Nutrient and water availability are two key requirements of plant survival. Mycorrhizal symbiosis helps orchids coexist in areas with apparently low soil nutrients and water availability, in this case, in Estonian mine tailings that contain little more than burnt oil soaked shale and ash. We extracted all DNA samples taken from multiple sites in the mine tailings in both the summer and the fall. The sample size for summer was 25 and the sample size for the fall was 62. We carried out Polymerase Chain Reaction (PCR) on purified DNA samples, and performed gel electrophoresis and Restriction Fragment Length Polymorphism (RFLP) pattern determination. We then sequenced-identical RFLP patterns and carried out Basic Local Alignment Search Tool (BLAST) searches to determine the organismal composition of the samples. We found unique RFLP patterns for the fall samples and 12 unique RFLP patterns for the summer samples. The dominant organisms found were mycorrhizal fungi in families Thelephoraceae and Cortinariaceae. The overall diversity of mycorrhizal fungi determined by Shannon’s Diversity Index shows that for the fall, H=2.33, and for the summer, H=1.84. This is higher than the mycorrhizal diversity in the Brazilian rainforest where H ranges from 1.2 to .87. This is important because although the fungi in the mine tailings only represent a small fraction of the fungi present in rainforests, they may play a similar role in the nutrient cycling of this area. The diversity of the Johvi mine tailings areas are compared to another environment with mycorrhizal fungi such as a Brazilian forest with an estimated value for mycorrhizal fungi ranging from 1.2 to 2.7 depending on the season and the level of deforestation, one can see that the diversity of these mine tailings areas is quite high (2.3 to 1.8) by comparison (Morrów et al. 2007). We must conclude our discussion with the idea that these results are not only controlled by the presence of mycorrhizal fungi, but also by the environment itself. It is possible that the environment itself may have a significant influence on the diversity of the fungi in the mine tailings. The site which the orchid root samples and mycorrhizal soil samples were taken is on a barren hill that was created through FF, but five samples were taken from each with four being proximal to each other and one being distant from them. In the following study, the orchid root samples and mycorrhizal soil samples were taken is on a barren hill that was created through FF, but five samples were taken from each with four being proximal to each other and one being distant from them. Though mycorrhizal fungi are obviously present in the soil of this area, their ability to colonize must be limited due to the scarcity of nutrients and scarcity of the soil. Thus, I believe there will be little diversity in the mycorrhizal fungi living in the sampled mine tailing areas.


**Results and Discussion**

We estimated the Shannon’s Diversity Index for the summer and fall samples to be 2 as unique RFLP patterns obtained from the samples (Tables 1 and 2) since each pattern can represent different species. The Richness for the summer samples is 10 and the species Richness for the fall samples is 18. To estimate diversity, we used Shannon’s diversity index which is determined by the formula:

\[ H = -\sum_i p_i \ln p_i \]

where, \( H \) is a taxon’s total number of species and \( p_i \) is the proportion of a species to the total. The richness of each species to the whole can be found in Table 1 and 2. The diversity of the summer samples is \( H = 1.84 \) while the diversity of the fall samples is \( H = 2.33 \).

We were able to obtain PCR product from 64% of the summer soil samples and from 70% of the fall soil samples. Many samples with strong PCR products contain multiple fungal species. This was expected as the dilution of fungal DNA is very low and for the mine tailings we would not be surprised if some of these reads were due to low-level contamination. It is not surprising that some of the samples did not produce informative RFLP patterns; of all of the samples, 30% of the summer samples and 43% of the fall samples were estimated for one or more reasons.

The evenness and overall diversity for the fall samples (\( H = 2.33 \)) is greater than that for the summer samples (\( H = 1.84 \)). This could be due to the sampling interval during the summer (20) than during the fall (30). However, it could also indicate that the diversity increases during the fall due to some storage in the environment, such as non-competitive interactions for the fungi to colonize. These observations are consistent with those made by Ono and colleagues (1996) who reported that fungal diversity may be increased by soil habitat diversity and general adaptations to new environments or conditions (Cowan 1996).

When the total fungal diversity of the mine tailing areas is compared to other environments with mycorrhizal fungi such as a Brazilian forest with an estimated value for mycorrhizal fungi ranging from 1.2 to 2.7 depending on the season and the level of deforestation, one can see that the diversity of these mine tailing areas is quite high (2.3 to 1.8) by comparison (Morrów et al. 2007). We must conclude our discussion with the idea that these results are not only controlled by the presence of mycorrhizal fungi, but also by the environment itself. It is possible that the environment itself may have a significant influence on the diversity of the fungi in the mine tailings.

A perennial barren area can support mycorrhizal fungi and their symbionts. Perhaps the goal of land recovery or woodland restoration efforts could be to create resting populations of fungi that can colonize other barren areas such as landfills and waste dumps can be useful. The next step in this project is to gain a better picture of the species diversity of this area through exhaustive sampling of the entire site. In addition, further research could be conducted on the diversity and distribution of mycorrhizal fungi in the area. With further picture of what species exist in the area, we can better estimate the diversity and thus the ecological stability of this area. Other projects that might interest would be to study landfills and contamination. Also, we could study other biologically similar areas such as landfills or waste dumps to sample for similar species.

**Conclusions**

The diversity of the John-Kose mine tailing areas does not seem to be very limited by the area's apparent scarcity of resources. This lack of resources can result in the higher diversity of the mycorrhizal communities. Increased understanding through the application of molecular techniques. Environmental Microbiology 6: 769-772


