Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 21 | No. 2 | pp. 775-778 | 2022

Original Research Paper

https://doi.org/10.46488/NEPT.2022.v21i02.041

Open Access Journal

Photosynthetic Microorganisms Consortium as Bioindicators for Heavy Metals

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 08-06-2021
Revised: 07-08-2021
Accepted: 26-08-2021

Key Words:
Bioindicators
Photosynthetic microbes
Environmental pollutants
Heavy metals

ABSTRACT

Heavy metals that are discharged through industrial and agricultural activities cause contamination, especially to the water sources, and bring about negative impacts on the flora and fauna in the ecosystem. The monitoring of heavy metals in the environment requires high technical skills with sophisticated equipment and is also time-consuming. In this study, the potential of using natural photosynthetic microorganism consortiums collected from natural water bodies as bioindicators for the screening of heavy metals was explored. The photosynthetic microorganism consortiums were first cultured in lab, immobilized, and then exposed to different heavy metals (Cd, Cu, Ni and Co) at different concentrations (0.01 $mg.L^{-1}$, 0.05 $mg.L^{-1}$, 0.10 $mg.L^{-1}$, 0.50 $mg.L^{-1}$, 1.00 $mg.L^{-1}$ and 5.00 $mg.L^{-1}$). The fluorometric responses before and after the exposure to heavy metals were measured. The results revealed that consortium cells responded to a wide range of heavy metals within a short period of exposure. The responses showed that the consortium cells can detect the presence of Cd, Cu, Ni, and Co within the range of 0.05-5.00 $mg.L^{-1}$. The study confirmed that the photosynthetic microorganism consortiums collected from natural water bodies could be used as bioindicators for the screening of heavy metals.

INTRODUCTION

Rapid urbanization and population increase result in significant water demands to meet varied development needs, with lakes and rivers serving as the primary supplies of water for domestic, industrial, and agricultural activities. Industrial, manufacturing, and agricultural sectors are the major contributors to economic development for many countries. Products generated from these industries discharge tonnes of waste-containing metals into the environment (Afroz & Rahman 2017). The most commonly found metals include cadmium, chromium, copper, lead, nickel, and zinc (Jaishankar et al. 2014). This has become a serious environmental problem that left toxic effects on the flora and fauna and even threatening human life.

Several studies have shown the involvement of microorganism consortiums (algae, cyanobacteria, bacteria) in the degradation of a toxic substrate, thiocyanate (Ryu et al. 2015) as well as in the treatment of organic pollutants (Mahdavi et al. 2015). Multiple species that co-exist in the ecosystem may provide robustness to environmental variation, stability for the species, ability to share metabolites and weather periods of nutrient limitations, and resistance to invasion by other species. Therefore, photosynthetic microorganism consortiums which are present abundantly in the environment and are sensitive to any environmental changes might be good indicators of heavy metals by providing biological responses upon contact with these heavy metals.

In this paper, the fluorometric responses of photosynthetic microorganism consortiums after exposure to heavy metals are reported. The potential of the responses to be used as bioindicators for the presence of heavy metals is discussed as well.

MATERIAL AND METHODS

Chemicals and Cell Cultures

Copper in the form of $CuSO_4$, cadmium in the form of $Cd(NO_3)_2$, nickel in the form of $Ni(NO_3)_2$, and cobalt in the form of $CoCl_2$ were procured from Sigma-Aldrich, Malaysia.

Bold Basal medium (50x stock) was obtained from Sigma-Aldrich, Malaysia, and Jaworski medium was prepared according to the information provided by the Culture Collection of Algae and Protozoa, United Kingdom. To provide nutrients to different species of photosynthetic microorganisms, 1 mL of Jaworski medium and 1 mL of Bold Basal medium were added to 98 mL of deionized water to produce 100 mL of medium.

The consortium of photosynthetic microorganisms was collected from two different locations in Malaysia, namely Taman Tasik Titiwangsa, Kuala Lumpur (L1) and Taman

Metropolitan Kepong, Selangor (L2). These two locations were selected for sample collection as they are surrounded by large water body which contains good populations of photosynthetic microorganisms. The consortium of microorganisms collected from different locations were cultured separately.

The cultures were placed on an orbitals shaker at a temperature of 20 ± 2°C with continuous aeration at 95 rpm to minimize cell clumping. Photoperiods of 16 hours in light and 8 hours in dark conditions were applied (Wong et al. 2012). The microalgae and cyanobacteria were identified through a simple light microscope (Eclipse E-100 LED, Nikon) at 400x magnification. The cell growth was determined for 15 days using a hemocytometer (Neubauer, Marienfeld) and a light microscope.

Heavy Metal Exposure

Cell density was determined with a spectrophotometer (GeneQuant 1300, GE) at a wavelength of 700 nm. The intensity of the fluorescence emission of the consortium cells was determined using a spectrofluorometer (Glomax Multi Jr., Promega).

A volume of 2 mL of cells from the day-10 culture with OD = 0.30 (λ = 700 nm) was exposed to different concentrations of Cd (0.05, 0.10, 0.50, 1.00, and 5.00 mg.L^{-1}). The fluorescence emission intensity was measured at wavelength = 648 nm, with excitation wavelength = 526 nm (Khishamuddin et al. 2018). Before and after the exposure, the intensity of the fluorescence emission was measured at t = 30, 60, 120, 240, 360, and 480 minutes. Cu, Ni, and Co were used in the same experiments. The reaction of cells that had not been exposed to heavy metals served as a negative control. For all of the exposure tests, a medium without any cells was employed as a blank. All of the exposure experiments were done three times.

RESULTS AND DISCUSSION

Before reaching the stationary phase, the cells in consortiums grew over the first 10 days in the culture (Fig. 1). Due to nutrient depletion and waste accumulation in the culture system, microbial growth generally reached a stationary phase. The cells from the consortiums on day-10 were selected for the exposure tests. The correlation between the OD measured with λ = 700 nm and cell density is portrayed in Fig. 2. The experimental results showed cells in consortiums with OD = 0.3 A yielded the highest fluorescence emission, with an average number of cells of 7.9 x 10^5 per 1 mL of culture. When the number of cells exceeded OD = 0.3 A, the fluorescence emission reduced, possibly due to non-photochemistry quenching, in which emitted fluorescence was reabsorbed by nearby cells, lowering the fluorescence intensity (Wong et al. 2013).

The changes in fluorescence emission of the cells in consortiums caused by the presence of Cd, Cu, Ni, and Co are illustrated in Fig. 3 and Fig. 4. The presence of Cd disrupted the photosynthesis system, which caused changes in the fluorescence emission from chlorophyll. Mera et al. (2016) and Cheng et al. (2016) reported that Cd reduced the chlorophyll concentration in microorganism cells, and this result is consistent with their findings. Furthermore, long-term exposure to Cd inhibits the proliferation of microalgae cells and causes their decrease. Photosynthetic microorganisms require modest amounts of copper as a nutrient. Cu in high quantities, on the other hand, interferes with the metabolic functions of cells, producing disruptions in ATP production, pigment synthesis, and cell division suppression (Kumar et

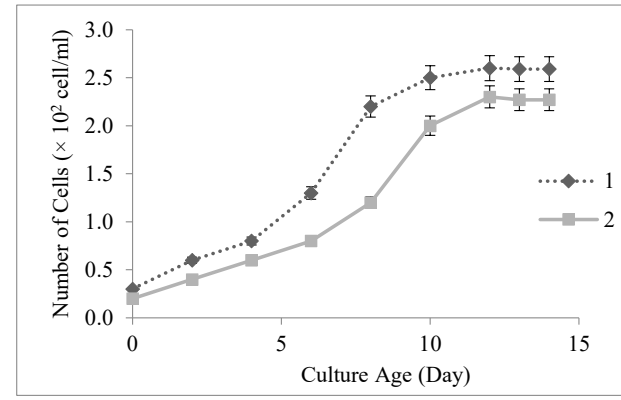

Fig. 1: Growth of consortium culture from L1: Taman Tasik Titiwangsa, L2: Taman Metropolitan.

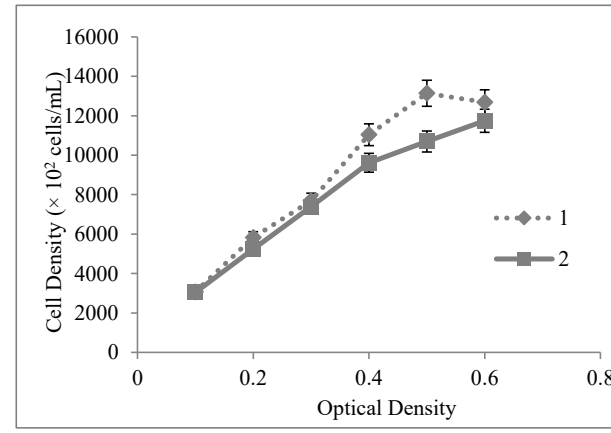

Fig. 2: The correlation between optical density measured at λ = 700 nm with cell density calculated using haemocytometer and light microscope for consortium cultures from L1: Taman Tasik Titiwangsa (linear equation: y = 20978x + 1564.3, R^2 = 0.9402), L2: Taman Metropolitan (linear equation: y = 17765x + 1737.3, R^2 = 0.9761).

al. 2015). The presence of Cu was found to affect the fluorescence emission by the chlorophyll as well (Wong et al. 2017).

According to Martínez-Ruiz et al. (2015) and Guo et al. (2017), the presence of Ni caused a reduction of chlorophyll. It disrupted the active site of the oxygen-evolving complex by reducing electron transport activity, which could impact photosynthetic bacteria' fluorescence emission (Boisvert et al. 2007). Co, like Ni, affects the concentration of chlorophyll (Guo et al. 2017). The presence of Ni affects the production of photosynthetic enzymes and the synthesis of macromolecules in photosynthetic organisms as well.

Despite the relevance of some heavy metals as nutrients for photosynthetic microorganisms, the presence of high concentrations (greater than 0.05 $mg.L^{-1}$) of Cd, Cu, Ni, and Co reduced the fluorescence emissions by photosynthetic microbes in consortiums. In general, heavy metals increased the number of reactive oxygen species (ROS) in photosynthetic microorganisms, which could disrupt the electron transport chain (Devi & Mehta 2014). After 120 minutes of exposure, the change in fluorescence emission intensity was dose-dependent. The research found that the natural consortium of photosynthetic microorganisms can be employed as a bioindicator for Cd, Cu, Ni, and Co screening.

CONCLUSION

This study reported the changes in fluorescence emission intensity due to the exposure of photosynthetic microbes in consortiums to heavy metals. The presence of heavy metals generally caused a decrease in fluorescence emission. The fluorescence responses by the photosynthetic microbes in the consortium confirmed the potential of these microbes to be used for screening Cd, Cu, Ni, and Co.

ACKNOWLEDGEMENTS

This work was supported by funding from the Ministry of Higher Education Malaysia (Grant No. FRGS/1/2020/

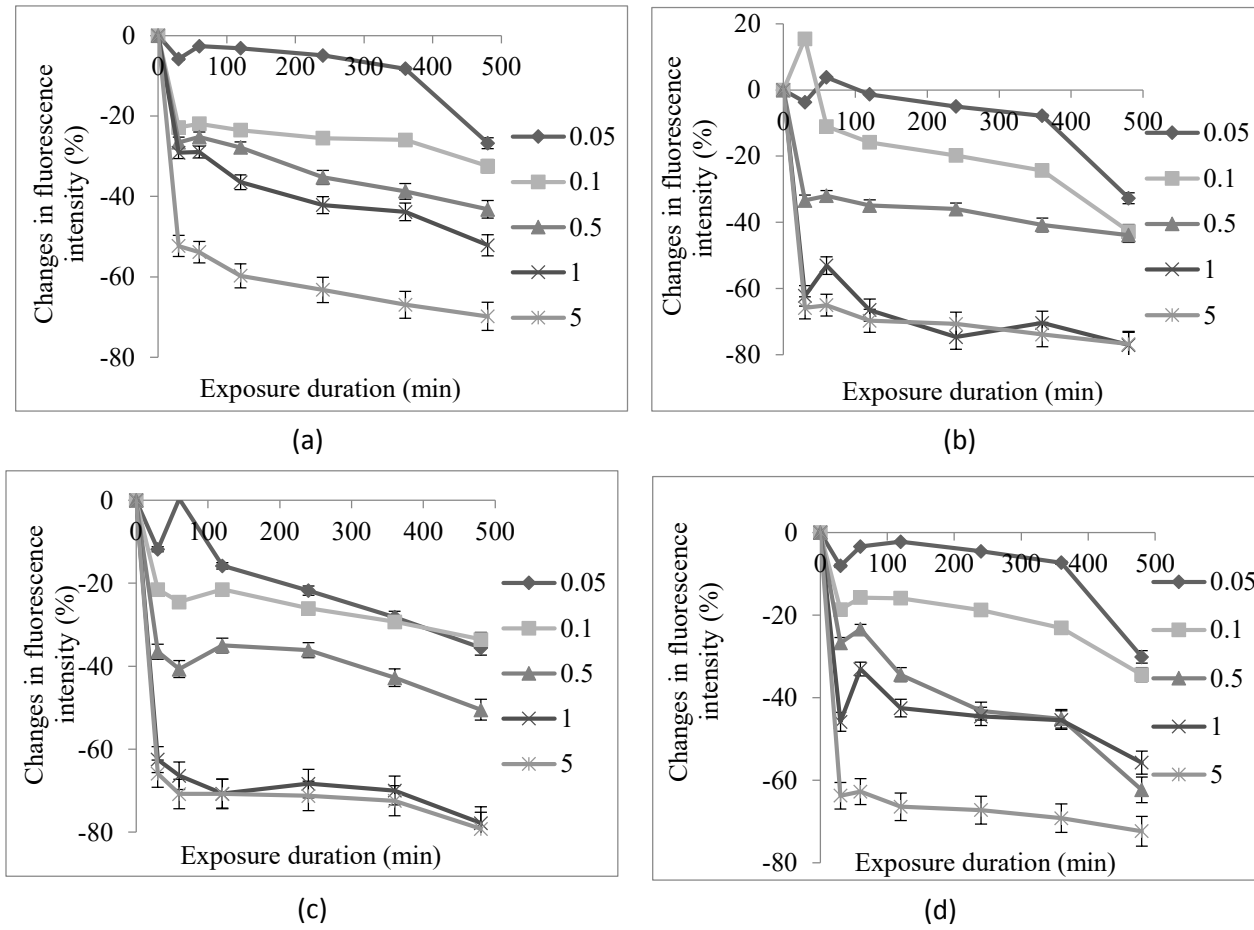

Fig. 3: Exposure of consortium of cells collected from L1 to (a) Cd, (b) Cu, (c) Ni and (d) Co.

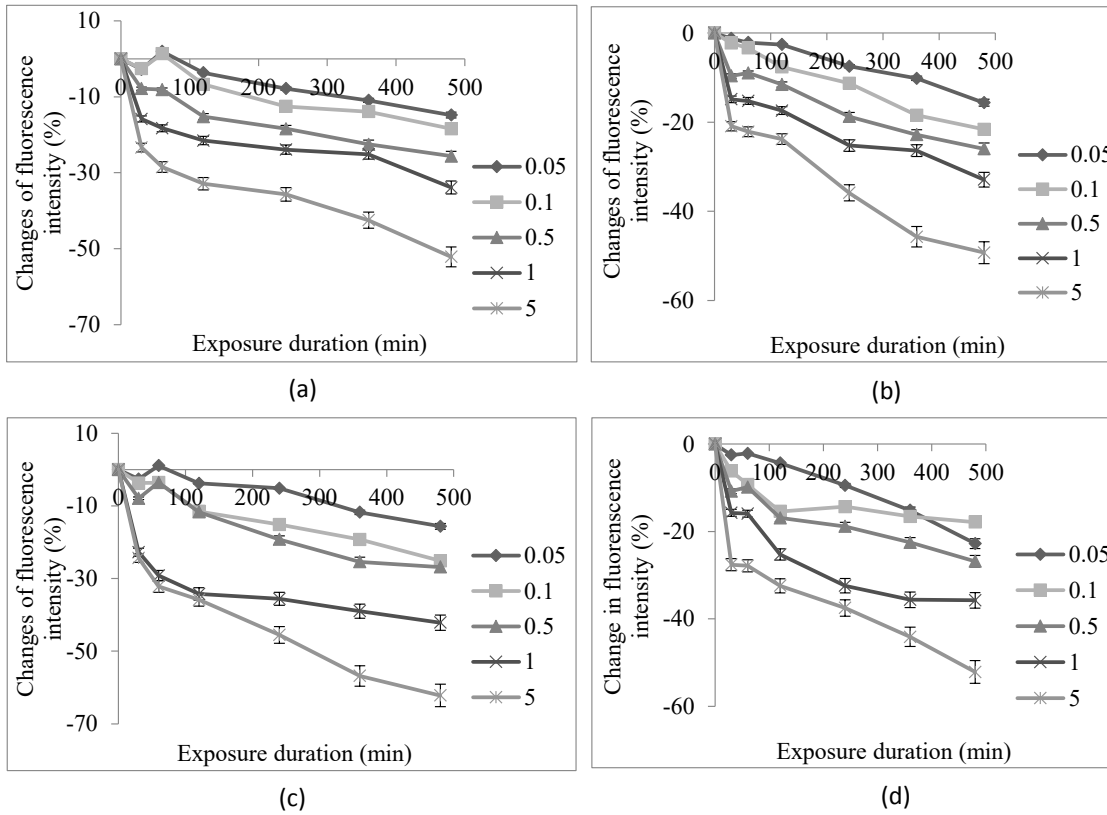

Fig. 4: Exposure of consortium of cells collected from L2 to (a) Cd, (b) Cu, (c) Ni, and (d) Co.

STG03/INTI/01/1), and funding from INTI International University, Malaysia (Grant No. INTI-FHLS-01-01-2016).

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