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# Responses of Bioluminescent Bacteria Isolated from Philippine Marine Fishes to Various Heavy Metals

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**ABSTRACT**

The Vibrio/photobacterium and Vibrio Anguillarum identified using 16s rRNA gene from Lutjanus argentimaculatus and Leiognathus equulus respectively were exposed to different concentrations of selected heavy metals. Heavy metals normally found in contaminated water and soil were magnified in three different concentrations 1000x, 100x, 10x. Baseline data for these metals were based on the Philippine National Standards for drinking water (PNS) and the US Environmental Quality Standards for Soil Pollution. Bioluminescent bacteria were streaked in petri dishes of equal sizes. Ten replicates were made for every concentration and distilled water served as the control. The luminosity of the bioluminescent bacteria exposed to the heavy metals were measured using Image Processing and Analysis in Java(ImageJ®) in normalized light units for one hundred twenty minutes. Luminosity were measured every 30 minutes. Results showed that bioluminescent bacteria from Lutjanus argentimaculatus and Leiognathus equulus have various reactions to different heavy metals at different concentrations.



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## INTRODUCTION

Bioluminescence is the ability of certain organisms to emit light. The bioluminescent reaction is primarily caused by the luciferin and luciferase (*Jarup, 2003)*. The introduction of oxygen to luciferin with the aid of the catalyze luciferase causes the organism to emit light (*Jarup, 2003)*. Bioluminescent organisms are diverse. The phenomena can be observed in bacteria, dinoflagellates, radiolarians, ctenophores, molluscs, cnidarians, annelids, crustaceans, tunicates, echinoderms, fish and other worms (Steven *et al.*, 2009*)*.

Bioluminescent bacteria only glows if there is a sufficient number of cells present. This reaction is called quorum sensing. Quorum sensing is due to the production of and response to autoinducers. It is responsible for different processes such as genetic competencies, virulence, sporulation and bioluminescence in the case of the bioluminescent bacteria. Now they are linking the quorum sensing of the bacteria with starvation of the bacteria itself (Lazazzera, 2000) It is only after reaching a critical number of cells can the signal molecules bind with signal receptor present inside the bacterial cell which would result to a glowing effect (Antunes *et al.*, 2010).

These bacteria are also symbiotic to its environment and other forms of living organisms. One example would be its symbiotic relationship to the Hawaiian bobtail squid were the squid uses the glow of the bacteria in deterring potential predators (Stabb, 2005). Another example of symbiosis is the proliferation of other bacterium with the aid of the luminosity of the bacteria. This works by attracting potential predators to eat the host thus introducing the bacteria as well as the other bacterium to different organisms (Zarubin *et al.*, 2011).

Bioluminescent bacteria is commonly used as a biosensor for the presence of different environmental pollutants found in the water, soil and the atmosphere (Gu, 2005). Usually bioluminescent bacteria are used for vectoring for a more detailed result on the toxicity of a sample (Gu, 2005). It is due to the sensitivity of the gene *lux operon* to external sources like pollutants and heavy metals. This affects the intensity of glow of the bioluminescent bacteria. This makes it as an effective biosensor (Goklahe *et al.*, 2012).

Bioluminescent bacteria can also be used as a metal sensor due to its sensitivity (Broers, 2004). Bioluminescent bacteria's ability to luminesce is

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being used as of today as an indicator for different contaminants and heavy metals (Boynton,2009; Petanen and Romantschuk, 2002; Girotti, *et.al*, 2002). Testing its ability to detect heavy metals present in the water and soil system could prove to be beneficial for human health in terms of monitoring the different levels of the heavy metal contaminants. Bioluminescent bioassay was described as one of the express methods because it only needs micro quantities of pollutants in order to garner positive results. It gives an integral estimation of the toxicity present in a sample and it also surpass other known bioassays in terms of speed, accuracy, sensitivity and simplicity (Medvedeva *et al.,* 2009).

According to World Health Organization, metallic elements can be stored in tissues and organs. Commonly the elements that were ingested with food, water or acquired from environmental sources would be excreted in the form of urine and faeces(WHO, 2006) It is also observed that as we age the levels of Cd,Fe,Pb,V increases in man, while levels of Hg and Mn decreases as a person age.

This study aimed to determine the reaction of the bioluminescent bacteria to various concentrations of water and soil contaminant using the standards set by the PNS/FDA and the US Environmental Quality Standards for Soil Pollution as the baseline proving that bioluminescent bacteria can be used an alternative test for detecting water and soil contaminants.

## MATERIALS AND METHODS

***Collection and Identification of fish host samples:***

Philippine marine fishes were collected from the public markets of Iloilo City (*Lutjanus argentimaculatus*) and Olongapo City (*Leiognithus equulus*). The gut of the fishes were dissected and swabbed into a bioluminescent bacteria agar plate.

After determining the fish samples with BLB symbionts, the standard protocol in morphological identificationwas performed usingthe following references: Rau and Rau (1980)Carpenter and Niem (2001).FishBase, (Froese and Pauly, 2014). The specimen and descriptions were then verified by Dr. Roberto C. Pagulayan, a zoologist.

***Isolation of BLB Strains:***

All fishes were dissected immediately after the collection, swabbing first the eyes and the skin using sterile cotton swabs. The fish ventral area was then cut open exposing the stomach and the intestines which were cut laterally making the mucosa exposed. These areas were swabbed as well.

All cotton swabs were streaked on standard BLB agar plates incubated at $4^{o}$ C and room temperature for 24 hours.

***Molecular identification and phylogenetic analysis of BLB isolates with anti-nosocomial activity:***

The Insta Gene Matrix ™ DNA extraction kit was used in the isolation of the genomic DNA of the selected BLB isolates. Agarose gel electrophoresis was performed using1% agarose at 100 volts for 30 min using Gel XL Enduro™.

The 16S rRNA gene was amplified using KAPATaq DNA Polymerase™ PCR Kit using the concentrations following the manufacturer's instruction. The universal primers 27F (3'AGAGTTTGATCCTGGCTCAG5') and 1492R (5'GGTTACCTTGTTACGACT3') were used (Arakaki, 2010). Reactions were carried out using MyGene™ Series Peltier Thermal Cycler Model MG25+, with the following PCR conditions from the manufacturer.

PCR products were sent to AITBiotechPte Ltd, Singapore for DNA sequencing. The chromatograms were edited using CHROMAS LITE version 2.1. DNA sequences were checked for the presence of chimera using DECIPHER (Wright *et al*., 2012). The DNA sequences were compared with DNA sequences deposited in public database using the Basic Local Alignment Search Tool (BLAST ®) (Johnson *et al*. 2008). FASTA file of the BLB DNA sequences together with the similar species to the query based on the BLAST analysis were aligned using ClustalW in MEGA 6 (Tamura *et al.* 2013). Trees were calculated using the neighbor joining method bootstrapped 1000 times.

***Testing of solution:***

The introduction of the different water and soil contaminants on a glowing plate media were observed. Two hundred seventy microliters of each solution were introduced to each plate where the diameter of swabbed bioluminescent bacteria was uniform. Distilled water served as the negative control. The samples were then observed with an interval of 5 minutes for 2 hours. A digital camera was used in gathering the photos. The same settings and conditions were used in all solutions.

The change on the intensity of luminosity of each sample were computed with the aid of ImageJ®. Ten replicates per contaminant were tested to ensure accurate data. The raw data or the measured light unit (MLU) were then analysed in order to get the normalized light unit (NLU). The normalized light unit were used in the presentation of data found in the graphs.

## RESULTS AND DISCUSSIONS

Bioluminescent bacteria is one of the emerging subjects of research today due to its promising future as a biosensor for the health of the environment (Goklahe *et al*., 2011), tests that have the ability to benefit both the environment and man using bioluminescent bacteria in the study had shown its

ability to discern between different concentrations of each tested contaminant.

Figure 1.a shows the reaction of bioluminescent bacteria to aluminum sulfate where the luminosity increased in reaction to certain amounts particularly at 0.2 mg/L, 2 mg/L and 20 mg/L. The bioluminescent bacteria with the highest concentration of aluminum sulfate (200 mg.L) has the lowest luminosity. Figure 1.b shows the reaction of bioluminescent bacteria to arsenic trioxide where there was an induced brightness in the bioluminescent bacteria in the following concentrations, 0.05 mg/L and 5 mg/L while 50 mg/L concentration lowered the brightness of the bacteria. It was also observed that after 120 minutes the brightness of the bacteria exposed to the different concentrations of the water contaminant were higher than that of the control (distilled water). The reaction of bioluminescent bacteria with Zinc sulfate is shown in Figure 1.c. lower concentrations of the water contaminant induced the luminosity of the bacteria (5 mg/L, 50 mg/L and 500 mg/L) while higher concentration (5000 mg/L) lower the luminosity of the bacteria. It was observed that the *Vibrio*/photobacterium exposed to the different concentrations of water contaminants recover their brightness minutes after exposure.

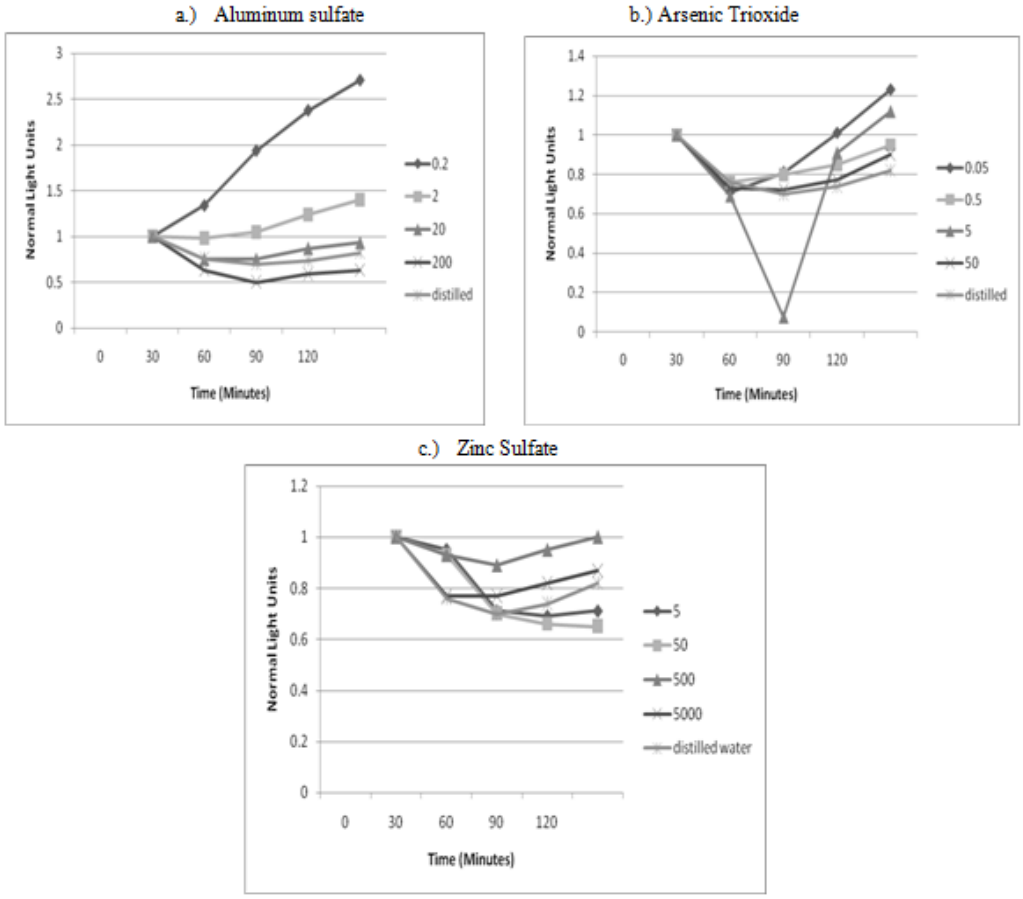


**Fig. 1:** The effect of the different concentrations of water contaminants a.) Aluminum sulfate b.) Arsenic Trioxide c.) Zinc Sulfate to *Vibrio/photobacterium* isolated from the gut of *Lutjanus argentimaculatus.*

Figure 2 shows a uniform pattern on the reaction of *Vibrio anguillarum* to the different concentrations of soil contaminants manganese (II) sulfate and silver nitrate. As seen in figure 2.a and 2b, the luminosity of the bioluminescent bacteria is lower as the concentration of the contaminant is higher. It is also evident that the luminosity of *Vibrio anguillarum* exposed to the soil contaminants is lower compared to the control (distilled water).

***Summary and Conclusion:***

In the tests regarding the contaminants in concentrations set by the Philippine National Standards and US Environmental Quality Standards for Soil Pollution, the reaction of the bioluminescent bacteria *Vibrio/photobacterium* and *Vibrio anguillarum* showed an immediate response after the addition of the contaminants.

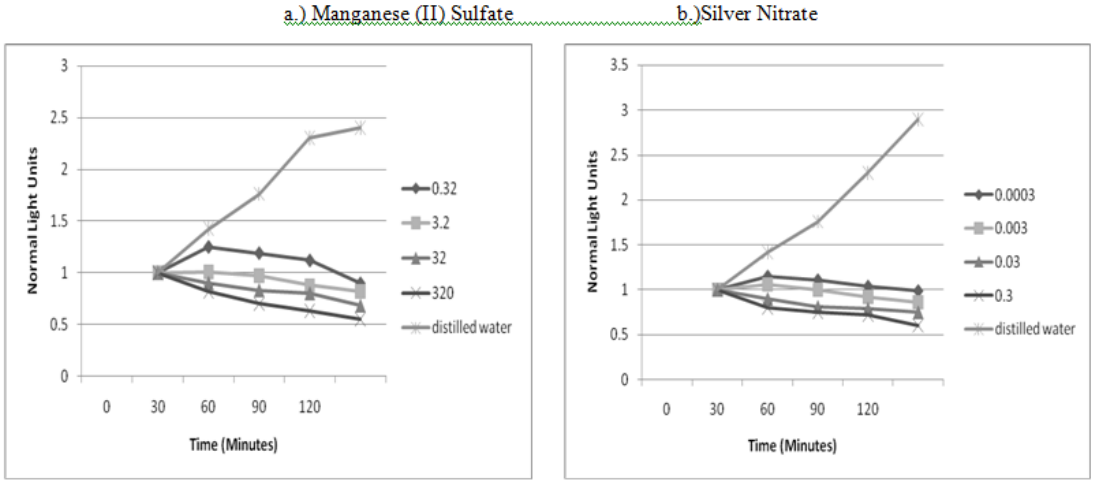


**Fig. 2:** The effect of the different concentrations of soil contaminants a.) Manganese (II) Sulfate b.) Silver Nitrate to *Vibrio anguillarum* isolated from the gut of *Leiognithus equulus.*

## REFERENCES

*Air quality guidelines global update, 2005. Particulate matter, ozone, nitrogen dioxide, and sulfur dioxide*. World Health Organization.

Antunes, L.C.M., R.B.R. Ferreira, M.C.C. Buckner, B.B. Finlay, 2010. Quorum sensing in bacterial virulence. Microbiology, 156: 2271-2282. DOI:10.1099/mic.0.038794-0.

Asuncion, S.B., 2012. Bioluminescence and the future of lighting. Manila, Philippines: Philippine Daily Inquirer.

Binaeian, E., A.M. Rashdi, MR. Toosi jamali, 2013Fabrication of biosensor for toxicity evaluation of some heavy metals and biocides. Sci.Int.(Lahore), 25(1): 103-110-106. ISSN 1013-5316.

Boynton, L., 2009. Using Bioluminescent Bacteria to Detect Water Contaminants. Journal of the U. S. SJWP, pp: 29-41.

Broers, C.A.M., J. Lappalainen, 2004. New developments in the bioluminescence assay. Courrier du Savoir, pp: 107-110.

Carpenter, K. E., V.H. Niem, 2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific.Volume 5.Bony fishes part 3 (Menidae to Pomacentridae). *FAO species identification guide for fishery purposes.The living marine resources of the Western Central Pacific.Volume 5. Bony fishes part 3 (Menidae to Pomacentridae)*, iv+-2791.

Charrier, T., M.J. Durrand, M. Affi, S. Jouanneau, H. Gezekel, G. Thouand, 2010. Baterial Bioluminescent Biosensor Characterisation for On-line Monitoring of Heavy metals Pollution in Waste Water Treatment Plant Effluents, Biosensor, Pier Andrea Serra (Ed.), ISBN:978-953- 7619-992.

Deng, H., MS. Li, YC. Zhou, 2012. Soil metal contamination and fractionation of tea plantations: case studies in a normal tea garden and in a restored mineland tea stand. *Environmental Studies*, 21(5): 1223.

Diels, L., M. De Smet, L. Hooyberghs, P. Corbisier, 1999. Heavy metals bioremediation of soil, *Mol Biotechnol*, 12(2):149-58.

Froese and Pauly, 2014. FishBase.World Wide Web electronic publication. www.fishbase.org, version (04/2014).

Girotti, S., L. Bolelli, A. Roda, G. Gentilomi, M. Musiani, 2002. Improved detection of toxic chemicals using bioluminescent bacteria. *Analytica Chimica Acta*, *471*(1): 113-120.

Goklahe, T., A. Wali, S. Parikh, N. Sood, 2012. Research of marine isolates in development of biosensors for environmental pollutants. Engineering review, 32(1): 17-22.

Gu, M.B., Environmental Biossensors Using Bioluminescent Bacteria.

Hagedorn, B., 2008. Acid extraction of sediments, sludges and soils. *Applied science engineering and technology laboratory*, pp: 3-4.

Harrison, R.N., 2003. What Yoi Should Know About Heavy Metals.

Hu, H., 2002. Human Health And Heavy Metals Exposure. MIT press

Jarup, L., 2003. Hazards of heavy metal contamination. British Medical Bulletin, 68: 167-172. DOI: 10.1093/bmb/Idg032.

Javed. M., N. Usmani, 2010. Accumulation of heavy metals in fishes: A human health concern. International journal of environmental sciences, 2(2): 659-670. ISSN 0976-4402

Johnson, M., I. Zaretskaya, Y. Raytselis, Y. S. Merezhuk, T.L. Madden, 2008. NCBI BLAST: a better web interface. *Nucleic acids research*, *36*(suppl 2): W5-W9.

Jomova, K., M. Valko, 2011. Advances in metal-induced oxidative stress and human disease. Toxicology, 283(2-3), doi: 10.1016/j.tox.2011.03.001.

Kamaruzzaman, B.Y., Z. Rina, B.A. John, K.C.A. Jadal, 2011. Heavy metal accumulation on commercially imporatant fishes of south west Malaysian coast. Research Journal on Environmental Science. DOI: 10.3823/rjes

Lazazzera, B.A., 2000. Quorum sensing and starvation: signals for entry into stationary phase. Current Opinion in Microbiology, 3: 177-182.

Lee, J.H., R.J. Mitchell, B.C. Kim, D.C. Cullen, M.B. Gu, 2005. A cell array biosensor for environmental toxicity analysis. *Biosensors and Bioelectronics*, *21*(3): 500-507.

Li, Y.F., F.Y. Li, C.L. Ho, V.H.C. Liao, 2008. Construction and comparison of fluorescence and bioluminescence bacterial biosensors for the detection of bioavailable toluene and related compounds. Environmental pollution, 152: 123-129. Retrieved from www.sciencedirect.com

Medvedeva, S.E., N.A. Tyulkova, A.M. Kuznetsov, E.K. Rodicheva, 2009. Bioluminescent Bioassays Based on Luminous Bacteria. Journal of Siberian Federal University, Biology, 4(2): 418-452, doi: 574.24:577.344:579.843

Mudga, V., M. Madaan, A. Mudgal, R.B. Singh, S. Mishra, 2010. Effect of Toxoc Metals on Human Health. The Open Nutraceuticals Journal, 3: 94-99.

Orengo, J.M., N. Eva, R. Marrero, C.R. Velazquez, 2010. Isolation and characterization of bioluminescent bacteria from marineenvirnments of Puerto Rico. Technology and education topics in applied microbiology and microbial technology.

Pastillas and Yema, Philippine National Standard. Recommended code of practice for the processing and handling of ethnic milk-based confectioneries. Standard parameters and values for drinking water (pp: 14-15). PNS/FDA 35:2011/ICS 67.100.10

Petänen, T., M. Romantschuk, 2002. Use of bioluminescent bacterial sensors as an alternative method for measuring heavy metals in soil extracts. *Analytica Chimica Acta*, *456*(1): 55-61.

Rau, N., A. Rau, 1980. *Commercial marine fishes of the central Philippines (bony fishes)*.

Robinson,G.M., K.M. Tonks, R.M.S. Thorn, D.M. Reynolds, 2011. Application of Bacterial Bioluminescence To Assess the Efficacy of Fast-Acting Biocides. American Society for Microbiology, 55(11): 5214-5219, doi:10.1128/AAC.00489-11

Sharma, D., A.K. Shukla, 2013. Analysis of Heavy Metals in Soil and Vegetables Irrigated with Wastewater in Meerut City Region. International Journal of Advanced Life Sciences (IJALS). ISSN 2277 – 758X

Stabb, E.V., D.S. Millikan, 2005. IS the Vibrio FIsheri- Euprymna scolopes symbiosis a defensive mutualism. In White, J. F. Jr, Torres, M. S(Eds), Defensive mutualism in microbial sysmbiosis, pp: 85-96.

Steven, H.D., Haddock, A.M. Mark, F. James Case, 2009. Bioluminescence in the Sea. Annu. Rev. Mar. Sci. 2010.2:443-93, doi: 10.1146/annurev-marin120308-081028

Stihi, C., C. Radulescu, G. Busuioc, I.V. Popescu, A. Gheboianu, A. Ene, 2009. Studies On Accumulation Of Heavy Metals From Substrate To Edible Wild Mushrooms. Rom. Journ. Phys, 56(1-2): 257-264.

Strosnider, H., 2003. Whole-Cell Bacterial Biosensors and the Detection of Bioavailable Arsenic.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30: 2725-2729.

Wannamaker, Anne Marie, 2013. "Quorum Sensing in Vibrios and Cross-Species Activation of Bioluminescence Lux Genes by VibrioHarveyi LuxR in an Arabinose-Inducible Escherichia Coli Expression System" (2013). Theses and Dissertations.Paper 177.

World Health Organization, 2006. World Health Organization. Regional Office for Europe.

Wright, 2012. "DECIPHER, A Search-Based Approach to Chimera Identification for 16S rRNA Sequences." Applied and Environmental Microbiology, doi:10.1128/AEM.06516-11.

Yoshizawa, S., M. Wada, K.K. Tsukamoto, A. Yokota, K. Kogure, 2009. Photobacterium aquimaris sp. Nov., a luminous marine bacterium isolated from seawater. International Journal of Systematic and Evolutionary Microbiology, 59: 1438-1442. DOI 10.1099/ijs.0.004309-0

Zarubin, M., S. Belkin, M. Ionescu, A. Genin, 2011. Bacterial bioluminescence as a lure for marine zooplankton and fish. Proceedings of the national academy of sciences of the United states of America. doi: 10.1073/pnas.1116683109