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Freshwater Green Microalga for Bioremediation of River Melaka Heavy-Metals Contamination

Muhammad Muhammad Nmaya[1]*, Mohd Arif Agam[1], Hazel Monica Matias-Peralta[2], Jibrin Alhaji Yabagi[1] and Mohammed Isah Kimpa[1]

[1]*Department of Science, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia.
[2] Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia

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Abstract: Heavy metals toxicity can adversely affect aquatic life and in some cases result in massive fish mortalities. Recent observation of fish mortalities floating on the surface of *Bandar Hilir* (city center) section of Melaka River (Latitude 2.194871, Longitude 102.248950) but none in the section of the river close to the open sea (latitude 2.193556, Longitude 102.247048) necessitated research into the cause. This work looks into heavy metal contamination as a possible cause. It also presents the use of wastewater culture method for growing an indigenous microalga *Scenedesmus* sp. while simultaneously serving as a means for bioremediation of the river water. Water sample from the two sections were collected for laboratory analysis. This research used Inductively coupled plasma mass spectrometry (ICP-MS) to detect the presence and concentration of selected heavy metals in the two different sections. The results show a significantly higher concentration of Zn in the section with fish mortalities than the section were no mortality was seen. The bioremoval experiment shows that the living cells of microalgae *Scenedesmus* sp. took up 97% and 99% of the Zn after three and seven days respectively. These results indicate (1) possibility of Zn contamination and (2) excellent bioremediation potential of this contamination with *Scenedesmus* sp.

Keyword: Melaka River, Water contamination, Fish mortality, Zinc, Bioremoval, *Scenedesmus* sp.

1. Introduction

Research reports have shown that the number of polluted river in Malaysia is rapidly increasing [1,2]. Water contamination by heavy metals is a threat to the well-being of humans and the natural environment. Heavy metals constitute a core group of aquatic pollutants possessing bio-accumulative and non-biodegradable properties in food. Humans can be easily affected by pollutants associated with aquatic systems through consumption of contaminated fish and other aquatic foods from this environment The availability, sustainability, efficiency and cost-effectiveness of a procedure to be used in obtaining clean water while ensuring environmental protection are key issues of importance. Water treatment through the use of chemicals come with a great cost to human life in particular and the environment in general [3, 4, 5].

The search for a cost-effective and relatively safe method for the removal of these harmful contaminants led researchers to develop interest in microorganisms that possess resistance to toxic metals. Bioprocesses, such as bioaccumulation and biosorption, which utilizes microorganisms that show resistance to these toxic metals can provide better alternatives to the physico-chemical methods [5, 6, 7]

Several decades of research in utilizing algae, especially microalgae, for the treatment of municipal and other wastewaters has shown that this has the potential to improve the water quality. Microalgae are known to be resistant to heavy metal toxicity and excellent in removal of nutrient and toxic metals from wastewaters [7, 8, 9].

The microalgae *Scenedesmus* is reported to be an efficient hybrid biosorbent for removal of variety of metals and can sequester metals both in living and nonliving condition [1, 9, 10, 11]. It has a phenotypic adaptability towards a wide range of heavy metals and the ability to survive in anthropogenically disturbed ecosystems [12, 13]. The growth and culture of the microalgae in wastewater whereby the wastewater serves as

*Corresponding author: mmnone@yahoo.com

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a potential and sustainable media of growth for algal feedstock has been reported [14,15,16,17,18]. This process is also utilized in high rate algal pond (HRAP) systems which has, in turn, enhanced the possibility for sewage treatment, removal of nutrients and several toxic metal ions from the wastewater by microalgal consumption. Several agro-industrial wastewaters can be treated by the use of this system [19, 20,21,22].

2. Materials and Method

2.1 Water Samples Collection

Water samples were collected from the city center section of the river where fish mortalities were seen (Latitude 2.194871, Longitude 102.248950) and labeled Sm while the sample from the section with no observed fish mortalities (latitude 2.193556, Longitude 102.247048) was labeled Sn.

2.2 Concentration of the Heavy-Metals

The presence and concentration of heavy metals in the water samples, before and after treatment with microalgae, was determined using PerkinElmer SCIEX Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN 9000 at the Analytical Laboratory of FKASS UTHM Johor, Malaysia.

2.3 Heavy-Metal Bioremoval Experiment

Scenedesmus sp. cells were grown in suitable medium. After two (2) weeks, cells were harvested by centrifugation at 6000rpm for 15min at 4 ◦C and washed three times with distilled water. The harvested cells were used in the heavy-metal removal experiment; seven (50ml) conical flasks were filled with 10 ml algae suspension and 20 ml of sample S_m. The same procedure was done for sample S_n with another set of seven conical flask. These were kept at room temperature for 7 days. The experiment was carried out in triplicate.

2.4 Growth of *Scenedesmus* Cells During Experiment

Thermo Scientific Biomate 3S UV-Vis Spectrophotometer was used to monitor the growth of *Scenedesmus* cells. Optical density (O.D) measurements at 685nm were take daily for the seven days of heavy-metal bioremoval experiment.

3. Results and Discussion

The metals present in the water samples and their concentrations were determined (Table 1a, b,c and d). Values are in mean±standard deviation

Table 1a Sample S_m after three (3) days of treatment

Metal	Conc. Before treatment (ppb)	Conc. after Treatment (ppb)	Percentage removed (%)
Copper (Cu)	1.3±0.261	1.17±0.416	10
Zinc (Zn)	16.4±0.035	0.414±0.18	97
Nickel (Ni)	1.92±.0.28	0.454±0.11	76
Iron (Fe)	59.5±0.056	41.5±1.44	19
Arsenic (As)	2.76±0.043	2.26±0.172	18

Table 1b Sample S_m after seven (7) days of treatment

Metal	Conc. before treatment (ppb)	Conc. after Treatment (ppb)	Percentage removed (%)
Copper (Cu)	1.3±0.261	0.842±0.22	35
Zinc (Zn)	16.4±0.035	0.147±0.406	99
Nickel (Ni)	1.92±.0.28	0.162±0.66	78
Iron (Fe)	59.5±0.056	24.1±1.24	59
Arsenic (As)	2.76±0.043	1.49 ±0.411	46

Table 1c Sample S_n after three days of treatment

Metal	Conc. before treatment (ppb)	Conc. after Treatment (ppb)	Percentage removed (%)
Copper (Cu)	1.39±0.239	0.989±0.301	29
Zinc (Zn)	1.97±0.416	-	100
Nickel (Ni)	2.1±0.46	0.573±0.	73
Iron (Fe)	48.2±0.29	26.6±0.	45
Arsenic (As)	3.7±0.71	2.62±0.	29

Table 1d Sample S_n after seven days of treatment

Metal	Conc. Before treatment (ppb)	Conc. after Treatment (ppb)	Percentage removed (%)
Copper (Cu)	1.39±0.239	0.783±0.21	44
Zinc (Zn)	1.97±0.416	-	100
Nickel (Ni)	2.1±0.46	2.1±0.168	80
Iron (Fe)	48.2±0.29	21.6±2.01	55
Arsenic (As)	3.7±0.71	1.69±0.607	52

When compared with the National Recommended Water Quality Criteria by U.S. EPA and Malaysian Interim Water Quality Standard (NWQS), concentration of the metals detected in both water samples are lower and within the range of EPA criteria and Class III of NWQS standards.

However, heavy-metals are very reactive and can be toxic even at low concentrations due to their ability for bio-magnification in the food Chain where they accumulate and inflict damage to living organisms or delicate body structures such as gills in fish [23,24, 25]

The concentration of Fe in S_m and S_n is higher than all the other metals but, unlike for Zn, there is no significant difference between these concentrations.

The remarkable difference between concentration of Zn in S_m and S_n may be a pointer to the fish mortalities observed in the section of the river from which S_m was obtained but none in the section where S_n was collected. The toxic effect of Zn on fish and other aquatic organisms has been widely reported by several researchers [1, 4, 25, 26, 27].

Zinc is a ubiquitous element and one of the most common contaminants in aquatic systems. It is biologically essential for normal growth, physiology, and development of fish in minute quantities but becomes toxic when in excess of cellular requirements. Zinc tends to occur in elevated concentrations adjacent to areas of urban run-off, industrial discharges, and soil erosion [26].

An increase in amount of water-borne Zn can lead to the amount entering the fish through the gills exceeding the requirement for this metal. This may result in direct toxic action of Zn on fish by precipitating the layer of mucus on the surface of the gill, causing suffocation. Toxic level of water-borne Zn can cause a variety of physiological and behavioral changes such loss of appetite, reduced growth and increased fish mortality. [27].

However, a complicating factor the assessment of the toxicity potential for any particular heavy metal is that multiple metals typically occur and interact in aquatic systems. A toxicological studies that focus on the effects of single metals may not be environmentally realistic in assessing the actual impacts on fish. Combinations of heavy metals have been found to behave in three ways: the first is additively (when one metal acts independently from the others, and the toxic effect of each metal in combination is the same as the effect of the individual metals). Secondly is synergistically (here, different metals interact, and the toxic effect of the combined metals is greater than the additive effects of the individual metals), or thirdly; antagonistically (different metals interact, but the toxic effect of the combined metals is less than the additive effects of the individual metal [28].

Bioremoval of the toxic metals

The ICP-MS result for metal concentration, before and after treatment, indicates an impressive ability for removal for the heavy metals with *Scenedesmus* sp. cells. The efficiency of the process for both samples is in the order Zn>Ni>Fe>As>Cu.

Scenedesmus growth during experiment

The growth curves (fig. 1) indicates that the cells in S_m where able to obtain nutrient and not only grow rapidly but also continued growing all through the seven days. This can be attributed to the ability of the microalgae to utilize nutrients in contaminated water for survival [12, 13]

On the other hand, the cells in S_n grew at a lower rate have started showing a decline from day six.

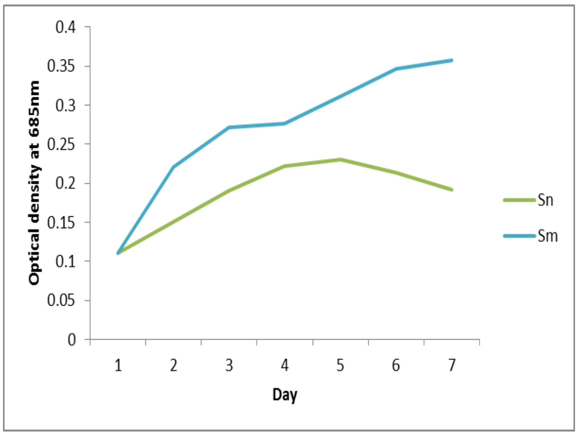

Fig. 1 Growth of *Scenedesmus* sp cells in the water samples.

4. Conclusion

The result of this work shows a possibility of zinc toxicity as the cause of fish mortarlities observed in the *Bandar Hilir* (city center) section of Melaka River. The result also shows that *Scenedesmus* sp. can be used to remove this and other potential metal pollutants detected in the river. The effectiveness of the microalgae to do this was observed through the bioremoval experiment carried out. A great amount of the Zn (97% and 99%). Further research can be conducted particularly on the fish mortalities.

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References

[1] Baharoma, Z. S., Ishaka, M. Y. (2015) "Determination of heavy metal accumulation in fish species in Galas River, Kelantan and Beranang mining pool, Selangor" Procedia Environmental Sciences 30: 320 – 325.

[2] Rahman MM, Awang MB, Jalal KCA, Aisha S, Kamaruzzaman BY. (2013). "Study on Toxic chemicals in Kuantan River during Pre and Post Monsoon season" Australian Journal of Basic and Applied Sciences 7(4):24-30.

[3] Nmaya, M. M., Ishaq, A. G., Agam, M. A., Matias-Peralta, H. M., Khaled, N., Yabagi, J. A., Kimpa, M. I. (2015) "Biosorption of Heavy Metals by *Scenedesmus* sp. Isolated from the Temporary Waters of Endau Rompin, Johor, Malaysia" Jurnal Teknologi (Sciences & Engineering) Vol. 77. No. 24 pp. 35–38.

[4] Rohasliney H, Tan HS, Noor Zuhartini Md.M, Tan PY. (2014). "Determination of Heavy Metal Fishes from the lower reach of Kelantan River, Kelantan, Malaysia" Tropical Life Sciences Research; 25(2):21-39.

[5] Kikuchi, T. and Tanaka, S. (2012). "Biological Removal and Recovery of Toxic Heavy Metals in Water Environment. Critical Reviews in Environmental Science and Technology. 42:10, 1007-1057.

[6] Viera, R.H.S.F. and Volesky, B. (2000). "Biosorption: a solution to pollution?". Internatl Microbiol 3:17-24

[7] Hernández, D., Riaño, B., Coca, M., García-González, M.C. (2013). "Treatment of agro-industrial wastewater using microalgae–bacteria consortium

combined with anaerobic digestion of the produced biomass" Bioresour. Technol. 135, 598–603.

[8] Cristina, M., Monteiro. And Castro, P. M. L. (2012). "Metal Uptake by Microalgae: Underlying Mechanisms and Practical Applications." American Institute of Chemical Engineers. 20(2): 299-311.

[9] Tuzen M, Sari A (2010). "Biosorption of selenium from aqueous solution by green algae (Cladophora hutchinsiae) biomass: equilibrium, thermodynamic and kinetic studies" Chem Eng J 158:200–206.

[10] Mirghaffari N, Moeini E, Farhadian O (2014). "Biosorption of Cd and Pb ions from aqueous solutions by biomass of the green microalga, Scenedesmus quadricauda." J Appl Phycol.

[11] Onalo, J. I., Matias-Peralta, H. M., Sunar, N. M. (2015). "Growth of Freshwater Microalga, Botryococcus sp. in Heavy Metal Contaminated Industrial Wastewater" Journal of Science and Technology

[12] Bayramoglu, G., Yakup, Arica M. (2009). "Construction a Hybrid Biosorbent using Scenedesmus quadricauda and Ca-alginate for Biosorption of Cu(II), Zn(II) and Ni(II): Kinetics and Equilibrium Studies." BioresourTechnol. 100: 186-193.

[13] Pena-Castro, J. M., Martinez, F. J., Esparza-Garcia, F. Canizares-Villanueva, R. O. (2004) "Heavy Metals Removal by the Microalga Scenedesmus incrassatulus in Continuous Cultures" Bioresour Technol. 94: 219-222.

[14] Ajayan, K. V., Selvaraju, M., & Thirugnanamoorthy, K. (2011). "Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and petrochemical effluents." Pakistan Journal of Biological Sciences, 14(16), 805-811.

[15] Mandal, S., & Mallick, N. (2011). "Waste utilization and biodiesel production by the green microalga Scenedesmus obliquus." Applied and Environmental Microbiology, 77(1), 374-377.

[16] Xin, L., Hong-ying, H., Ke, G., & Jia, Y. (2010). "Growth and nutrient removal properties of a freshwater microalga Scendesmus sp. LX1 under different kinds of nitrogen sources." Ecological Engineering, 102, 379-381

[17] Park, K. Y., Lim, B.-R., & Lee, K. (2009). "Growth of microalgae in diluted process water of the animal wastewater treatment plant." Water Science & Technology, 59(11), 2111-2116.

[18] Ramanan, R., Kim, B., Cho, D., Oh, H. and Kima, H. (2016). "Algae–bacteria interactions: Evolution, ecology and emerging applications" Biotechnology Advances 34: 14–29.

[19] Kim, B.H., Kang, Z., Ramanan, R., Choi, J.E., Cho, D.H., Oh, H.M. (2014). "Nutrient removal and biofuel production in high rate algal pond (HRAP) using real municipal wastewater." J. Microbiol. Biotechnol. 24, 1123–1132.

[20] Craggs, R.J., Heubeck, S., Lundquist, T.J., Benemann, J.R., (2011). "Algal biofuels from wastewater treatment high rate algal ponds." Water Sci. Technol. 63, 660–665.

[21] Kim, B.H., Kang, Z., Ramanan, R., Choi, J.E., Cho, D.H., Oh, H.M. (2014). "Nutrient removal and biofuel production in high rate algal pond (HRAP) using real municipal wastewater." J. Microbiol. Biotechnol. 24, 1123–1132.

[22] Craggs, R.J., Heubeck, S., Lundquist, T.J., Benemann, J.R., (2011). "Algal biofuels from wastewater treatment high rate algal ponds." Water Sci. Technol. 63, 660–665.

[23] Ayangbenro, A. S., Babalola, O. O. (2017). "A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents" Int. J. Environ. Res. Public Health 2017, 14, 94.

[24] Mani, D.; Kumar, C. (2014). "Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: An overview with special reference to phytoremediation." Int. J. Environ. Sci. Technol. 11, 843–872

[25] Price, M.H.H. (2013). Sub-lethal metal toxicity effects on salmonids: a review. Report prepared for Skeena Wild Conservation Trust. Smithers, BC.

[26] Afshan, S., Ali, S., Ameen, U. S., Farid, M., Bharwana, S. A., Hannan, F., Ahmad, R. (2014). "Effect of Different Heavy Metal Pollution on Fish" Res. J. Chem. Env. Sci., Volume 2 Issue 1 February 2014: 74-79.

[27] Rani, S., Gupta, R. K., Rani, M. (2015) "Heavy Metal Induced Toxicity in Fish with Special Reference to Zinc and

Cadmium" International Journal of Fisheries and Aquatic Studies 2015; 3(2): 118-123.

[28] Boyd, R.S. (2010). "Heavy metal pollutants and chemical ecology: exploring new frontiers." Journal of Chemical Ecology 36: 46-58.