1. Contestant profile

▪ Contestant name:	**Assery Mbonea Mdoe**
▪ Contestant occupation:	Student
▪ University / Organisation	Sokoine University of Agriculture
▪ Number of people in your team:	**3**

2. Project overview

Title:	The use of mycelium in soil detoxification and Understanding fungi diversity a case of wazo hill quarry
Contest: (Research/Community)	Research
Quarry name:	Wazo Hill Quarry

ABSTRACT

The Macrofungi are known as common bioremediators especially in quarrying and searching of raw materials required for cement industries. At Wazo hill in Tanzania quarry activities have resulted to soil degradation leading to lose the diversity of the area due to deposition of heavy metals such as calcium, Aluminium and Silicate and traces of lead, cordium, iron, Titanium and silica which lead to soil toxicity. Nitrate. This study aims to understand the diversity of macrofungi, in rehabilitated and undisturbed area of wazo hill quarry, also understanding the soil characteristics and implications of mycoremediation in quarry soils. The diversity was assessed through, transect method in dry and wet season, soil samples we collected before and after mycoremediation using trowel technique. The shannon wiener indice of diversity shows that there is a significant difference in diversity of macrofungi between the rehabilitated area and undisturbed area where H= 3.7 and 3.1 respectively with t = 7.4861, df= 137.87 p< 0.05. Soil analysis was conducted bay a two-way analysis of variance yielded a main effect for the soil parameters, $F(2,96) = 3.09$, $p > .05$, such that the average measurement was not significantly higher for Undisturbed (M = 15.3%, SD = 4.44) than for Rehabilitated (M = 12.6%, SD = 6.18). The main effect soil parameter was significant, $F(3, 96) = 2.699$, $p < .05$. However, the interaction effect was not significant, $F(6, 196) = 2.195$, $p >.05$, indicating that the soil parameter effect was less in the undisturbed than in the rehabilitated

Moreover the mycorestoration process was conducted after recording the soil characteristics and soil were then collected and analysed after restoration. The Independent t-test showed that was no significant difference in the soil Ph before mycoremediation (M= 7.4625, SD= 0.423) and after remediation (M= 7.3975, SD= 0.495) conditions; t=0.28df =14, p = 0.782”

INTRODUCTION

The mining and searching of raw materials required for cement production in cement industries at Wazo hill in Tanzania has resulted to soil degradation leading to loose the diversity of the area due to the deposition of heavy metal substances such as calcium, aluminum, and silicate and traces of lead (Pb), cordium (cd), iron (Fe), Titanium (Ti) and silica which leads to soil toxicity.

Mycorestoration process involves the use of fungi mycelium to remove the toxic materials from the soil, hence introduction of these mycelium will result to reviving of the soil. The introduction of the mycelium done by first introducing the organic growth media such as sawdust or rice straw in the area to provide a media for growing the cultured mushroom mycelium that was introduced in the area and the management was provided for them to grow.

This will revive the soil by breaking and absorbing all the heavy metals from the soil. The mycorestoration process will result to detoxification of Wazo hill area and attracts the occurrence or growth of the vegetation in the area which will also attract other organisms hence reviving biodiversity.

Main objective

- To assess and detoxify the soil contaminants by the use of Fungi, Mycelium and its diversity

Specific Objectives

➢ To assess the amount of toxins (PH, Electric conductivity, Nitrate ions, Sulphate ions, and calcium ions) in the soil at selected sites (quarry area , undisturbed and rehabilitated area area) in Wazo hill

➢ To assess the Fungi Diversity species at Wazo hill undisturbed area

➢ To Promote and establish diversity of organisms from mycelium as the root of ecology (mycoremediation)

Methodology

- **Description of the study Site**

Wazo Hill is located near Dar es Salaam city at Tegeta approximately 25km from the city Centre between latitude 6° 34' south and longitudes 39° 24' east, and an altitude between 100 and 200 above mean sea level (Kebede & Nicholls, 2011). The climate of Dar es Salaam is very much influenced by its closeness to the equator and the adjacent Indian Ocean. Thus, it has tropical climatic conditions with hot and humid weather throughout the year. It receives the rainfall of around 1000mm per annum and the average temperature is 26 degree centigrade. The area is under Tanzania Portland Cement Company Limited (TPCC) which is a large company which is in the business of manufacturing, selling and distribution of high quality construction cement in Tanzania (Gastory, 2012). To produce high production of cements, TPCC requires raw materials including limestone, clay

and mudstone or shale. The quarried landscape looks barren while heaps of topsoil is deposited on the edges of mined blocks as source of materials for planned rehabilitation.

- **Experimental Design**

The use of mycelium in soil detoxification and enhancement of biodiversity was involving the fieldwork, laboratory work and deskwork. The fieldwork was important for collection of the information for the data input in the restoration and identification. While the lab work was involved the identification of the Fungi species collected at Wazo Hill quarry. The fieldworks also were providing all factors affecting the fungi species available in Wazo Hill quarry

- **Soil sample collection**

The field of study at Wazo hill quarry was divided into different homogenous units based on the visual observation such a low areas and high areas and closeness to the sources of contamination. The upper surface containing dead organic matter and other dirty materials were removed from the sampling spot. A trowel was drived and ploughed to a depth of 15cm and soil sample was drawn. Soil samples were collected from different sampling units and placed in a single container and mixed properly to create uniformity, the samples were properly parked in plastic bags and taken to the laboratory for analysis.

- **Soil Analysis**

For the determination of the physiochemical properties of the contaminated soil samples 600 model Plaintest micro was used to determine the pH, TDS, and electric conductivity respectively (600 modle Plaintest micro, UK). In addition, for the determination of the anions concentration Sulphate and Nitrate in the contaminated water samples, UV/Visible Spectrophotometer was employed (a modle 6705 UV/Vis Jenway, UK). While, for the Cations concentration which are (Ca+2, Pb+2), instrumental techniques were employed to determine the concentrations. These instruments are respectively flame photometer for calcium determination (Model PFP7 Jenway, UK), and Atomic absorption spectrophotometer for Lead Concentration (Model 210/211/ Buck Scientific) [1221].

- **Assessment of Fungi Diversity species available in undisturbed areas**

Macrofungi were collected along 3 laid transects in each sites of undisturbed and rehabilitated at Wazo hill quarry each measuring 15 × 20 m marked with fixed ribbons on pegs and on site trees. The plots were

approximately 10 m apart. Surveys in the plots were done in two seasons the rainy season, March to May, and one dry season July to August. Sampling methods complies with that of Tibuhwa et al. (2010) and consists of collecting the basidiomata randomly throughout each specified habitat recording each collection point using the global positioning system" (GARMIN etrex 11). The macrofungi nomenclature is based on Kirk and Ansell (1992) as well as the web site of CABI bioscience databases (http://www.speciesfungorum.org/Names/Names.asp).

Scientific names are those recognized by the index fungorum. Each observed mushroom was photographed in situ, prior to picking from its substrate, Picking was done with the aid of the scalpel for some cases a hoe and bush knife for hard wood inhabiting mushrooms. Picked mushrooms were then packed into sampling ziplock bags which were accurately labeled with collection number, collecting date, name of the collector as well as few field identification tips such as sporocarp shape, color, odor, color changing on bruising, and tentative name then taken to Sokoine Agricultural University for further identification. The collected macro fungi were identified using colored field guide books, monographs (Arora, 1986; Härkönen et al., 1995, 2003; Kirk et al., 2001; Lodge et al., 2004) and internet facility Statistical analyses on diversity was done using Shannon Wiener species diversity index among the habitats of the studied sites were carried out according to Magurran (1988) using PAlaeontological Statistics (PAST 3) for species diversity and richness.

- **Mycoremediation**

This is the method that was used to remove the toxic materials from the soil by the use of fungi / mushroom mycelium. Local native mushroom species were collected from areas around the quarry. Spores were set to inoculate the pasteurized straw and kept In a favorable conditions that allowed growth of mycelium and allowed to develop well. After proper development mycelium was introduced into hardwood chips that was introduced as growth media in the affected area at Wazo hill quarry.

RESULTS

For assessing the amount of toxins (PH, Electric conductivity, Nitrate ions, Sulphate ions, and calcium ions) in the soil at selected sites (undisturbed and rehabilitated area) in Wazo hill,

A two-way analysis of variance yielded a main effect for the soil parameters, $F(2,96) = 3.09$, $p > .05$, such that the average measurement was not significantly higher for Undisturbed ($M = 15.3\%$, $SD = 4.44$) than for Rehabilitated ($M = 12.6\%$, $SD = 6.18$). The main effect soil parameter was significant, $F(3, 96) = 2.699$, $p < .05$.

However, the interaction effect was not significant, F(6, 196) = 2.195 , p >.05, indicating that the soil parameter effect was less in the undisturbed than in the rehabilitated

Macrofungi diversity

For assessment of fungi diversity (specie identification, morphological observation) mushroom samples were collected at random from the undisturbed area and restored area during the rain season at Wazo hill quarry.

In the study area, more than 253 macro fungi individuals were observed as shown in Table 1. The specie diversity index differed among the two habitats (Table 2) which are the undisturbed areas and the restored areas. There is a significant difference on diversity of species between undisturbed area and rehabilitated areas t = 7.4861, df= 137.87 p< 0.05,

	Undisturbed Area	Rehabilitated Area
Individuals	188	68
Shannon_H	**3.796**	**3.101**

Table 1: There is a significant difference in specie diversity between undisturbed and Rehabilitated area.

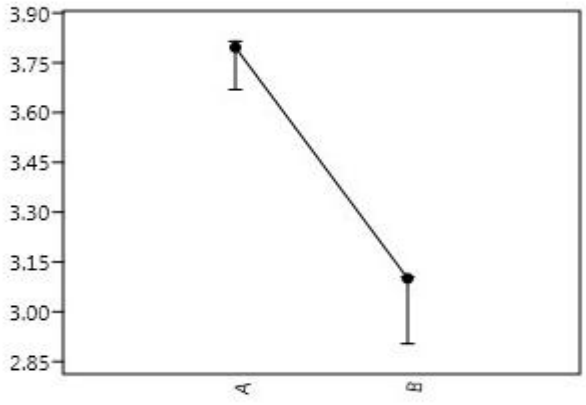
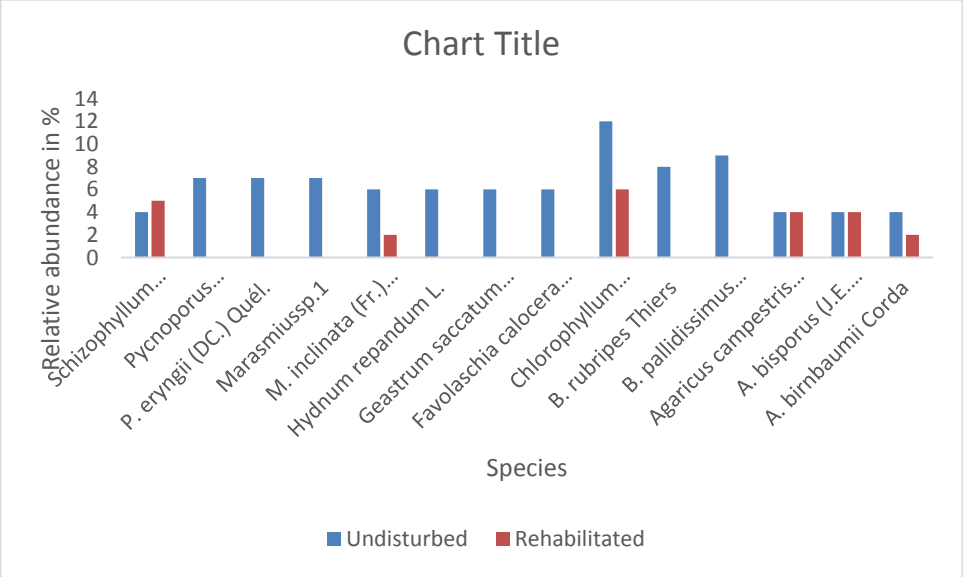


Figure 3: A plot showing shannon diversity indices for rehabilitated and undisturbed areas



	Undisturbed	Rehabilitated
Schizophyllum commune Fr.	+	+
Pycnoporus sanguineus (L.) Murrill	+	
P. eryngii (DC.) Quél.	+	-
Marasmiussp.1	+	-
M. inclinata (Fr.) Quél.	+	+
Hydnum repandum L.	+	-
Geastrum saccatum sensu auct. brit.	+	-
Favolaschia calocera R. Heim	+	-
Chlorophyllum molybdites (G. Mey.) Massee	+	+
B. rubripes Thiers	+	-
B. pallidissimus Watling	+	-
Agaricus campestris L.:Fr.	+	+
A. bisporus (J.E. Lange) Imbach	+	+
A. birnbaumii Corda	+	+

Table 2: Presence and absence of dominant species from rehabilitated and rehabilitated area

Mycoremediation

There was no significant difference in the soil Ph before mycoremediation (M= 7.4625, SD= 0.423) and after remediation (M= 7.3975, SD= 0.495) conditions; t=0.28df =14, p = 0.782"

Discussion

This study shows that the soil of the studied area could be categorized as moderately to heavily pollute. The value of different parameter in soil samples, showed the pH and electrical conductivity values are suitable for growth.

Soil analysis

After the soil analysis by a two way analysis of variance the results show that, in the different in measurement of the soil parameters there was no significantly difference in the undisturbed area compared to the disturbed area, however the results showed that the level of soil toxicity in the undisturbed area is less compared to the restored area and this is because of the presence of the natural soil layers and the presence of microbial activities that helps in the decomposition of organic matter improving the soil properties in the undisturbed area

Macrofungi diversity

After collection and identification of the mushroom species from the rehabilitated and undisturbed area. The shannon wiener indice of diversity shows that there is high diverse in undisturbed area compared to rehabilitated area with values of H= 3.7 and H= 3.1 respectively. Furthermore the diversity t-test confirmed that there is a significant difference in diversity of macrofungi between the rehabilitated area and undisturbed area $t = 7.4861$, $df= 137.87$, $p< 0.05$. This shows that there is a difference between the two areas where samples were collected where the undisturbed area contains many species of fungi compared to the disturbed areas.

The simulated and observed results revealed that there was a variation in Fungi species diversity within the disturbed area. This is because within the disturbed area the natural vegetation cover was lost during mining activities and the area is low in organic matter and less moisture hence difficult for fungi to grow.

Mycorestoration

mycorestoration process wa conducted after recording the soil characteristics and soil were then collected and analysed after restoration. The Anova test showed that was no significant difference in the soil PH before mycoremediation (M= 7.4625, SD= 0.423) and after remediation (M= 7.3975, SD= 0.495) conditions; $t=0.28$ $df =14$, $p = 0.782$”

The mycorestoration process did not show a significant difference between the soil samples before mycorestoration and after mycorestoration and this is because the mushroom species that were introduced for the mycorestoration process did not develop to a large extent as required due to unfavourable conditions of dusts

from the quarries and quick dryness of the soils. The mushroom developed at a low extent reducing a small amount of toxins only.

Conclusion

- The soil toxicity as assessed through soil samples collected at undisturbed and disturbed of wazo hill quarry and soil laboratory analysis shows that the undisturbed areas soils are less affected by toxic materials compared to mined areas and this is due to removal of the organic materials and limestone mining which affects soil properties.
- The mycorestoration process of soil at Wazo Hill quarry was conducted with field data collection in the restored sites. The process was successful at a very low level, but the soil properties indicated after mycorestoration showed some decrease in soil toxicity hence if the process can be conducted successful Toxic materials from the soil would be reduced.
- The specie diversity assessment as conducted at Wazo hill Quarry through observation, data collection and laboratory observation it indicated that the undisturbed area contained a many fungi species compared to the disturbed area. This is because the undisturbed areas contain the natural material high organic matter and moisture that favors fungi growth.

Recommendation

We recommend first, the mined areas of the quarry should be restored to ensure that the soils toxicity is reduced. This can be done by introducing a top layer of soil or organic materials that will decompose through fungi and termite activities and add nutrient that will allow plants to grow hence reviving it fertility. Second, cement plants should control the dust by fine water sprays. Third for all vehicle and big machine engines should use low sulphur diesel oil. Forth, the plant should cover of building materials like cement and put them in a place where they will not be washed into waterways or drainage areas.

Referennces

1. Arora D (1986). Mushrooms demystified: A comprehensive guide to the fleshy fungi. Berkeley: Ten Speed Press, p. 959.
2. Härkönen M, Niemelä T, Mwasumbi L (1995). Edible Mushrooms ofTanzania. Karstenia, p. 92.
3. Härkönen M, Niemelä T, Mwasumbi L (2003). Tanzanian Mushrooms: Edible, Harmful and Other Fungi. Norrlinia, p. 200
4. Kirk PM, Ansell AM (1992). Authors of fungal names. A list of authors of scientific names of fungi with recommended standard forms of their

names, including abbreviations. Index of Fungi Supplement. CAB International, Kew, Great Britain, p. 95.

5. Kirk PM, Cannon PF, David JC, Stalfers JA (2001). Ainsworth and Bisby‟s Dictionary of the Fungi. 9th Edn., CABI Publishing.Electronicversion: htpp://www.speciesfungorum.org/AuthorsoffungalNames.htmWallingford, UK.

6. Lodge JD, Ammirati FJ, O‟Dell ET, Mueller MJ (2004). Collecting and Describing Macrofungi. In Biodiversity of Fungi: Inventory and Monitoring Methods, Mueller, MG, Bills FG, and Foster SM (Eds.). Elsevier Academic Press, San Diego, CA., pp. 128-158.

7. Tibuhwa DD (2011). Morphology and taxonomy of *Sarcoscypha ololosokwaniensis* sp. nov.: A new Ascomycota species from Serengeti National Park-Tanzania. J. Yeast Fungal Res., 2(1): 1 – 6

Appendix

Fungi species

1. Termitomyces microcarpus

2. ganodermataceae

3. Schizophyllum commune

Appears to be this species growing on dead wood

4. Lentinus sp., Polyporaceae,

5. Oyster mushrooms – Pleurotus ostreatus

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6. lactarius or milk mushroom.

One of many woodland species. Some edible, some not. All are mycorrhizal.

7. Ringless Honey Mushrooms

8. ***Coprinus sp.***

9. **Coprinus comatus**

To be kept and filled in at the end of your report

Project tags (select all appropriate):

This will be use to classify your project in the project archive (that is also available online)

Project focus:

- ☐Beyond quarry borders
- ☐Biodiversity management
- ☐Cooperation programmes
- ☐Connecting with local communities
- ☐Education and Raising awareness
- ☐Invasive species
- ☐Landscape management
- ☐Pollination
- ☒Rehabilitation & habitat research
- ☐Scientific research
- ☒Soil management
- ☒Species research
- ☐Student class project
- ☐Urban ecology
- ☐Water management

Flora:

- ☐Trees & shrubs
- ☐Ferns
- ☐Flowering plants
- ☒Fungi
- ☐Mosses and liverworts

Fauna:

- ☐Amphibians
- ☐Birds
- ☐Insects
- ☐Fish
- ☐Mammals
- ☐Reptiles
- ☐Other invertebrates
- ☐Other insects
- ☐Other species

Habitat:

- ☐Artificial / cultivated land
- ☐Cave
- ☐Coastal
- ☐Grassland
- ☐Human settlement
- ☐Open areas of rocky grounds
- ☐Recreational areas
- ☐Sandy and rocky habitat
- ☐Screes
- ☐Shrub & groves
- ☒Soil
- ☐Wander biotopes
- ☐Water bodies (flowing, standing)
- ☐Wetland
- ☐Woodland

Stakeholders:

- ☐Authorities
- ☐Local community
- ☐NGOs
- ☐Schools
- ☐Universities