees by species, organ, and soil depth,” *Forestist* 73(3), 257-263. DOI: 10.5152/forestist.2023.22069

Ghoma, W. E. O., Sevik, H., and Isinkaralar, K. (2023). “Comparison of the rate of certain trace metals accumulation in indoor plants for smoking and non-smoking areas,” *Environmental Science and Pollution Research* 30, 75768-75776. DOI: 10.1007/s11356-023-27790-9

Helander, M. L. (1995). “Responses of pine needle endophytes to air pollution,” *New Phytologist* 131(2), 223-229. DOI: 10.1111/j.1469-8137.1995.tb05723.x

Hsieh, C. Y., Tsai, M. H., Ryan, D. K., and Pancorbo, O. C. (2004). “Toxicity of the 13 priority pollutant metals to *Vibrio fisheri* in the Microtox® chronic toxicity test,” *Science of The Total Environment* 320(1), 37-50. DOI: 10.1016/S0048-9697(03)00451-0

Isinkaralar, K., Isinkaralar, O., Koc, I., Ozel, H. B., and Sevik, H. (2023). “Assessing the possibility of airborne bismuth accumulation and spatial distribution in an urban area by tree bark: A case study in Düzce, Türkiye,” *Biomass Conversion and Biorefinery* 2023(Online), 1-12. DOI: 10.1007/s13399-023-04399-z

Istanbullu, S. N., Sevik, H., Isinkaralar, K., and Isinkaralar, O. (2023). “Spatial distribution of heavy metal contamination in road dust samples from an urban environment in Samsun, Turkey,” *Bulletin of Environmental Contamination and Toxicology* 110(4), article 78. DOI: 10.1007/s00128-023-03720-w

Karacocuk, T., Sevik, H., Isinkaralar, K., Turkyilmaz, A., and Cetin, M. (2022). “The change of Cr and Mn concentrations in selected plants in Samsun city center depending on traffic density,” *Landscape and Ecological Engineering* 18, 75-83. DOI: 10.1007/s11355-021-00483-6

Key, K., Koc, I., Kulac, S., and Sevik, H. (2021). “Determination of the air Pb pollution in Kastamonu with the help of *Picea pungens* needles,” in: *6th International Conference on Material Science and Technology,* Cappadocia, Turkey, pp. 497.

Khan, A. R., Waqas, M., Ullah, I., Khan, A. L., Khan, M. A., Lee, I. J., and Shin, J. H. (2017). “Culturable endophytic fungal diversity in the cadmium hyperaccumulator *Solanum nigrum* L. and their role in enhancing phytoremediation,” *Environmental and Experimental Botany* 135, 126-135. DOI: 10.1016/j.envexpbot.2016.03.005

Koc, I., Cobanoglu, H., Canturk, U., Key, K., Kulac, S., and Sevik, H. (2023). “Change of Cr concentration from past to present in areas with elevated air pollution,” *International Journal of Environmental Science and Technology* 2023, 1-12. DOI: 10.1007/s13762-023-05239-3

Kumar, V., and Dwivedi, S. K. (2021). "Mycoremediation of heavy metals: Processes, mechanisms, and affecting factors," *Environmental Science and Pollution Research* 28(9), 10375-10412. DOI: 10.1007/s11356-020-11491-8

Kuzmina, N., Menshchikov, S., Mohnachev, P., Zavyalov, K., Petrova, I., Ozel, H. B., Aricak, B., Onat, S. M., and Sevik, H. (2023). "Change of aluminum concentrations in specific plants by species, organ, washing, and traffic density," *BioResources* 18(1), 792-803. DOI: 10.15376/biores.18.1.792-803

Li, H. Y., Li, D. W., He, C. M., Zhou, Z. P., Mei, T., and Xu, H. M. (2012). "Diversity and heavy metal tolerance of endophytic fungi from six dominant plant species in a Pb–Zn mine wasteland in China," *Fungal Ecology* 5(3), 309-315. DOI: 10.1016/j.funeco.2011.06.002

Lindblom, S. D., Wangeline, A. L., Valdez Barillas, J. R., Devilbiss, B., Fakra, S. C., and Pilon-Smits, E. A. (2018). "Fungal endophyte *Alternaria tenuissima* can affect growth and selenium accumulation in its hyperaccumulator host *Astragalus bisulcatus*," *Frontiers in Plant Science* 9, article 1213. DOI: 10.3389/fpls.2018.01213

Mowll, J. L., and Gadd, G. M. (1984). "Cadmium uptake by *Aureobasidium pullulans*," *Microbiology* 130(2), 279-284. DOI: 10.1099/00221287-130-2-279

Refaey, M., Abdel-Azeem, A. M., Abo Nahas, H. H., Abdel-Azeem, M. A., and El-Saharty, A. A. (2021). "Role of fungi in bioremediation of soil contaminated with heavy metals," in: *Industrially Important Fungi for Sustainable Development: Volume 1: Biodiversity and Ecological Perspectives,* Springer International Publishing, Cham, Switzerland, pp. 509-540. DOI: 10.1007/978-3-030-67561-5_16

Savas, D. S., Sevik, H., Isinkaralar, K. Turkyilmaz, A., and Cetin, M. (2021). "The potential of using *Cedrus atlantica* as a biomonitor in the concentrations of Cr and Mn. *Environmental Science and Pollution Research* 28(39), 55446-55453. DOI: 10.1007/s11356-021-14826-1

Shourie, A., and Vijayalakshmi, U. (2022). "Fungal diversity and its role in mycoremediation," *Geomicrobiology Journal* 39(3-5), 426-444. DOI: 10.1080/01490451.2022.2032883

Singh, V., Singh, M. P., and Mishra, V. (2020). "Bioremediation of toxic metal ions from coal washery effluent Desalin," *Desalination and Water Treatment* 197, 300-318. DOI: 10.5004/dwt.2020.25996

Soanes, D., and Richards, T. A. (2014). "Horizontal gene transfer in eukaryotic plant pathogens," *Annual Review of Phytopathology* 52, 583-614. DOI: 10.1146/annurev-phyto-102313-050127

Sulhan, O. F., Sevik, H., and Isinkaralar, K. (2023). "Assessment of Cr and Zn deposition on *Picea pungens* Engelm. in urban air of Ankara, Turkiye," *Environment, Development and Sustainability* 25(5), 4365-4384. DOI: 10.1007/s10668-022-02647-2

Turkkan, M., Cebi Kilicoglu, M., and Erper, I. (2020). "Characterization and pathogenicity of *Rhizoctonia* isolates collected from *Brassica oleracea* var. *acephala* in Ordu, Turkey," *Phytoparasitica* 48(2), 273-286. DOI: 10.1007/s12600-020-00793-9

Ucun Ozel, H., Ozel, H. B., Cetin, M., Sevik, H., Gemici, B. T., and Varol, T. (2019). "Base alteration of some heavy metal concentrations on local and seasonal in Bartin River," *Environmental Monitoring and Assessment* 191, article 594. DOI: 10.1007/s10661-019-7753-0

Vaid, N., Sudan, J., Dave, S., Mangla, H., and Pathak, H. (2022). "Insight into microbes and plants ability for bioremediation of heavy metals," *Current Microbiology* 79(5), article 141. DOI: 10.1007/s00284-022-02829-1

White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," *PCR Protocols: A Guide to Methods and Applications* 18(1), 315-322.

Yayla, E. E., Sevik, H., and Isinkaralar, K. (2022). "Detection of landscape species as a low-cost biomonitoring study: Cr, Mn, and Zn pollution in an urban air quality," *Environmental Monitoring and Assessment* 194(10), article 687. DOI: 10.1007/s10661-022-10356-6

Zhang, J., Fan, X., Wang, X., Tang, Y., Zhang, H., Yuan, Z., Zhou, J., Han, Y., and Li, T. (2022). "Bioremediation of a saline-alkali soil polluted with Zn using ryegrass associated with *Fusarium incarnatum*," *Environmental Pollution* 312, article ID 119929. DOI: 10.1016/j.envpol.2022.119929

Zheng, J., Xie, X., Li, C., Wang, H., Yu, Y., and Huang, B. (2023). "Regulation mechanism of plant response to heavy metal stress mediated by endophytic fungi," *International Journal of Phytoremediation* 25(12), 1596-1613. DOI: 10.1080/15226514.2023.2176466

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