Potential of Wild Fungi (*Amanita princeps* & *Tylopilus felleus*) in Mycoremediation of Selected Heavy Metals in Soil

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**Abstract.** This study was conducted to determine the potential of wild fungi, namely *Amanita princeps* and *Tylopilus felleus* in mycoremediation of selected heavy metals (Cadmium, Chromium, Copper, Lead and Zinc) in soil. The wild fungi were collected in two different location which are at Sabah Agricultural Park, Lagud Seberang, Tenom and Universiti Malaysia Sabah (UMS) peak, respectively. The classification of fungi species and its characteristics was done by identifying their morphology and habitat. The uptake and distribution of heavy metals in their parts (root, stem and cap) were determined using ICP-OES instrument. The samples were digested using an acid digestion method; mixture of nitric acid (HNO₃), hydrogen peroxide (H₂O₂) and hydrochloric acid (HCL). The result shows that the uptakes of heavy metal by both *Amanita princeps* and *Tylopilus felleus* in all plant parts were highest by Zn>Cu>Cr>Pb respectively. Study on phytoremediation mechanism shows that the enrichment factor (EF) for both fungi was recorded below 1 (EF<1) indicating the low ability to absorb and accumulate heavy metals. On the other hand, results on translocation factor (TF) shows that heavy metal Zn was higher with value more than 1 (TF>1) on both species while Cu recorded high (TF>1) in *Amanita princeps*. The results also show that the TF value for most heavy metals are below 1 (TF<1), which suggested as both plants used as phytostabilization mechanism in reduction of bioavailability of heavy metals in soil.

1. Introduction
Heavy metals have recently been recognized as important pollutants due to the toxicity at low concentrations and can be more concentrated in living organisms than in the environment. According to [1], soil heavy metal pollution has become a serious issue in many parts of the world. The rapid growth of urbanization, usage of chemicals in agriculture, and improper waste disposal practices has accelerated soil contamination around the globe [2]. Although most heavy metal pollution was from anthropogenic sources, some forest soils contain large terrestrial pools of trace metals and metalloids naturally [3]. It has the potential to be taken up by terrestrial organisms, leached into groundwater, or transferred to surface waters. The widespread contamination of soil with heavy metals currently represents one of the most severe environmental problems that can seriously affect environmental quality and human health. As the number of soil pollution by heavy metal keep increasing day by day, a few methods have been employed with the intention to minimize the pollution. Heavy metal removal is usually approached with physicochemical processes that can be expensive and inefficient [4]. In pursuing the sustainable
development, this study proposed a method of removing heavy metal in soil by using wild fungi which is more environmentally friendly. Fungi are also known to accumulate high concentration of heavy metal [5]. Fungi use mechanisms like extracellular precipitation, valence transformation, and active uptake (e.g., biosorption to cell wall and pigments, intracellular compartmentation, complexation and crystallization, and sequestration) and therefore could be used to degrade, accumulate or remove metal pollutants [6]. This study facilitates better understanding on heavy metal uptakes on wild fungi with the species-dependent mechanism and other environmental factor influencing. The mycoremediation using wild fungi has become an alternative way to minimize the heavy metal pollution in the environment and this technique is known to be environmentally friendly and cost-effective. The abundance of species of wild fungi in Malaysia also encourages more research to utilize the country’s resources beneficially. Therefore, this research focused on investigating the potential of wild fungi, namely *Amanita princeps* & *Tylopilus felleus* in uptakes selected heavy metals (Pb, Cu, Cd, Cr, Zn) from the soil. The study is to provide information for a quality assessment as well as to seek the potential of species wild fungi in Sabah as an agent of heavy metal removal. Besides that, wild fungi are chosen in this study because the diversity and abundance of wild fungi in Sabah as the tropical climate allows many species to grow in tropical climate.

2. Methodology

2.1 Samples Collection
Sampling was done in two different localities which were Sabah Agricultural Park, Lagud Seberang, Tenom and University Malaysia Sabah (UMS) peak, Kota Kinabalu. At least three replicates of the wild fungi species and soil samples were collected from sampling site in the inner part of the forest. The samples were kept in a box to maintain its condition and further analyses in Toxicology Lab, Faculty of Science and Natural Resources, Universiti Malaysia Sabah.

2.2 Preparation of Wild Fungi and Soil Samples
Samples of wild fungi were washed with tap water and rinsed with distilled water for the purpose of cleaning surface debris particles that attach to it. Then, the samples were dried in an air-oven at 80°C for 24 hours. The dried sample were cut into a smaller piece. After that, it was stored in polyethylene bottles. Then it was sealed, labeled, and keep in desiccators to keep it away from moisture. For soil samples, larger organic material was removed from the samples to avoid disturbances on the accuracy of reading. Then, the soil was placed in a crucible and was heated in an air-oven at 80°C for 24 hours. The samples then left to cool before it is sieved through a pore size of 63 µm of sieve mesh.

2.3 Identification of Wild Fungi
The identification of the species was done by identifying their morphology and habitat with the guide of a books entitled “The larger Fungi of Borneo” [7] and “Checklist of Fungi of Malaysia” [8]. Some online research for picture comparison purposes also have been done.

2.4 Digestion of Samples
The digestion of wild fungi was conducted in a hood designed for safety purpose. 3 mL of 68% concentrated nitric acid (HNO₃) and 9 mL of 36% concentrated hydrochloric acid (HCl) was added to 1 g of small pieces of wild fungi. The sample then was heated in range 85-95°C. 5 mL of nitric acid was added until there was no brown fume generated indicating complete reaction with HNO₃. As the colour of the liquid turn into light yellow, the volume of the sample decreased to 5 mL. The sample was then left to cool down. 3 mL of 30% hydrogen peroxide (H₂O₂) and 2 mL of H₂O was added. Then the mixture was placed on the block heater again to start the peroxide reaction. 1 mL of 30% H₂O₂ was continue added while on the block heater until the effervescence is minimal. The sample then was waited to cool down and proceed by filtering the sample. The filtered samples then were diluted to a volume to 100 ml in volumetric flask using distilled water and store in polyethylene bottles at 4 °C prior to analysis. For soil samples the digestion process was carried out using nitric acid (HNO₃), hydrochloric acid (HCL) and hydrogen peroxide (H₂O₂), digestion according to USEPA 3050B standard method similar to fungi samples digestion.
2.5 Determination of Enrichment Factor (EF)
The enrichment factor, as mentioned by [9], is to measure the degree of enrichment and transfer of metals into the fungi samples through the soil. This data was evaluated by the ratio of the heavy metal concentration in fungi (ppm) to the heavy metal concentration in the soil (ppm). The enrichment factor was calculated by using the formula below:

\[
EF = \frac{\text{Concentration of heavy metal in fungi sample (mg/kg)}}{\text{Concentration of heavy metal in soil sample (mg/kg)}}
\]  

(1)

2.6 Determination of Translocation Factor
Translocation factor (TF) or mobilization ratio is used as indicators to determine the plant’s ability to tolerate and accumulate heavy metals. This factor is calculated using the ratio of metal concentration in plant shoots to the roots [10]. Basically, this ratio is calculated to determine relative translocation of metals from soils to root and shoot of particular plant of interest [9]. The translocation factor was calculated by using the formula below:

\[
TF = \frac{\text{Concentration of heavy metal in plant shoot (mg/kg)}}{\text{Concentration of heavy metal in root (mg/kg)}}
\]  

(2)

3. Results and Discussion
3.1 Wild Fungi Identification and Characterization
Figure 1 (a-b) showed wild fungi species that have been found and collected from Sabah Agricultural Park, Tenom and Universiti Malaysia Sabah (UMS). The wild fungi that collected in Sabah Agricultural Park, Tenom was identified as *Amanita Princeps*, whereas wild fungi collected in Universiti Malaysia Sabah (UMS) peak was identified as *Tylopilus felleus*. It was noted that both species found are known to be native species in Sabah.

![Amanita princeps](image1)

(a)

![Tylopilus felleus](image2)

(b)

Figure 1. Wild Fungi species that have been found from two different localities; (a) *Amanita princeps* collected from Sabah Agricultural Park, Tenom and (b) *Tylopilus felleus* collected from UMS peak, Sabah

*Amanita princeps* was found in the deep forest of Sabah Agricultural Park, Tenom. The size of the cap of *Amanita princeps* varies from 7 centimeters to 15 centimeters. The colours of the cap are brown to yellowish brown with dirty greyish mombrous volval remnants and have free crowded and white gills [8]. The stem is a cylindrical shape and tapering upwards a little bit, of which the annulus is often broken during cap expansion [11]. *Amanita princeps* is one of the most toxic known mushrooms found worldwide; however certain tribes or Orang Asli in Peninsular Malaysia eat it as food [8]. Another species *Tylopilus felleus* was found at the peak of Universiti Malaysia abah’s (UMS) hill. It has a life
span varies from 10 – 14 days. The common name of this species is bitter boletus. The cap of this fungi is semi-globular with grey-brown in colour. The size of the cap varies from 4 cm to 12 cm. underneath the cap was a white pore and it will turn pink upon maturity. The stem of this fungi is brownish in colour and have 1 cm to 2 cm diameter with a cylindrical shape [12]. This mushroom is an exotic food, especially among the elderly. On the reason of its taste is bitter, the mushroom was neither to be eaten raw nor simply cooked as the commercial mushroom [12]. Table 1 showed the summary characteristics for both wild fungi found at two different localities.

### Table 1. Characteristic of wild fungi collected at two different localities.

| Species          | Class             | Characteristic                                      | Life span | References |
|------------------|-------------------|----------------------------------------------------|-----------|------------|
| Amanita princeps | Basidio mycota    | - 7cm to 15cm - large plane - Yellowish brown      | 6 – 11 days | [11, 13]   |
|                  |                   | - The flesh and crowded gills are white.            |           |            |
|                  |                   | - Dirty white to grey in colour - Cylindrical in shape |           |            |
| Tylopilus s felleus | Basidio mycota  | - 4cm to 12cm - Semi-globular - Grey-brown         | 10 – 14 days | [8, 12]    |
|                  |                   | - It has pores underneath - White at first, turn pinkish with maturity |           |            |
|                  |                   | - Brownish stem - 1 cm to 2 cm diameter. -Cylindrical in shape |           |            |

3.2 Distribution of Heavy Metals (Cd, Cr, Cu, Pb and Zn) in soil and wild fungi parts (root, stem, cap) of Amanita princeps and Tylopilus felleus

Figure 2 showed the distribution of selected heavy metals in the soil and fungi parts (root, stem, cap) of Amanita princeps and Tylopilus felleus. Based on the analysis, it was found that heavy metal Zn shows the highest distribution in the root, stem and cap in Amanita princeps as compared to other metals. The order of heavy metal distribution within the three wild fungi parts of Amanita princeps was Zn > Cu > Cr > Pb. The highest concentration of Zn within the fungi part of Amanita princeps was found in the cap (66.280 mg/kg), while the lowest was in the stem (38.001 mg/kg). The heavy metal (Zn, Cu, Pb) were distributed randomly throughout the fungi parts of Amanita princeps except for Cr. It is in decreasing order from roots to stem. For Pb, it does not translocate to the higher part (cap) of Amanita princeps. It can be explained by the low mobility of Pb from stem to cap due to barriers or lack of transport mechanism [14]. In addition, Pb does not play any beneficial role in the growth and development of the plant [15]. The Zn and Cu in the cap of Amanita princeps were 1.53 folds and 1.29 folds higher than the root, respectively. According to [16], when analyzing heavy metal concentration in caps and roots of fungi, 60 percent of the sample showed a higher concentration of Cu and Zn in the cap. This explained why the concentration of Cu and Zn is higher in Amanita princeps and supports the present study. For Tylopilus felleus, heavy metal Zn showed the highest distribution in all fungi parts (root, stem and cap) as compared to other metals. This is due to heavy metal Zn plays a part in the basic roles of cellular functions and indispensable micronutrient in all living organisms for their development [17]. The order of heavy metal distribution within the three wild fungi parts of Tylopilus felleus was Zn > Cu > Cr > Pb. It has the same trend as Amanita Princeps. The highest distribution Zn within the fungi of Tylopilus felleus was found in the stem (39.456 mg/kg). It was 1.1-fold higher as compared to the lowest distribution Zn that was found in the root (31.505 mg/Kg). The distribution of Cu and Zn were fluctuating from the root to the cap of Tylopilus felleus. Whereas the initial concentrations of Cr in the soil has decreased as it moves towards the cap. These observations show that the uptake of essential
metal from the soil was distributed from the root to the stem and cap, where it is necessarily needed for the growth, spores-bearing, or other biochemical activity.

Figure 2. The distribution of heavy metal in the soil and wild fungi parts (root, stem, cap) of *Amanita princeps* and *Tylopilus felleus* at two different localities.

In this study, results of heavy metal Cd was detected in the root system of *Tylopilus felleus* with a small value of (2.442 mg/kg). The plant tends to sequesters it in the roots to avoid damage to the upper part since Cd is a toxic, non-essential element for the growth of fungi. [15} deduce that the accumulation of Cd that found only in root suggested that Cd uptake is restricted and not readily translocated in the fruiting body of fungi. The Cu in the root of *Tylopilus felleus* was 2.24 folds higher than in the cap, which was 29.060 mg/kg and 12.916 mg/kg, respectively. [18] reported that within roots, Cu is associated mainly with cell and is largely immobile. The study shows that trend of heavy metal in soil for *Amanita princeps* decreases according to this trend: Zn (173.612 mg/kg) > Cr (41.533 mg/kg) > Cu (36.756 mg/kg) > Pb (28.303 mg/kg). Whereas, heavy metals in *Tylopilus felleus* decreases according to this trend: Zn (50.983 mg/kg) > Cr (47.701 mg/kg) > Cu (35.561 mg/kg) > Pb (26.854 mg/kg) > Cd (3.4231 mg/kg). Cd was not detectable for *Amanita princeps* due to a low level of concentration in the soil. All these heavy metals in the soil have a high concentration compare to the concentration in fungi parts. The result agrees with [19], which reported that the accumulation of heavy metal in soil is higher than in fungi. This indicates that heavy metals are not absorbed thoroughly into the fungi. Cr was found high in the soil for *Amanita princeps* and *Tylopilus felleus*, which were (41.533 mg/kg) and (47.701 mg/kg) respectively. But, the concentration of Cr in the fungi part is so little, which was in the range of 4.904 mg/kg to 1.223 mg/kg. Cr at low concentration was found to promote growth and increase yield in plants, but it is not considered an essential element; it is highly toxic compound in high concentration and disturbed the growth and development of the plant [20]. This result agrees with a previous study by [21], which stated that *Tylopilus felleus* is not a good accumulator of Cr. [22] also revealed that Cr has low solubility in soil with strong barrier and hence not significantly translocated to the fungi parts, which agree with the present study. The Zn content in both wild fungi was primarily high as it is one of the most important elements needed for the growth and development of organisms. Soil fungi are also known as Zn accumulator and sporophore, where the substrate ratio for Zn ranges from 1 to 10 mg/kg [23]. Although other heavy metals also present in the soil, it does not accumulate much in the fungi parts. Through this result, it can infer that both species uptakes a higher concentration of essential elements while a lower concentration for less essential elements.

3.3 Enrichment Factor (EF) of *Amanita princeps* and *Tylopilus felleus*.
Based on Figure 3, the results showed that in *Amanita Princeps*, the highest EF is Cu while in *Tylopilus felleus*, Zn show the highest value of EF. The trend of enrichment factor for *Amanita princeps* was in the order of Cu > Zn > Cr, whereas in *Tylopilus felleus* was Zn > Cu > Cr > Pb. The enrichment factor for Pb in *Amanita Princeps* and Cd show 0 due to its concentration that is too low below the detection
limit. The EF values of the wild fungi study are relatively low; this indicates that the heavy metal is not abundantly accumulated in the fungi. According to numerous researchers, establishing an enrichment factor is an alternative method used to categorize the metal sources between anthropogenic and naturally occurring [24, 25]. Based on the graph, it shows that all enrichment factor values are below unity, and it indicates that the origin of heavy metal is most probably from natural occurrence. The interpretation of heavy metals accumulation in soil based on enrichment factor is depletion to minimal enrichment of heavy metal [26]. This is because of both of the wild fungi from the unpolluted forest area.

![Image](Figure 3. The Enrichment Factor (EF) of wild fungi from soil to cap for two different localities)

### 3.4 Translocation Factor (TF) of Amanita princeps and Tylopilus felleus.

The result shows that the TF is <2 for both species studied from root to cap. Translocation of Cd is not detected in the study. This is due to the low concentration of Cd value, thus make it undetectable. For *Amanita princeps*, the translocation factor was in the order of Zn (1.540) > Cu (1.294) > and Cr (0.249).

![Image](Figure 4. The Translocation Factor (TF) of wild fungi from root to the cap for two different localities.)

The translocation factor for Pb in *Amanita princeps* is 0 concentration, which was below the detection limit. Whereas the ranking order of translocation factor for *Tylopilus felleus* was Zn (1.072) > Pb (0.747) > Cr (0.585) > Cu (0.444). According to [27], TF>1 indicates rapid heavy metal transportation from root to cap. In contrast, the TF<1 indicates the root to cap heavy metal transportation is limited, thus labelling these fungi as trace metal excluders. For *Amanita princeps*, Zn showed the highest value for
translocation factor from root to cap and the lowest is Cr. In *Tylopilus felleus*, Zn is the highest with 1.072 and Cu is the lowest with a value of 0.444. This result indicates that both species *Amanita princeps* and *Tylopilus felleus* are considered as good mobility for Zn. [28] reported that both translocation factor (TF) and enrichment factor (EF) of the plant must be higher than 1 to indicate the capability of the plant to transport metal to cap and the ability to uptake heavy metal more than the sediment, respectively. In this study, Zn and Cu are higher than unity for *Amanita princeps* and Zn for *Tylopilus felleus*. Since Zn and Cu is an essential element for the growth and development of the organism in all parts, thus it is understandable for these elements can rapidly be transported from root to the cap. Also, it can be deduced that both species can be used as possible bioindicator for Zn pollution. The graphs depict Cr in *Amanita princeps*, Cr, Pb and Cu in *Tylopilus felleus* show limited translocation to the cap of the soil fungi. In addition, the high concentration of these metals in the root of the fungi and low translocation in above-ground parts indicated their potential to be used as phytostabilization of metal in the study area. The phytostabilization mechanism involved the immobilization metals in fungi, thus reducing their bioavailability via erosion and leaching [29].

4. Conclusions
The study concludes that both species found in selected localities, namely *Amanita princeps* and *Tylopilus felleus* hold a promise in mycoremediation of heavy metals from soil. It was found that both species have the potential in uptakes a high level of Zn as compared to other metals. This study also suggested that both species have the potential to be used as phytostabilization plants. The data obtained through this study on the distribution of Cd, Cr, Cu, Pb and Zn in *Amanita princeps* and *Tylopilus felleus* plant parts could be used as baseline study for the possible use of other soil fungi in mycoremediation other pollutants or environmentally monitoring for future research.

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