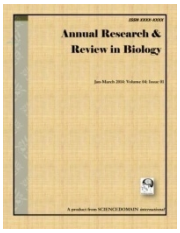

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Isolation and Colonial Morphological Characterization of Fungal Species in Copper Laden Sediments of Calancan Bay, Marinduque

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Authors' contributions

This work was carried out in collaboration between all authors. Author GSS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ELF, MMM and MAS managed the analyses of the study. Authors MLSS, ER and AAB managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of our study was to isolate and characterized the potential fungal species, which can be used for future rehabilitation of these environment.
Study Design: Cross-sectional study.
Place and Duration of Study: Calancan Bay, Marinduque, between November 2011 and December 2012.
Methodology: Copper-laden sediments were obtained in the Calancan Bay; cultured and fungal species were examined morphologically by both macroscopic and microscopic means. Edaphic factors (soil temperature, moisture, pH, organic matter, and texture) were also determined in all randomly selected stations.

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Results: Five fungal genera were isolated from the Calancan Bay, namely, *Aspergillus sp., Penicillium sp., Fonsecaea sp., Trichoderma sp. and Fusarium sp.*
Conclusion: The diversity and occurrence of the isolated fungal species were dependent on the edaphic factors emanating in the area.

Keywords: Fungi; copper; edaphic; heavy metal.

1. INTRODUCTION

The presence of heavy metals in the environment remains to be a global problem that needs immediate attention. Although heavy metals occur naturally, anthropogenic activities continue to contribute to the release of huge amounts of heavy metals in the environment. The Calancan Bay in Marinduque is one example of this phenomenon where the continuous discharge of copper mine tailings has filled up the bottom of the bay and eventually created a causeway [1]. A previous study [2] has indicated that, over the years, the sediments and waters surrounding the Calancan Bay contain high amounts of heavy metals, particularly total copper (Cu), total zinc (Zn) and total lead (Pb), even though the area has already been abandoned. The persistence of these heavy metals, particularly Cu, poses a grave threat not just to the organisms inhabiting the area but also to the people who continuously depend on the bay for their day-to-day subsistence [3]. People's continuous exposure to heavy metals can lead to various morbidities and mortalities. Hence, there is a need to address the problem by utilizing means that would facilitate in the remediation of these heavy metals in the environment.

Various technologies are used to manage heavy metal-contaminated environments. However, the use of these technologies proved to be very expensive; hence, biological control methods are preferred. Instituting biological control methods like plants and microorganisms [4] provide an efficient and economical means to reduce environmental toxins. Fungi are one of the commonly used biological control methods in contaminated environments. Leitao [5] has indicated that fungi play an important role in the natural remediation of metal and aromatic compounds present in the environment. The marked tolerance of these microorganisms to survive in adverse conditions as such makes them suitable for this particular purpose. Fungi are known to accumulate heavy metals in the environment, and the bioremediation capacities of these microorganisms likewise make them very good bioindicator organisms of heavy metal pollution [6]. In the Philippines, there is a paucity of literature on fungal species present in metal-laden sediments particularly in Calancan Bay, Marinduque. This study aims to isolate and characterized the potential fungal species, which can be used for future rehabilitation of these environment.

2. MATERIAL AND METHODS

2.1 Study Site Description and Sampling

The Calancan Bay in Marinduque is situated 60 km north of the center of the Philippines and 160 km southeast of Manila Fig. 1. The study site is positioned at 13º32'20" north latitude and 121º58'46" east longitude. The causeway in Calancan Bay is about 5 km long running through the bay. The causeway bay is formed from the deposition of mine tailings in Marinduque. Five sampling sites were identified along the causeway. The causeway was divided into five regions, from the farthest end of the bay with each region being

approximately 1 km long. In each region, the sampling station was identified randomly based on the direction determined by the watch's second hand upon the glance of the researcher. Fig. 2 shows the sampling stations in Calancan Bay.

Fig. 1. Calancan Bay, Marinduque

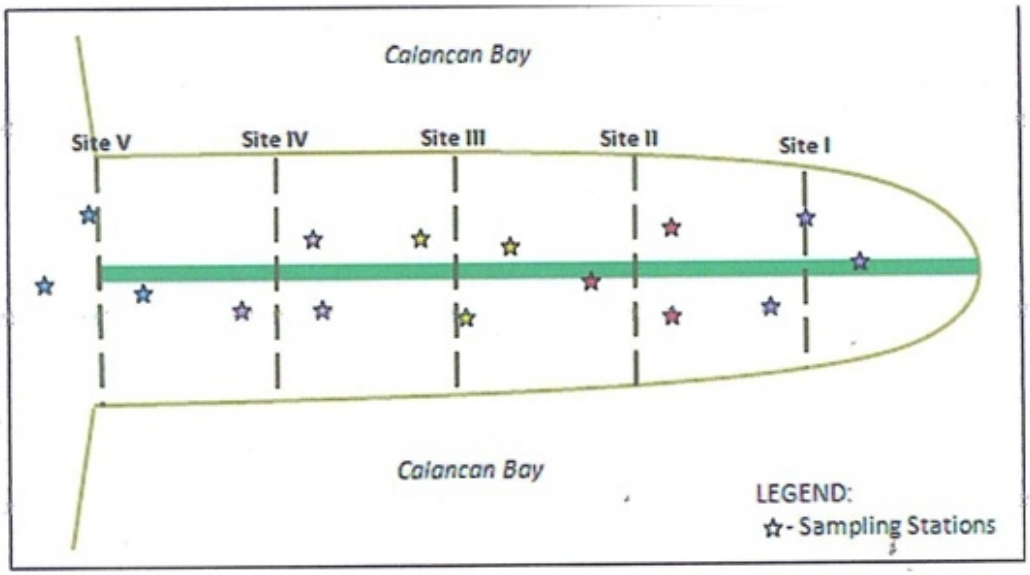

Fig. 2. Sampling stations in the causeway bay of Calancan

2.2 Soil Collection, Handling and Processing

The soil surface debris was brushed off, and a presterilized spatula was used to collect soil from a depth of approximately 15-20 cm from the soil surface. Soil samples for mycological assessment were placed in presterilized glass bottles and were properly labeled. Additional soil samples were collected for the processing of the edaphic factors (pH, moisture, organic matter and texture). The temperature of the soil was determined in situ.

2.3 Edaphic Factors Determination

The soil moisture was determined following the procedures indicated in ASTM D2216 [7]. The soil texture determination was performed following the feel method as indicated by Thien [8]. The soil organic matter was determined following the procedures of ASTM D2974 [9]. The soil pH was determined by mixing a gram of the soil sample to 5 ml of distilled water, making a suspension and a pH paper (Merck, Germany) was used to measure the pH of the soil suspension.

2.4 Statistical Analysis

Results of the edaphic factors (soil temperature, pH, moisture and organic matter content) were analyzed for significant differences across all the sites sampled using the analysis of variance. A test with a $P = 0.05$ indicates that all statistical analyses are significant. All statistical analyses were performed using the IBM Statistical Package for Social Sciences software.

2.5 Fungal Culture, Isolation and Morphological Characterization

A soil suspension was made from mixing a gram of the soil sample to 5 ml of sterile distilled water. The soil suspension was diluted fivefold. Ten microliters of the last three dilutions prepared were spread plated in prepared potato dextrose agar (PDA) containing 30 ppm copper sulfate based on a previous study [2] conducted in the study site. The plates were incubated at 25ºC for 4-7 days. In each plate, all distinct colonies were isolated and inoculated in separate PDA containing copper sulfate. The macroscopic characteristics such as the colony features, growth rate, and pigmentation of the isolated fungal species were observed and noted. Isolated colonies were also cultured onto low carbon agar (LCA) plates for fungal sporulation. Slide preparations of the fungal isolates were prepared, stained with lactophenol cotton blue (LCPB), viewed microscopically at 400-1000x and compared to manuals [10,11].

3. RESULTS AND DISCUSSION

The edaphic conditions in Calancan Bay, Marinduque, are shown in Table 1. The soil temperature readings in the study sites range from 23ºC to 28ºC. The soil pH in the study sites ranged from 5 to 6.5, indicating that the soil conditions are slightly acidic. Higher soil moisture percentages were evident in site 4, and the lowest soil moisture is at site 2. The soil texture of all the examined study sites showed varied results. The soils in all the study sites were a mixture. Among all examined sites, site 1 had the highest percentage of soil organic matter content while site 2 had the lowest percentage of soil organic matter.

Results of the analysis of variance indicated that the soil temperature collected in situ at different sampling stations varied significantly (F = 26.70; P = 0.000). However, the soil pH (F=3.313; P = 0.057), soil moisture (F=0.135; P=0.966) and soil organic matter (F=0.732; P=0.590) did not show any significant differences among the different study sites collected.

Table 1. Edaphic factors in Calancan Bay, Marinduque

Study Sites	Edaphic Factors (Mean ± SEM)				Soil Texture
	Temperature (°C)	pH	Moisture (%)	Total Organic Matter (%)	
1	24.00±0.58	5.00±0.00	11.5 ±5.10	4.33±2.30	Silty clay, loam–sandy
2	23.00±0.29	5.67±0.33	10.0 ±1.05	0.5 ±0.22	Sandy clay–clay loam
3	28.00±0.00	5.83±0.17	11.6 ±0.48	1.6 ±0.33	Sandy–loamy
4	27.00±0.58	6.00±0.29	13.5 ±3.28	4.0 ±3.58	Sandy clay–clay
5	24.50±0.29	5.50±0.00	11.1 ±4.74	4.0 ±1.18	Clay loamy

Table 2 shows the listing of fungal isolates obtained per sampled study site. The fungal isolates obtained on the copper-laden sediments of Calancan Bay, Marinduque, are classified under five genera, namely *Aspergillus, Penicillium, Fonsecaea, Trichoderma,* and *Fusarium.* Among those belonging to the *Aspergillus* genera, the following species identified were *Aspergillus niger, Aspergillus fumigates* and *Aspergillus flavus.* Another *Aspergillus sp.* was unidentified up to the species level.

Table 2. Mycological assessment in Calancan Bay, Marinduque

Study Sites	*Fungal Isolates*
1	*Penicillium* sp., *Fonsecaea* sp., *Trichoderma* sp., *Aspergillus flavus*, *Aspergillus* sp.
2	*Penicillium* sp., *Aspergillus fumigatus, Aspergillus* sp.
3	*Penicillium* sp., *Aspergillus fumigatus, Aspergillus niger, Aspergillus* sp.
4	*Fusarium* sp., *Aspergillus fumigatus, Aspergillus flavus, Aspergillus* sp.
5	*Penicillium* sp., *Trichoderma* sp., *Aspergillus niger, Aspergillus fumigatus, Aspergillus* sp.

This study was cross-sectional in nature. The mycological assessment was only done in the copper-laden sediments of the causeway bay of Calancan, Marinduque. The edaphic factors in the study area influence the abundance and diversity of the isolated soil fungi. In all study sites, the soil organic matter content of the study area shows that the type of soil in the causeway of Calancan Bay is a mineral soil since the organic matter content was less than 30% [12]. The edaphic conditions in the study area showed favorable conditions for the growth of fungal microorganisms. The soil temperatures in the study sites indicate the best temperature to grow for mesophilic organisms like fungi. Likewise, the soil pH showed slightly acidic soils and fungi favor growing in acidic soils particularly at pH of 4-6 [13]. A study [12] has indicated that fungal microorganisms prefer areas with low soil moisture, because too much water in the soil can lead to poor soil aeration.

Different species of fungi were isolated from the metal-laden sediments. Among the sites assessed, only study site 2 gave the least abundance and diversity of fungal species

isolated. This result may be because, among all the sites examined, sites 2 showed the lowest in soil moisture percentage and soil organic matter percentage. The edaphic conditions in the study sites greatly favored the growth of the five genera of fungi, namely, *Aspergillus, Penicillium, Fonseceae, Trichoderma,* and *Fusarium.* In all study sites examined, the predominant isolated fungi were those of the *Aspergillus sp.* This particular observation is not surprising, as a study [14] has indicated that the *Aspergillus sp.* can be isolated in a wide variety of environments. Despite the presence of heavy metals in the laden sediments of the causeway bay of Calancan, a number of fungal species were still isolated. According to Ezzouhri et al. [15], fungi can thrive in contaminated sites even in mine sites. It is likely that these microorganisms thrived in these areas because of their abilities to uptake metals [16]. Apart from the *Aspergillus sp.* in degrading various xenobiotic compounds [5]. In all study sites examined, the *Fonseceae sp.* was only evident in site 1. The high organic matter present in site 1 can be likely attributed to the occurrence of the saprophyte. The *Fusarium sp.* was observed in site 4. The occurrence of the microorganism may be likely due to the high organic matter and high soil moisture [17] present in the study site, particularly that these conditions are the evident favorable growing conditions of the microorganism. Likewise, the ability of this microorganism to tolerate contaminated soils, especially those containing metals, may also be the reason for the occurrence of the microorganism in metal-laden sediments [15]. The *Trichoderma sp.* was observed in sites 1 and 5. The occurrence of the *Trichoderms sp.* in both sites shows that the fungus is capable of surviving in sediments containing high metal concentrations, as they have the capacity to uptake the metals [18].

4. CONCLUSION

The results of our study showed that different species of fungi were isolated in all the copper-laden sediments of the study sites in Calancan. The fungi were able to grow and adapt even in such conditions. It is likely that their occurrence may be due to their adaptation and associated metal sorption capacities. It is also that the emanating edaphic factors and metals present in the sediments of the causeway bay of Calancan may influence their occurrence in the study sites.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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