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Human impact on underground cultural and natural heritage sites, biological parameters of monitoring and remediation actions for insensitive surfaces: Case of Slovenian show caves

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ABSTRACT

Underground tourist cultural and natural heritage sites in Slovenia that share similar management problems with other such sites worldwide include Postojna Cave with more than a half million annual visitors and the UNESCO World Heritage Site Škocjan Caves. The underground environment is challenged by ultrasonic noise derived from different electric devices in a broad range (10–123 kHz) that can be minimised with protective housings. Lamps which increase temperature and lower relative humidity should be omitted. *Chlorella vulgaris* thrived very well under a halogen lamp and LEDs whose spectra were modified to give a natural appearance to illuminated features and emitted photon quanta close to the photosynthetic compensation point (~20 μmol photons/m^2 s). Remediation of insensitive calcite surfaces colonised by lampenflora with a 15% (v/v) solution of hydrogen peroxide (pH 7.0–7.5) was successful. Because visitors introduce and spread, especially by footprints, many live microorganisms (>10 000 colony-forming units per 100 cm^2), measures to reduce such input should be implemented. Bacterial counts expressed as colony-forming units per m^3 were more indicative for the presence/absence of tourists than were changes in carbon dioxide concentration. Not only tourists, but also external climatic conditions influenced the concentration of airborne bacteria. Microbiological parameters should be included in estimating tourist carrying capacity for sensitive underground sites.

Introduction

Underground and other light-devoid environments hold a very special place in human history. Early humans used natural cavities for rituals, shelters, storage, and places of inspiration (Bahn 2010). Later in history humans started to build underground tunnels and labyrinths such as catacombs (Adams 1886). Today in many of these places remnants of prehistoric man are found linked to burials, artefacts, daily life, drawings and inscriptions (Ziegert 2002). The most important natural and man-made underground cavities of natural and cultural importance are listed on the United Nations Educational, Scientific, and Cultural Organisation (UNESCO) World Heritage List (http://whc.unesco.org/en/list/; Williams 2011). There are also many other underground objects of regional and local importance offered on display to the public. Opening these places to the public bring changes that on occasions irreversibly alter the environment, such as construction of walking surfaces and infrastructure for electricity and water.

The most obvious effect of tourist use is artificial lighting and development of heterotrophic biofilms (Jurado et al. 2009; Saiz-Jimenez et al. 2011) and phototrophic communities which serve as primary producers (Bastian & Alabouvette 2009). This community, termed lampenflora and composed of different microbes, algae, and sometimes also mosses and ferns, is usually strongly adhered to the substratum and deteriorates speleothems and other objects (Mulec 2012). Biomass fixed due to light energy and other organic matter brought by tourists on clothing, skin, etc. become available for cave organisms. To attract visitors' attention, "artificial" waterfalls in caves are frequently introduced, resulting in additional boosting of microbial biofilms. Generally, the organic input in underground environments is not high (Simon et al. 2007), and higher nutrient input into cave environments enables newcomers to be more competitive than the originally present troglomorphic organisms (Mulec & Kosi 2009). Changes in underground environment may cause some animals, such as bats, to abandon their natural habitat (Fong 2011).

Some managers of underground places ignore the impact of microorganisms and biofilms until this problem becomes very obvious and difficult to solve. A few prehistoric places are now limited or even closed to the public, for example the UNESCO sites Altamira (Spain) and Lascaux (France) with famous Palaeolithic

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cave paintings. For instance, in Altamira Cave visitors provoked aerosolisation of fine particles from cave floor sediments including bacterial and fungal spores, and because of biodeterioration of cave paintings, Altamira Cave was closed to the public. Installation of a thermally insulated door reduced the entry of airborne particles, the condensation rate in the entrance area, and the metabolic activity of microbial colonies (Saiz-Jimenez et al. 2011). In Lascaux Cave chemical products and antibiotics were applied to address this problem, but consequently more problems arose (Bastian et al. 2010). A wide range of biocidal chemicals have already been applied in different caves, but hydrogen peroxide seems to be currently the most favourable one (Faimon et al. 2003; Mulec and Kosi 2009). Remediation actions that are done without careful planning and forethought may not solve, and may even exacerbate, the problem. Another example connected with management of underground sites is installation of LED lamps without previous studies, and expectation that this step will be enough to stop proliferation of lampenflora.

Loss or irreversible damage of paintings and other objects of cultural and natural value in the underground due to biological activities is an urgent problem. Based on experiments from two Slovenian show caves – the highly visited Postojna Cave and the UNESCO site Škocjan Caves – the paper presents evidence, solutions for remediation of insensitive surfaces and a monitoring plan for light eutrophication, ultrasonic smog, and microclimatic changes and surface contamination due to tourist use.

Materials and methods

Experimental sites

The experiments and measurements were conducted in a natural cave environment, in two show caves in Slovenia. Škocjan Caves (Škocjanske jame, total length 5.8 km) are on the UNESCO World Heritage List and were recognised as the first underground karst wetland under the Ramsar Wetland Classification System. This place is visited by approximately 100 000 tourists per year. A subset of experiments were conducted in Postojna Cave (Postojnska jama, total length 20.6 km) which attracts more than 500 000 visitors annually (Fig. 1). Both caves share similar problems with other underground caves around the world exploited for tourism, including climate changes, introduction of tourist-originated particles, surface contamination, light eutrophication, lampenflora and ultrasonic smog due to various electric devices installed in the underground.

Atmospheric parameters

Temperature (accuracy, ±1.0 °C; range −45 to 125 °C) and relative humidity (RH, accuracy ±3% RH, range 0.0–100.0%) were measured using a portable Kestrel 4500 PocketWeather Tracker (USA) to observe effects of different lights and tourists on cave atmosphere. In addition, atmospheric carbon dioxide was measured during tourist visits with a MI70 Vaisala handheld carbon dioxide metre (Finland; probe GMP222, accuracy ±1.5% of range + 2% of reading; range 0–3000 ppm).

Culture media

Liquid Jaworski medium (Warren et al. 1997) was adopted to estimate effects of different lights on algal growth. To estimate the culturable airborne microbiota introduced by tourists, a 1.5% nutrient agar (NA, Sigma, USA) was used. NA was selected because it is frequently used to isolate bacteria, and because bacteria grown on NA are good estimators of introduced microbes during tourist visits in caves (Mulec et al., 2012a). No antimicrobial substances were added to the media. Conspicuous colony-forming units (CFU) were screened under a zoom stereomicroscope (Nikon SMZ800).

Growth and analyses of phototrophs with different light regimes

To study growth of lampenflora at underground temperature conditions under different quality of lights, 10^4 cells per millilitre of *Chlorella vulgaris* SAG 211-12 (DSMZ, German collection of microorganisms and cell cultures) in exponential phase were inoculated into liquid medium and cultured under controlled lighting conditions in an unvisited portion of Postojna Cave (Fig. 1). *C. vulgaris* was selected as a proxy organism in order to compare results with previous studies (Mulec et al. 2008) and because this organism is frequent in lampenflora community (Mulec 2012; Roldán and Hernández-Mariné, 2009; Smith and Olson 2007). Three different lamps that are currently being used in show caves were selected as light sources: a 200 W halogen, (Osram, Germany, abbreviated as Hal); a 12 W cool-white LED (Cree, USA, abbreviated as LED Cool); and, a 22 W 61Y10G1B LED (Enlux, USA) which uses a combination of yellow, green and blue LEDs (LED ygb). These LED lamps were also selected in the experiment because their light resembles natural light, from white (LED Cool) to yellowish (LED ygb). Cultivation conditions included an 8:16 light/dark period with a photosynthetic photon flux density (PPFD) < 20 μmol photons/m^2 s or PPFD > 20 μmol photons/m^2 s for 24 weeks. This PPFD threshold was selected to allow observation of the response of *C. vulgaris* when growing below and close/above the photosynthetic compensation point (PCP). PCP for green algae lies at about 21 μmol photons/m^2 s (Stevenson et al. 1996). On a LI-1000 datalogger (LI-COR, USA) were plugged a LI-190SA quantum sensor (range 400–700 nm, sensitivity 8 μA per 1000 μmol/m^2 s) to measure PPFDs (μmol photons/m^2 s) and a LI200 SA pyranometer sensor (range 400–1100 nm, sensitivity 80 μA per 1000 W/m^2) that served to measure irradiances (W/m^2). Illumination levels (Lux) were measured by a Miniluxmeter 4 (Optronik, Germany, range 400–700 nm, sensitivity typically 20 μA per 100 kLux) and were adjusted by insertion of screens and by adjustment of the distance from cultures to the light source. Cultures were cultivated in triplicates, mixed daily, and counted under a microscope (Nikon Eclipse 600) with a hemocytometer. Emission spectra of lights used in the study were measured in the cave with a Jaz spectrometer (Ocean Optics, USA; detector 200–1000 nm, sensitivity 75 photons/count at 400 nm, 41 photons/count at 600 nm).

After incubation, a certain volume of *C. vulgaris* was filtered through a glass fibre (GF) filter (Millipore, USA) and dried at 105 °C to estimate dry weight. The remained culture was filtered through another GF filter. Cells attached on a GF filter with cold 90% acetone were mechanically disrupted in a mortar. Extract with photosynthetic pigments was centrifuged 10 min at 4000 RPM, and supernatant was used to measure absorption spectra from 350 to 750 nm with a Lambda 25 UV–Vis Spectrometer (Perkin-Elmer, USA). Concentration of photosynthetic pigments chlorophylls *a* and *b* (Chl *a*, Chl *b*) and carotenoids as β-carotene were determined as previously described (Wetzel and Likens 1995), and evaluated.

Remediation of lampenflora locations

At carefully selected sites around lamps in caves, a 15% solution of hydrogen peroxide was tested to observe the efficient removal/oxidation of organic biofilms formed by lampenflora and dead lampenflora incrusted within the calcite. Before application, inspection of the site was needed to check for the presence of cave terrestrial fauna, and to remove mosses and ferns from lampenflora community to ensure good contact between biocidal hydrogen peroxide and lampenflora algae that were attached or incrusted