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MICROBIAL AIR AND WATER QUALITY ASSESSMENT OF A FRESHWATER LIMESTONE CAVE IN THE PHILIPPINES AND ITS IMPLICATIONS FOR ECOTOURISM MANAGEMENT

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ABSTRACT – The Philippines has an extensive cave system owing to its geologic history. However, as human populations and tourism expand, this unique ecosystem is increasingly threatened by anthropogenic influences. The extent of these human impacts and the suitability of ecosystems for human use can be assessed through measurement of indices. Cultivable microbial groups were used as quality indices for air and water habitats in the Cacupangan Cave, Pangasinan, Philippines. Air quality was measured by determining the Index of Microbial Air Contamination (IMA) using settle plates for enumeration of air-borne fungal spores. Water quality was measured by determining the concentration of coliforms expressed as Most Probable Number (MPN) using a multiple tube fermentation technique. Results showed that air quality ranges from good to very poor. IMA based on a cave assessment index indicates that the cave is already "threatened by fungi" signifying possible degradation of cave structures due to fungal activities. The most common genera identified were *Aspergillus* and *Penicillium*. These are commonly associated with epigean sources such as soil and plants. They are ubiquitous in the environment and their spores are easily carried by wind currents. Water quality, on the other hand, is low because of high coliform content indicating fecal contamination and therefore the possible presence of pathogens. Coliforms may have come from bats as well as epigean and anthropogenic sources. *Escherichia*, *Klebsiella*, and *Enterobacter* were the genera most commonly identified. Continuous regular monitoring of this ecotourism site is important for its sustainable management. Control of activities is necessary to maintain the integrity of the system.

Keywords: air-borne fungi, Cave environmental monitoring, coliforms Index of Microbial Air Contamination, Most Probable Number

INTRODUCTION

Caves are important natural resources that provide a wide variety of ecosystem goods and services. Unfortunately, this unique subterranean ecosystem is often taken for granted because it is out-of-sight and its connection with the surface is often overlooked. It is therefore very prone to over-extraction

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of materials, as well as excessive exposure to runoffs from industrial, agricultural, and human settlements, to name a few. Recently, inadequate management of ecotourism activities has also impacted this vulnerable ecosystem. These various factors are especially intense in the Philippines given the various socio-economic and political problems besetting the country. Recognizing these threats, the Philippine government enacted Republic Act 9072, the National Caves and Cave Resources Management and Protection Act. It mandates the Department of Environment and Natural Resources (DENR) to formulate, develop, and implement a national program for the management, protection, and conservation of caves and its resources as well as call upon the community to assist in the fulfillment of this goal (http://policy.denr.gov.ph/2001/Ref_Act_9072.pdf).

The Philippines has an extensive cave system owing to its geologic history. The government's DENR has recorded around 1,800 caves with more yet to be discovered (http://www.denr.gov.ph/news-and-features/latest-news/288-caves-and-cave-resources.html). However, as the resident human population and tourism expand, this unique ecosystem is becoming increasingly threatened by anthropogenic influences. DENR has identified the following reasons: "the lack of specific statutory protection, increased demand for recreational sites, treasure hunting, mining, pollution, illegal collection of cave resources, and rapid urbanization" (DENR-PAWB, 2009). The extent of these human impacts as well as the suitability of certain ecosystems for human use can be monitored through measurement of certain microbiological parameters, among others.

Environmental quality assessment can be done using microorganisms as biological indicators. Indicators are generally used as pointers or signs that give valuable information about something. In this case, microorganisms are used to indicate quality of a particular resource as well as its suitability for human consumption. Microbial indicators are often used to indicate the status of an area in terms of cleanliness, such as in production areas or clean rooms; or of contamination source such as in drinking water systems. (Check out DENR AO 2016-08 Section 5) The same can be used for environmental monitoring of key ecosystems such as caves. It is an important component of adaptable management as it helps to spot potential problems early on. This is of particular interest in the Philippines because of the rapid decline of ecosystems as well as in other countries that experience the same problems.

In this study, air and water quality inside the Cacupangan Cave, Pangasinan, Philippines was assessed by measuring the Index of Microbial Air Contamination (IMA) by fungi and the concentration of coliforms expressed as Most Probable Number (MPN). Correlation of contributing factors was determined and possible connections with epigean sources were identified.

MATERIALS AND METHODS

Study site and collaborators

Cacupangan Cave is located in Mabini, Pangasinan in Luzon, northern part of the Philippines (16.056170, 119.954932; Fig 1). This cave is traversed by Balincaguin River and is part of a forested karst landscape. Sampling was conducted during dry (April 2015) and wet (October 2015 and January 2016) seasons. Cultivable microbial groups were used as quality indices for air and water habitats. The study was conducted in cooperation with the Local Government Unit (LGU) of Mabini, the Balincaguin Conservancy group, the Cave Ecology class of the Institute of Biological Sciences, University of the Philippines Los Baños (UPLB) during the 2nd Semester AY 2014-2015 and Mr. John Mark A. Encinares who conducted his M.S. Environmental Science thesis in the same area in 2015-2016. The collaborators conducted the mapping of the area, biodiversity survey of cave fauna, as well as some of the interviews with tour guides and locals. Identification of isolates was conducted in collaboration with the Museum of Natural History, UPLB.

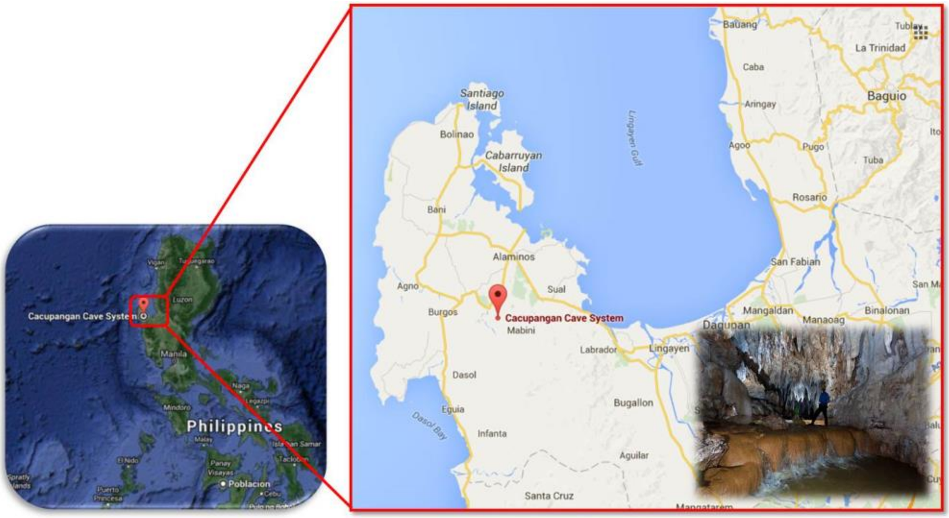

Figure 1. Location of the Cacupangan Cave System, Mabini, Pangasinan in Luzon, northern part of the Philippines (16.056170, 119.954932). Inset shows one of the microbial assessment sites inside the cave.

Gathering of relevant information

Other relevant information were gathered from observations in the area, unstructured interviews with locals and perusal of the comprehensive land use map and other information regarding the Cacupangan Cave from the LGU and the DENR.

Air quality monitoring

A total of eight (8) sampling points were identified inside the 820-m stretch of the mapped portion of the cave (Fig 2). In addition, one (1) sampling point immediately outside the cave near its entrance was included. Air quality was measured by determining the Index of Microbial Air Contamination (IMA) through a passive sampling procedure using settle plates for enumeration of fungal propagules in the air according to Pasquarella et al. (2000) with some modifications. Three Potato Dextrose Agar (PDA; Pronadisa) plates supplemented with 0.005% Rose Bengal and 100 ppm chloramphenicol were exposed at each of the eight designated sampling points for one (1) hour and then brought to the lab for incubation at room temperature (25-28°C) for 7 days. The number of fungal colony forming units was counted for all plates and the IMA computed as the average fungal colony-forming units (CFU) per plate per hour of exposure. The IMA was then converted into fungal concentration in colony forming units per cubic meter (CFU m^{-3}) according to the equivalencies set by the European Good Manufacturing Processes (EU GMP 1997 as cited by Pasquarella et al. 2000) where 200, 100, and 10 CFU m^{-3} of fungal propagules in the air is equivalent to 25, 12.5 and 1.25 colonies per plate per hour, respectively, using the settle plate method.

This is considered as an estimated fungal concentration only since it is just a derivation from the settle plate counts. The estimate fungal propagule concentration in the air was computed as IMA/0.125.

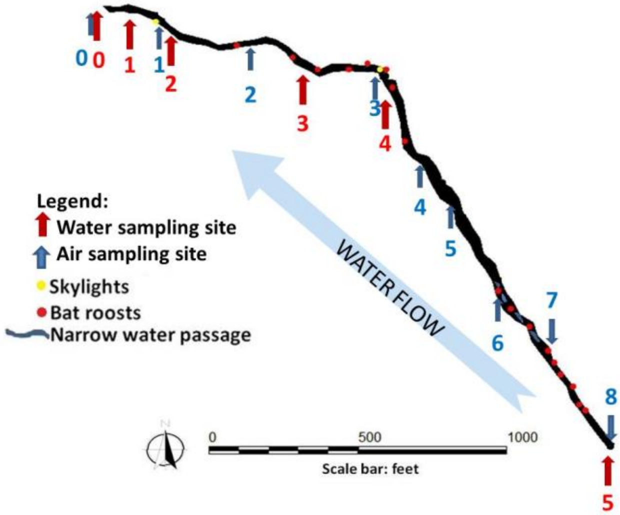

Figure 2. Sampling points for air and water quality assessment inside Cacupangan Cave, Mabini, Pangasinan, Philippines. The first red and blue arrows at the upper left-most part of the diagram are located just outside of the cave entrance while the last red and blue arrows at the lower right-most part of the diagram are located at the innermost passable and mapped portion of the cave which is 820 m from the entrance.

High-resolution pictures were taken of each plate for digital documentation and later use for data analysis. Distinct colony forms were coded, isolated and morphologically characterized to determine putative identities. Identification was carried out using diagnostic keys (Samson et al. 2002; Pitt and Hocking 2009). The putative identities and counts of each of the different colony forms present in each plate were recorded based on the pictures taken. Computations of the following indices were likewise based on the pictures of the settle plates. The Jaccard Index of Similarity (JS) was computed for each sampling point to compare fungal profiles based on the occurrence of each colony form in settle plates using the formula JS (A,B)= $\frac{(A \cap B)}{(A \cup B)}$. Relative abundance (Pi) of small-spored genera was determined by dividing the number of colonies of small-spored genera over the total number of colonies in plates for each sampling station. Assignment of colonies into either small-spored (≤10 µm) or large-spored (>10 µm) were based on literature (Golan and Pringle 2017; Reponen et al. 2001; Yamamoto et al. 2012). Species Richness (S) was determined by counting the number of different genera occurring on settle plates in each sampling point. Correlation analyses (in Microsoft Excel) were done to determine relationships between distance from the entrance and the other computed indices (IMA, JS, Pi, and S). Air temperature and relative humidity inside the cave were also measured using a thermometer and a whirling psychrometer, respectively.

Water quality monitoring

Water quality was assessed from five (5) sampling points within the cave and one (1) sampling point from the pipe immediately outside the cave. Assessment was done by determining coliform levels using a multiple tube fermentation technique (MTFT) for non-potable water (5-tube method) where the results are statistically expressed in terms of the Most Probable Number (MPN) of coliforms. This is a standard three-stage procedure composed of the presumptive stage, confirmatory stage, and the completed test conducted according to the Standard Methods Committee, A–E (1999) specifically the section on "Water of Other than Drinking Water Quality". The tests are done using lauryl sulfate tryptose broth (LSTB), brilliant green lactose bile broth (BGLB), and eosin methylene blue agar (EMB). After the completed test, representative EMB colony types were isolated and putatively identified using microscopic examination of Gram-stained smears, catalase test, IMViC tests (indole-methyl red-voges proskauer-citrate), motility test, and determination of growth characteristics in triple sugar iron (TSI) agar. Putative genus-level identities were determined using an identification scheme (Fig. 3) based on the keys from Bergey's Manual (Garrity, 2005). Possible sources of and threats posed by the species identified were determined from literature.

Relative abundance (Pi) of identified isolates was computed as follows:

$$Pi = \frac{\#of\ isolates\ identified\ as\ species\ x}{total\ \#\ of\ isolates\ identified}$$

Occurrence of identified isolates was determined by taking note of their presence or absence in each of the sampling areas such that 100% occurrence indicates that the isolate was detected in all 6 sampling areas.

Other water quality parameters such as dissolved oxygen, electrical conductivity, pH, and temperature were measured using various measuring equipment [DO meter (Yellow Spring model 57); SCT meter (SX713 Model); pH meter (Milwaukee pH600)].

RESULTS AND DISCUSSION

Relevant information about the study site

Cacupangan Cave is a 4-km karst cave with a water system that flows into the Balincaguin River. This cave is one of the explored caves in Pangasinan as it is being promoted by the Pangasinan Tourism Council. It has been made conducive for entry of tourists as a staircase has been constructed along the entrance. Stalactites, stalagmites, fine rimstone pools, and calcite crystals are some of the formations seen in the cave. Also residing within the cave are colonies of bats of the genera *Miniopterus* sp., *Myotis* sp., *Hipposideros* sp., and *Rhinolophus* sp. (unpublished data from the Cave Ecology class). However, the cave is prone to be littered and at certain spots, vandalized, with the entry of visitors. In fact, local interviews revealed and on-site observations indicated that there were signs of both guano and speleothem harvesting or extraction as shown by thin guano deposits despite the presence of 17 bat roosts and extensive destruction of structures in certain areas (called the "logging room" by the locals), respectively. Residents around the area were also seen utilizing the water flowing out of the cave for bathing, washing, or collection for storage (based on unstructured interviews). Some locals who were interviewed also mentioned using the water for cleaning, watering plants, and sometimes even for cooking. These observations and informal interviews are consistent with the results of semi-structured recorded interviews conducted by Encinares

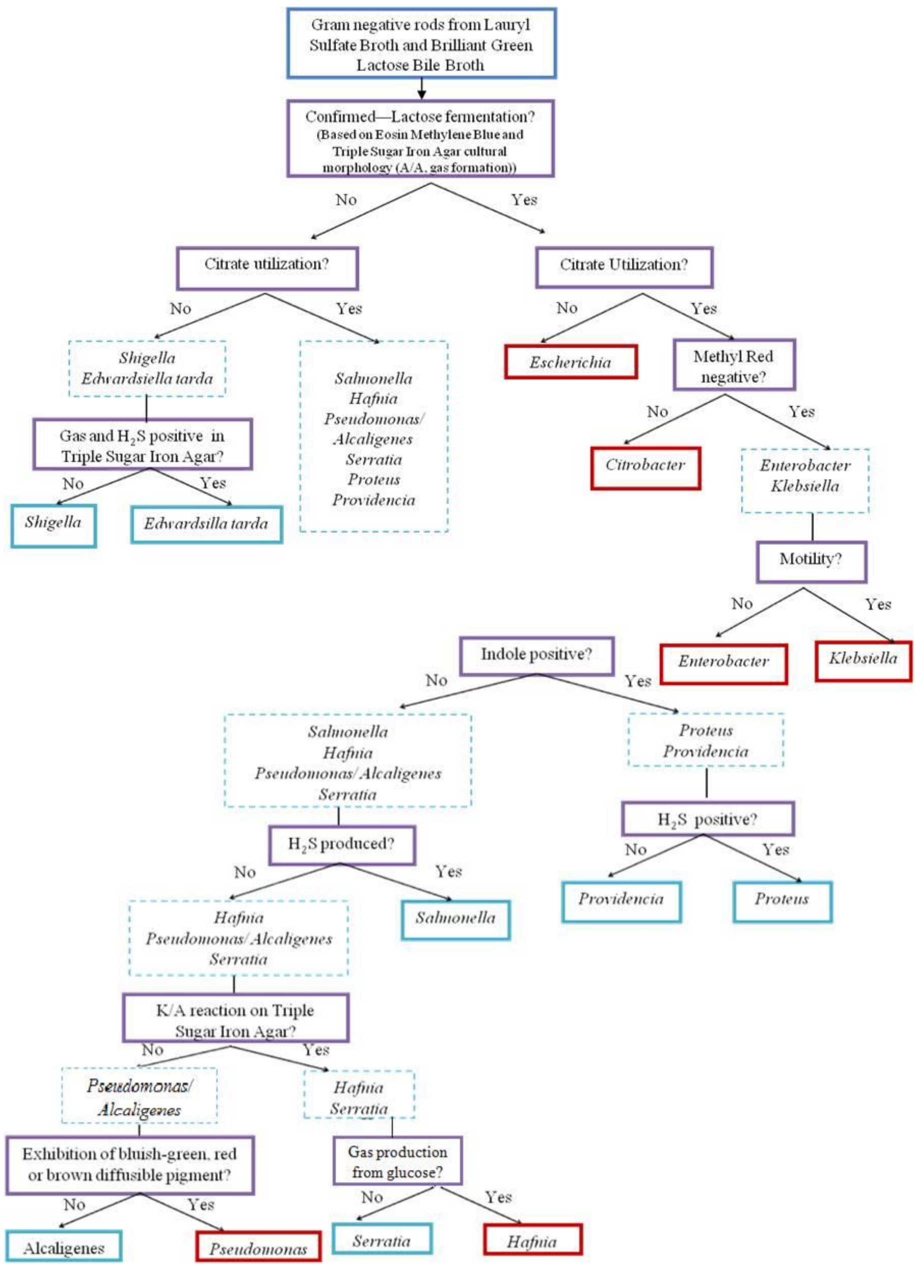

Figure 3. Identification scheme used for determining putative identities of colonies isolated from Eosin Methylene Blue agar plates used in the multiple tube fermentation technique completed test for coliforms in the subterranean stream water of Cacupangan Cave, Mabini, Pangasinan, Philippines.

(2016) to gauge the knowledge, attitudes, and practices of locals pertaining to cave resources. He enumerated several external and internal disturbance indicators that were similar to those observed in this study.

Inside the caves, some areas were seen to have makeshift "dams" to create areas deep enough for swimming. At the entrance of the cave are picnic huts, barbecue pits, tables, chairs and parking spaces. The surface landowner developed the area around the cave into a commercial tourist area making the cave a private resort. The Comprehensive Land Use Map obtained from the LGU as well as an examination of the area through Google Earth show that nearby regions consist of agricultural areas (rice fields and livestock farms) as well as human settlements through which the Balincaguin river waters pass going into the cave. These are all indicators of external disturbances that affect the cave.

Encinares (2016) reported an inference of anthropogenic disturbance in Cacupangan Cave. The level of disturbance was gauged by determining the Inferred Degree of Disturbance (IDD) from observed external and internal indicators of disturbance. The index used was adapted from Quibod (2013), which was modified and developed according to the local situation from other disturbance indices used by Van Beynen and Townsend (2005) and Phelps and Kingston (2010). The reports of Encinares (2016) indicate that Cacupangan Cave is moderately disturbed and that the disturbance is evident up to 400 m into the cave. At 600 m from the entrance there is only low disturbance. This is consistent with the observations of disturbance indicators in this study at least based on immediate visual observations.

Air quality

Data summarized in Table 1 show that air quality ranges from very poor to good with an average of fair rating based on the IMA in fungal colony forming units per hour per plate (Pasquarella et al. 2000). This means that the air inside the cave is still "fair" for humans. However, when an air quality scale used specifically for cave systems was used (Porca et al. 2011), the index showed that the cave environment is already threatened by fungal contamination and that management and controls are necessary. In particular, regions of the cave near the entrance (within 176 m from the entrance) show very high fungal counts which probably indicate massive visits and frequent disturbances (Porca et al. 2011). Consistent with the IDD computations of Encinares (2016), the IMA values at sampling sites 6-8 (605 m from the entrance and onwards) and the fungal concentrations at sampling sites 7-8 (691 m from the entrance and onwards) imply low anthropogenic disturbance in these areas. In general, the present study indicates that, based on aeromycological (air-borne fungi) indices, sites 7 and 8 (691-820 m from the entrance) show good air quality but that some periodic controls and studies may be required to monitor the area.

Table 1. Aeromycological indices at each air sampling station inside the Cacupangan Cave.

Station	Distance from the Entrance (m)	IMA (CFU/hr)	Remarks (Pasquarella et al. 2000)	Fungal concentration (CFU / m^3)	Remarks (Porca et al. 2011)
1	62	144	very poor	1152	V
2	176	79	very poor	632	IV
3	319	43	fair	344	III
4	438	33	fair	264	III
5	491	32	fair	256	III
6	605	19	good	152	III
7	691	18	good	144	II
8	820	17	good	136	II
Mean		**48**	**fair**	**385**	**III***

* threatened by fungi, management and controls necessary

Correlation between distance from the cave entrance and IMA (Fig 4) showed a strong negative exponential relationship where 95% of total variation in IMA can be explained by differences in distance from the cave entrance. This suggests that fungal propagules in the air of caves originated from outside the cave. This may indicate that presence of air-borne fungal propagules is strongly influenced by air ventilation such that greater air movements outside the cave and around the cave entrance are associated with higher IMA and vice versa. These air movements may be facilitated by various factors including cave ventilation, number of visitors, and stream flow, among others (Dredge et al. 2013).

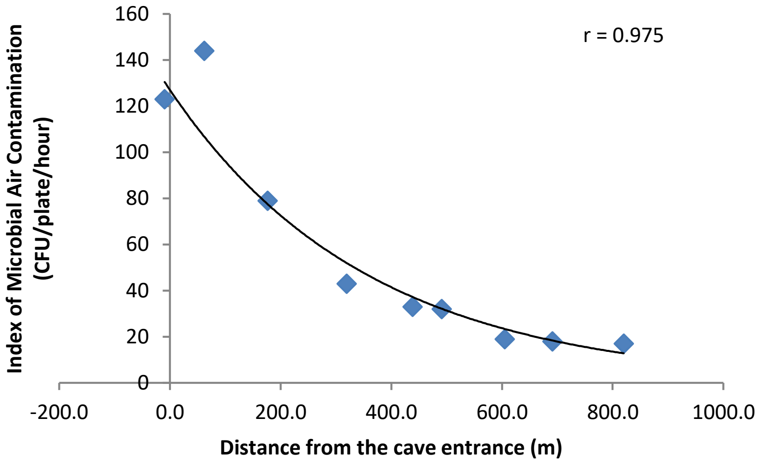

Figure 4. Negative exponential correlation between distance from the cave entrance and fungal propagule deposition rate or the Index of Microbial Air Contamination.

A total of 26 distinct fungal colony forms belonging to at least 11 genera were putatively identified based on microscopic observations and cultural morphology. Fungal genera identified are *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Mucor*, *Geotrichum*, *Alternaria*, *Trichoderma*, *Microsporum*, *Rhizopus* and *Acremonium*. Isolates of *Aspergillus* and *Penicillium* constitute more than half of the total number of identified distinct colonies with *Cladosporium* contributing another 14%. These genera are ubiquitous fungi commonly isolated from natural environments such as soil, plants and decaying materials. They are saprotrophic and contribute greatly to nutrient cycling in the environment. Samplings conducted during the dry season and those conducted during the wet season showed similar trends wherein *Aspergillus* and *Penicillium* were dominant species and sampling points near the entrance had the highest IMA. Previous researches from other regions likewise show the prevalence of *Aspergillus* and *Penicillium* in cave air (Docampo et al. 2011; Porca et al. 2011; Martin-Sanchez et al. 2014). An unpublished research in the same cave has documented the presence of *Aspergillus*, *Penicillium*, and *Cladosporium* from swabs from the dermal layer of bats roosting inside the cave. These may form part of the source propagules in certain areas of the cave. A study by Borda et al. (2014) has identified that bats and bat guano are sources of aerosolized microorganisms inside caves.

There was high similarity of airborne fungal propagule profiles between Stations 1-3 and Station 0 located outside the cave (Table 2). The similarity with the outside environment decreases with increasing distance from the entrance. In addition, presence of skylights in some areas increases similarity with the outside environment. The fungal profile of stations (5-8) located further away from the entrance were different from those found in the first few stations nearer to the entrance. These observations indicate that a large percentage of fungal propagules inside the cave come from outside and probably brought in by air currents. The study by Garcia-Anton et al. (2014) supports the decisive role of cave entrances and other air passages in the entry and transport of microorganisms into the cave. Likewise, fungal species richness in the air decreases with increasing distance from the cave entrance (Fig 5) and that small-spored fungal genera (*Aspergillus, Penicillium, Trichoderma, Acremonium, Mucor*, and *Rhizopus*) were carried further into the cave than big-spored genera (*Geotrichum, Alternaria, Fusarium, Cladosporium*, and *Microsporum*) (Fig 6). A moderate positive linear correlation exists between proportion of small-spored genera and distance from the entrance; only 41% of the variation in relative abundance of small-spored genera is related to distance from the entrance. This indicates that although air currents may be the primary influence of fungal propagule transport into the cave, other factors such as drag, aerodynamics of the spore (including its structure and ornamentation), humidity, how long the particles remain airborne, etc., also have an influence on which types of spores are carried further into the cave (Reponen et al. 2001; Dredge et al. 2013). In particular, how long the particles remain airborne depends on their density, their aerodynamic diameters, air movement, and the nature of the immediate environment. Nonetheless, Chi Square tests indicate significant difference between relative abundance of ‘small-spored’ genera at the entrance and those at the farthest stations (70% at the entrance, 100% at the farthest stations). Genera producing larger spores were more frequently seen in sites nearer to the entrance while those producing smaller spores were transported deeper into the cave.

Table 2. Jaccard Index of Similarity between fungal colony types isolated from the air using settle plates located at sampling stations inside Cacupangan Cave, Mabini, Pangasinan, Philippines with station 0 located just outside the cave entrance.

	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0	100	90	74	79	69	45	23	15	12
1*	90	100	75	72	56	29	25	20	15
2	74	75	100	60	89	40	20	25	20
3*	79	72	60	100	89	19	19	29	25
4	69	56	89	89	100	76	20	56	20
5	45	40	40	19	76	100	80	72	55
6**	23	20	20	19	20	80	100	75	40
7**	15	20	25	29	56	72	75	100	70
8**	12	15	20	25	20	55	40	70	100

* with skylight
** low ceiling

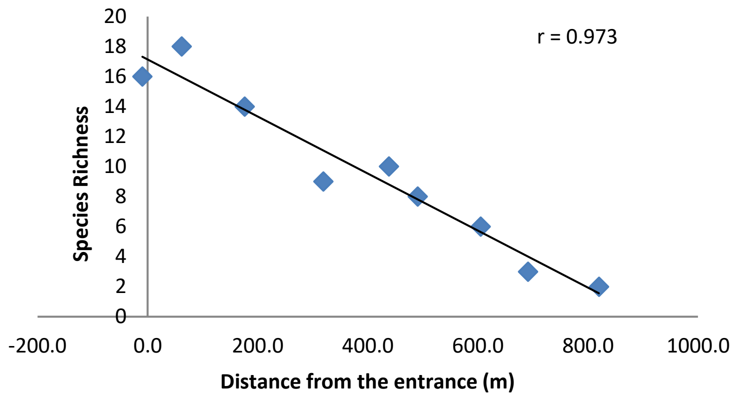

Figure 5. Negative linear correlation between distance from the entrance and fungal species richness in the air of Cacupangan Cave, Mabini, Pangasinan, Philippines.

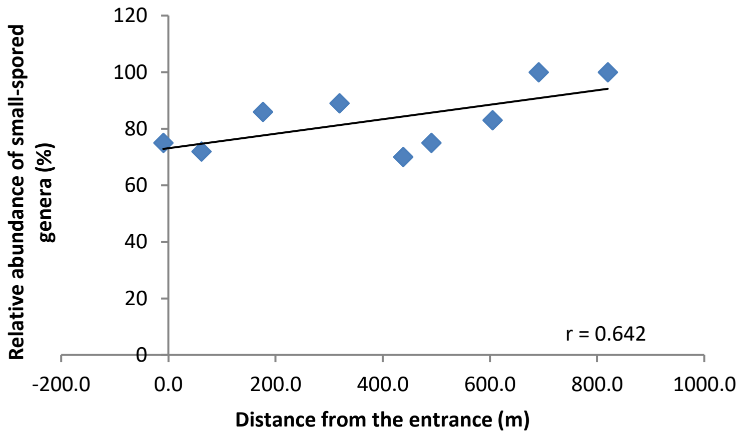

Figure 6. Moderate positive linear correlation between distance from the cave entrance and proportion of small-spored genera isolated from the air of Cacupangan Cave.

Water quality

Water quality, on the other hand, is low because of high coliform content. Tables 3 and 4 summarize the physico-chemical parameters measured for the water inside the cave as well as the MPN of coliforms determined using the multiple tube fermentation technique. These indicate that although the stream water inside the cave may have physico-chemical parameters that fall within the normal range for stream waters, the MPN of coliforms indicate that it is actually already heavily contaminated by fecal matter possibly from various sources. It has been reported that the primary factor associated with impairment in lotic ecosystems are pathogens and its indicators (Meals et al. 2013).

Table 3. Average measurements of physico-chemical water parameters in Cacupangan cave, Pangasinan during the dry season (DS) and wet season (WS). (DS values are the average from the 6 sampling points whereas WS values are the average from 6 sampling points of 2 trials; where indicated, numbers in parentheses are standard deviations, otherwise it is zero).

Sampling season	DO (mg L^{-1})	pH	Cond (µS)	Salinity (ppt)	Temp (˚C)
DS	5.35 (0.15)	8.03 (0.64)	370.80 (35.76)	0.18	26.48 (0.98)
WS	6.61 (0.26)	7.54 (0.73)	369.04 (61.93)	0.20	24.33 (1.54)

Table 4. Most Probable Number (MPN) of Coliforms obtained from different sampling sites in Cacupangan cave, Pangasinan during the dry season (DS) and wet season (WS). (DS values are obtained from one trial whereas WS values are the average of 2 trials; where indicated, number in parenthesis is the standard deviation, otherwise it is zero).

Sampling Site	0 (just outside the entrance)	1	2	3	4	5 (innermost sampling point)
MPN / 100 mL (DS)	1600	920	>1600	>1600	1600	920
MPN / 100 mL (WS)	1600	920	>1600	>1600	>1600	545 (530)

The data on coliform levels indicate that the water samples from the cave exceed the fecal coliform levels for Class D (navigable water), the lowest class of freshwater bodies in the Water Quality Guidelines (WQG) for Primary Parameters (Department Administrative Order 2016-08 of the DENR). However, water quality monitoring should be conducted for three consecutive months to generate data on coliform levels computed as geometric mean. Although the high coliform levels were recorded in two separate seasons (once in DS and twice in WS) these are insufficient to have a definitive evaluation of the water quality. As stated in Section 6.3 DAO 2016-08, there should be at least three datasets per quarter (three month-period) from which the geometric mean will be computed in order to determine if the coliform levels conform to the WQG. However, the data presented do indicate fecal contamination and hence, monitoring of the fecal coliform levels for at least three months, preferably in both dry and wet seasons is recommended for Cacupangan Cave.

The presence of coliforms, particularly *Escherichia coli*, indicates fecal contamination, a high chance of presence of pathogens, and is a good predictor of swimming-associated gastroenteritis. More than 38% of the isolates identified are *E. coli* (both typical and atypical strains), with both *E. coli* and *Klebsiella* having 100% occurrence. In the sampling site, coliforms may have come from bat guano as well as epigean sources such as soil, vegetation, flowing water, and various human influences such as tourists who enter the caves and runoffs from human settlements and agricultural areas. Since the Cacupangan Cave is promoted as a tourist spot, and is the most visited cave in the town of Mabini, Pangasinan (as recorded in the Physical Environment portion under Volume 3, Sectoral Studies, of Mabini) the anthropogenic impact brought about by caving of tourists may contribute to the high coliform levels. Other studies similarly suggest the potential contribution of visitors to the high coliform counts of cave waters (Hunter et al. 2004; Campbell et al. 2011; Mulec et al. 2012). A study on groundwater quality in another cave in the Philippines revealed the huge effect of human activities on water quality as shown by very high coliform counts (Husana and Kikuchi, 2013). These were attributed to tourist activities and septic tank leakage, among others. The land use map of Mabini, Pangasinan show that there are several rice fields, poultry and cattle farms nearby as well as residential areas that may contribute to the high coliform counts. High coliform counts indicate degradation of water quality. Land use changes, in particular, affect water quality in varying degrees. Uriarte et al. (2011) identified that pasture development and urbanization contribute to water quality degradation. Their study also determined that agriculture had mixed impacts contributing both positive and negative effects.

Aside from *E. coli*, genera of bacteria putatively identified were *Klebsiella*, *Enterobacter*, *Citrobacter*, *Hafnia*, and a few isolates of *Shigella*, *Alcaligenes* and *Pseudomonas* (Fig 7). These genera include organisms that contribute to nutrient cycling in the environment most especially in the Nitrogen Cycle as diazotrophs and nitrifyers (Garrity 2005). Species belonging to these genera, however, are also opportunistic pathogens that cause various diseases in humans including, but not limited to, septicemia, diarrhea and gastroenteritis (Meals et al. 2013). Contamination is usually through the fecal-oral route. The presence of these organisms indicates fecal contamination and therefore increases the likelihood that other pathogens may be present as well. In particular, the detection of *Shigella* indicates high likelihood of direct human-derived contamination because there is no other known source of *Shigella* but humans; implication of food and water in Shigellosis incidences are likewise derived from direct human contact and contact with excreta of infected humans (https://www.cdc.gov/shigella/infection-sources.html).

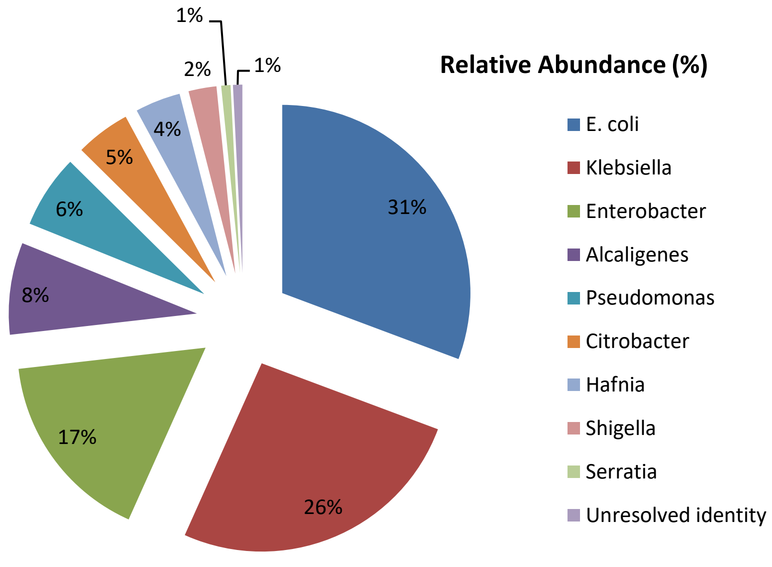

Figure 7. Relative abundance of putatively identified bacteria isolated from the coliform test of stream waters inside Cacupangan Cave, Mabini, Pangasinan, Philippines based on data from six sampling points for both dry and wet seasons of 2015.

Implications for ecotourism management

The IMA and MPN of coliforms clearly indicate that there is cause for concern in the Cacupangan Cave and that this has implications for management of ecotourism activities. Although one index of air contamination indicates that the cave air is still "fair" for humans, another index also indicates that the cave is already threatened by fungal contamination. In fact, the indices in areas near the cave entrance already imply massive visits and indicate the need for urgent attention and changes in management. Likewise, the MPN of coliforms indicate heavy contamination of the cave waters. Although physico-chemical parameters are within normal range and can support freshwater species, the high numbers of coliforms indicate that the water is neither fit for drinking nor for any contact recreation activities.

There is a need to restrict ecotourism activities in the Cacupangan Cave. Perhaps a study on carrying capacity of the cave to determine the limit of tourist visits in the site needs to be conducted. Limiting tourist visits may help reduce fungal and coliform contamination.

In order to adequately protect the subterranean environment, the surface environment needs to be protected as well. The forest located above the cave system needs to be maintained and agricultural activities in nearby areas need to be properly managed to reduce harmful run-offs. In addition, regular monitoring of the area needs to be conducted as part of a natural resource adaptive management scheme that is able to address potential problems as soon as they are detected.

The study by Encinares (2016) revealed that the whole Mabini Karst Landscape to which the Cacupangan Cave belongs is moderately disturbed by human activities based on the Karst Disturbance Index (KDI) developed by Phelps and Kingston (2010). Disturbance indicators used were quarrying, vegetation removal, urbanization, infrastructure development, construction inside caves, bat/guano/nest collection, debris dumping, vandalism, and visitation rate. Proper management of the site needs to consider all of these factors.

CONCLUSION AND RECOMMENDATIONS

Concentration, richness, and similarity of air fungi with the outside environment decreases as one go further into the cave. Airborne fungal propagules from outside the cave are carried inside by air currents. Smaller fungal spores and propagules are brought deeper into the cave than larger ones but transport inside the cave is only partly related to its size. Although one index indicates that the cave air is still "fair" for humans, another index also indicates that the cave is already threatened by fungal contamination.

There is high coliform concentration in the Cacupangan Cave stream water. This indicates fecal contamination possibly from various sources. The cave water is neither fit for drinking nor for any contact recreation or activities.

Microbial assessment helps us see unseen threats to both caves and humans entering the caves. Continuous regular monitoring of this ecotourism site is important for its sustainable management and continuous assessment of possible contaminant sources and human health risks. Good adaptive management of a natural resource requires excellent monitoring systems that are able to indicate potential problems early.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

STATEMENT OF AUTHORSHIP

MIA and MGP helped conceptualize the study, did the data gathering in the field, identified the isolates in the laboratory, and wrote portions of the manuscript draft. MPDL helped conceptualize the study, supervised the conduct of the field and laboratory activities, and gave comments on the manuscript draft. CGBB helped conceptualize the study, gave advice on the conduct of field and laboratory activities, gave comments on the manuscript draft, analyzed the data, and wrote the final manuscript.

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