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Biomonitoring of Water Bodies in Metro Manila, Philippines Using Heavy Metal Analysis and Erythrocyte Micronucleus Assay in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Environmental biomonitoring of water bodies is routinely done to assess the ecological state of aquatic systems by detecting the hazardous and genotoxic pollutants. In this study, a combination of atomic absorption spectroscopy and the fish micronucleus assay was used to determine and compare the genotoxic potential of water bodies specifically the two Esteros namely, Estero de Vitas and Estero de Paco, which are part of the Pasig River System, Philippines. As part of the strategy, the Esteros are being rehabilitated to control pollution in the river systems whereby Estero de Paco was recently rehabilitated, whereas, Estero de Vitas is still largely neglected. The elevated levels of micronuclei and nuclear abnormalities were observed in the erythrocytes of the genetic model, the Nile tilapia (*Oreochromis niloticus*) exposed to water samples from the two sources tested when compared to a control group indicating the presence of genotoxic and hazardous pollutants in the water bodies of Estero de Paco and Estero de Vitas. Further, the water samples from Estero de Vitas were found to be far more genotoxic as compared to the water samples from Estero de Paco ($p<0.05$). The observed genotoxic effects of the water samples appeared to be related to the physico-chemical characteristics studied using atomic absorption spectroscopy, which showed the presence of heavy metals in the water samples from both the sources. The AAS results also confirm the presence of heavy metals in the fish tissue exposed to the water samples from the two locations. Hence, the tilapia fish (a component of Filipino diet) should be consumed with precautions as it can absorb the heavy metals present in ecosystems. The results establish that the fish micronucleus test is an effective assay for environmental biomonitoring. The lower genotoxicity potential of Estero de Paco clearly demonstrates that the restoration of the Esteros can be an effective approach to control pollution of the water bodies, especially the Pasig river system.

INTRODUCTION

In the capital of Philippines, Metro Manila, the Pasig River System is notoriously known for its polluted state (Gorme et al. 2010, Arcega 2017). There are 47 Esteros (estuaries) that drain into the Pasig River and almost all of these tributaries are polluted, thereby further making the Pasig River more polluted. For this reason one of the strategies adopted by the authorities was, to clean these Esteros that contain the pollution in the Pasig River (Citiscope 2016). In 2010, the Pasig River Rehabilitation Commission along with other partner agencies and organizations undertook the task of cleaning up Estero de Pacoas part of the greater campaign to rehabilitate and clean the Pasig River and its tributaries (Asian Development Bank 2012). The efforts to clean Estero de Paco, lead to an aesthetically better looking Estero as compared to its former state (Fig. 1A and 1B). Contrary to Estero de Paco, no efforts have been made to rehabilitate the Estero de Vitas, which is located at Tondo, Manila (Fig. 2B). It is close to the Smokey Mountain dumpsite where electronic waste recycling and salvaging of garbage is rampant, especially in the dumpsite drainage, termed Smokey Mountain, causing an increase in heavy metals such as chromium, copper, lead, and zinc, and other pollutants in the waters of the Estero de Vitas (Yoshimura et al. 2015).

The restoration efforts should also lead to an improvement in the quality of water with respect to its genotoxic and cytotoxic potential as this parameter is of utmost importance in terms of the safety to the living beings. There is very scanty data available on the mutagenic and genotoxic effects of water bodies in Philippines (Tuddao & Gozales 2016). The genotoxicity potential of water samples from Pasig and Marikina River and Estero de Vitas was established by studying the inhibition of root growth, decrease in mitotic index, and presence of chromosomal aberrations in *Allium cepa* root cells (Alam et al. 2016). Heavy metals were reported to cause elevated levels of micronuclei and DNA damage in the fish erythrocytes of *Oreochromis niloticus* or the Nile tilapia caught at different sites within

Fig. 1(A): Estero de Paco before Rehabilitation, (B) Estero de Paco after rehabilitation (photo from ©ABS-CBN Foundation Inc.).

Taal Lake and Pampanga river (Hallare et al. 2016). Though Estero de Paco has been restored, it is important to study if the water of the Estero de Paco is safe in terms of its genotoxic and cytotoxic potential.

Environmental biomonitoring is one of the efficient methods, which can be used to keep track of the environment's status and determine changes in the environment that elicit a biological response. One of the ways of biologically monitoring the environment is through the micronucleus assay (Cavas & Ergene-Gozukara 2003). The micronucleus assay has been used to assess the genotoxicity of water from rivers or lakes by making use of aquatic species, mainly fishes (Ergene et al. 2007, Obiakor et al. 2012, Dar et al. 2016, Authman et al. 2015, Hallare et al. 2016).

Heavy metals are known to be genotoxic and cytotoxic even at low concentrations and have adverse effects on the aquatic life (Karbassi et al. 2006, Guo et al. 2009, Jakimska et al. 2011). These metals are ingested through the gills, skin and the alimentary tract (Fazio et al. 2014) and further transported through the bloodstream into organs and tissues, where they accumulate at high concentrations (Dupuy et al. 2014). At the molecular level, the heavy metal cytotoxicity includes damage to plasma membranes, inhibition of Na and K dependent ATPases, oxidative DNA damage with reduction of antioxidant enzymes via reactive oxygen species (Stohs & Bagchi 1995, Leonard et al. 2004). For this reason, several of the heavy metals have been reported to have genotoxic effects on the fish genome (Bucker et al. 2012). As fishes are at the end of the aquatic food chain, human consumption of fishes that accumulate metals may cause various diseases.

Therefore, it is important to study the presence of heavy metals in the water samples besides other pollutants to comprehend the genotoxic effects especially pertaining to fish as the experimental organism. The presence of heavy metals as stated is persistent and reflects, the past exposure of the fish to contaminated water, allowing the fish to show the current situation of toxicity in the aquatic environment (Javed et al. 2015). Fishes have also been identified as a sentinel organism that can be used to assess the risk of human exposure to drinking water contamination with genotoxic chemicals (AI-Sabti & Metcalfe 1995). Out of many varieties, *O. niloticus* (Nile tilapia) is known to be a good indicator of different toxic pollutants and hazardous substances present in the aquatic ecosystems (Mohamed et al. 2012).

Hence, in the present study, the micronucleus assay and the heavy metal analysis using atomic absorption spectroscopy in the Nile tilapia (*Oreochromis niloticus)* was used to compare the genotoxic potential of the water samples from Estero de Paco with that of Estero de Vitas and further ascertain the affectivity of the restoration efforts of various stakeholders. The physico-chemical analysis of water samples was also carried out to further validate the results. Should the water quality of Estero de Paco be better than that of Estero de Vitas in terms of its genotoxicity, it

Fig. 2A: Satellite image of Estero de Paco.

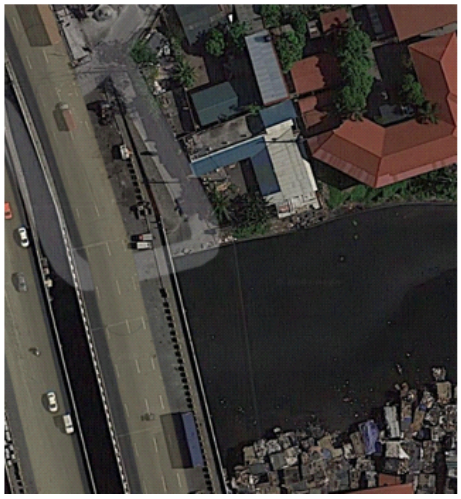

Fig. 2B: Satellite image of Estero de Vitas.

Fig. 3 A: Riverbank along Estero de Paco

Fig. 3B: Riverbank along Estero de Vitas.

can be established that the proper rehabilitation and restoration of inland waters will consequently bring down levels of pollution and restore the overall cleanliness of the environment.

MATERIALS AND METHODS

Collection of samples: Water samples were collected along the banks of Estero de Paco (Fig. 2A) and Estero de Vitas (Fig. 2B), both tributaries of the Pasig River System.

Despite Estero de Paco's recent restoration, the stream still appears to be polluted and is aesthetically unpleasant (Fig. 3A). Estero de Vitas, on the other hand, has not been restored, and expectedly appears to be highly polluted along the banks (Fig. 3B).

Acclimatization and treatment of the samples: Juvenile Nile tilapia (*Oreochromis niloticus)* (size range from 4-5 inches) was procured from Cartimar pet market, Manila. Separate aquaria were used with individual water filter and air pumps. Five fish were placed in each aquarium with tap water from the lab's faucet to be acclimatized for 24 hours. After acclimatization, the fish were then transferred to treatment water from two Esteros. The water filter attachment was removed but the air pump remained in the aquarium. They were fed daily for 48 hours after being introduced to the water treatment and were kept at a constant temperature (30±3°C), and at a natural photoperiod of 12 hr light: 12 hr dark. After a 48 hours exposure, one sample was prepped for blood collection through cardiac/caudal vein puncture. To determine increasing genotoxicity over time, the same procedure was repeated after 72 and 96 hours exposure to the treatment.

Fish micronucleus test: Blood samples were collected through cardiac/caudal vein puncture using a sterilized needle based on the procedure recommended by Bucker and Conceição (2012) with a few modifications. The blood sample was smeared onto five clean glass slides. After 24 hours of air-drying, the glass slides were fixed with methanol for 10 minutes and then stained by 5% Giemsa solution for 15 minutes. After staining, the slides were blot dried using paper towels and viewed in a light compound microscope under

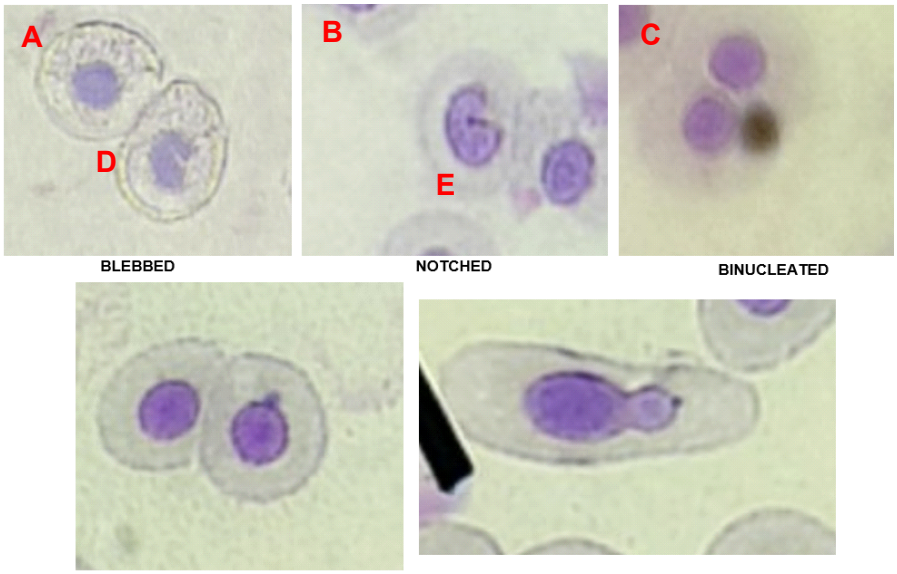

Fig. 4: Types of Nuclear Abnormalities: (A) Blebbed, (B) Notched, (C) Binucleated, (D) Micronucleus, (E) Lobed

HPO objective at 1000x magnification. Approximately 2000 cells were evaluated and observed per fish. The number of normal cells and cells with abnormalities (micronucleus and nuclear abnormalities) were scored per fish in each treatment and then, the aberrant percentage was calculated. The erythrocytes nuclear abnormalities were also identified as micronucleus, binucleated, notched, blebbed and lobed cells (Fig. 4).

Analysis of physico-chemical properties of water samples: Three parameters were assessed to study the physico-chemical properties of the water samples: pH level, amount of dissolved oxygen (DO) present and the biochemical oxygen demand (BOD), which are primary parameters set by DENR. The pH level was checked by submerging pH strips in the water samples, and counter-checking the equivalent value of the resulting colour change with the standards. Meanwhile, the DO and BOD were obtained using the Winkler titration method (Department of Biology 2015).

Atomic absorption spectroscopy (AAS) of water samples and fish tissue: The AAS test was performed to detect the presence of heavy metals (Pb, Hg, Cu, Cd, Zn) in the water samples and fish tissue samples by using the atomic absorption spectrophotometer (model AA-6300 Shimadzu) and the standard procedure was followed (Sharma et al. 2013). Analysis of the presence of mercury was done at the Philippine Institute of Pure and Applied Chemistry (PIPAC), Ateneo de Manila University using cold vapour atomic absorption spectroscopy (CVAA).

Acid digestion of water samples: For the sample to be digested and dissolved into a solution, the standard procedure of acid digestion of water samples was followed (Siraj et al. 2013). 5 mL of HNO_3 was added into the 250 mL beaker with 50 mL of the water sample. It was slowly boiled using a hot plate until 20 mL of the solution was left. After cooling it down for 5 minutes, 5 mL of HNO_3 was added once again for the final digestion. It was further boiled until 10 mL of the solution was left. It was filtered and diluted in a 100 mL volumetric flask with distilled water and stored in the refrigerator until used.

Acid digestion of fish tissue: To detect the levels of heavy metals in the gills and scales of the fish, the acid digestion procedure by Akintujoye et al. (2013) was followed. The scales and gills of fish per treatment were placed and wrapped using paper towels and newspaper for oven drying. The oven was set to 150°C for at least 5 hours. The samples were then transferred into 250 mL beakers. 15 mL of concentrated nitric acid was added to each beaker and left at room temperature for at least 24 hours with an aluminium cover. After the pre-digestion, the samples were heated to at least 100°C for 4 hours and adding 10 mL of nitric acid to prevent the samples from drying. After heating, the samples were cooled off before transferring into small propylene plastic containers with labels.

Data analysis/statistical tool: One-way ANOVA test was performed to determine the difference in the means of micronuclei/nuclear abnormalities between the treatments significant at 95% confidence level. Further, t-test was used to compare the difference between the control group and the experimental groups comprised of treatments with water from Estero de Paco and Estero de Vitas. Any significance

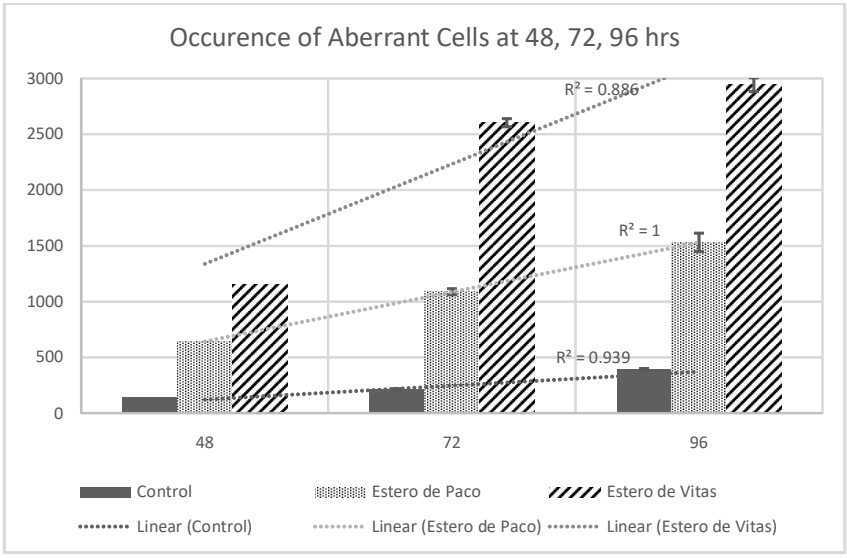

Fig. 5: Frequency of aberrant cells in Control, Estero de Paco and Estero de Vitas at 48, 72 and 96-hour exposure.

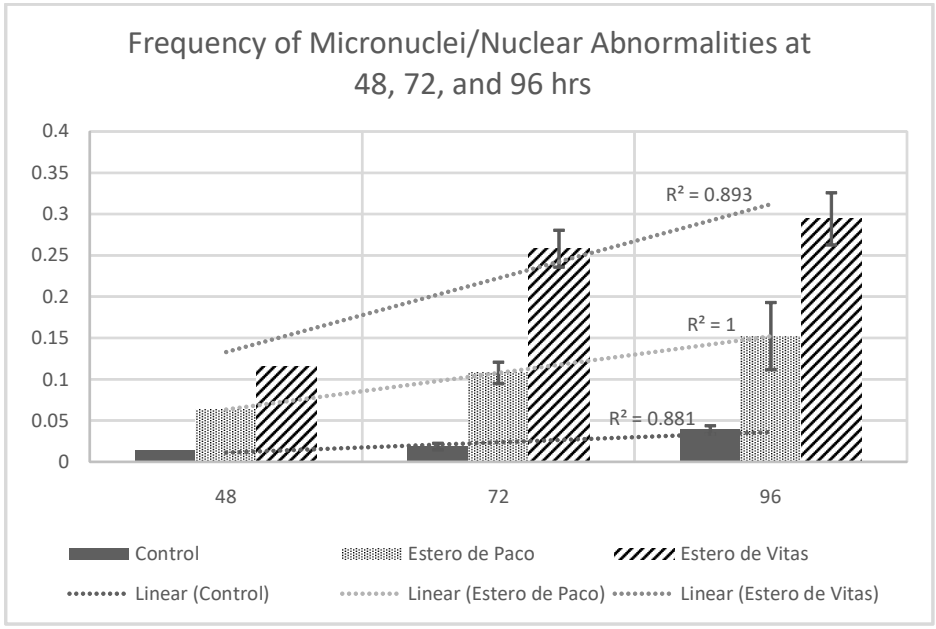

Fig. 6: Total micronuclei/abnormalities in Control, Estero dePaco and Estero de Vitas at 48, 72 and 96-hour exposure.

was determined with an alpha of at least 0.05 (95% confidence). Aside from this, linear regression analysis was also performed to study the trends in the occurrence of aberrant cells and micronuclei with the increase in the exposure duration through 48, 72 and 96 hours. The difference in the levels of the heavy metals analysed using AAS in the water was compared with the standard values fixed by the Department of Environment and Natural Resources, whereas for fish tissue the comparison was made with the standard values suggested by JEFCA (Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food additives).

RESULTS AND DISCUSSION

The number of aberrant erythrocytes along with the appearance of micronucleus and other nuclear abnormalities were

Table 1: Comparison of aberrant erythrocytes after 48, 72 and 96 hours exposure to Estero de Vitas and Estero de Paco water samples.

	Treatments			p-values		
Duration	Control	Estero de Paco	Estero de Vitas	*p-value	**p-value	***p-value
48 hrs	0.01±0.004	0.06±0.01	0.13±0.02	0.0007542	0.0008124	0.00604239
72 hrs	0.01±0.005	0.10±0.04	0.26±0.03	0.012352	0.0001155	0.00038046
96 hrs	0.04±0.006	0.15±0.01	0.29±0.03	3.18E-06	3.784E-05	5.31E-05

*p-value between Control and Estero de Paco water samples; **p-value between Control and Estero de Vitas water samples
***p-value between Estero de Paco and Estero de Vitas water samples

Table 2: Comparison of total micronuclei/nuclear abnormalities after 48, 72 and 96 hours exposure to Estero de Paco and Estero de Vitas water samples.

	Treatments			p-values		
Duration	Control	Estero de Paco	Estero de Vitas	*p-value	**p-value	***p-value
48 hrs	28 ± 7.97	128.6 ± 27.32	230.6 ± 36.26	0.000877	0.0004	0.002821
72 hrs	42 ± 8.97	217.4 ± 83.13	520.6 ± 61.76	0.013746	0.000105	0.000627
96 hrs	78 ± 11.12	306 ± 33.66	588.8 ± 51.76	5.06E-05	4.25E-05	3.8E-05

*p-value between Control and Estero de Paco water samples; **p-value between Control and Estero de Vitas water samples
***p-value between Estero de Paco and Estero de Vitas water samples

found to be higher in the tilapia exposed to water samples of Estero de Paco and Estero de Vitas in comparison to the control treatment at $p<0.05$ (Tables 1 & 2). This indicates that the aquatic environments in each site possess genotoxic/mutagenic contaminants. The duration of exposure appears to affect the occurrence of micronucleus and erythrocyte nuclear abnormalities, as there was generally an increase in the occurrence of the nuclear abnormalities with the increase in the exposure time ranging from 48 hours to 96 hours.

Analysis of aberrant erythrocytes/cells in fish: There was an increase in the number of aberrant erythrocytes in the Nile tilapia with the increase in the exposure time to water samples from both Estero de Paco and Estero de Vitas (Fig. 5).

The appearance of aberrant cells in the erythrocytes of fish treated with water from both Estero de Paco and Estero de Vitas after 48, 72 and 96 hours was found to be significantly higher as compared to the control group (Table 1). Further, the occurrence of aberrant cells in the Estero de Vitas treatment was found to be significantly higher than the occurrence of aberrations in the Estero de Paco treatment at $p<0.05$ (Table 1). The correlation of the total aberrant number of cells over time at each treatment was found to have high R^2 value, with Estero de Paco having the highest R^2 value of 1. Estero de Vitas treatment, on the other hand, still had a high R^2 value of 0.8867 albeit being lower than Estero de Paco treated fishes. The negative control also had a high R^2 value of 0.9394. Overall, the R^2 value of each treatment was high indicating the positive correlation between the occurrence and increase of the frequency of aberrations/total number of cells over time (Fig. 5).

Micronuclei/nuclear abnormalities in fish erythrocytes analysis: The *O. niloticus* micronucleus test showed that the water samples collected from both locations was significantly genotoxic as compared to tap water used as the negative control. The total micronuclei/nuclear abnormalities observed in the erythrocytes of Nile tilapia exposed to water samples from Estero de Paco and Estero de Vitas was found to be significantly higher as compared to the aberrations found in the control group at 48, 72 and 96 hours (Table 2). Total nuclear abnormalities observed in the fishes treated with Estero de Vitas water samples was found to be statistically significant as compared to the total aberrations found in fishes treated with Estero de Paco water at (Table 2). The micronucleus frequency observed in the negative control in the present study is in concurrence with similar results reported in other studies using Nile tilapia (Matsumoto et al. 2006).

The erythrocyte nuclear abnormalities (Fig. 4) were classified as blebbed, notched and lobed (Carrasco et al. 1990, Ayylon & Garcia-Vazquez 2000, Çavas & Ergene-Gozukara 2003).

The occurrence of erythrocyte nuclear abnormalities over time was found to have a positive correlation in all treatments, with very high R^2 values, indicating the general increase of the percentage of aberrations in each treatment over time at 48, 72, and 96-hour exposure. Estero de Paco

water treated fish samples had the highest R^2 value of 1 while Estero de Vitas treated fishes had an R^2 value of 0.8939. The negative control fishes also had a high R^2 value of 0.8817, indicating a gradual increase in percentage of abnormalities. Overall, there was a high R^2 value in all treatments, indicating the positive correlation between the general increase of abnormalities over time in each treatment (Fig. 6).

At 48 hours exposure, micronucleus and blebbed cells were generally high in frequency among the aberrant cells, with blebbed cells having an average of 39% and micronucleus having an average of 31% in occurrence (Table 3). At 48 hours of exposure, it can also be seen that the occurrence of notched cells is high at the Estero de Vitas treatment in comparison with the control and Estero de Paco. At 72 hours, the general occurrence of the aberrations is in the order of - micronucleus > notched > blebbed > lobed > binucleated, respectively. At 72 hours, there was a general increase in notched cells, causing close means between the occurrence of micronucleus, notched, and blebbed cells. At 96 hours, there was a dominant occurrence of micronucleus and blebbed cells, with the occurrence ranging from blebbed > micronucleus > notched > lobed > binucleated, decreasing respectively.

Micronucleus assay has certain advantages over other DNA damage techniques, such as, it can be performed rapidly, is low cost and the preparation and analysis is simpler and faster than conventional chromosomal aberration studies. Since, research on environmental biomonitoring requires fast, reliable and reproducible results, micronucleus assay in fish species should be standardized to improve the sensitivity of this assay to assessment of genotoxicity. The results in the present study confirm the suitability of using Nile tilapia micronuclei as one of the appropriate assays in environmental biomonitoring.

Physico-chemical analysis of water samples: The biochemical oxygen demand (BOD) values for Estero de Vitas far exceeded the Department of Environment and Natural resources (DENR) standard value of 7 mg/L, so much so that it was impossible to measure it (Table 4). This is indicative of the untreated pollutants and waste present in the Esteros de vitas waters. The BOD values for water samples from Estero de Paco is less than Estero de Vitas, and is somewhat closer to the DENR standards pointing to the successful rehabilitation of this Estero and the urgent need of constant monitoring to further improve the quality of water and prevent any deterioration of the Estero de Paco. The low levels of dissolved oxygen (DO) in the water samples of both locations could be the reason of their inability to support any aquatic life (Table 4).

Since, there are human settlements close to these Esteros, the discharge of household detergents and cleaning chemicals along with the other human waste could be responsible for the rise of pH levels in the water samples (Table 4). The excessive number of bacteria in the untreated sewage, and the organic and anoxic discharges generally use up dissolved oxygen and lead to an increase in the biological oxygen demand. This is exactly the trend observed in the present study (Table 4).

Heavy Metals Analysis

Heavy metals in water samples: The heavy metal contamination has been reported from other locations such as Pampanga in Philippines as well (Marvin et al. 2017). Our results also clearly establish the presence of heavy metals in the water samples collected from the two locations in Metro Manila.

A minimum detection limit of 0.05 ppm is established through standard readings prior to the heavy metal analysis of the water samples, with the exception of mercury readings, whose minimum detection limit is set at 0.0005 ppm as the analysis was done by an external source. In the control samples, the negative values for zinc and copper signify the presence of the said heavy metals to be below 0.05 ppm (Table 4). Generally, the control sample had the least presence of all the metals tested. For Estero de Paco, among the four metals tested, only copper was the one detected with a value lower than 0.05 ppm. Meanwhile, Estero de Vitas had the highest concentration of all the four heavy metals tested among the samples, with only copper being read under the minimum detection limit.

Three metals tested namely, Zn, Cd and Pb were found to be significantly higher in the water samples from Estero de Paco as well as Estero de Vitas as compared to the control ($p<0.05$, Table 4). Also, there was a significant difference between the heavy metal contents in the water samples of Estero de Paco and Estero de Vitas with heavy metals concentration being much higher in the water samples from Estero de Vitas at $p<0.05$ (Table 4). Levels of zinc, cadmium and lead were higher than the recommended DENR Class C standards in the water samples from Estero de Paco. As compared to the control, the presence of these heavy metals was significantly higher in the Estero de Paco water samples, with the exception of copper, which was not identified in both the water samples. The Estero is situated beside a market, indicating possible wastes from the market leaching into the waters. Mercury was also not detected in the water samples of control, Estero de Paco and Estero de Vitas. The levels of the heavy metals zinc, cadmium and lead for Estero de Vitas far exceeded the recommended lev-

Table 3: The range of nuclear abnormalities observed in the Nile tilapia erythrocytes exposed to Control, Estero de Paco and Estero de Vitas water treatments at 48, 72 and 96-hour exposure.

		Micronucleus	Binucleated	Notched	Lobed	Blebbed
	Control	8 ± 3.16	0.6 ± 0.49	3 ± 1.1	4 ± 0.89	12.6 ± 5.00
48 hrs	Estero de Paco	45.6 ± 18.28	0.6 ± 0.8	10.6 ± 2.06	15.6 ± 5.12	56.2 ± 6.79
	Estero de Vitas	66.2 ± 16.38	6.6 ± 2.58	58.4 ± 9.85	32.6 ± 6.95	66.8 ± 8.3
	Control	9.2 ± 2.64	1.4 ± 1.49	12 ± 1.09	10.2 ± 2.71	9.2 ± 5.19
72 hrs	Estero de Paco	63.6 ± 31.53	5.8 ± 5.94	60 ± 16.21	39.8 ± 13.35	48.2 ± 23.88
	Estero de Vitas	173.4 ± 31.63	9.8 ± 4.99	108.6 ± 17.3	101.2 ± 29.62	127.6 ± 20.4
	Control	22.8 ± 7.14	1.6 ± 1.4	12.2 ± 7.05	13.4 ± 7.5	29.2 ± 7.96
96 hrs	Estero de Paco	120.2 ± 24.6	5 ± 4.3	35.4 ± 13.26	18.4 ± 5.54	127.8 ± 19.53
	Estero de Vitas	203.8 ± 38.5	9.6 ± 3.3	76.4 ± 12.64	38.4 ± 11.15	263 ± 25.12

Table 4: Comparison of physico-chemical properties and heavy metal profile of water samples: Control, Estero de Paco and Estero de Vitas versus DENR Class C Standard.

	Treatments				p-values		
	Control	Estero de Paco	Estero de Vitas	DENR Class C Standard	*p-value	**p-value	***p-value
B.O.D (mg/L)	4.5	8	-NA-	7	-	-	-
D.O. (minimum; mg/L)	4	3	-NA-	5	-	-	-
pH	7	7	8	6.5-9.0	-	-	-
Zinc (ppm)	-0.2004	1.4817	5.8928	2	1.37E-08	4.58E-08	1.22E-07
Cadmium (ppm)	0.1191	0.9807	2.6487	0.005	0.000228521	0.000139787	0.000374848
Lead (ppm)	0.3384	1.3535	3.5868	0.05	0.010631731	0.001092761	0.004232585
Copper (ppm)	-0.0669	-0.0713	0.0089	0.02	0.93393979	0.01907959	0.012584121
Mercury (ppm)	-	<0.0005	<0.0005	0.004	-	-	-

*p-value between Control and Estero de Paco water samples; **p-value between Control and Estero de Vitas water samples; ***p-value between Estero de Paco and Estero de Vitas water samples

els stated by DENR for Class C waters. In addition to this, the concentration of heavy metals in Estero de Vitas water samples was significantly higher than that found in Estero de Paco and the control treatment at $p < 0.05$ (Table 4).

The fact that no restoration efforts have been made to clean up the Estero de Vitas and the continued disposal of untreated solid waste, particularly the e-waste leachates from the dumpsites located in the smokey mountain area could be responsible for the presence of high concentration of heavy metals (Alam et al. 2016). Electronic waste recycling and salvaging of garbage is rampant in the Smokey Mountain/Tondo area, especially in the dumpsite drainage, causing an increase in heavy metals such as chromium, lead, and zinc, and other pollutants in the flow of the river and its estuary, Estero de Vitas (Yoshimura et al. 2015)

Heavy metals are known to cause chromosomal damage and micronuclei, which is indicative of genotoxicity (Morales et al. 2016). The heavy metal contamination in aquatic ecosystem is a serious problem that has direct impact on the quality of water sediments and fish quality especially with respect to their safety for human consumption (Tarrio et al. 1991, Khayatzadeh & Abbasi 2010). In general, the contamination of water bodies by heavy metals lead to disastrous effects on the ecological balance of the aquatic environment and may reduce the diversity of aquatic organisms (Ayandiran et al. 2009).

The observation of diminished genotoxicity of the water samples of Estero de Paco after its rehabilitation as compared to the water samples from the untreated Estero de Vitas confirms that the rehabilitation efforts to control the pollution levels in the Estero de Paco have been successful to some extent and can be one of the strategies to contain pollution in the Pasig river. It is important to consider factors such as the duration of exposure, age and the presence of mutagenic pollutants in treatment, as these factors may affect the occurrence of abnormalities, as well as the survivability of the fish in harmful environments. Hence, a constant environmental biomonitoring is the need of the hour. The results also confirm that Nile tilapia erythrocytes micronuclei assay is a sensitive method that can be used for environmental biomonitoring.

Heavy metals analysis of fish tissue samples: Fish (Nile tilapia) exposed to water samples from each site also exhibited the presence of heavy metals in their two tissue samples

Table 5: Comparison of heavy metal concentration found in gills and scales of fishes treated with water samples from Estero de Paco and Estero de Vitas. a Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA).

*MDL = 0.5 ppm	Control		Estero de Paco		Estero de Vitas		JECFA[a] Tolerable Daily Intake	p-values		
	Gills	Scales	Gills	Scales	Gills	Scales		*p-value	**p-value	***p-value
Zinc (ppm)	<0.5	<0.5	<0.5	<0.5	0.8371	1.0133	0.3-1.0	-	-	-
Cadmium (ppm)	<0.5	<0.5	<0.5	0.8459	1.8244	2.1307	-	-	-	-
Lead (ppm)	0.1993	0.259	0.8853	1.2131	1.9746	2.7876	0.025	Gills:3.52 E-08 Scales:3.81 E-10	Gills:1.59 E-12 Scales:4.62 E-07	Gills:5.21 E-09 Scales: 3.09 E-06
Copper (ppm)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.05-0.5	-	-	-
Nickel (ppm)	2.1209	3.2324	2.9002	4.574	3.6924	5.8899	0.07	Gills: 0.002644 Scales: 0.004439	Gills: 4.23E-07 Scales: 0.000326	Gills: 0.002556 Scales: 4.64E-07

*MDL-Minimum Detection Limit; *p-value between Control and Estero de Paco fish tissue samples; **p-value between Control and Estero de Vitas fish tissue samples; ***p-value between Estero de Paco and Estero de Vitas fish tissue samples

tested namely gills and scales. Levels of zinc, nickel and lead are higher than the tolerable daily heavy metal intake of humans as per the standard of Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) in the fish samples treated with water from both the sites (WHO, 1982, 1993, 2000, 2005). The fish treated with water samples from Estero de Vitas were observed to have the most concentration of heavy metals zinc, cadmium, lead and nickel in their gills and scales, with the exception of copper metal (Table 5). Fish treated with water from Estero de Paco, on the other hand, only exhibited the presence of cadmium, lead and nickel in their gills and scales. Interestingly, the presence of cadmium was detected in the scales, but not detected in the gills of the fish treated with Estero de Paco water. This may have been due to the presence of heavy metals in gills having lower concentration as compared to the presence of heavy metals in fish scales, as seen in all the specimens tested (Table 5). As compared to the control, the presence of the these heavy metals was significantly higher in the fish exposed to Estero de Paco water samples, with the exception of copper, which was not identified in the fish tissues exposed to both the water samples ($p<0.05$). Estero de Vitas, when compared to the control also had a significantly higher concentration of heavy metals ($p<0.05$). When fish from the two sampling sites were compared, there was a significant difference, indicating the overall higher concentration of heavy metals in fish tissue of the fishes treated with Estero de Vitas as compared to fishes treated with Estero de Paco water ($p<0.05$).

The presence of heavy metals, both in the water and the fish in the present study, indicates the possible reasons for the difficulty in the survivability of the fish in its habitat. In the fish, accumulation of heavy metals can cause different harmful effects depending on the heavy metal intake (Jezierska & Witeska 2006). Cadmium exposure causes histological and pathological alterations in the liver, intestine and kidneys; and has an overall negative effect on the growth rate, meat quality and blood physiology of the Nile tilapia (Omer et al. 2012, Abbas & Ali 2007). Nickel (Ni) is considered to be immunotoxic, genotoxic and carcinogenic agent to living organisms (Kasprzak et al. 2003). Another heavy metal tested in this study namely lead is reported to accumulate in the fish organs liver, kidneys, spleen and gills and throughout in the digestive tract upon exposure to contaminated water (Creti et al. 2010). Specifically, the exposure to lead is also reported to severely damage erythrocytes and leuckocytes as well as the nervous system in Nile tilapia (El-Badawi 2005). Zinc though an essential micronutrient, is toxic at high concentration causing mortality, damage to gills and other organs in the Nile tilapia (Abd El-Gawad 1999).

The bioaccumulation of heavy metals through aquatic food webs is known to cause environmental and human health hazards (Dehn et al. 2006). The fish and the crustacean species are known to be the most significant vectors of pollutants, and therefore, their consumption by human becomes the major route of exposure to toxicants such as heavy metals (Al-Sabti & Metcalfe 1995). Fish are also the top consumers in the aquatic food chain, consequently, the pres-

ence of heavy metals in their tissues is an indication of health hazards posed to the populations consuming fish in their diet (Chezhian et al. 2010).

It was found that zinc, nickel and lead levels in the fish tissue were beyond the tolerable daily intake standards by JECFA indicating the possible excessive presence of these heavy metals in the Nile tilapia found in local markets. Assuming fishes of this quality are consumed, the excessive presence of these heavy metals will have detrimental effects on the human health. The cytotoxicity studies of heavy metals establish their potential for induction of mutagenicity and formation of tumours in experimental organisms and humans upon exposure (Garcia-Rodríguez et al. 2001). Zinc, in general, is considered non-toxic, however, at excessive levels, it is known to cause lethargy, neuronal deficits, metal fume fever, nausea, epigastric pain, diarrhoea and an elevated risk of prostate cancer (Plum et al. 2010). Lead is a significant heavy metal known to cause lead poisoning, which generally cause dysfunctions in the reproductive, cardiovascular, nervous and the digestive system. Specifically, lead poisoning can cause inhibition of haemoglobin production, bloody urine, neurological disorders and brain damage; while in children, lead poisoning can cause poor development of the brains' gray matter (Duruibe et al. 2007). Studies have shown that excessive nickel exposure causes spontaneous abortions among females at nickel refinery (Denkhaus & Salnikow 2002).

According to Food and Agricultural Organization (2005), Nile tilapia prefers living in shallow waters and in a temperature of 31 to 36°C. Hence, this species is easily bred in tropical countries such as the Philippines, wherein recently it was listed as one of the countries considered as a primary producer of the Nile tilapia (FAO Fisheries Statistics 2005). The polluted aquatic environment can possibly lead to local breeding of Nile tilapia and produce substandard fish for consumption. The presence of heavy metals in the fish tissue, as detected by AAS in the present study, highlights the necessity of continuous monitoring of fish population in the Philippines, specifically all the varieties of tilapia, as it is one of the major constituents of Filipino diet.

CONCLUSION

The micronucleus assay results confirm the genotoxic potential of waters from Estero de Paco and Estero de Vitas, while the atomic absorption spectroscopy (AAS) confirms the presence of several heavy metals that could be one of the possible source of the genotoxicity. The fish micronucleus assay and physico-chemical analysis of water samples along with the heavy metal detection in the water samples and fish tissue, clearly establish that the rehabilitation efforts to control and improve the water quality of Estero de Paco have been effective to some extent. The results also establish that the fish micronucleus test is an effective assay for evaluating the presence of genotoxic pollutants in aquatic environments as a tool for environmental biomonitoring. It is also advised that a relocation of inhabitants settling along the banks be accomplished, to prevent or minimize the exposure of these inhabitants to the genotoxic contaminants present in these water bodies. Further, a continuous biomonitoring including more number of sites and all other Esteros is recommended to achieve the goal of cleaning up the water bodies present in Metro Manila.

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