Investigating the Effects of Dyes on *Perna viridis* and the Methods for the Removal of Dyes

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Abstract

Many industries pollute the water by discharging coloured effluents, presenting a major environmental problem as they are typically toxic, carcinogenic and mutagenic to life forms. Activated carbon is commonly used to purify water commercially, but it is economically unviable. This study aims to investigate the ecological effects of waste dyes on *Perna viridis* and explore the effectiveness of utilising dragon fruit peels to remove waste dyes from water. The byssus thread count, clearance rate and neutral red retention time assays were conducted to study the physiological and cellular effects the dyes had on the health of the *Perna viridis.* Dragon fruit peels were then added to methylene blue solutions as an adsorbent and compared with activated carbon. Results showed that the presence of 10 ppm of methylene blue negatively impacted *Perna viridis* in all assays except byssus thread count, highlighting the importance of keeping the seas unpolluted. Dragon fruit peels have also proved to be effective adsorbents which are comparable to activated carbon and can potentially be a cost-effective alternative in the bioremediation of water as compared to activated carbon.

1. Introduction

Researchers have been investigating the ecological effects of industrial waste dyes on aquatic life such as fishes and algae. Many of these dyes contain chemicals, which are toxic or will pollute the water (Kant, 2012), and organic materials that react with many disinfectants such as chlorine to form products that are often toxic, carcinogenic or mutagenic (Suteu et al., 2009). Moreover, dyes also contain colour pigments and oily scums which not only gives the water it is in a foul smell and bad appearance but also blocks the penetration of light into the water, which reduces the rate of photosynthesis of plants in polluted water. This inevitably reduced the level of oxygen in the water, thus affecting aquatic life (Kant, 2012; Gita, Hussan & Choudhury, 2017). Textile effluents containing dyes discharged into water caused certain fishes to develop physically abnormal behaviours (Gita et al., 2017), unusual internal organs (Karthikeyan, Jambulingam,

Sivakumar, Shekhar & Krithika, 2006) and production of thick mucus on their bodies as observed (Selvaraj, Leena & Kamal, 2015).

Byssus threads are secreted by the byssus gland at the base of the foot of mussels (Yonge, 1962). The byssus thread secretion in mussels is influenced by many factors, both exogenous and endogenous factors, including environmental factors (Zardi, Mcquaid & Nicastro, 2007). Variations in byssus thread production with changes in environmental conditions have been reported for several species of mussels (Winkle, 1970; Price, 1980; Meadows & Shand, 1989), and this could potentially serve as a biomonitoring tool for environmental contamination (Goh, Lai, Tan, Yap & Dissanayake, 2014).

Being sessile filter feeders, water processing capabilities of *Perna viridis* directly translates to feeding efficacy and is integral to organismal health and survival (Goh et al., 2014). Studies on physiological responses of bivalves have shown reduction in feeding rates with exposure to environmental stress factors like sedimentation (Bricelj & Malouf, 1984; Ward & MacDonald, 1996; Baco, MacDonald & Ward, 1998) or heavy metals (Watling, 1981; Grace & Grainey, 1987). To find out the effect of pollutants in the water on mussels, the volume of seawater filtered by the mussels can be found out which can be assessed by means of microalga feed removal (clearance rate) from a fixed volume of water (Coughlan, 1969).

Molluscs such as mussels have organs and tissues which are highly dependent on an extensively developed lysosomal system: subcellular organelles surrounded by a semipermeable membrane that contain numerous hydrolytic enzymes involved in a range of cellular processes including digestion, defense, and reproduction (Moore, 1980; Pipe, 1992; Ferreira & Dolder, 2003). Lysosomes play an important role in the detoxification and defence in marine organisms (Moore, 1980; Lowe et al., 1995a, b), and may accumulate a variety of toxic metals and organic chemicals. The stability of the lysosomal membrane can then be used to determine their efficiency in performing these functions, giving rise to the neutral red retention assay. (Lowe et al., 1992, 1995a, b; Goh et al., 2014).

As the impact of dyes on marine organisms cannot be underestimated, it is paramount to find solutions to remove waste dyes from water. Activated carbon is commonly used to purify water commercially, but it may not be cost-effective (EPA Alumni Association, n.d.). Hence, this study explored the possibility of using dragon fruit peels as an economically viable adsorbent. In

Vietnam, the total annual production of dragon fruit is around 700,000 tonnes (FFTC, 2018). As 22-44% of a dragon fruit is made up of the rind (Cheok, 2016), usually discarded, this results in huge amounts of waste, which can be harvested for the bioremediation of polluted water. Ground dragon fruit peel was also embedded in alginate beads to improve the ease of retrieval after use.

2. Objectives and Hypotheses

The objectives of this study are to investigate the ecological effects of waste dyes on *Perna viridis* as well as the effectiveness of dragon fruit peels in removing waste dyes from water.

The hypotheses of this study are that exposure of mussels to methylene blue treated water will reduce clearance rate, neutral red retention time, and byssus thread count of *Perna viridis*; and that dragon fruit peels are comparable to activated carbon in the bioremediation of water treated with methylene blue.

3. Methods and Materials

3.1. Materials

For the investigation of the ecological effects of waste dyes on *Perna viridis*, the *Perna viridis* were bought from local sources. For the investigation of the effectiveness of dragon fruit peels in the bioremediation of polluted water, the dragon fruit peels were sourced from a local supermarket. Instant Ocean aquarium salt was bought from Aquarium Systems, while microalga feed was bought from Reed Mariculture.

3.2. Methods

The experimental procedure is split into 3 segments. Firstly, *Perna viridis* was pre-treated in artificial seawater containing methylene blue at 10 parts per million (ppm). Afterwards, three biological assays (byssus thread count, clearance rate, neutral red retention time) were conducted. In the second part of the experiment, water with methylene blue was bioremediated with dragon fruit peels and activated carbon separately and compared. The possibility of using sodium alginate beads to encapsulate the dragon fruit peels was also explored.

3.2.1. Preparation of artificial seawater

357.89 grams of Instant Ocean aquarium salt was dissolved in 10 litres of deionised water to form artificial seawater which would then be stored in an air-tight container for later use.

3.3. Byssus thread count assay

3.3.1. Pre-treatment

Five crystallisation dishes were set up each for the control set-up, which contained 1 litre of artificial seawater per dish; and the treatment set-up, which contained methylene blue at 10ppm in addition to artificial seawater. Three *Perna viridis* with their byssus threads removed at the start of the experiment were placed into each dish with their dorsal sides facing the side of the dish. 1 ml of Reed Mariculture microalga feed was provided in each dish, along with constant aeration.

3.3.2. Counting of byssus thread

After 24 hours of pre-treatment, the number of byssus threads produced by each mussel was counted. Every individual thread was considered as one thread. If the threads were bundled up, every thread that could be identified as an individual thread was considered as one thread, and the rest of the bundle was considered as one thread.

3.4. Clearance rate assay

3.4.1. Pre-treatment

Two crystallisation dishes were set up each for the following set-ups: the control, which contained 1 litre of artificial seawater per dish; the 5ppm methylene blue and 10ppm methylene blue set-ups, which contained the corresponding concentration of methylene blue per dish in addition to seawater. Three *Perna viridis* and 1 ml of microalga feed was added into each dish, along with constant aeration.

3.4.2. Feeding of mussels

After 24 hours of pre-treatment, the mussels in each crystallisation dish were transferred into separate clean crystallisation dishes which each contained 1 litre of artificial seawater and 1 ml of microalga feed, along with constant aeration. After making sure that the microalga feed was spread evenly across the seawater, 200 µl of the mixture was aliquoted from each dish into Eppendorf tubes. At the end of 1 hour of feeding, another 200 µl of the mixture was aliquoted.

3.4.3. Counting of microalga cells

In each Eppendorf tube, 20 µl of the mixture was loaded into a haemocytometer. The haemocytometer was placed under the microscope so the microalga cells could be enumerated.

3.5. Neutral red retention time assay

3.5.1. Pre-treatment

One crystallisation dish was set up each for the following set-ups: the control, which contained 1 litre of artificial seawater per dish; the 5ppm methylene blue and 10ppm methylene blue set-ups, which contained the corresponding concentration of methylene blue per dish in addition to seawater. In the crystallisation dish belonging to the control set-up, 1 litre of artificial seawater was added. Five *Perna viridis* and 2 ml of microalga feed was added into each dish, along with constant aeration.

3.5.2. Pre-experiment preparations

Firstly, the microscope slides that would be used for this assay had to be coated with Poly-L-Lysine diluted with deionised water at a ratio of 1:10 and air-dried. Secondly, Marine Bivalve Physiological Saline was prepared so that the mussel cells could be transferred from place to place without feeling stressed. Deionised water was added to 4.77g of hepes, 25.48g of sodium chloride, 13.06g of magnesium sulfate, 0.75g of potassium chloride and 1.47g of calcium chloride until the volume of the mixture is 1 litre. Thirdly, the Neutral Red stain (working solution) was prepared. The stock solution prepared prior to the experiment was removed from the fridge so that it could reach room temperature. 995 µl of physiological saline and 5µl of the NR stock solution was added into a 2ml smoked vial before they were mixed by vortexing.

3.5.3. Recording of neutral red retention time

After 1 hour of pre-treatment, the mussels (N = 15) were opened using a fixed scalpel blade and the excess seawater was drained out from the shell cavity. 0.5 ml of haemolymph was then drawn from each mussel into a syringe which already contained 0.5 ml of physiological saline. The haemolymph was transferred into pre-siliconised microcentrifuge tubes. 40 µl of the haemolymph mixture from each tube was transferred onto the centre of a Poly-L-Lysine coated microscope slide. All the slides were then incubated in a light proof humidity chamber for 15 minutes. Following the incubation period, the excess physiological saline/haemocyte mixture was tapped off the slides and 40µl of the working neutral red solution was added to the cells on the slide. All the slides were incubated in the light proof humidity chamber for another 15 minutes to allow the dye to penetrate the lysosomal compartment of the cells. Afterwards, each slide was

periodically examined under the light microscope and the time taken for 50% of the cells in view to release dye from their lysosomes was recorded. Cells which have released dye from their lysosomes tend to be the more stressed cells. They are typically rounder in shape, smaller in size, and have darker and larger lysosomes.

3.6. Bioremediation of dyes using dragon fruit peels

3.6.1. Preparation of dragon fruit peels

The dragon fruit peels were cut into smaller pieces, washed with deionised water and then dried out in the sun. Using a blender, the dragon fruit peels were blended into granular sizes.

3.6.2. Preparation of sodium alginate beads

8 ml of sodium alginate solution and 1 g of adsorbent were added to a beaker containing 8 ml of deionised water. The contents were mixed thoroughly using a glass rod. Then, a syringe was used to suck in the mixture before the mixture was dripped into another beaker containing 100 ml of calcium chloride solution. Beads averaging 5 mm in diameter were produced with every drip. After the mixture in the syringe had been depleted, the beads were sieved out of the calcium chloride solution and rinsed with deionised water twice. The beads were finally stored in a beaker containing deionised water to prevent them from drying out.

3.6.3. Bioremediation

Six groups of three beakers were set up, each containing 100 ml of 10 ppm methylene blue. 1 ml of the solution in each bottle was transferred into cuvettes which were analysed using a UV-Visible Spectrophotometer at 664 nm. Each beaker in every group of beakers except the control carried added substances: 1 g of dragon fruit peels, 1 g of activated carbon, sodium alginate beads carrying a total of 1 g of dragon fruit peels, sodium alginate beads carrying a total of 1 g of activated carbon and empty sodium alginate beads were added to each of the bottles. The bottles were left on the magnetic stirrer to stir for 24 hours. 10 ml of the solution in each bottle was then transferred into centrifuge tubes. The solutions which originated from bottles without the addition of alginate beads were centrifuged at 8500 rpm for 10 minutes and filtered. The supernatant in each tube was transferred into cuvettes which were analysed using the spectrophotometer at 664 nm.

4. Results and Discussion

4.1. Byssus Thread Count

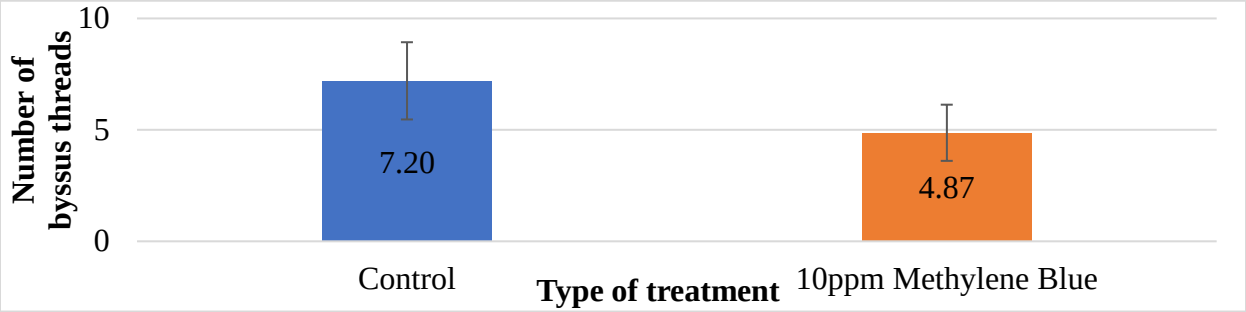

Figure 1: Graph showing the effect of 10 ppm methylene blue on the average number of byssus threads produced by the mussels

Figure 1 shows that the mussels that were exposed to 10ppm methylene blue dye grew an average of 4.87 threads while those that were exposed to artificial seawater without methylene blue grew an average of 7.20 threads. The mussels in the control set-up grew an observably greater number of byssus threads than those in the treated set-up. A Mann-Whitney U-Test was carried out which revealed that $p > 0.05$ which showed that the results are not statistically significant. Being an environmental stressor, the 10ppm methylene blue dye likely caused the mussels close their valves and subsist on anaerobic respiration to reduce exposure. Thus, in order to survive, the energy supplied to various physiological processes not critical for survival, including byssus thread production, would be limited (Goh et al., 2014). The result is similar to that reported by Winkle, (1970) and Price, (1980), when the mussels were subjected to other environmental stressors.

4.2. Clearance Rate

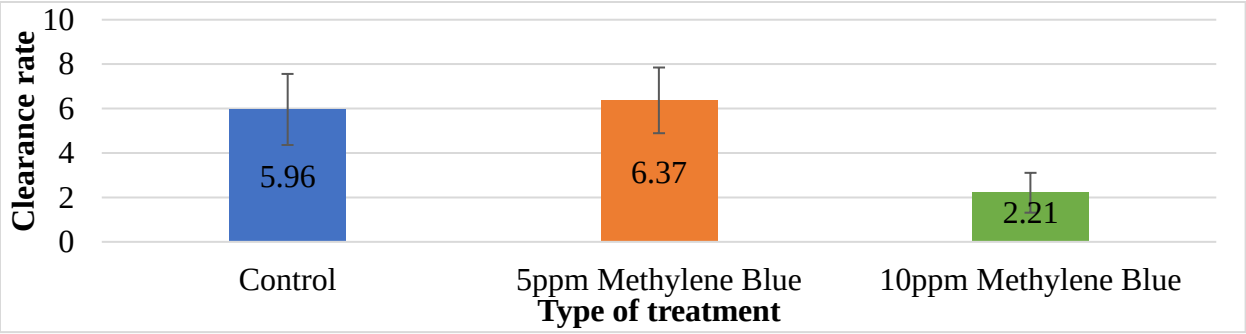

Figure 2: Graph showing the effect of different concentrations of methylene blue on the clearance rate of mussels.

Figure 2 shows that the mussels that were exposed to artificial seawater without methylene blue had an average clearance rate of 5.96. The mussels that were exposed to artificial seawater with methylene blue at 5 ppm and 10 ppm had average clearance rates of 6.37 and 2.21 respectively. A Kruskal-Wallis Test was conducted on the three set-ups which revealed that $p < 0.05$. A Mann-Whitney U-Test was carried out with the control and the set-up with methylene blue added at 5 ppm which revealed that $p > 0.05$. Another Mann-Whitney U-Test was carried out with the control and the set-up with methylene blue added at 10 ppm which revealed that $p < 0.05$. In addition to the reduced energy supplied for feeding when the mussels were subjected to the 10ppm methylene blue dye environmental stressor due to their subsistence on anaerobic respiration, feeding is also decreased due to reduced filtering when the valves are closed to reduce exposure to the environmental stressor. This is similar to the results when the mussels were subjected to other types of pollution, as reported by Bacon et al., (1998), Wang et al. (2011), Watling, (1981).

4.3. Neutral Red Retention Time

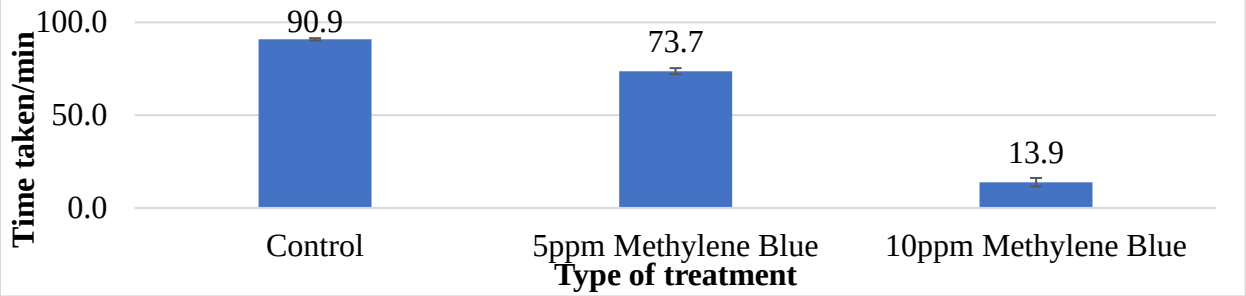

Figure 3: Graph showing the effect of different concentrations of methylene blue on the time taken for 50% of the cells to lyse when treated with neutral red

Figure 3 shows that the mussels that were exposed to artificial seawater without methylene blue had an average neutral red retention time of 13.9 minutes. The mussels that were exposed to artificial seawater with methylene blue at 5 ppm and 10 ppm had average neutral red retention times of 73.7 and 90.9 minutes respectively. A Kruskal-Wallis Test was conducted on the three set-ups which revealed that $p < 0.05$. The methylene blue has affected the lysosomes of the cells not only directly on the membrane but also by activating (or downregulating) calcium- and tyrosine kinase-dependent cell signalling pathways. This destabilised the lysosomal membrane, as they are membrane dependant processes, which in turn decreased the ability of the lysosomes to retain the Neutral Red dye. Under physiological stress, the lysosomes lysed readily releasing neutral red to

the rest of the cytoplasm. As a result, the lysosomes of the mussels exposed to 10 ppm methylene blue retained the neutral red for the shortest duration after uptake. This trend has also been observed in similar studies such as those by Moore, (1980), Lowe et al., (1995a, b).

4.4. Bioremediation of dyes using dragon fruit peels

4.4.1. Standard curve

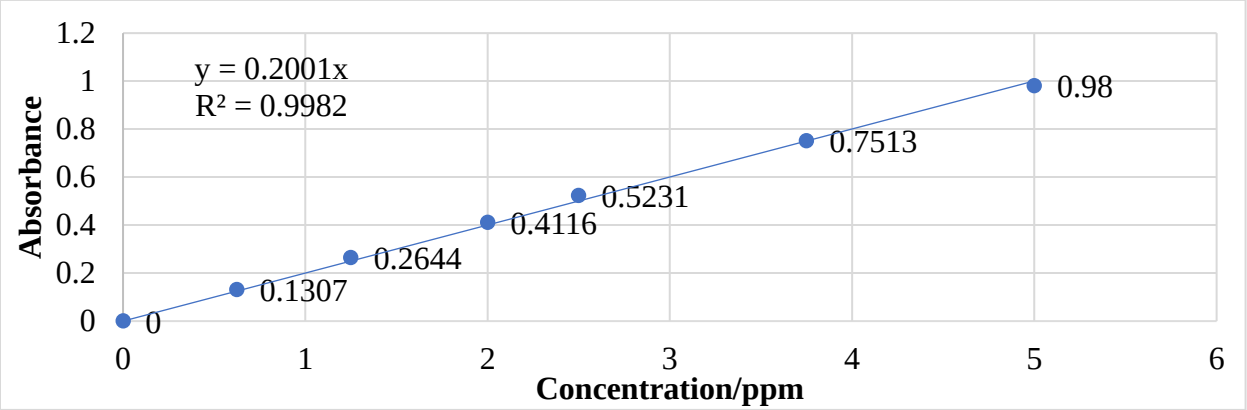

Figure 4: Calibration curve showing the concentrations corresponding to absorbance

4.4.2. Percentage of methylene blue dye adsorbed by adsorbents

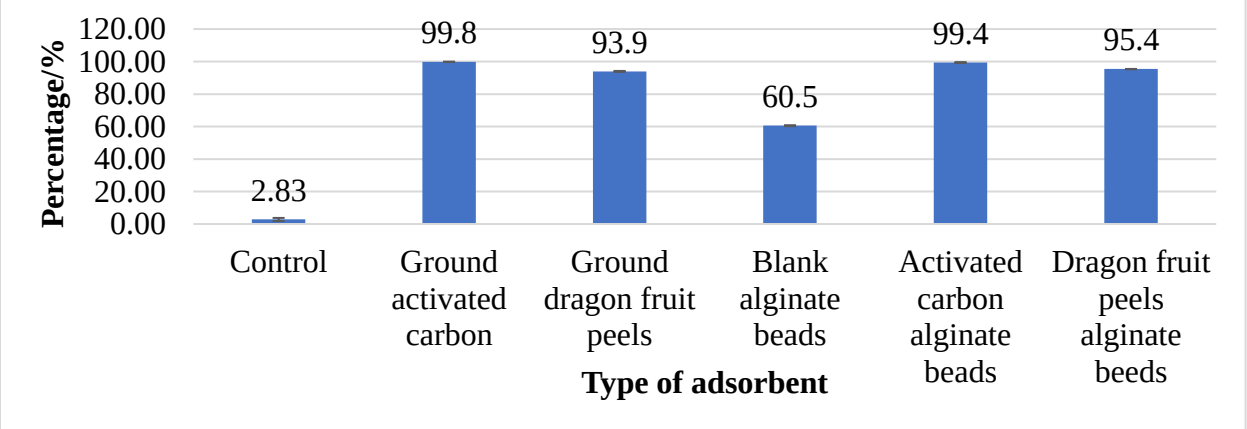

Figure 5: Percentage of methylene blue dye adsorbed by adsorbents

Figure 5 shows that dragon fruit peels adsorbed 93.9% of the methylene blue dye which is comparable to activated carbon which adsorbed 99.8% of the dye, suggesting that dragon fruit peels has great potential to be further developed into a commercial product. A Kruskal-Wallis test was conducted on all six sets of data which revealed that $p < 0.05$. Using sodium alginate beads to contain the adsorbents had negligible effect on the percentage of dye except in the case of the

control, as the blank alginate beads had adsorbed some methylene blue. Cellulose found in dragon fruit peels has hydroxyl groups, which is anionic, it can attract the cationic ions found in methylene blue, which makes dragon fruit peels a good adsorbent of methylene blue. Moreover, using alginate beads has not compromised the ability of dragon fruit peels to adsorb methylene blue. Hence, the dragon fruit peels embedded in alginate beads can be easily retrieved after use.

5. Conclusion

Perna viridis experienced a significant reduction in clearance rate and neutral red retention time when exposed to methylene blue dye. There was an observable reduction in byssus thread count in mussels exposed to 10 ppm methylene blue dye, but the large variation made the results statistically insignificant. It can be concluded that when methylene blue dye is present in seawater, *Perna viridis* will face many ecological impacts that are detrimental to its survival. It is possible that other marine organisms such as crustaceans and fish will also be affected by methylene blue as *Perna viridis* is a reliable bioindicator of pollution. The ecological impact of pollution of seawater has dire consequences especially if the effect is bioamplified as it progresses up the food chains. Water pollution must be curbed to deter the mass extinction of marine organisms. In addition, this study has shown that dragon fruit peel is comparable in effectiveness to commercial activated carbon in the bioremediation of water polluted by methylene blue dye. Dragon fruit peels can hence potentially be a more economically viable alternative to commercial activated carbon.

6. Future Work

Due to bioamplification, predators of *Perna viridis* will accumulate exponentially higher concentrations of pollutants. Hence, it is necessary to investigate the effects of both consuming *Perna viridis* which have consumed dyes and other pollutants such as nitrates, as well as the effects of direct exposure to pollutants on other organisms. More assays can also be conducted such as phagocytosis, cell viability and glycogen analysis to further investigate the potential effects of dyes on humans.

References

Abu-El-Halawa, R., Zabin, S. A., & Abu-Sittah, H. H. (2016). Investigation of Methylene Blue Dye Adsorption from Polluted Water Using *Oleander* Plant (*Al Defla*) Tissues as Sorbent. *American Journal of Environmental Sciences, 12*(3), 213-224. doi:10.3844/ajessp.2016.213.224

Bacon, G. S., MacDonald, B. A., & Ward, J. (1998). Physiological responses of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles: I. Feeding activity and selection. *Journal of Experimental Marine Biology and Ecology, 219*(1-2), 105-125. doi:10.1016/s0022-0981(97)00177-9

Bricelj, V. M., & Malouf, R. E. (1984). Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. *Marine Biology, 84*(2), 155-165. doi:10.1007/bf00393000

Cheok, C. Y., Adzahan, N. M., Rahman, R. A., Abedin, N. H., Hussain, N., Sulaiman, R., & Chong, G. H. (2016). Current trends of tropical fruit waste utilization. *Critical Reviews in Food Science and Nutrition,* 1-27. doi:10.1080/10408398.2016.1176009

Coughlan, J. (1969). The estimation of filtering rate from the clearance of suspensions. *Marine Biology, 2*(4), 356-358. doi:10.1007/bf00355716

Dillon, E. C., Wilton, J. H., Barlow, J. C., & Watson, W. A. (1989). Large surface area activated charcoal and the inhibition of aspirin absorption. *Annals of Emergency Medicine, 18*(5), 547-552. doi:10.1016/s0196-0644(89)80841-8

EPA Alumni Association. (n.d.). Senior EPA officials discuss early implementation of the Safe Drinking Water Act of 1974. Retrieved from https://www.epaalumni.org/history/video/interview.cfm?id=13

Ferreira, A., & Dolder, H. (2003). Cytochemical study of spermiogenesis and mature spermatozoa in the lizard *Tropidurus itambere* (Reptilia, Squamata). *Acta Histochemica, 105*(4), 339-352. doi:10.1078/0065-1281-00731

FFTC. (2018, June 28). Asia-Pacific Information Platform on Agricultural Policy-Dragon Fruit Production in Vietnam: Achievements and Challenges. Retrieved from http://ap.fftc.agnet.org/ap_db.php?id=873

Goh, B.P.L., Lai, C.H., Tan, L.T., Yap, N.W.L. & Dissanayake, A. (2014) Handbook of Marine Ecotoxicology Techniques. National Institute of Education, Nanyang Technological University. National Parks Board, Singapore, 110 pp.

Gita, S., Hussan, A., & Choudhury, T. G. (2017). Impact of Textile Dyes Waste on Aquatic Environments and its Treatment. *Environment & Ecology, 35*(3C). Retrieved from https://www.researchgate.net/profile/Ajmal_Hussan2/publication/321443064_Impact_of_Textile_Dyes_Waste_on_Aquatic_Environments_and_its_Treatment/links/5a21b08baca2727dd87ac403/Impact-of-Textile-Dyes-Waste-on-Aquatic-Environments-and-its-Treatment.pdf.

Grace, A. L., & Gainey, L. F. (1987). The effects of copper on the heart rate and filtration rate of *Mytilus edulis*. *Marine Pollution Bulletin, 18*(2), 87-91. doi:10.1016/0025-326x(87)90574-1

Green, R. B., Moskovitz, R. L., & Dalal, N. S. (1979). Detection of Metal Ions by Quenching of Dye Laser Emission. *Spectroscopy Letters, 12*(11-12), 849-856. doi:10.1080/00387017908069209

India, National Institution for Transforming India (NITI Aayog). (2018, June 12). *Composite Water Resources Management: Performance of States*. Retrieved August 3, 2019, from https://niti.gov.in/writereaddata/files/document_publication/2018-05-18-Water-Index-Report_vS8-compressed.pdf

Kant, R. (2012). Textile dyeing industry an environmental hazard. *Natural Science, 04*(01), 22-26. doi:10.4236/ns.2012.41004

Karthikeyan, S., Jambulingam, M., Sivakumar, P., Shekhar, A. P., & Krithika, J. (2006). Impact of Textile Effluents on Fresh Water Fish *Mastacembelus Armatus* (Cuv. & Val). *E-Journal of Chemistry, 3*(4), 303-306. doi:10.1155/2006/701612

Lachance, A., Myrand, B., Tremblay, R., Koutitonsky, V., & Carrington, E. (2008). Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. *Aquatic Biology, 2*, 119-129. doi:10.3354/ab00041

Lowe, D. M., Fossato, V., & Depledge, M. (1995a). Contaminant-induced lysosomal membrane damage in blood cells of mussels *Mytilus galloprovincialis* from the Venice Lagoon: an in vitro study. *Marine Ecology Progress Series, 129*, 189-196. doi:10.3354/meps129189

Lowe, D. M., Moore, M. N., & Evans, B. M. (1992). Contaminant impact on interactions of molecular probes with lysosomes in living hepatocytes from dab *Limanda limanda*. *Marine Ecology Progress Series, 91,* 135-140. doi:10.3354/meps091135

Lowe, D. M., Soverchia, C., & Moore, M. N. (1995b). Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. *Aquatic Toxicology, 33*(2), 105-112. doi:10.1016/0166-445x(95)00015-v

Meadows, P. S., & Shand, P. (1989). Experimental analysis of byssus thread production by *Mytilus edulis* and *Modiolus modiolus* in sediments. *Marine Biology, 101*(2), 219-226. doi:10.1007/bf00391461

Moore, M.N. (1980). Cytochemical determination of cellular responses to environmental stressors in marine organisms. Rapports et Proces-verbaux des Réunions. *Conseil International pour l'Éxploration de la Mer 170,* 7–15.

Pipe, R. K. (1992). Generation of reactive oxygen metabolites by the haemocytes of the mussel *Mytilus edulis*. *Developmental & Comparative Immunology, 16*(2-3), 111-122. doi:10.1016/0145-305x(92)90012-2

Price, H. A. (1980). Seasonal variation in the strength of byssal attachment of the common mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom, 60*(4), 1035-1037. doi:10.1017/s0025315400042065

Rafatullah, M., Sulaiman, O., Hashim, R., & Ahmad, A. (2010). Adsorption of methylene blue on low-cost adsorbents: A review. *Journal of Hazardous Materials, 177*(1-3), 70-80. doi:10.1016/j.jhazmat.2009.12.047

Seglen, P. O. (1983). [59] Inhibitors of lysosomal function. *Methods in Enzymology Biomembranes Part J: Membrane Biogenesis: Assembly and Targeting (General Methods, Eukaryotes),* 737-764. doi:10.1016/s0076-6879(83)96063-9

Selvaraj, D., Leena, R., & Kamal, D. C. (2015). Toxicological and histopathological impacts of textile dyeing industry effluent on a selected teleost fish *Poecilia reticulata*. *Asian Journal of Pharmacology and Toxicology, 3*(10), 26-30. Retrieved from http://www.asianpharmacology.com/uploads/351-963-1-PB.pdf

Suteu, D., Zaharia, C., Bilba, D., Muresan, R., Popescu, A., & Muresan, A. (2009). Decolorization wastewaters from the textile industry – physical methods, chemical methods. *Industria textilă 60*, 254-263.

Tan, Leo W. H. & Ng, Peter K. L., 1988. Green Mussel (*Perna viridis*) A Guide to Seashore Life. The Singapore Science Centre, Singapore. 160 pp. Retrieved from: http://mangrove.nus.edu.sg/pub/seashore/text/158.htm

Wang, Y., Hu, M., Wong, W. H., Shin, P. K., & Cheung, S. G. (2011). The combined effects of oxygen availability and salinity on physiological responses and scope for growth in the green-lipped mussel *Perna viridis*. *Marine Pollution Bulletin, 63*(5-12), 255-261. doi:10.1016/j.marpolbul.2011.02.004

Ward, J. E., & MacDonald, B. A. (1996). Pre-ingestive feeding behaviours of two sub-tropical bivalves (*Pinctada imbricata* and *Arca zebra*): Responses to an acute increase in suspended sediment concentration. *Bulletin of Marine Science, 57*, 417-432.

Watling, H. (1981). The Effects Of Metals On Mollusc Filtering Rates. *Transactions of the Royal Society of South Africa, 44*(3), 441-451. doi:10.1080/00359198109520587

Winkle, W. V. (1970). Effect of environmental factors on byssal thread formation. *Marine Biology, 7*(2), 143-148. doi:10.1007/bf00354918

Yonge, C. M. (1962). On The Primitive Significance of the Byssus in the Bivalvia and its Effects in Evolution. *Journal of the Marine Biological Association of the United Kingdom, 42*(1), 113-125. doi:10.1017/s0025315400004495

Zardi, G. D., Mcquaid, C. D., & Nicastro, K. R. (2007). Balancing survival and reproduction: Seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Marine Ecology Progress Series, 334*, 155-163.doi:10.3354/meps334155