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TOXICITY AND WATER QUALITY ASSESSMENT OF PASIG RIVER, PHILIPPINES USING ZEBRA FISH (*Danio rerio*) EMBRYO MODEL

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ABSTRACT

Daniorerio embryos at segmentation phase were exposed to water samples collected from five selected ferry stations of the Pasig River in the Philippines. The mortality, hatchability, growth retardation, tail malfomation rates were determined and the morphological phenotypes of exposed embryos were examined. The physico-chemical characteristics of the river water were also determined. Results revealed that Pasig River water had a maximum temperature of 30.7°C, pH range of 7.8 to 8.5, dissolved oxygen range of 0.9 to 8.1 mg/l, phosphate content range of -0.80 to 0.28 mg/l andmaximum level of silicate of 8.54 mg/l. Embryos exposed to water samples collected from Escolta and PinagbuhatanStations at 24 hours exposure showed 16.67% and 8.33% mortality, respectively. Mortality of Escolta Station water exposed embryos increased to 41.67% after 36 hours and 66.67% after 48 hours, which recorded the highest mortality. PinagbuhatanStation water-exposed embryos recorded the highest percentage hatchability of 83.33% whereas the lowest percentage hatchability of embryos was found in Escolta water with 16.67%. Embryos exposed to Escolta water had the highest percentage of growth retardation of 50% and highest percentage tail malformation of 41.67%.Several abnormal phenotypes were observed in river water-exposed embryos, including hypo-pigmentation, wavy somites, bent tail, irregular shape of tail, lengthy tail, deformed yolk, pericardial edema, girth between head and body

region.Overall, the aforementioned results indicate that Pasig River water is polluted with chemical or substances which may cause toxic and teratogenic effect in aquatic organisms.

Keywords: Ecotoxicology, *Daniorerio*, Pasig River, Aquatic toxicity

INTRODUCTION

Ecotoxicology provides better understanding on the mechanism and attributes of aquatic toxicity which are essential for risk assessment and environmental protection. In several studies conducted, aquatic toxicity assessment is carried out using different model organisms such as zebrafish (*Daniorerio*), medaka (*Oryziaslatipes*), fat-head minnow (*Pimephalespromelas*) and three-spinedstickleback (*Gasterosteusaculeatus*), guppy (*Poeciliareticulata*), frog (Ranapipiens) tadpoles, and water flea (*Daphnia magna*)**[1, 2, 3, 4]**. In this context, results of toxicity test using aquatic organisms would represent the effect of a wide range of toxic compounds and pollutants in order to characterizethe water quality.

Among fish models, zebrafish,a tropical freshwater fish belonging to family Cyprinidae, has emerged as a popular vertebrate model in biology, oncology, toxicology, teratology, genetics, environmental sciences**[5, 6, 7, 8]**due to the unique and advantageous characteristics. Zebrafish has very rapid developmental processes, high fecundity, transparency, easy maintenance in the laboratory,accessible to experimental manipulation and similarity to the embryonic development of higher forms of vertebrates. In addition, zebrafishis a suitable vertebrate model in toxicity assessment*in-vivo* and serve as an intermediate between cell-based and mammalian testing**[9]**.

Pasig River is one of the major rivers in the Philippines and forms the most important natural water system in greater Metro Manila area. The river passes through the urban areas of the metropolis from its upstream portion west of Laguna De Bay, moving downstream to east of Manila Bay. It traverses five cities (Manila, Mandaluyong, Makati, Taguig and Pasig) and one municipality of Rizal (Taytay). Due to the industrialization and urbanization in Metro Manila, Pasig River transformed into sewage and a depot for industrial wastes. Pasig River is filled with oil slicks, has unpleasant odor, dark colored water, hyacinth blooms, and floating garbage and feces. It is also known to have high organic loads and contaminated with heavy metals, pesticides, nitrates and phosphates. The presence of these materials has degraded the water quality of Pasig

River consequently upsetting its ecological balance **[10]**.

To the best of our knowledge,there were no reported data on the aquatic toxicity and teraogenicity assessment of Pasig River water using zebrafish embryo as model. Thus, this present work was carried-out in this context. The physico-chemical characteristics of Pasig River water were likewise determined and its effect on the developing zebrafish embryos was correlated.

MATERIALS AND METHODS

Sampling Sites

At present, the Pasig River Ferry System which is under the operation of Metro Manila Development Authority (MMDA) is being operated with eleven stations. To cover the conditions of the 11 station, the following stations were considered in the present study:Pinagbuhatan, Guadalupe, PUP, Hulo, and Escolta. Water sample each stationwas collected using water sampler and placed in empty bottles. The study was done on July 24, 2015 between 8:00 AM to 1:30 PM under a fair weather condition.The water samples were stored in the dark room at 5^{o}C until the analysis.

Determination of Physico-chemical Characteristics

The dissolved oxygen and water temperature were measured using a Jenway 980 Oxygen meter. The probe was immersed2 inches below the surface of the waterfor 2 minutes until the reading was stabilized. Using a digital pH meter, the pH of the water was determined by filling-up a 500 ml capacity beaker and immersed the probe of the meter for few seconds until reading was determined. The probes were washed with distilled water in every test conducted. The two important chemical attributes,phosphate and silicate, of water samples were analyzed using atomic absorption spectrophotometer at 880 nm and 820 nm absorbance, respectively.

Danio rerio Embryo

Adult zebra fish (7 females and 14 males) were confined in a plastic mesh in an aquarium covered with black plastic for 12 hours. After which, the light was turned on for 12 hours to allow fertilization.Fertilized eggs were siphoned out of the aquarium using a hose and were rinsed three times with embryo water. Embryos were examined using a microscope to ensure the uniformity of developmental phase and normal condition of the embryos. Unfertilized and coagulated eggs were discarded.

Embryo-toxicity and Teratogenicity Assay

Three ml of each water sample together with embryo water (control) were dispensed into the well of the 12-well ELISA plate. Triplicate per treatment was

carried out. Four embryos at segmentation phase were exposed to the different treatments. The plates were incubated at 26°C ± 1°C. Teratogenic activity was examined using a dissecting microscope after 12, 24, 36, and 48h of incubation. Morphological endpoint evaluation of zebra fish was based on the parameters established by Nagel **[11]**: embryo-toxic (coagulation, tail not detached, no somites, and no heart-beat), teratogenic (malformation of head, tail and heart, scoliosis, deformity of yolk, and growth retardation), and normal. Hatchability, malformation and mortality rates were recorded, and death was defined as coagulated embryos and as no visual heartbeat. Tests were repeated three times.

RESULTS AND DISCUSSION

Physico-chemical Characteristics

Thephysico-chemical attributes play important roles which influence the quality of water body. Thus, the different water attributes such as temperature, pH, dissolved oxygen, phosphate and silicate of Pasig River water were determined in this study and the data are shown in Table 1. The maximum water temperature of 30.7°C was recorded in Escolta while the lowest water temperature of 29.7°C was noted in Hulo.pH is one of the most important factors that influence the aquatic production. The pH of water was in the range of 7.8 to 8.5, which is still acceptable as per guideline suggested by World Health Organization (WHO).This indicates that water was slightly alkaline. The slight alkaline state of the water might be due to the enhanced chemical interaction that leads to buffering and release of alkaline ions (bicarbonate and carbonate ions) or salts in the river water.

As per WHO, dissolved oxygen (DO) permissible limit is 4.6-6.0mg/l.DOs of the five stations were out of the acceptable range. Four stations namely Escolta, PUP, Hulo and Guadalupe were found lower than the acceptable limit while Pinagbuhatan had higher than the allowable limit.These results depict water pollution largely due to the presence of organic waste materials.The determination of phosphate and silicate ions in water is important for managing the water quality and investigating the environmental factors that affect their concentration and flux**[12]**. The phosphate content of the water was found in the range of -0.80 to 0.28 mg/l. The phosphate content in PUP Station (0.28 mg/l) was found higher than the standard limit in drinking water which is 1 mg/l. On the other hand, silicate was also found in the different stations. The maximum level of silicate of 8.54 mg/l was also found in PUP, followed by Escolta with 7.23 mg/l.Thesehigh amounts of phosphate and

silicate in PUP Station could be attributed to the discharge from the biological sewage treatment plant, surface water runoff, andhumansactivities that significantly increased the amount ofinorganic phosphate and silicate. The presence of Phosphate and silicate, however, are required nutrient compounds for aquatic organisms.

Table 1. Physico-chemical attributes of the Pasig River water

Station	Temperature (°C)	pH	DO (mg/l)	Phosphate (mg/l)	Silicate (mg/l)
Escolta	**30.7**	**8.20**	**0.9**	**-0.24**	**7.23**
PUP	**30.2**	**8.06**	**3.7**	**0.28**	**8.54**
Hulo	**29.7**	**7.83**	**4.4**	**-0.52**	**1.78**
Guadalupe	**30.1**	**7.80**	**4.5**	**-0.53**	**1.65**
Pinagbuhatan	**30.4**	**7.96**	**8.1**	**-0.80**	**-3.71**

Mortality of *D. rerio*Embryos Exposed to Pasig River Water

Aquatic toxicity using*D. rerio* embryois a reliable modelin the assessment of water quality due to their high sensitivity and specificity. This method provides a rapid and relatively accurate tool for toxicity and teratogenicity evaluation for possible hazard monitoring and identification in ecotoxicology studies. In the present work, toxic and teratogenic substances present in the water samples from the five stations of Pasig River were investigated. Table 2 presents the percentage mortality of embryos exposed to the different water samples of Pasig River. Mortality was defined as coagulation and no visual heartbeat of embryos. Apparently, after 12 hours of exposure, no mortality was observed in all water samples. However, at 24 hours exposure, embryos exposed to Escolta and Pinagbuhatanwater samples showed 16.67% and 8.33% mortality, respectively. Mortality of Escolta water exposed embryos increased to 41.67% after 36 hours and 66.67% after 48 hours. Among the water samples, Escolta recorded the highest mortality while Pinagbuhatan registered the lowest mortality. These significant results obtained in the present study strongly indicate that indeed there are chemical substances present in the water samples that caused toxicity to *D. rerio* embryos. It was reported by Pasig River Rehabilitation Commission **[10]** that Pasig River has high organic loads and contaminated with heavy metals, pesticides and other toxic substances.In the similar aquatic toxicity assessment using *D. rerio* embryos conducted by Pan et al. **[9]**, single-walled carbon nanotubes (CNT), which is one of the most widely explored nano-materials with great potential applications in electronics, chemistry and biomedicine that may possibly released into the environment during the processes of

manufacturing, modification and/or use, at ≥5 μg/ml showed significant reduction onthe survival rates of embryos.

Table 2. Mortality of *D. rerio* embryos exposed to water from the different stations of Pasig River

Station	Mortality (%)			
	12 hour	24 hour	36 hour	48 hour
Escolta	0.00	16.67	41.67	66.67
PUP	0.00	0.00	0.00	16.67
Hulo	0.00	0.00	0.00	16.67
Guadalupe	0.00	0.00	25.00	33.33
Pinagbuhatan	0.00	8.33	8.33	8.33
Control	0.00	0.00	0.00	0.00

Hatchability and Teratogenic Effects of *D. rerio* Embryos

Hatching dictates the successful developmental processes of the embryos. The assessment of hatch rate is a standard toxicity measurement of zebrafish embryo.The percentage hatchability of embryos exposed to water samples from the five stations of Pasig River at 48 hours post treatment application (hpta) is depicted in Table 3. Apparently, 100% hatching of control embryoswas observed at 48 hpta. Among water samples, embryos exposed to Pinagbuhatan water recorded the highest percentage hatchability of 83.33%, followed by Hulo with 75%. The lowest percentage hatchability of embryos was found in Escolta water with 16.67%. Accordingly, hatching of embryos was affected by the different water samples from Pasig River. This difference on the percentage hatching could possibly be explained by the level and variety of toxic substances present in the water.Hatching process of the embryo requires secretion of proteolytic enzymes to soften the chorion and embryonic movement to break the chorion. This failure of hatching would suggest a disruption in one of these processes.

Table 3. Hatchability, growth retardation, and tail malformation of *D. rerio* embryos exposed to water from the different stations of Pasig River

Station	Hatchability (%)	Growth retardation (%)	Tail malformation (%)
Escolta	16.67	50.00	41.67
PUP	66.67	41.67	33.33
Hulo	75.00	25.00	16.67
Guadalupe	66.67	33.33	25.00
Pinagbuhatan	83.33	25.00	16.67
Control	100.00	0.00	0.00

Growth retardation is also obvious in this study as one of the teratogenic effects of the Pasig River water. It can be seen in Table 3 that embryos exposed to Escolta water had the highest percentage of growth retardation with a mean of 50%, followed by those exposed to PUP water with 41.67%. The lowest percentage of growth retardation was noted in embryos exposed to both Hulo and Pinagbuhatan water

samples having 25%. These results clearly dictate that water samples from Pasig River contained chemical or substances that disturb the normal embryonic developmental processes of the *D. rerio*. As important endpoint of the delayed growth, tail malformation is apparently another teratogenic effect of the five water samples from Pasig River observed in the present study. Similar with the growth retardation, embryos exposed to Escolta water registered the highest percentage tail malformation of 41.67%. This was followed by those embryos at PUP and Gaudalupewater samples having means of 33.33% and 25.00%, respectively. The lowest was observed in embryos exposed to Hulo and Pinagbuhatan water samples with both 16.67% tail malformation. Tail malformation is considered as the morphological fingerprint in the teratogenic evaluation of toxic substances using *D. rerio* and this is also true in the present study (Figure 1). Therefore, water samples are contained with toxic compounds or substances.

Phenotypes of *D. rerio* Embryos Exposed to Pasig River Water

Control embryos completed the normal morphogenesis of primary organ systems and start to hatch out of the chorion at 48 hpta. The different developmental features such as eye development, somite formation, blood circulation, heartbeat, pigmentation in head-body and in tail, pectoral fin formation, hatching and protruding mouth were observed. The phenotypic endpoints of embryos exposed to water samples from the five stations of Pasig River are shown in Figure 1. Apparently, the abnormal phenotypes were observed in those embryos exposed to the water samples of Pasig River. These abnormalities included hypo-pigmentation, wavy somites, bent tail, irregular shape of tail, lengthy tail, deformed yolk, pericardial edema, girth between head and body region. Among these dysmorphologies, tail malformation and wavy somites were the most common in all Pasig River water samples exposed embryos. These abnormal phenotypes were also noticed in amide functionalized single-walled carbon nanotubes (SWCNTs)-exposed embryos **[9]**.

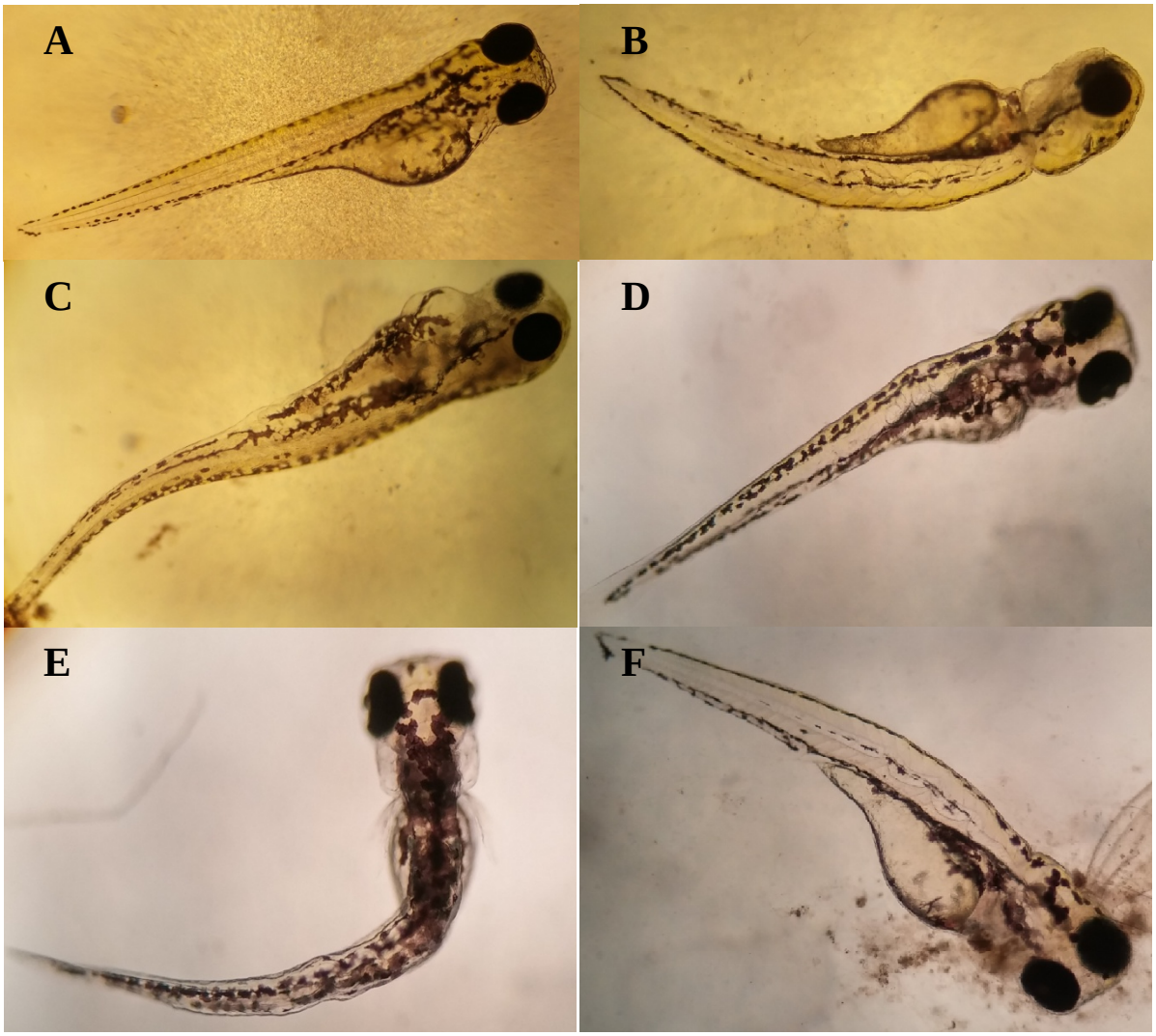

Figure 1: Phenotypic endpoints of *D. rerio* exposed to (A) Embryo water, (B) Escolta, (C) PUP, (D) Hulo, (E) Guadalupe, and (F) Pinagbuhatan water samples.

CONCLUSION

Based on the data gathered in present work, it is evident that the water from Pasig River could induce toxicity and teratogenicity in *D. rerio* embryos by reducing their survival rates and affecting their normal embryonic development. Therefore, it is necessary to elucidate the toxic and teratogenic compounds present in the Pasig River water in the future studies.

REFERENCES

[1]Norrgren L. Fish models for ecotoxicology. Acta Veterinaria Scandinavica, 2012, 54(Suppl 1):S14.

[2]Fraker SL, Smith GR. Direct and interactive effects of ecologically relevant concentrations of organic wastewater contaminants on *Ranapipiens* tadpoles. Environ Toxicol,2004, 19: 250–256.

[3]Hawkins WE, Walker WW, Fournie JW, Manning CS, Krol RM. Use of the Japanese medaka (*Oryziaslatipes*) and

guppy (*Poeciliareticulata*) in carcinogenesis testing under national toxicology program protocols. ToxicolPathol,2003, 31: 88-91.

[4]Poynton HC, Varshavsky JR, Chang B, Cavigiolio G, Chan S, Holman PS, Loguinov AV, Bauer DJ, Komachi K, Theil EC, Perkins EJ, Hughes O, Vulpe CD. *Daphnia magna* ecotoxico genomics provides mechanistic insights into metal toxicity. Environ Sci. Technol, 2007, 41:1044-1050.

[5] Nagel R. DarT: the embryo test with the Zebrafish *Danio rerio* – a general modeline cotoxicology and toxicology. Altex, 2002, 19: 38–47.

[6]Dulay RMR, Kalaw SP, Reyes RG, Alfonso NF, Eguchi F. Teratogenic and toxic effects of lingzhi or reishi medicinal mushroom, *Ganoderm alucidum* (W.Curt.:Fr.) P. Karst. (Higher basidiomycetes), on zebrafish embryo as model.International Journal of Medicinal Mushrooms, 2012, 14(5): 507–512.

[7]Peterson SM, Freeman JL.Cancer cytogenetics in the zebrafish. Zebrafish, 2009, 6: 355-360.

[8]Mione MC, Trede NS. The zebrafish as a model for cancer. Dis Model Mech,2010, 3: 517–523.

[9] Pan H, Lin YJ, Li MW, Chuang HN, ChouCC. Aquatic toxicity assessment of single-walled carbon nanotubes using zebrafish embryos. Journal of Physics: Conference Series, 2011, 304: 1-7.

[10]2012 Annual Report: Pasig River Rehabilitation Commission. 2012.

[11]Nagel R. In W. Heger, S. Jung, S. Martin et al. (eds.), UBA Texteb and 58/98 (80-93). Berlin: Umweltbundesamt. 1998.

[12]Nakatani N, Masuda W, Kozaki D, Goto R, Nakagoshi N, Mori M, Hasebe K, Tanaka K. Simultaneous spectrophotometric determination of orthophosphate and silicate ions in river water using ion- exclusion chromatography with an ascorbate solution as both eluent and reducing agent, followed by post column derivatization with molybdate. Analytical Sciences,2009, 25: 379-383.