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ECO-FRIENDLY REMEDIATION OF LAMPENFLORA ON SPELEOTHEMS IN TROPICAL KARST CAVES

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Abstract

This paper presents an experiment on lampenflora removal in show caves located in a tropical monsoon climate in southeast Asia. Lampenflora thrive in wet conditions on surfaces directly illuminated by white light. They colonize different levels in show caves, from the cave ceiling, with a biota characterized of mainly cyanobacteria (Oscillatoria, Spirulina), algae (*Chlorella, Oedoclarium*), and mosses (*Cyathodium, Thuidium*), to near the cave floor, with a more complex biota including higher plants like ferns (*Asplenium*) and flowering plants (*Centella*). Mature lampenflora mats also harbor non-phototrophic fungi and bacteria. With the use of environmental scanning electron microscopy, speleothem surfaces were found severely damaged by lampenflora and their associates. In this study, we used H_2O_2 as an environmentally friendly chemical to exterminate lampenflora. The applied solution should be at least 15% H_2O_2 to efficiently destroy microbiota such as green algae, diatoms, and bacteria. For a complex community including mosses, fungi, and vascular plants, repeated spraying of chemical and, if possible, water jet washing at carefully selected places are required to recover the aesthetic characteristics of speleothems. Only a combination of such cleaning practices, and then some modification of the illumination regime, can minimize lampenflora development in show caves.

Introduction

Lampenflora is a community of phototrophic organisms and their associates developing at sites where light is provided from artificial sources rather than natural circumstances (Mulec and Kosi, 2009). It is a widespread problem in many show caves, as it modifies the appearance of the cave's interior, and more importantly, bio-deteriorates the various types of substrate onto which it is attached (Roldán and Hernández-Mariné, 2009). Thus, removal of lampenflora is of particular interest to cave conservation and management (Piano et al., 2015).

In general, removal of lampenflora should be complete, practicable, and done harmlessly to the cave environment. Since caves are confined spaces, the use of herbicides, a usual method applied in agriculture, should not be considered. Mechanical cleansing with the use of brush or water jet is not effectual, as mature and complex lampenflora may entangle or intertwine with abiotic substrates that are not easily detached. The use of strong oxidating agents to remove lampenflora has been reported, and biota have been removed by a sodium hypochlorite solution (Mulec and Kosi, 2009), which effectively eradicates any presence of cyanobacteria, algae, and mosses. During the cleansing operation, however, measurable amounts of chlorine as well as other compounds are released into the atmosphere and pollute the cave environment. The disappearance of some insect species in some show caves could be connected with these toxic substances (Faimon et al., 2003).

Alternatively, hydrogen peroxide (H_2O_2) has recently been recently introduced and is thought environmentally friendly (Kubesova, 2000). Oxidation of organic matter, simplified as $(CH_2O)_n$, can be expressed as $(CH_2O)_n + 2nH_2O_2 \rightarrow nCO_2 + 3nH_2O$. Here, H_2O_2 appears to be a more eco-friendly agent than hypochlorites or herbicides, as fewer oxidation byproducts are released to the environment.

Our review concludes that previous studies of treatment by H_2O_2 were applied to caves in temperate region (e.g., Mulec and Kosi, 2009), and as H_2O_2 is a strong corrosive and low-pH solvent that could dissolve substrate, the studies established a threshold of H_2O_2 application compromising between halting lampenflora growth and avoiding damaging the substrate (Faimon et al., 2003). There is a possibility that such protocols and thresholds may not be optimal for every region and climate, since lampenflora should be representative of the local biological community and substrate in caves may varying depending on the regional hydrogeology and cave microclimate. The thresholds obtained from these studies need to be validated for tropical caves where biota, cave microclimate, and illumination systems are very different from temperate ones. For instances, autotrophs in tropical climate grow well throughout the year, while in temperate region, they grow mostly in spring and summer. Hydrology and biogeochemistry in tropics can result in caves of gigantic size with large entrances and usually water constantly flowing inside (Limbert et al., 2016). In these caves, connection between the outside atmosphere and cave interior is strong. Surrounded by tropical jungle of highly diverse biology,

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caves in this region have maintained some exquisite cave organisms (Luong and Holinska, 2015). It is also expected that anthropogenic impacts to the show caves in tropical countries like Vietnam are different from the ones in temperate regions of Europe or the US, where advanced technologies and infrastructures are usually employed to reduce undesired impacts. In tropical countries, illumination systems installed into the show caves are patchy (Ngoc et al., 2014), construction to facilitate sightseeing does not help cave preservation (Trinh and Guinea, 2014), or there is no control of number of visitors every day (Dukhach, 2016). Thus, the objectives of this study are characterization of the show-cave microclimate that supports lampenflora development in a tropical coastal region, central of Vietnam, assessment of the lampenflora impact on speleothems inside humid-tropical show caves, and re-evaluation and adjustment of an environmentally friendly lampenflora cleansing practice that was previously tested in case studies in temperate regions (Faimon et al., 2003; Mulec and Kosi, 2009).

Materials and Methods

Study Site Description

The Phong Nha-Ke Bang National Park covers an area of 857.54 km^2 and is a UNESCO World Heritage Site, reflecting its global importance. The park came under UNESCO protection in 2003 because of its extraordinary stratigraphical diversity from the Precambrian to the present day, the long development of its topography from the Oligocene to the present day, and the intensively developed tropical karst formations (Fig. 1). Over three hundred karst caves have been recorded in the park (Limbert et al., 2012). Along with geological and geomorphological diversity, the park has considerable biodiversity in fauna and flora and extraordinarily well conserved tropical karst forests.

The central limestone area is bordered by impermeable strata that collect water on the surface and in the southern part of the park discharge it towards the Chay River lying farther north. This inflow of allogenous water is the main factor for development of the underground caves explored to date. Excellent examples of caves of this type are the Phong Nha show cave and the Hang Vom system. With the entrenchment of the Chay River, the underground flows shift lower and lower and leave fossil caves at the higher levels. Examples of such caves are Tien Son Cave, rich in calcite deposits and open to tourists as a show cave, and Thien Duong Cave, part of the Hang Vom system. The caves follow the bedding planes into the thickly stratified Devonian–Carboniferous–Permian limestone and numerous faults tied to the predominantly north-south faults in the Alpine Orogeny (World Heritage List Nomination Form 2000) throughout a long history of karstification in limestone strata over 1,000 meters thick. This study targets three show caves, Phong Nha, Tien Son, and Thien Duong (Fig. 1; Table 1). The first two caves have been open for frequent visits since 1995, and the last one was opened in 2006. They receive thousands of visitors every day (Phongnhakebang, 2014; Dukhach, 2016) with highest numbers during summer and national holidays, and that number has increased over the years (Vietnamtourism, 2016).

Surveys and Sampling

Since 2014, five surveys of cave microclimate have been conducted in spring (March–May) and autumn (August–September). In each cave, the monitoring took approximately one working day from 10 a.m. to 4 p.m. from the cave entrance to the end of passages open to visitors. Variables of temperature (°C, accuracy: ±0.3 °C), relative humidity (RH, accuracy: ±0.1 %), wind speed (accuracy: ±2% rdg, range: 0–30 m s^{-1}), and CO_2 (accuracy: ±1 ppm) were monitored with the use of a GreyWolf Toxic Gas TG 501 (USA). Since all stairs are fenced to prevent visitors from crossing out of the paths, air measurements were taken a few meters away from the stairs to avoid direct interference from visitors. The Gas TG 501 was left to stabilize for a few minutes and then programmed to record five results at 2 minute intervals. For the whole monitoring period, only one person in charge of microclimate monitoring was within 1 m of the sensor to limit possible interference. The sensor was placed approximately 1 m above the cave floor. All microclimate data were recorded between 10 and 11:30 a.m. and 2:30 and 4 p.m. when visitor number was peaking during a day.

Sampling of speleothems covered with lampenflora was conducted in the 2014 autumn and 2015 spring surveys. At each cave, three representative experimental sites were selected near the cave entrance, around the middle of the show section, and near the end of the show section (Fig. 1 g,h,i). Depending on the caves' morphologies and their entrance sizes, the sites near entrances were chosen between 50 and 100 m from them to guarantee no natural light interference at the sites. Sites near the end of exhibited passages were about 50 m to the end of show path. It should be stated that in show caves in Phong Nha–Ke Bang, visiting pathways consist of wooden, plastic, and steel stairs or steps standing on top of the cave floor, and lamps were consistently positioned along the passages with the light beam directed away from the pathways into the speleothems. Around massive and exquisite speleothems along the visiting passages, additional lamps were placed to give visitors a better view. In fact, the representative experimental sites are where attractive speleothems are concentrated in the proximity of visiting passages. Therefore, the speleothems were well illuminated and accessible.

Samples were taken between 0.5 and 1.5 m above the ground at the most trophic and prolific surfaces that were

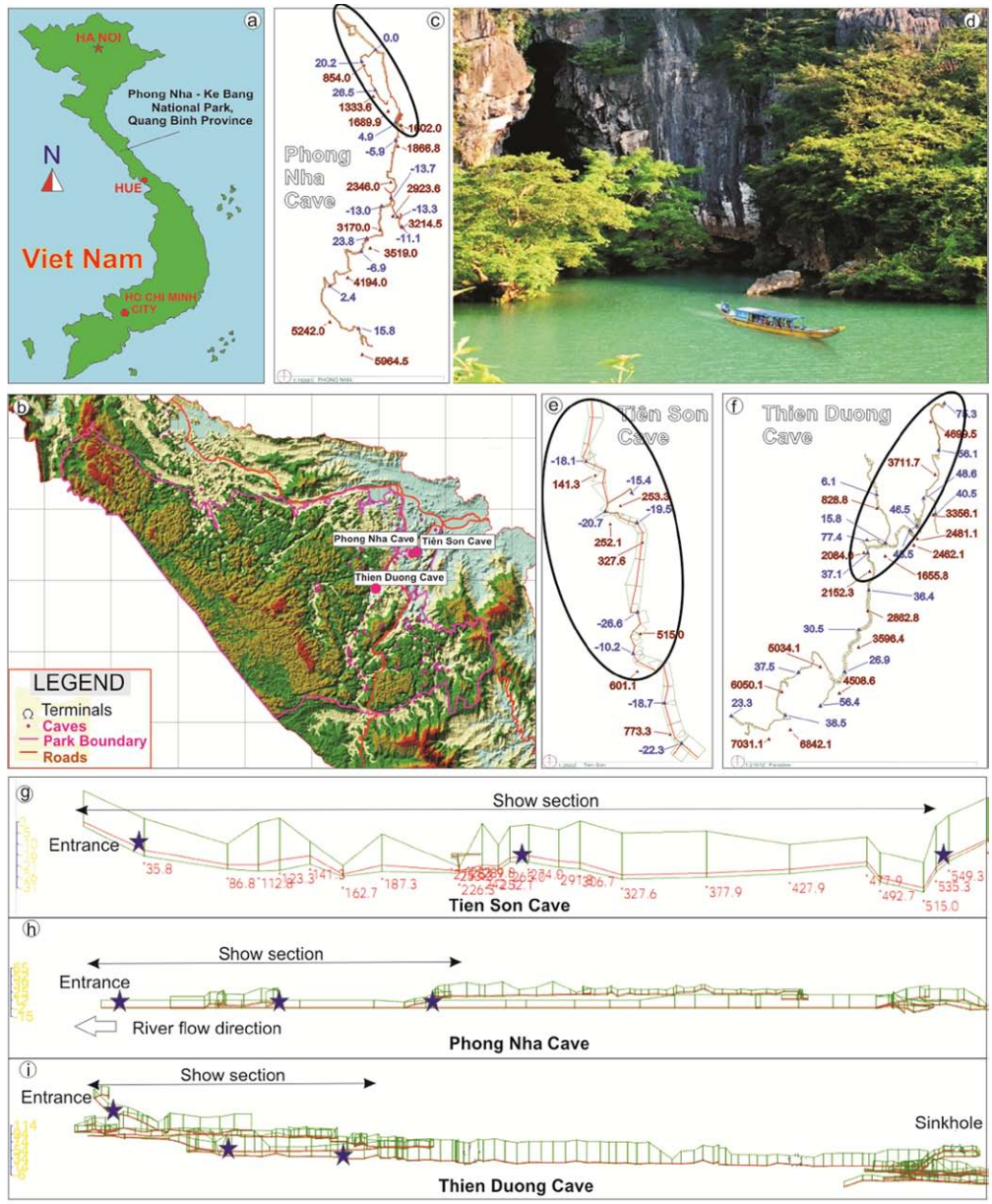

Figure 1. Map of the Phong Nha–Ke Bang National park and cave locations. (a) Vietnam Map and location of the Phong Nha–Ke Bang National Park, (b) Map of the Phong Nha–Ke Bang National Park and relative positions of the show caves studied in this research (Thien Duong, Tien Son, and Phong Nha), (c) Phong Nha Cave, (d) a photo taken in front of the Phong Nha Cave showing its enormous entrance with underground flow, (e) Tien Son Cave, (f) Thien Duong Cave, (g) cross-section of Tien Son Cave, (h) cross-section of Phong Nha Cave, (i) cross-section of Thien Duong Cave. Note: Oval shapes in (c), (e), and (f), indicate the artificially illuminated show-cave sections; Blue numbers represents elevation (m) as compared to the entrance; red numbers are relative distance (m) from entrance. Stars in (g), (h), and (i) indicate the locations of monitoring and sampling inside the caves.

constantly wet and directly illuminated with white lamps. All samplings were from hard substrates with comparable mineral composition of secondary microcrystalline calcite. With approval from the Phong Nha–Ke Bang National Park Management Board, hammers and knifes were used to detach the samples from the host speleothems. Each sample is about 1 cm thick and 5 cm across and fully covered with lampenflora. Samples were separately put in plastic bags, numbered, and kept in the dark and insulated cool box for transport to the laboratory. In the laboratory, they were stored in a refrigerator prior to analysis and experiments. It should be stated that microclimate conditions were monitored around the sampling speleothems andadjacent points where no lampenflora was observed for references. A Hioki 3423 Lux HiTester (Japan) was placed on the speleothem surface, and light intensity was averaged from five readings at 1 minute intervals.

Vascular plants (flowering plants and ferns) are identified by photographs taken at sites. Microbiota were identified according to morphology using light-microscope observation (Olympus BX51 at ×200/400 magnification). Identification of cyanobacteria was achieved by the use of the taxonomic literature of Komárek and Anagnostidis (1989, 1999, 2005). Diatom identification was based on the classification of Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

Environmentally Friendly Removal of Lamplenflora

In this study, industrial H_2O_2, 30% and medical H_2O_2, 3% (CPC1, Vietnam) were used for this treatment experiment. From the industrial H_2O_2, 30%, two solutions of H_2O_2, 10% and 15% were prepared, making up a total of four testing

solutions; 3%, 10%, 15%, and 30%. Concentrated NaOH solution was used to adjust pH of the H_2O_2 solutions to 7–7.5 immediately before the treatment. pH paper was used to ensure the neutral pH of the solution.

Four sub-samples weighing within 0.5 and 1.5 g and size of less than 15 mm wide and 3 mm thick were placed in petri dishes and separately sprayed with the prepared H_2O_2 solutions. Two hours after spraying, the sub-samples were dried at 95 °C in atmospheric pressure for 24 hours and then subject to microscopic and mass-loss analyses. The procedure was then repeated with the same sub-samples after 2 weeks. The repeated treatments reflected a possible future practice when the remediation would be carried out twice per year.

Before and after each treatment step, all sub-samples were subjected to electron microscopic analysis to examine effect of H_2O_2 on lampenflora removal and substrate dissolution. The environmental scanning electron microscope (ESEM) XL30 microscope from FEI (Field Emission and Ion Company) is a low-vacuum ESEM (model Quanta) that enables high-resolution inspection and chemical analysis of non-conductive specimens.

In parallel, the sub-sample mass before and after each treatment step was weighed by micro balance (R200D Sartorius) to quantitatively evaluate the lampenflora removal efficiency by H_2O_2. Mass loss comparison was also made between untreated and treated sub-samples to evaluate the hydrogen peroxide dissolution effect on $CaCO_3$ substrate. In total, mass balances of sub-samples were checked before treatment (1), after first treatment (2), after second treatment (3), and after incineration at 550 °C (4). The reference sub-sample without treatment was also weighed, incinerated at 550 °C, and weighed again (5). Thus by comparing (1) and (2), (2) and (3), (3) and (4), and (4) and (5), we respectively have the removal fraction after the first spraying of H_2O_2, the removal fraction after the second spraying of H_2O_2, the lampenflora biomass remained in substrate (speleothem sub-sample) after treatments with H_2O_2, and the substrate fraction removed by H_2O_2. It is necessary to note that the removed substrate makes up a part of the removal fraction and is basically inorganic $CaCO_3$, not organic matter. The removed lampenflora organic matter is thus equal the removal fraction after treatment the second spraying minus the removed substrate. To avoid systematic errors during the heating procedures, substrate may gain or lose weight during drying or incinerating due to other causes unrelated to the H_2O_2 treatment. Pieces from inner sections of the samples not covered with lampenflora were sent to thermal-gravity analysis with the furnace set in atmospheric air environment with no purge gas. The mass change obtained by TGA at 100 °C and 550 °C was included in the calculation to achieve the exact mass loss after each treatment and incineration.

Results

Microclimates

During our surveys, temperatures inside the caves were generally lower than outside atmospheric temperature (Table 1). Inside the caves, temperatures measured near entrances and at deeper parts of the caves were similar during the sampling period, attesting the thermic stability of the caves and the consequent general atmosphere stability inside the caves. As shown in Figure 1, the cave entrances are typically high above the cave floor. Due to this morphology, a faster exchange of air between cave exterior and interior occurs in winter, when cave air is hotter and has a lower density than outside air. During that period, hot cave air blows out of the cave and cold exterior air descends into the cave. The cave's air temperature reduces quickly. When outside temperature starts to increase after winter, cave air is heavier and trapped inside the cave. A lower-than-outside temperature in cave air continues until the next winter (Mattey et al., 2016; Faimon and Lang, 2013). This explains why during our surveys in spring and autumn the cave air was found to be colder than the outside atmosphere. The Phong Nha Cave, characterized by an enormous entrance at a similar elevation with its interior, has a temperature more equilibrated with the outside temperature than other caves (Fig. 1).

The high humidity, a typical characteristic of tropical monsoon, has contributed to the caves' microclimates. Temperatures colder than outside increased relative humidity inside the cave. Depending on the entrance size, relative humidity would slightly fluctuate near the entrance, but it was always stable and close to the saturation level deep inside the caves. It should be noted that the water flow running inside the wet caves is another stabilizing factor for humidity in cave air. This factor could also be used to explain why in the dry cave of Tien Son humidity was somewhat lower than in the wet caves.

Monitored air circulation was fairly steady all over the show sections thanks to the large and multiple entrances and

Table 1. Atmospheric conditions in the surveyed caves in Phong Nha – Ke Bang. (ND = not detected)

Atmospheric Parameter	Thien Duong	Phong Nha	Tien Son	Outside
Temperature, °C	21.3 − 24.3	26.2 − 29.5	22.2 − 25.6	30.9 – 32.1
Relative humidity, %	89.5 − 99.5	85.8 − 98.5	78.4 − 94.5	75 – 81
Wind speed, m s^{-1}	0.0 − 0.2	0.0 − 0.1	0.0 − 0.1	0.3 – 0.4
Light, Lx	ND − 60	ND − 80	ND − 270	16800 − 42800
CO_2 concentration, ppmv	420 − 640	430 − 960	820 – 2500	240 − 590

the underground river. In addition, since most sampling-monitoring sites are in the vicinity of visitor pathways, cave air was also disturbed by thousands of visitors per day. There is an exception in term of air circulation in the Tien Son. This cave has only one entrance and has no underground water flow. Its deepest gallery is now closed to visits, thus yielding virtually non-detectable air flow there (Fig. 1c).

The illumination system inside Phong Nha–Ke Bang is generally equipped with two different color lamps; white (5500–6000K) and yellow (3700–4000K) (Ngoc et al., 2014). The white lamp spectrum in the visible range superimposed on the typical absorption peak of chlorophyll-a (660 nm; Piano et al., 2015) favors photosynthesis. The typical arrangement of illumination in the caves is to concentrate lamps around attractive speleothems, which has enhanced light intensity up to hundreds of lux on directly illuminated surface (Table 1).

Like other caves in karst regions (Fernandez-Cortes et al., 2015), the cave air CO_2 concentrations are higher than background atmospheric value (Table 1). In caves, CO_2 emitted from soil and epikarst, dripping water and visitors tends to be trapped due to a higher density of cave air compared with exterior, reaching value as high as 2500 ppm. Our surveys of the caves show that while temperature and humidity are stable and similar, CO_2 concentrations varied among galleries and peculiarly different among caves. In Tien Son, a dry and single-entrance cave, CO_2 reached 3 times higher in the deepest gallery than near the cave entrance section and 10 times higher than outside atmosphere. Carbon dioxide content was also highest among the surveyed caves. Since CO_2 was highly concentrated in the single-entrance cave and relatively low concentrated in the multiple-entrance ones, it is wise to conclude that CO_2 reflects exchange of cave air with the outside environment.

Lampenflora on Speleothems

Usually, light is switched on in the show caves every day from early morning (7 a.m.) till the afternoon (6 p.m.). In all show sections, lampenflora was observed from ceiling to floor where light and other microclimate conditions were favorable. In some parts, it covers an area of hundreds of square meters (Fig. 2).

Compared to the notion that lampenflora organisms are ubiquitous, fast-reproducing, and adaptable soil algae (Piano et al., 2015), lampenflora in the show caves of Phong Nha–Ke Bang appear to be more complex. In some parts, it was a lightly green, thin layer (Fig. 3a) or a dark green, thick layer (Fig. 3b). In other parts, it was a greenish yellow (Fig. 3c) or dark brown mat (Fig. 3d). Near the cave floor, where speleothems are usually covered by dirt or soil brought from outside by flows or visitors, lampenflora were more complex, with flowering plants (Fig. 3e) and ferns (Fig. 3f) in germination stage.

We observed a light-color preference of lampenflora growth. Between white and yellow, it was noticed that lampenflora grew well on surfaces directly illuminated with white light. The growth usually spread within 5 m from the light sources where light intensity was detected as between 50 and 200 lx. In several places where illumination was amplified by multiple light sources, lampenflora could be as far as 20 m from the lamps. Apart from light, water appears to be another essential factor for lampenflora growth. All lampenflora speleothems were soaked with drip water. Altogether, speleothems that are constantly wet, directly illuminated with white light, and within 5 m from the light source are likely covered with lampenflora.

Optical microscopic analysis reveals that, apart from green algae and cyanobacteria, diatoms, a class that are usually found on silicate based substrates, were abundant in the collected samples (Table 2, Supplement Fig. A). Filaments of fungi were also observed in colonies detached from white, yellow, and dark-brown mats.

The environmental scanning electron microscope images clearly show lampenflora as a complex community

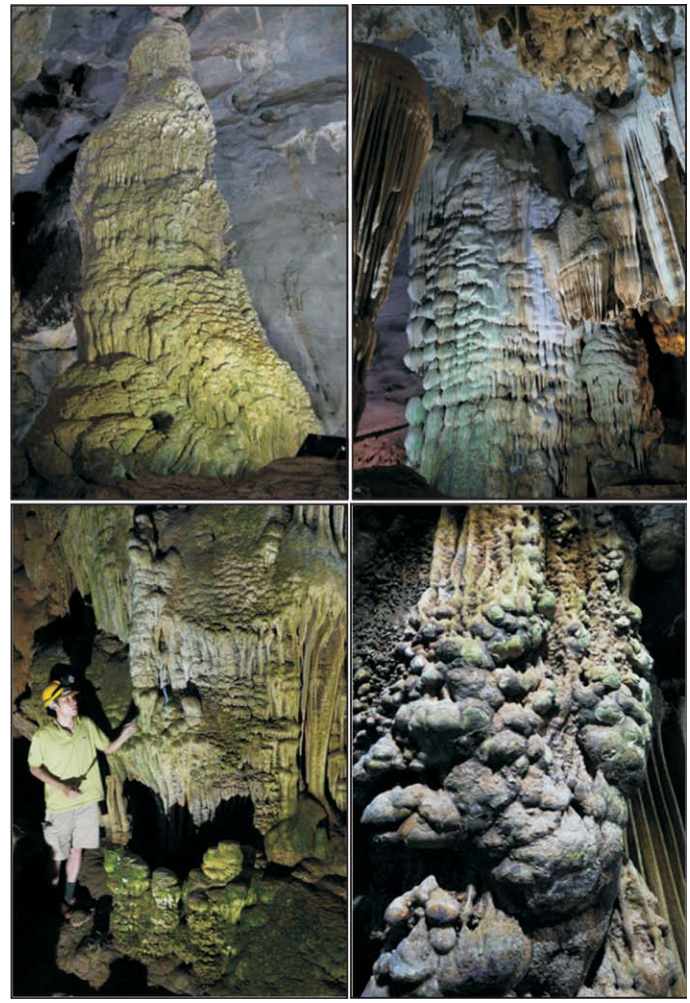

Figure 2. Lampenflora thrive on wet and white-light directly illuminated surfaces from stalactites near ceiling to flowstones and stalagmites on the ground of caves.

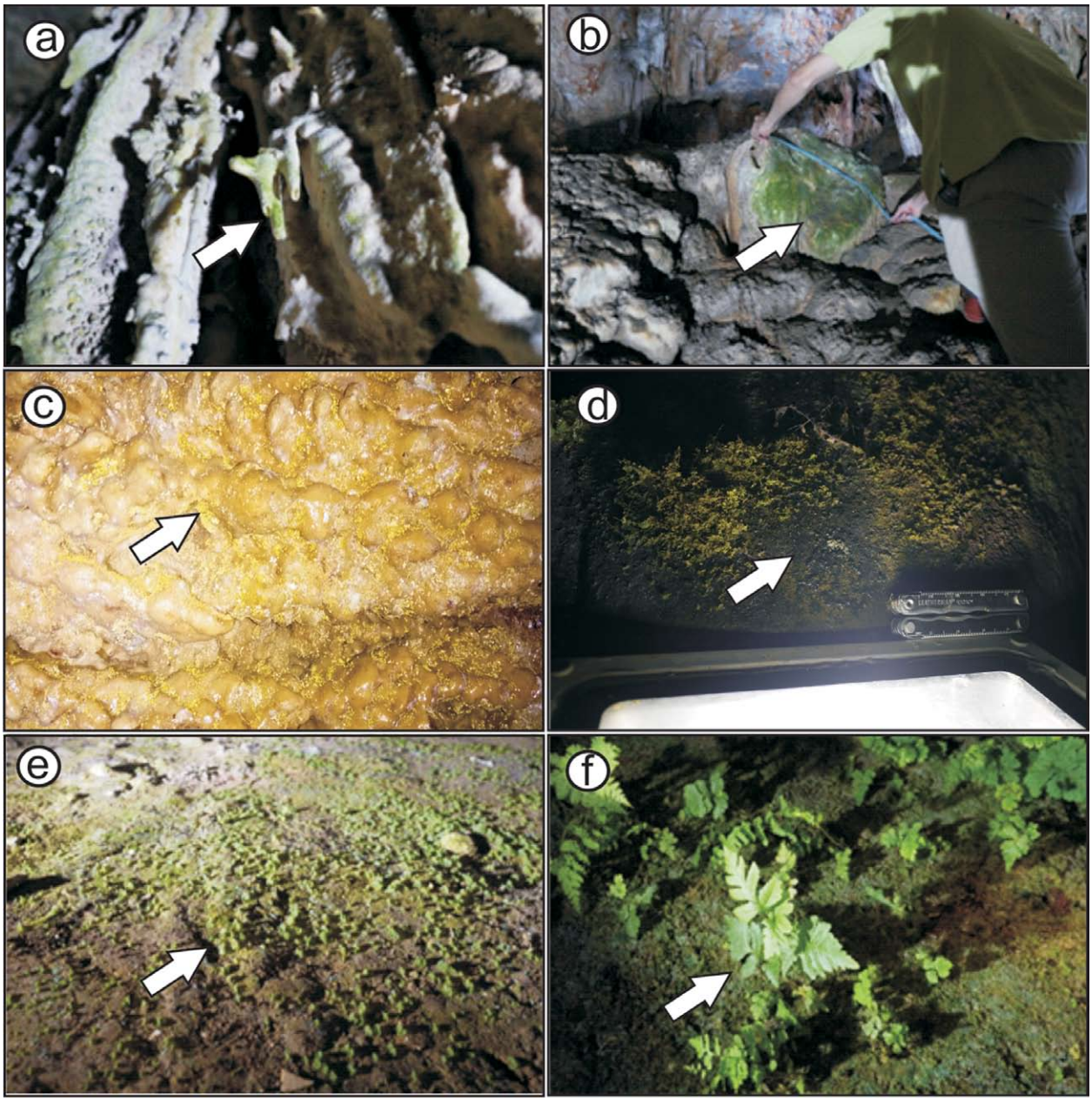

Figure 3. Images showing complex lampenflora biota: (a) light green thin layer, (b) dark green thick layer, (c) greenish yellow, (d) black and sticky mat, (e) germinating flowering plants, (f) germinating ferns

Table 2. Typical genus of lampenflora found in the caves in Phong Nha – Ke Bang.

Vascular Plants		Non-Vascular Plants				
Angiospermae (Phanerogams)	**Chryptogams (Ferns)**	**Bryophytes (Mosses)**	**Algae**		**Bacteria**	**Fungi**
			Chlorophyta	Bacillariophyte		
...	...	...	(Green algae)	(Diatoms)	Cyanobacteria	...
Centella	*Asplenium*	*Cyathodium*	*Chlorella*	*Nitzschia*	*Oscillatoria*	*Filamentous*
...	...	*Thuidium*	*Oedoclatium*	*Pinnularia*	*Spirulina*	...
...	...	...	*Microthamnion*	*Navicula spp.*	*Synechocystis*	...
...	...	...	...	*Achnanthes*	...	...
...	...	...	...	*Diploneis*	...	...

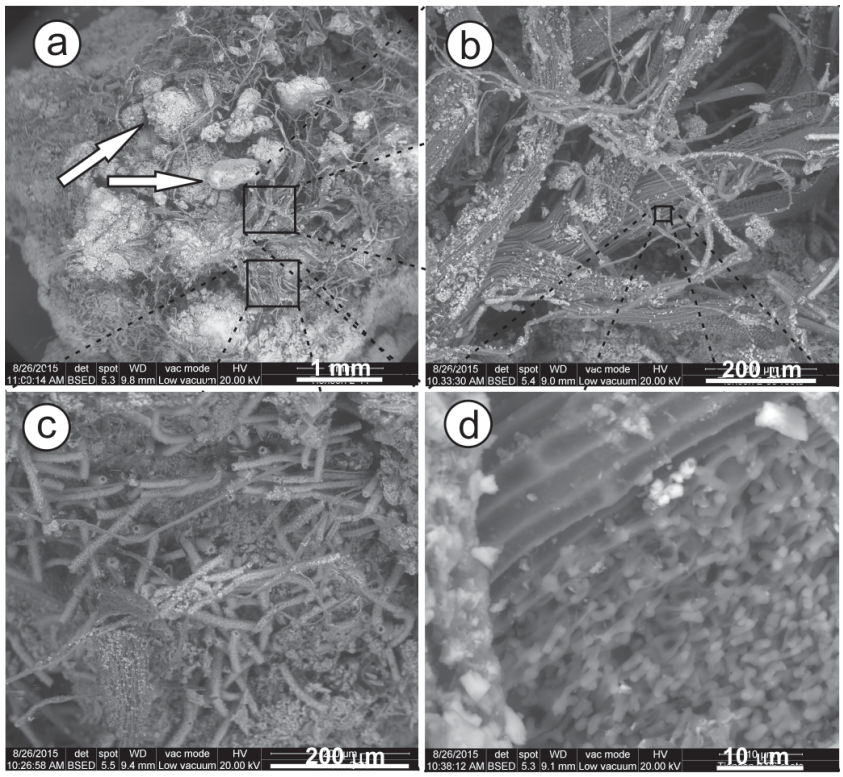

Figure 4. Microscopic images: (a) overview of speleothem surface destroyed by lampenflora (arrows point to the large $CaCO_3$ fragments detached from substrate), (b) large segment of fresh vascular roots, (c) calcite encrusted filaments (tubiform), and (d) fresh roots associated with calcite crystal fragments

that has a profound impact on speleothems (Fig. 4). There existed microbial assemblages directly or indirectly able to mediate a variety of constructive and destructive processes that resulted in the formation of distinctive fabrics. Destructive processes include dissolution of $CaCO_3$ due to respiration in rhizoids, microbes, moss, fungi, and lichens (Groth et al., 1999). It led to broken limestone pieces intertwined with root hairs and rhizoids as shown in Figure 4 a,b,c. Constructive processes include calcified microbes and bio-induced crystalline precipitates (Sanchez-Moral et al., 2003) as shown in Figure 4 d. Both processes, though opposing each other, impact the speleothem surfaces in undesirable ways, amplifying the porosity and crumbliness of the substrate. The smooth and scintillating surface of attractive speleothems is obliterated.

It is interesting to note that biogenic calcite crystals, which are not usually found in a highly oligotrophic environment, were abundant in collected samples here. The most abundant form of biogenic calcites results from the filamentous cyanobacterial calcification (Fig. 4d), with other forms of biogenic crystals (e.g., spherical) being less common (Supplement Fig. B). The calcification mechanism is probably sheath impregnation (Couradeau et a, 2013). The mechanism of cyanobacterial calcification by sheath impregnation in several occurrences, including the oldest calcified microfossils, remains controversial. Based on petrographic evidence, some authors have proposed that the calcification took place post-mortem (Pratt, 2001); others suggest it is a result of cell metabolic activity (Arp et al., 2002). Microscopic images here prove this calcification is a result of cell metabolic activity in which the filamentous crystals are in various types such as dendrite, needle, rhombohedra (Supplement Fig. B) and, in particular, always consists of an organic inner layer and a freshly formed $CaCO_3$ outer layer (Supplement Fig. C).

Hydrogen Peroxide Treatment

Organic Matter Removal

It was expected that concentrated H_2O_2 would largely remove lampenflora, but the results here did not confirm it. Microscopic images (Fig. 5) show that, at all H_2O_2 concentrations, lampenflora are dead and broken down but not massively oxidized. With H_2O_2 at 3% and 10 %, the filamentous lampenflora were still visible after repeated sprayings (Supplement Fig. D). With H_2O_2 at 15% and 30%, microbiota groups like algae and cyanobacteria were largely removed, but other groups like mosses, lichens, or ferns were only partly removed. Their remnant roots and stems were still seen on substrates.

The mass loss analysis confirms the conclusion derived from the microscopic analysis that all tested H_2O_2 solutions could not completely cleanse lampenflora (Table 3). With 3% H_2O_2 there is very little effect on lampenflora, with a mass loss of few percent. At 10%, for some types of lampenflora such as biofilms, the removal efficiency could be as high as 25.2% after the first spraying, but removal efficiency of vascular plants, mosses, or fens was low. Removal efficiency improved with the use of 15% and 30% H_2O_2, and a first spraying could remove up to 61.5% of total lampenflora mass.

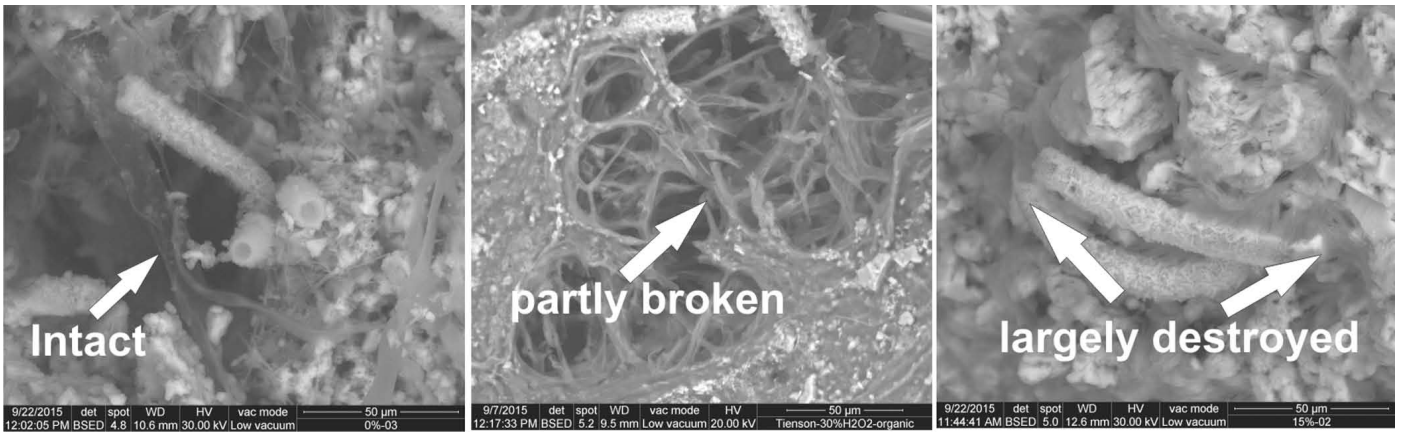

Figure 5. BSED images: Microbial mats at different stages of treatment, (a) before treatment, (b) after first treatment with H_2O_2, 30%, and (c) after second treatment with H_2O_2, 30%. Arrows point to the organic mat (a) or its remnants after treatments (b and c)

However, even after two sprayings about 30% of lampenflora mass remain in place.

This result is in agreement with previous studies stating that H_2O_2, 15% could efficiently destroy microbiota. For instance, Faimon et al. (2003) revealed that repeated applications of 15% peroxide solution with 2 or 3 weeks rest periods in between led to a total destruction of the algae, cyanobacteria, and slightly developed mosses. The highly developed growths of mosses yellowed and dried after these applications. They concluded the threshold hydrogen peroxide concentration for lampenflora destruction was 15%.

Another observation is that mass-loss analysis indicated a lower removal efficiency following the second spraying than the first (Table 3). This could be explained that the first spraying has removed the most labile and easily oxidized fraction of lampenflora. In the second spraying, the less oxidizeable one needed more H_2O_2 to be removed, leading to lower removal efficiency.

Dissolution of $CaCO_3$

One concern with the application of H_2O_2 for lampenflora removal is the potential risk of speleothem damage by chemical dissolution of $CaCO_3$ or bio-physical detachment of abiogenic and biogenic fragments associated with lampenflora mats (Fig. 4). We observed the effects of H_2O_2 on selected calcified filaments that were relatively in the same size and form and abundant in lampenflora substrates. We find that concentrated H_2O_2 (15% and 30%) has slightly reduced the thickness of calcified filaments (Fig. 6), indicating a possible $CaCO_3$ dissolution. Microscopic analysis also leads to a belief that calcite fragments (Figs. 4 a-c) as well as calcified microorganisms (Fig. 4 d) mixed inside the lampenflora mat were detached from the substrate when organic matters were cleansed by H_2O_2. Microscopic images also indicated that repeated sprayings increased the substrate dissolution. The mass-loss analysis taken between the treated and the non-treated sub-samples gives an averaged ratio of removed lampenflora biomass over removed $CaCO_3$ as 0.813. This number indicates that a substantial fraction (more than half) of removed lampenflora was actually inorganic $CaCO_3$. Probably this removed $CaCO_3$ consists mainly of the easily detachable biogenic $CaCO_3$, rather than the solid abiotic speleothems.

Discussion

The Show-Cave Environment and Lampenflora Growth

Usually cave fauna and flora are rare and perhaps adapt to extremely oligotrophic conditions (Holsinger, 1988). Our surveys, indicate that trophic condition in the show caves in Phong Nha–Ke Bang was not extremely oligotrophic. Irradiation, nutrients, and water, three essential conditions for autotroph growth, inside show sections were enough to sustain many groups of autotrophs. The condition is derived from both natural and anthropogenic factors. Indeed, natural factors such as large and multiple entrances, underground flow, and complicated seeping or dripping water patterns, leave the cave air and water well exposed to atmosphere. Our surveys, as well as another study on cave fauna (Luong and Holinska, 2015), found plankton and fishes in cave pools, lizards and spiders on cave walls, and bats flying near cave entrances. Anthropogenic impacts such as cave illumination, stairway construction, and visitors also accelerate caves' interior-exterior exchange. For instance, large or multiple entrances, together with a high frequency of visitors, facilitate air circulation. Typically, underground flows annually inundate some part of the caves. The flows have enough nutrients, such as 5 mg NO_3 and 0.03 mg L^{-1} PO_4, to sustain autotroph growth in inundated speleothems. Visiting activities increase the cave-air CO_2 content, as well as bring more material and organisms from outside into the caves. The caves' illumination systems have no motion sensors to restrict illumination to only when there are visitors. Lamps are

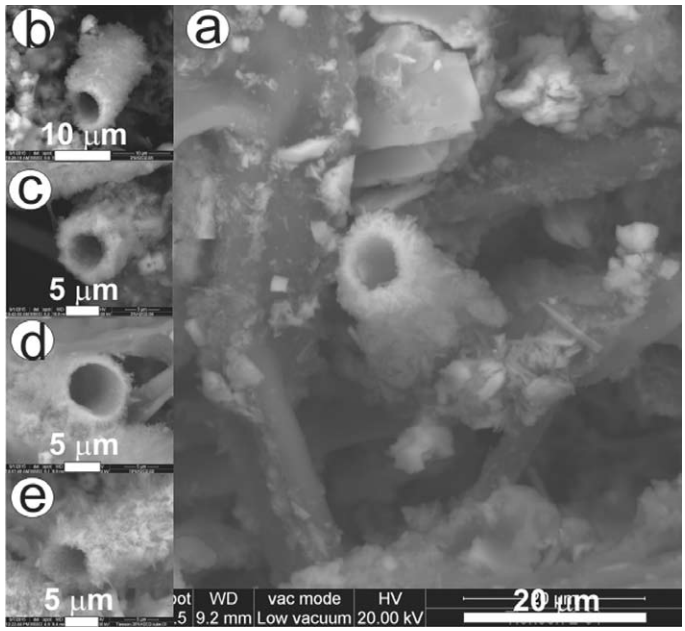

Figure 6: Fossilized tubes at the original and after spraying treatments with H_2O_2; from bottom to top are respectively, H_2O_2 3%, 10%, 15%, and 30%, showing relatively the effect of H_2O_2 on calcite dissolution.

Table 3. Lampenflora removal efficiency (% dw) after treatments with the use of H_2O_2.

H_2O_2	3%	10%	15%	30%
First treatment	1 – 4.4	2.8 – 25.2	30.5 – 61.5	48.1– 49.5
Second treatment	0.6 – 2.7	1.0 – 20.2	15.1 – 20.5	16.0 – 26.5

usually concentrated around beautiful speleothems, which intensifies photosynthesis (Table 1, Fig. 2).

Light intensity in show caves in Phong Nha–Ke Bang detected at sampling sites was more or less in the same range detected in show caves elsewhere (Piano et al., 2015). Here we found that light color was relatively decisive to lampenflora development. As stated in the result section, among the two main light colors used in the caves, white light is apparently more supportive to lampenflora development than yellow light. Basically, white light includes blue (about 450 nm wavelength) and red light (650 nm wavelength). They are the most favored wavelengths of photosynthesis. In particular, blue light is important during the germination stage of plant growth (Arnim and Deng, 1996). Yellow (wavelength around 550 nm) is less favorable to plants than blue and red. From the management point of view, our study suggests that particular attention should be devoted to implementing illumination system that would be less favorable for the growth of photosynthetic organisms. White lights should be replaced with colored lights with a spectrum out of the absorption peak of chlorophyll a, like green and yellow lights (Ngoc et al., 2014), and their distance from the speleothems should be increased to reduce direct illumination. The provision of motion sensors, at least along the tourist pathway, could be used to reduce the time of light exposure (Grobbelaar, 2000). A simple solution is to use appropriate LED lights, since LED light has a potential of tuning the desirable emission spectrum (D'Agostino et al., 2015).

Our macro- and microscopic analyses both reveal the importance of water. For instance, the abundance of cyanobacteria and its calcified structures (Fig. 4) is an indicator for the relationship between water and lampenflora in the show caves in Phong Nha–Ke Bang because the colonization of cyanobacteria (*Oscillatoria and Spirulina*) has been known to be dependent on water (Dayner and Johansen, 1991). Algae and flowering plants in their germination stage also require a lot of water (Fig. 3). Another effect of the presence of seeping water could be its role in transporting microorganisms inside the cave (Mulec et al., 2008). From this point of view, microorganisms, together with flora seeds, can enter the caves with the water seeping through the rock fractures, reaching the illuminated substrates. For management purpose, as seepage is a natural process, illumination should be limited in sections that are constantly wet.

As shown in Figures 2 and 3, mineral composition of substrates is another factor defining the lampenflora biodiversity. Generally, there is a stratification of the substrate mineral composition from purely limestone near the ceiling to a substrate contaminated with weathered or man-made materials near the bottom. Substrate that consists of a purely dense calcareous matrix allows mainly microbiota colonization. At mature stages, there will be lichens and fungal association. Near the ground, speleothems covered with significant amounts of weathering minerals sustains a more diverse biota (Warscheid et al., 1993). In addition, man-made materials, such as brick, mortar, or concrete brought to build the pathways, and sometimes artificial speleothems, are also susceptible to different forms of autotrophs and heterotrophs that are usually not found in karst caves. The degree of contamination will depend on the pore size distribution, as well as on the alkalinity of the artificial stones (Gu et al., 1998). For this mineral-composition issue, from a management point of view the advice is to keep clean the cave interior by daily washing, cleaning, and removing garbage, and avoid construction and new installations.

An Environmentally Friendly Treatment Protocol for Lampenflora Removal

The proliferation of lampenflora is a major threat for the conservation of show caves, since phototrophic organisms and their associates cause physical, chemical, and aesthetic damage to speleothems. To recover the aesthetic characteristics of speleothems, one apparently needs to eliminate completely lampenflora and its associates and in the meantime restore the physical appearance of speleothem's surface. Both microscopic and mass-loss analyses taken immediately after the second treatment reveals that the H_2O_2 chemical treatment alone has not met such expectation because organic matter are not completely removed and there are still $CaCO_3$-encrusted filaments and spores. The treated samples look yellow, dark, and even more crumbly than before treatment. Thus, it is impossible that sprayings with H_2O_2 will restore the aesthetic characteristics of speleothems.

In detail, our experimental results show clearly that treatment with diluted H_2O_2 (<15 %) has not much impact on lampenflora and their associates. The H_2O_2 treatment took effect only with more concentrated H_2O_2. The concentrated H_2O_2 treatments were able to remove microorganisms and destroy macroflora such as mosses and ferns. Together with that, many biogenic calcites were damaged because the dead organic-matter skeletons were burned by H_2O_2. Nevertheless, large fragments of roots and plant debris were still found after the concentrated H_2O_2 treatments (Fig. 5). Our results are, indeed, in agreement with a conclusion from Faimon et al. (2003) that lampenflora, mosses, and fungi died out and lost their structural integrity upon being sprayed with concentrated H_2O_2. This assumption leads to a suggestion to use water jets to clean the dead lampenflora and biogenic $CaCO_3$ on treated speleothems at carefully selected places. We recommend applying water jets to places near the cave floor where lampenflora are found more mature and complex and more difficult to chemically remove than the ones in higher places, and thus badly need further treatment after being chemically treated. Speleothems like flowstones and stalagmites are usually sturdy under a water jet, and due to natural and anthropogenic factors, speleothems near the floor are contaminated or covered with dirt and alluvium; an application of water to such surfaces would help washing as well. We believe that water jet application would not drastically enhance spreading of lampenflora inoculum in caves because the exchange of biota between the cave inner and exterior is already naturally strong. Their long history of karstification has made the caves spacious and highly accessible and, particularly, all studied caves are parts of active or inactive underground streams/rivers; Phong Nha and Thien Duong have large active rivers. Several studies have found invasive organisms to be abundant deep inside the caves (Luong and Holinska, 2015). In other pristine caves in Phong Nha–Thien Duong, such as Son Dong, life is thriving wherever the light reaches (CNN, 2016).

To conclude, the complete procedure for environmentally friendly removal of lampenflora in show caves in Phong Nha–Ke Bang consists of spraying at least two times with H_2O_2, 15% (no need to use a more concentrated solution), water-jet washing after chemical spraying at selected places, and illuminating caves with LED lamps of a different color such as yellow, installing motion sensors to illuminate only when necessary, and avoiding near and direct illumination onto constantly wet surfaces.

Conclusions

Caves in Phong Nha–Ke Bang National Park represent geomorphologic, geologic, biologic, historical, and paleoclimatic laboratories locating in a tropical monsoon coastal setting. This is, to our knowledge, the first time for tropical caves that lampenflora were studied and a remediation practice was proposed.

Our first conclusion is that with the addition of artificial light, white color in particular, lampenflora can easily develop in karst caves in tropical monsoon climate owing to a strong exchange between cave interior and exterior. A mature lampenflora community also harbors heterotrophs such as fungi and bacteria. Such complex biota is similar to the one in subsurface soil, where light is fairly limited but nutrients and water are available.

Second, remediation of lampenflora and its associates is not as simple as removal of soil-algae formed biofilm. In addition, the surfaces of speleothems colonized by mature lampenflora mat suffered from both destructive processes that break or dissolve carbonate and biogenic calcification that formed a porous and crumbly crystal layer.

Third, only a combination of chemical treatment, mechanical cleansing, and illumination modification can effectively eliminate lampenflora and recover the aesthetic properties of speleothems in show caves. This proposed protocol is cost effective and within the capability of local authorities.

It should be stated that as a strong oxidizer, H_2O_2 could be harmful to the indigenous cave fauna and flora. In Phong Nha–Ke Bang National Park, several new fauna species have recently been discovered in the caves (Lourenco and Pham, 2010, 2012; Luong and Holinska, 2015). All newly discovered species are highly vulnerable and rare cave dwellers. A direct hit of this chemical agent when sprayed could dispatch small invertebrates like scorpions, spiders, and millipedes or chase away vertebrates like lizards, which are found colonizing near the light sources for a better hunting. Thus it is compulsory that before applying H_2O_2 a thorough investigation of environment and biodiversity should be conducted at the sites. Preventive measures such as evacuation and dispersion of rare organisms should be taken. Spraying practice should be conducted carefully and avoid over-spraying. Water-jet should only be performed on solid

flowstone, rimstone, and stalagmites near the cave floor.

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