



ISSN NO. 2320-5407

Journal Homepage: -www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI:10.21474/IJAR01/1356
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/1356>

**RESEARCH ARTICLE**

**THE EVALUATION OF GENOTOXICITY POTENTIAL OF WATER BODIES IN AND AROUND
 METRO MANILA, PHILIPPINES USING *ALLIUM CEPA* METHOD.**

*Zeba Farooqi Alam. Keith Alexius Wangkay and Saejin Oh.

Biology Department, College of Science, De La Salle University, Taft Avenue, Manila, Philippines.

Manuscript Info**Manuscript History**

Received: 12 June 2016
 Final Accepted: 16 July 2016
 Published: August 2016

Key words:-

Alliumcepa Assay, Genotoxicity, Cytotoxicity, Pasig River, Marikina River, *Estero de Vitas* Chromosome Aberrations, Heavy Metals, Bio Monitoring

Abstract

Screening for hazardous chemicals and potential mutagens in water bodies, which are often the recipients of contaminated and polluted substances, is being accepted as a routine method in environmental monitoring programs. In this study, *Alliumcepa* method was used to evaluate the genotoxic potential of various water bodies namely- Pasig and Marikina Rivers and the *Estero de Vitas* located in and around *Metro Manila, Philippines*. Morphological modifications of the *Alliumcepa* roots, inhibition of root growth, mitotic index and the induction of high frequency of chromosomal aberrations comprising of chromosome fragments, laggards, anaphase and telophase bridges and C- mitotic effect were observed in the *Alliumcepa* roots exposed to water samples from three sources tested as compared to the control. The observed biological effects of the water samples appeared to be related to the physicochemical characteristics studied using Atomic absorption spectroscopy. The results of the investigation demonstrate that the hazardous substances and pollutants present in the water bodies has genotoxic and cytotoxic effects on *Alliumcepa* cells and there could be a potential threat to the human health as well as to the water ecosystems in and around Metro Manila. Hence the *Alliumcepa* test could be used to get preliminary information about the presence of genotoxic and or cytotoxic substances in the water bodies while performing the environmental monitoring.

Copy Right, IJAR, 2016,. All rights reserved.

Introduction:-

The pollution of the water bodies in and around urban areas all around the world including Philippines has become a huge problem with the pollutants with genotoxic and mutagenic effects posing direct threat to the human health as well as to the overall water ecosystems (O'Toole et al., 2009). The bodies of water in and around Metro Manila such as Pasig and Marikina River are notorious for being polluted. The Pasig River System, which is composed of river tributaries and several creeks and *Esteros*, was once inhabited by 25 varieties of fish and 13 types of aquatic plants. Due to rapid industrialization, and the increase in the population of Metro Manila, only 6 fish species, which includes Janitor fish (*Pterygoplichthysdisjunctivus*), Tilapia (*Oreochromisniloticus*), knife fish (*Apterotonusalbifrons*), sea catfish (*Ariidae*), Bighead Carp (*Hypophthalmichthysnobilis*) and only 2 plant species one of them being the water Hyacinth (*Eichorniacrassipes*), managed to survive in the polluted waters by the year

Corresponding Author:-ZebaFarooqiAlam.

Address:-Biology Department, College of Science, De La Salle University, Taft Avenue, Manila, Philippines.

1997 (Helmer&Hespanhol, 1997). In spite of the efforts and initiatives taken by the government and various organizations like the Pasig River Rehabilitation Commission¹, Asian Development Bank, Danish International Development Agency (DANIDA), ABS-CBN Foundation Inc.etc.to clean and rehabilitate the Pasig River, a worsening trend was reported with the presence of heavy metals (Greenpeace, 2001; Velasquez, 2002) along with the decrease in Dissolved Oxygen from 1999 to 2006 (Asian Development Bank's report, 2010).Between 1999-2009, the continuous decline in the water quality with respect to the amount of Dissolved Oxygen (D.O) and Biochemical Oxygen Demand (B.O.D.) continued (Gorme et al., 2010) and, the river never reached the Department of Environment and National Resource's (DENR) Class C standards (Table 1). Class "C" standard refers to quality of water that can be used for industrial purposes and recreational activities such as boating, fishing etc. Similar trends were reported for the connecting Marikina River, where both dissolved Oxygen levels and Oil and Grease concentrations did not meet Class C river standards of less than 5 mg/L and 2 mg/L respectively (Republic of The Philippines Department of Public Works and Highways, 2011).

Further, the Payatas dumpsite (the newly relocated Smoky Mountain, Pier 18) drainage became the major source of heavy metals such as chromium, copper, lead and zinc in the river, as well as other pollutants (Yoshimura et al., 2014). The presence of these heavy metals is quite alarming as prolonged exposure to these can cause many ailments including cancer, and mental retardation (Obligacion, 2015). Hence it is important to investigate if these polluted rivers and water bodies have the potential to cause genotoxicity and cytotoxicity especially post rehabilitation efforts by the Asian Development Bank and the Pasig River Rehabilitation Commission.

Since the pollution especially in the water bodies located in the urban areas can expose the environment and populations to many health risks, there is a need for rigorous environmental monitoring, where the sensitive but inexpensive biomonitors can be used to alert the surrounding populations of environmental contamination and pollutants. The merits of using *Alliumcepa* assay to detect environmental contamination are now well established. The USEPA GeneTox Program has classified the *Alliumcepa* method as one of the most well established plant assay systems for Environmental monitoring (Grant, 1982). *Allium* has small number of chromosomes (2n=16), relative low cost, and easy to obtain root meristems. The test is also done *in vivo* which means data can be extrapolated to other plants and even animals (Tedesco &Laughinghouse, 2012). Despite the typical use of it as a preliminary test, the accuracy and legitimacy of using the *Alliumcepa* assay has been reported to be very accurate with 84% correlation compared to carcinogenicity tests in rodents, and was even concluded to be more sensitive than the *Ames test* to check for mutagenic potential of chemicals (Rank and Nielsen, 1998). The onion being in direct contact with the substance of interest while growing is a major reason for its validity, especially in terms of measuring cytotoxicity as well as genotoxicity (Vicentini et al., 2001).

Therefore, it is now being routinely used to evaluate the genotoxic, cytotoxic or mutagenic effects of chemical compounds, nanomaterials, phytochemicals, pollutants and contaminants such as pesticides, herbicides, metals and heavy metals using the induction of DNA damages, chromosomal aberrations and alterations in the mitotic index as endpoints (Khallef et al., 2013; Klauck et al., 2013; Leme and Marin-Morales, 2008). Studies have indicated a correlation between pollution (through chemical composition) and inhibition of root growth and decrease in mitotic index (Radic et al., 2010).

Since the water bodies in and around Manila have been identified as reservoirs of all kinds of domestic waste and contaminants hence, *Alliumcepa* test is being used for environmental biomonitoring of these water bodies and rivers to assess the genotoxic potential of the environmental toxins if any present in the water.

Materials And Methods:-

Collection of Samples:-

Samples were taken from different locations along the rivers (Figure 1 and 2), and the equal samples were mixed together to get an average of the river's water composition.

¹ Presidential Decree Number 274, Pertaining to the Preservation, Beautification, Improvement, and Gainful Utilization of the Pasig River, Providing for the Regulation and Control of the Pollution of the River and Its Banks In Order to Enhance Its Development, Thereby Maximizing Its Utilization for Socio-Economic Purposes

In addition, *Estero de Vitas* which is also connected to Pasig river, was also sampled being in a former dumpsite known as Smokey Mountain (Figure 3). The estuary is now surrounded by land reclaimed areas, presumably made with some of the garbage that used to accumulate there.

Laboratory Analysis:-

A. *Alliumcepa* Test:-

The standard *Alliumcepa* method was adopted to carry out the genotoxicity analysis as used in many similar studies (Fiskesjo, 1997; Babatunde and Bakare, 2006). Briefly, medium sized (15-22 mm diameter) healthy bulbs of common onion, *Alliumcepa* were purchased from the local market. The outer scales of the onions were removed and had their roots cut without destroying the root primordial to allow the new roots to grow. The bases of the onions were then submerged in equally sized containers with tap water for 48 hours. The lengths of the new rootlets were measured. To test the effect of the river water samples, the onion roots were grown for 72 hours in treatments of 10%, 20%, 40%, 60%, 80% and 100% (v/v) concentrations of the sample waters collected. The treatment water samples were replaced every 24 hours. Just as tap water was used as negative control, methylmethanesulfonate (MMS, 10 ppm) was used as positive control. MMS is widely recommended as a positive control chemical in mutagenicity testing (Rank and Nielsen, 1998; Caritá and Marin-Morales, 2008). For each treatment, a series of 5 replicates were set up.

Following the exposure to the various treatments, the root length of the onions was measured at 24, 48 and 72 hours of growth. To study the chromosomal aberrations and mitotic Index, the slides were prepared for microscopy using the standard procedure. The roots, particularly the freshest and healthiest, were cut from each onion and were hydrolyzed in 1 N HCL at 60 °C for 12 min and then stained with acetocarmine stain solution prepared in 45% acetic acid. The microscope slides were prepared by placing the stained onion roots on the slide and squashing them in 45% (v/v) glacial acetic acid. The slides were observed using optical microscope at an overall magnification of 100X. Calculation of the mitotic index was done using the standard formula:

$$\frac{P + M + A + T}{\text{Total Cells}}$$

The types of aberrations observed were anaphase bridges, chromosomal breakages, and laggards, stickiness (Figures 12). The standard formula used for calculating proportion of chromosomal aberration is as follows:

$$\frac{P_a + M_a + A_a + T_a}{P + M + A + T}$$

Where Pa, Ma, Aa and Ta are the abnormal mitotic cells, and P, M, A and T are all mitotic cells.

B. Physicochemical Analysis:-

Atomic Absorption Spectrometry was performed to determine the presences of heavy metals such as Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn), Iron (Fe), Nickel (Ni) and Mercury (Hg). Biochemical Oxygen Demand and Dissolved Oxygen were also measured along with measurements for pH and turbidity.

C. Statistical Analysis:-

River treatments of different concentrations were compared to both positive and negative control through t-tests (for root length) and z-tests (for mitotic index and aberration proportion). Any significance was determined at an alpha of at least 0.05 (95% confidence). Linear regression was also done to determine if there were any trends between root growth, mitotic index and aberration versus increasing concentrations of the treatments. PHSTAT 4.0 was used for these statistical analyses.

Results And Discussion:-

Root Growth:-

The root growth for Pasig river water samples has a noticeable decreasing trend as the concentration of the treatment increases, with a high R² value of 0.8916 (Figure 4). This suggests a very good inverse correlation between root growths versus concentration of Pasig river water samples tested. Marikina river samples on the other hand showed a low R² value of 0.0012 suggesting poor correlation (Figure 5). It's noteworthy to point out that for both the Pasig and Marikina treatments, the onion roots of the 10% concentrations grew better than the negative control itself. For

the Pasig river sample, there was a significant difference at an alpha of 0.05 whereas 10% Marikina water treatment sample did not show the same significance ($P > 0.05$). This may be attributed to nutrients found in the river samples, which the tap water lacks. Human waste that goes into the rivers for example are rich in inorganic nitrate that is readily absorbed by plant roots (Crawford, 1995) and at low concentrations, may have assisted in root growth. Similar results have been reported where roots grew better in 20% sewage wastewater treatment than their negative control (Shashank and Suresh, 2012).

All other concentrations tested, from 20% concentrations onwards were observed to have lower means than the negative control, inducing significant inhibition of the root growth (Table 2). Significant differences were observed in Pasig 80% and 100% water samples, and in Marikina 40% and 100% water samples ($P < 0.05$). These results aggregately suggest that the river waters do inhibit root growth, starting from 20% concentration onwards with the rotting of roots observed at 80% and 100% concentration of Pasig river and at 100% concentration of Marikina, which is indicative of the cytotoxic effects of the water samples (Figure 7).

Estero de Vitas showed the best inverse trend of the three sample sites with a very high R^2 value of 0.9408. It should be pointed out that the decaying of roots in the onions was observed starting at 60% concentration water sample. The 80% and 100% concentrations were very highly toxic in particular (Figure 6), as the roots actually fell off due to rotting causing the lower average root length after 72 hours compared to their initial length. The onions treated with *Estero de Vitas* waters fared the worst over all of the three sample waters tested, with an average of 97.9% inhibition in root length (46.3% for Pasig, 56.2% for Marikina).

B. Mitotic Index:-

The mitotic index for Pasig river samples showed a good trend with a very high R^2 value of 0.9259 (Figure 8). Mitotic index was observed to decrease with the increasing concentrations of Pasig water samples. The Marikina samples expectedly showed a low trend ($R^2 = 0.0812$) for its mitotic index much like its root growth (Figure 9). Both Pasig river water mitotic index and Marikina water mitotic index was found to be significantly lower than the negative control at 20% concentrations onwards ($P < 0.0001$).

Estero de Vitas concentrations from 20% to 60% were also found to be very significantly lower, compared to the negative control ($P < 0.0001$, Figure 11). The 80% and 100% *Vitas* treatments showed indeterminate mitotic indices as cells in these concentrations were observed to be dead (Figure 10).

C. Chromosomal Aberrations:-

All concentrations of water samples from Pasig, Marikina River and *Estero de Vitas* tested, induced chromosomal aberrations such as chromosomal fragments, anaphase and telophase bridges and laggards in the *Allium cepa* root cells (Table 4). The lagging chromosomes are induced by a weak C- mitotic effect and are indicative of disturbance in the mitotic spindle or the centromere (Fiskesjo, 1994).

Pasig river samples showed a low inverse trend at an R^2 of 0.1122 (Figure 13). At 100%, the toxicity of the water suppressed the mitotic index significantly and since there were no viable cells, no mitotic aberrations were observed at all (Figure 14).

Marikina river water samples showed good trend (R^2 value of 0.6592) in terms of increase in aberrations with increase in concentrations of water samples (Figure 15). This shift in correlations may be explained due to the number of viable cells in Marikina treatments being comparable in amount as the root growths and mitotic index were about equal, where at the same concentrations, the Pasig river samples had dying or dead cells. The low value of chromosomal aberrations observed at Pasig 100% water treatment is indicative of the cytotoxicity of the water sample to the onion root cells. Similar results were reported by Radic et al. (2010), who found that cytotoxicity (cell killing) was not necessarily correlated to genotoxicity (gene damaging) due to the presence of nitrate, nitrite, ammonium and phosphate. Therefore, lower root growth/mitotic index does not necessarily mean higher chromosomal aberrations rate. In 2010, Samuel, Osuala & Odeigah, using the same assay had similar results in terms of correlation between concentrations versus mitotic index, where low concentrations had lower mitotic index or even higher aberrations compared to higher concentrations. Regardless, overall there was significant difference found at all concentrations showing the river water samples to have a genotoxic effect ($P < 0.005$). *Estero de Vitas* water samples also showed significantly higher chromosomal aberrations compared to the negative control at 10%, 20%, 40% and 60% concentrations ($P < 0.005$, Figure 16). The presence of sticky chromosomes indicates that the

pollutants and hazardous substances present in the river water samples are affecting the organization of the chromatin in the cells, which can ultimately lead to cell death. Many studies have reported the occurrence of sticky chromosomes in *Allium* roots after treatment with various heavy metals such as Hg, Ni and Cu (Fiskesjö, 1993, 1997; Egitto et al., 2007).

Overall, the results using the *Alliumcepa* test show that the water samples from the *Estero devitas* caused maximum genotoxicity as reported by other studies as well (Orozco & Zafaralla, 2012; Enguito, et al., 2013). The human activities have made these *Esteros* or creeks a major source of pollution with the waste dumped in these water ways obstructing the smooth flow of water and causing major flooding during the rainy season (Gilbuena et al., 2013). To improve the water quality of Pasig and Marikina river, it is important to clean these *Esteros* or creeks hence the decision of the Pasig River Rehabilitation Commission to focus on cleaning one by one these *Esteros* is a step in the right direction. According to the Pasig River Rehabilitation Commission, efforts are on to clean all 48 tributaries/*Esteros* of Pasig river by 2019.

D. Physico-chemical Characteristics of Water Samples:-

The Atomic absorption spectroscopy carried out on the water samples collected from Pasig, Marikina rivers and *Estero de vitas* locations indicate the presence of heavy metals such as Lead (Pb), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe) and Mercury. Some of these metals such as Cadmium (Cd), Chromium (Cr), and lead (Pb) were found to be present in excess of the standard accepted values set by the various local state agencies (Table 3). The presence of heavy metals in the water samples is not surprising as heavy metals are among the most common inorganic pollutants in water (Chandra et al., 2005). The source of heavy metals could be from the human waste dumped by the informal settlers living all along the river banks as well as commercial and industrial establishments operating near the river. Another possible source of the heavy metals could be the e waste leachates from some of the formal and informal electronic waste dumpsites and recycling sites located in and around Manila. The studies have indicated the presence of heavy metals in the soil and dust surface matrices from these electronic recycling sites which due to rain and flooding can easily flow out into the water bodies in and around these dumpsites (Fujimori et al., 2012). The presence of heavy metals such as Lead and Cadmium in excess of allowable limits set by the United States Environmental Protection Agency was reported even in the community tap water samples of Manila (Solidum and Solidum, 2012). The possibility of the leachates from the 19-hectare sanitary landfill in San Mateo, Rizal being the source of hazardous chemicals in the Marikina river should also be investigated.

The Biological oxygen demand (BOD) at 29 mg/L was significantly higher than the DENR Class C DENR Standard values of 7 mg/L. Similarly, the Dissolved Oxygen (DO) at 0.1 mg/L was much lower than the DENR Standard of 5 mg/L (Table 3). Though Pasig River Commission has been claiming steady improvement in the water quality of the rivers however according to some reports, the water tested at 14 stations set up along the route of Pasig River, failed to pass the DENR acceptable standards (Obligacion, 2015). The pH of the water samples from Pasig and Marikina rivers was found to be with in the DENR standard limits however the water samples from *Estero de vitas* were slightly acidic with pH of 6.7. The toxicity in terms of biological damage by complex mixtures is reported to enhance by extreme pH values (Fiskesjo, 1985). There is correlation between our physico-chemical analysis results of water samples and the genotoxicity observed in terms of inhibition of root growth and mitotic index and increase in the chromosomal aberrations with increase in the concentrations of water samples tested.

Heavy metals as pollutants can cause DNA damage and thereby cause carcinogenic and mutagenic effects with increase in the frequency of chromosome breaks and mutations in various test systems and also in humans (Minissi et al., 1997; Ivanova et al., 2008). The studies have also shown that long-term exposure to even small concentrations of heavy metals increases the risk of quite a few diseases, including cancers (Zhuang et al., 2009). Many studies have indicated that a variety of ecotoxicity tests should be used to detect chemicals that are capable of causing environmental mutagenesis as only chemical analysis is not sufficient to reveal their toxicity (Chen et al., 2004, White et al., 2004, Fuentes et al., 2006). It has also been established that *Alliumcepa* is one of the useful test systems for the detection of these potential genotoxic substances in water screening programs (Fiskesjo, 1997; Ivanova et al., 2002^a, 2002^b). The results also conclude that the *Alliumcepa* test system can be used very effectively to perform preliminary biomonitoring of contaminated water sources in the Philippines as well.

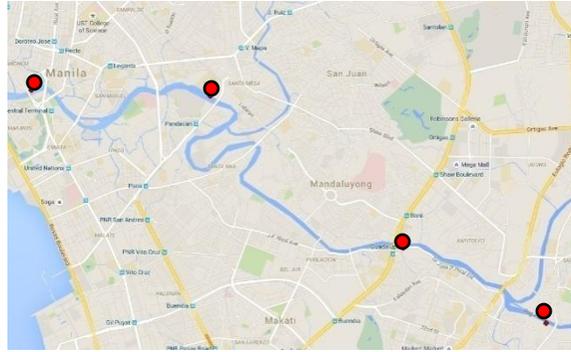


Figure 1:-Location of samples collected for Pasig River (Red Dots)

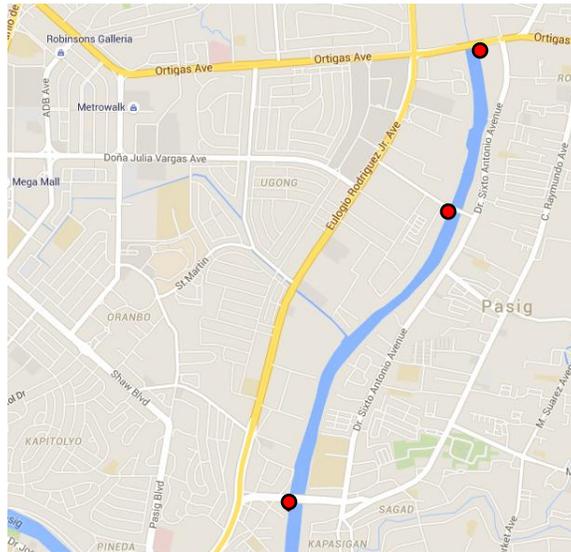


Figure 2:-Location of samples collected for Marikina River (Red Dots)

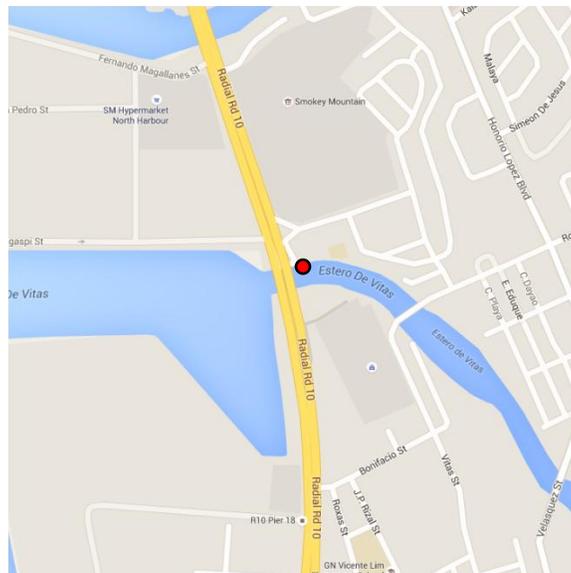


Figure 3:-Location of sample collected for Estero de Vitas (Red Dot)

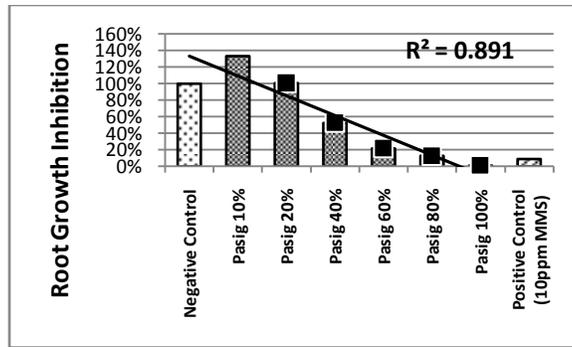


Figure 4:-The Root growth inhibition with increase in Pasig River water concentration treatment
 * – Lower root length at P < 0.05 compared to negative control. % Root Growth is length of growth after 72 hours. % Relative Root Length is length compared to negative control. % Inhibition is negative control minus relative root length.

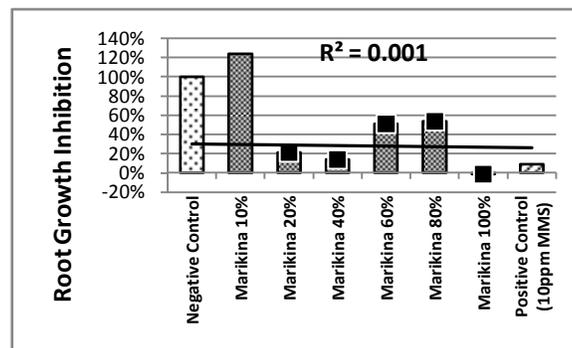


Figure 5:-Inhibition of Root length with increasing concentration of Marikina River Water Treatment

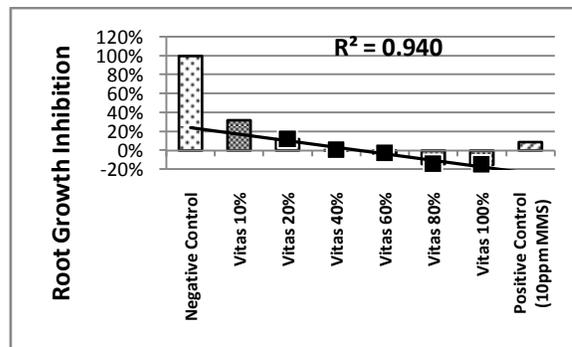


Figure 6:-Estero de Vitas Treatment – Inhibition of root growth with increase in the concentrations of the water samples



Figure 7:-Morphological comparison of onion roots and their growth after 72 hour

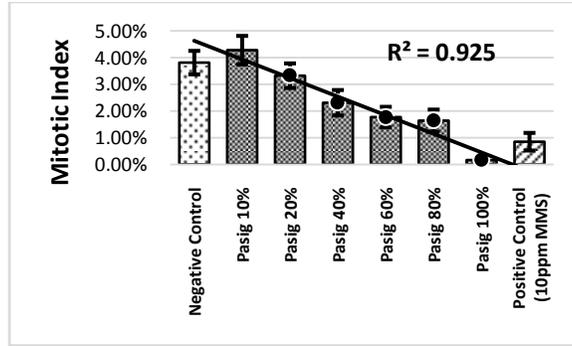


Figure 8:-Mitotic Index for Pasig River Water Treatments. Trend line is for concentrations from 20% to 100% compared to negative and positive control samples.

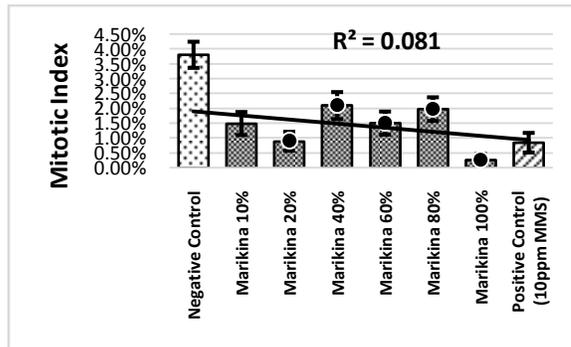


Figure 9:-Reduction in Mitotic Index for Marikina River Water Treatments. Trend line is for concentrations from 20% to 100% as compared to negative and positive control.

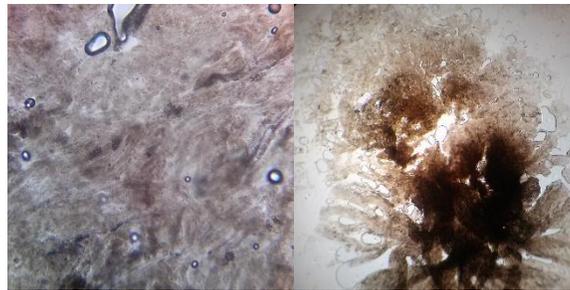


Figure 10:-Estero de Vitas 80% Water Sample Treatment with Root cells observed at 10X (Left) and 40X (Right). Cytotoxicity with no viable cells was observed in all replicates at these concentrations.

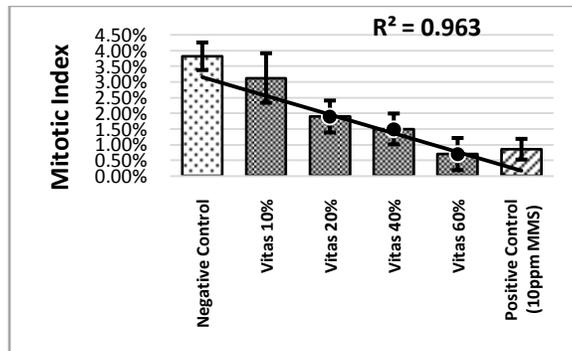


Figure 11:-Mitotic Index for Estero de Vitas Treatments. Concentrations at 80% and 100% were excluded due to no observable viable cells.

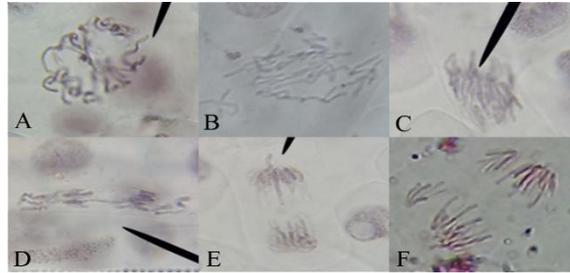


Figure 12:-Chromosomal aberrations observed in various treatments. A – C mitosis, B – Broken Spindle Fibers, C – Sticky Chromosome, D – Anaphasic Bridge, E – Laggard Chromosome, F – Damaged Centriole

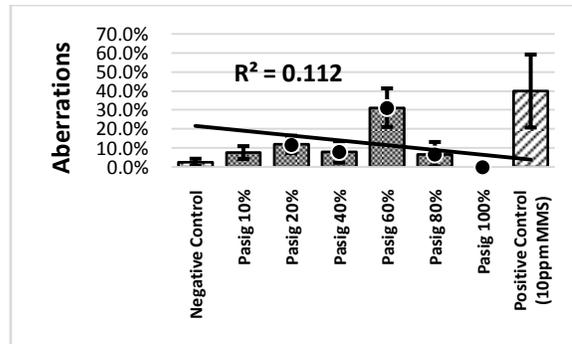


Figure 13:-Aberration Proportion for Pasig River Treatments

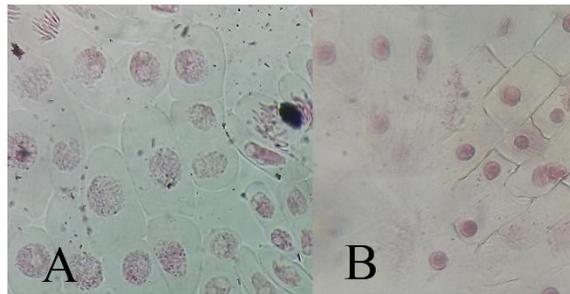


Figure 14:- A – Normal cells observed. B – Interphase Cells at 100% Concentration (Pasig River water): Very Dense Nucleus

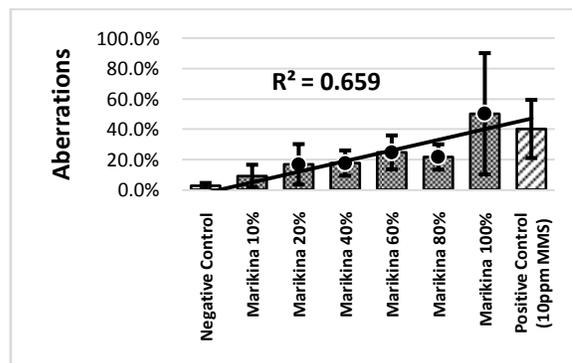


Figure 15:-Aberration Proportion for Marikina River Treatments

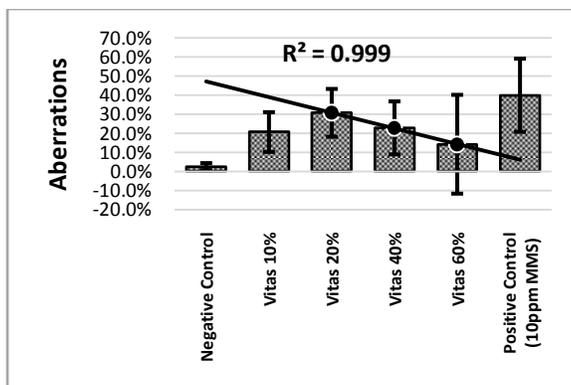


Figure 16:-Aberration Proportion for *Estero de Vitas* Treatments Concentrations at 80% and 100% were excluded due to no observable viable cells.

Table 1:-DENR Class C Fresh Water Classification

pH	6.5 – 8.5
D.O. (mg/L)	5.0
B.O.D. (mg/L)	7.0
Chromium (mg/L)	0.05
Oil and Grease (mg/L)	2.0
Lead (mg/L)	0.05
Mercury (mg/L)	0.002

Table 2:-Summary of Root Growth and Inhibition

Treatment	% Root Growth	% Relative Length	% Inhibition
Negative Control	301 ± 106	100	—
Pasig 10%	401 ± 123	133	-33
Pasig 20%	303 ± 102	101	-1
Pasig 40%	158 ± 81	52	48
Pasig 60%	65 ± 9	22	78
Pasig 80%*	38 ± 35	13	87
Pasig 100%*	3 ± 20	1	99
Marikina 10%	372 ± 154	124	-24
Marikina 20%	64 ± 82	21	79
Marikina 40%*	42 ± 46	14	86
Marikina 60%	154 ± 67	51	49
Marikina 80%	162 ± 110	54	46
Marikina 100%*	-4 ± 88	-1	101
Vitas 10%	96 ± 102	32	68
Vitas 20%*	36 ± 55	12	88
Vitas 40%*	1 ± 33	0.5	99.5
Vitas 60%*	-9 ± 4	-3	103
Vitas 80%*	-43 ± 4	-14	114
Vitas 100%*	-46 ± 16	-15	115
Positive Control (MMS)	27 ± 22	9	91

Table 3:-Comparison of Pasig and Marikina: Physico-chemical Characteristics versus different standards

Metal	Pasig River	Marikina River	<i>Estero de Vitas</i>	DENR Class C Standard
Cadmium (Cd)	0.0220	0.0275	0.0427	0.01
Chromium (Cr)	0.2266	0.2540	0.0130	0.05
Copper (Cu)	0.0217	0.0179	0.0203	0.05
Iron (Fe)	0.6360	0.6112	0.0424	-
Lead (Pb)	0.3176	0.4557	0.1608	0.05
Mercury (Hg)	<0.001	<0.001	<0.001	0.002
B.O.D.	29	30	-	7
D.O.	0.10	1.10	-	60
pH	7.5- 8.04	7.2-8.2	6.7	6.5-8.5

Table 4:-Breakdown of Results for total Chromosomal abnormalities for Pasig and Marikina at Different Concentrations of water samples tested

Treatment	Total Cells Examined	Total Mitoses	M.I. (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)	Abnormalities				Total Abnormalities
								M	P	A	T	
Negative Control	7257	277	3.82	1.60	0.74	0.52	0.95	0	0	7	0	7
Positive Control	2925	25	0.85	0.27	0.44	0.10	0.03	2	5	3	0	10
P10	5272	236	4.28	1.99	0.91	0.61	0.97	7	5	5	1	18 ^b
P20	5609	193	3.33	1.59	0.68	0.50	0.68	6	8	6	3	23 ^b
P40	3720	88 ^a	2.31	0.65	0.94	0.40	0.38	0	2	5	0	7 ^b
P60	4431	80 ^a	1.77	0.59	0.41	0.41	0.41	3	3	13	6	25 ^b
P80*	3538	59 ^a	1.64	0.54	0.54	0.31	0.28	0	0	3	1	4 ^b
P100*	2466	4 ^a	0.16	0.16	0.00	0.00	0.00	0	0	0	0	0 ^b
M10	3684	56 ^a	1.50	1.19	0.03	0.08	0.22	3	0	0	2	5 ^b
M20	3312	30 ^a	0.90	0.45	0.12	0.18	0.15	2	1	2	0	5 ^b
M40*	3725	80 ^a	2.10	0.45	0.12	0.18	0.15	7	5	2	0	14 ^b
M60	3713	57 ^a	1.51	1.02	0.40	0.32	0.40	4	5	5	0	14 ^b
M80	4597	93 ^a	1.98	0.78	0.30	0.30	0.16	5	6	9	0	20 ^b
M100*	2283	6 ^a	0.26	0.04	0.18	0.00	0.04	0	3	0	0	3 ^b
V10	1857	58 ^a	3.12	0.43	1.08	0.86	0.75	1	7	0	4	12 ^b
V20*	2745	52 ^a	1.89	0.55	0.62	0.40	0.33	3	2	8	3	16 ^b
V40*	2337	35 ^a	1.50	0.04	0.51	0.30	0.64	1	0	1	6	8 ^b
V60*	1007	7 ^a	0.70	0.30	0.20	0.20	0.00	0	1	0	0	1 ^b

Conclusion:-

The inhibition of root growth, the decrease in the mitotic index and the presence of chromosomal aberrations in the *Allium cepa* root cells exposed and grown in the water samples of Pasig river, Marikina river and *Estero de Vitas* is indicative of the presence of hazardous substances and pollutants capable of causing genotoxicity in these water bodies. Since the water samples of Marikina River and *Estero de Vitas* have been also included in this study, the results give more comprehensive information about the genotoxicity of the water bodies in and around Manila. There are 48 esteros linked to the Pasig River and these have been identified as the major source of pollution to the river due to human settlements around these tributaries. Only with the chemical analysis of water, it is not possible to determine the eco-toxicological risk that chemicals present in it can cause, since such analysis alone does not indicate toxicity (Fuentes et al., 2006). Therefore, ecotoxicity tests that can detect environmental mutagenesis, have been proposed and applied to understand the genetic and physiological responses of exposed organisms (White et al., 2004; Chen et al., 2004). The results clearly demonstrate that the *Allium cepa* assay in combination with the physicochemical analysis of the water bodies can be very effectively used as a preliminary screening test in the environmental monitoring to evaluate the presence of genotoxic and cytotoxic pollutants and environmental contaminants in the water bodies which will aid to safe guard the human populations as well as other organisms.

References:-

1. Asian Development Bank. (2010): Philippines: Pasig river environmental management and rehabilitation sector development program.
2. Babatunde, B.B. and Bakare, A.A. (2006): Genotoxicity screening of wastewaters from Agbara industrial estate, Nigeria evaluated with the *Allium* test. *Poll Res*, 25: 227–234.
3. Caritá, R. and Marin-Morales MA. (2008): Induction of chromosome aberrations in the *Alliumcepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*, 72: 722–5.
4. Chandra, S., Chauhan L.K.S., Murthy R.C., Saxena P.N., Pande P.N. and Gupta S.K. (2005): Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Science of the Total Environment*, 347: 46–52.
5. Chen, G., and White, P.A. (2004): The mutagenic hazards of aquatic sediments: A review. *Mutation Res*. 567:151–225.
6. Crawford, N.M. (1995): Nitrate: Nutrient and Signal for Plant Growth. *The Plant Cell*, 7: 859-868.
7. Egitto, L.M., Medeiros, M.D.G., Medeiros, S.R.B. and Agnez-Lima, L.F. (2007): Cytotoxic and genotoxic potential of surface water from the Pitimbu River, Northeastern/RN Brazil. *Gen Mol Biol*, 30: 435-431.
8. Enguito M.R., Matunog V., Bala J.J. and Villantes Y. (2013): Water quality assessment of Carangan Estero in Ozamiz City, Philippines. *Journal of Multidisciplinary Studies*, 1: 19-36.
9. Fiskesjö, G. (1985): The *Allium* test as a standard in environmental monitoring. *Hereditas*, 102: 99-112.
10. Fiskesjö, G. (1993): The *Alliumcepa* in wastewater monitoring. *Environ Toxicol Water*, 8: 291–8.
11. Fiskesjö G. (1994): *Allium* test II: assessment of a chemical's genotoxic potential by recording aberrations in chromosomes and cell division in root tips of *Alliumcepa*. *An International Journal of Environmental Toxicology and Water Quality*, 9: 235-241.
12. Fiskesjö G. (1997): *Allium* Test for Screening Chemicals; Evaluation of Cytological Parameters, In: Wang W, Gorsuch JW, Hughes JS (Eds.). *Plants for Environmental Studies*, New York: Lewis Publishers: pp. 308–333.
13. Fuentes, A., Llorén, M., Sáez, J., Aguilar, M.I., Pérez-Marín, A.B., Ortuño, J.F. and Meseguer, V.F. (2006): Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. *Environ Pollut*. 143:355-360.
14. Fujimori, T., Takigami H., Agusab T., Eguchi A., Bekki K., Yoshida A., Terazono A. and Ballesteros F.C. Jr. (2012): Impact of metals in surface matrices from formal and informal electronic-waste recycling around Metro Manila, the Philippines, and intra-Asian comparison, *Journal of Hazardous Materials*, 221–222:139–146.
15. Gilbuena R. Jr., Kawamura A., Medina R., Amaguchi H., Nakagawa N. and Bui, D. D. (2013): Environmental impact assessment of structural flood mitigation measures by a rapid impact assessment matrix (RIAM) technique: A case study in Metro Manila, Philippines. *Science of the Total Environment*, 456: 137-147.
16. Gorme J., Maniquiz M., Song P. and Kim L., The Water Quality of the Pasig River in the City of Manila, Philippines: Current Status, Management and Future Recovery, *Environmental Engineering Research*, 15(3), 2010, 173-179.
17. Grant W.F. (1982): Chromosome aberration assays in *Allium*. *Mutation Research*, 99: 273–291.
18. Greenpeace. (2001): Industrial Pollution in Pasig River.
19. Helmer, R. and Hespagnol I. (1997): Case Study III – The Pasig River, Philippines. *Water Pollution Control – A Guide to Use of Water Quality Management Principles*. World Health Organization.
20. Ivanova E, Staikova T., Velcheva I. (2002)^a: Mutagenic effect of water polluted with heavy metals and cyanides on *Pisum sativum* plant in vivo. *Journal of Balkan Ecology*, 3: 307-310.
21. Ivanova E., Staykova T. and Velcheva I. (2008): Cytotoxicity and genotoxicity of heavy metal and cyanide-contaminated waters in some regions for production and processing of ore in Bulgaria. *Bulgarian Journal of Agricultural Science*, 14: 262-268.
22. Ivanova, E., Velcheva, I., Staikova, T. and Kostadinova, P. (2002)^b: Somatostatic effect of heavy metal and cyanide contaminated waters on a *Pisum sativum* L. plant system in vivo, *J. Balkan Ecol*, 4: 443-446.
23. Khallef M., Liman R., Konuk M., Ciğerci İ.H., Benouareth D., Tabet M. and Abda A. (2013): Genotoxicity of drinking water disinfection by-products (bromoform and chloroform) by using both *Allium* anaphase-telophase and comet tests. *Cytotechnology*.
24. Klauck, C.R., Rodrigues M. A. S. and Basso Da Silva L. (2013): Toxicological evaluation of landfill leachate using plant (*Alliumcepa*) and fish (*Leporinus obtusidens*) bioassays. *Waste Management and Research*, 31: 1148–1153.
25. Leme D.M. and Marin-Morales M.A. (2008): Chromosome aberration and micronucleus frequencies in *Alliumcepa* cells exposed to petroleum polluted water - A case study. *Mutation Res*. 650:80-86.

26. Minissi, S. and Lombi E. (1997): Heavy metal content and mutagenic activity, evaluated by *ViciaFaba* micronucleus test, of Tiber river sediments. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 393: 17-21.
27. O'Toole, A. C. O., Hanso, K. C. and Cooke, S. J. (2009): The effects of shoreline recreational angling activities on aquatic and riparian habitat within an urban environment: implications for conservation and management. *Environmental Management*, 44: 324-334.
28. Obligacion, F. (2015): A layperson's guide to the environmental impact assessment of Pasig River cluster one esteros in the pre-rehabilitation phase.
29. Orozco, G. P. and Zafaralla, M. T. (2012): Biophysico-chemical and socioeconomic study of two major Manila esteros. *Biology Education for Social and Sustainable Development*, 2012, 161- 171.
30. Pasig River Rehabilitation Commission. (2014): Annual report: Pasig River Rehabilitation Commission.
31. Radic, S., Stipanicev, D., Vujcic, V., Rajcic, M., Sirac, S. and Pevalek-Kozlina, B. (2010): The evaluation of surface and wastewater genotoxicity using the *Alliumcepa* test. *Science of Total Environment*, 408: 1228-1233.
32. Rank, J. and Nielsen, M.H. (1998): Genotoxicity testing of wastewater sludge using the *Alliumcepa* anaphase-telophase chromosome aberration assay. *Mutat Res*, 418: 113-9.
33. Republic of the Philippines Department of Public Works and Highways. (2011): Supplemental environmental impact statement.
34. Samuel O.B., Osuala F.I. and Odeigah P.G.C. (2010): Cytogenotoxicity evaluation of two industrial effluents using *Alliumcepa* assay. *African Journal of Environmental Science and Technology*, 4: 21-27
35. Shashank, B. and Suresh, D. (2013): Toxicity effects of municipal sewage on onion roots. *Journal of Environmental Research and Development*, 7 No. 4A.
36. Solidum J.N. and Solidum G.G. (2015): Assessment and remediation of heavy metals in community tap water from Manila, Philippines. *International Conference on Environment Science and Engineering IPCBEE*, 32. IACSIT Press, Singapore.
37. Tedesco, S.B. and Laughinghouse H.D. (2012): Bioindicator of genotoxicity: The *Alliumcepa* test. Dr. JatinSrivastava (Ed.).
38. Velasquez I.B., Jacinto G.S. and Valera F.S. (2002): The speciation of dissolved copper, cadmium and zinc in Manila Bay, Philippines. *Marine Pollution Bulletin*, 45: 210-7.
39. Vicentini V.E.P., Comparoto M.L., Teixeira R.O. and Mantovani M.S. (2001): *Averrhoacarambola* L., *Syzygiumcumini* (L.) Skeels, *Cissussicyoides* L.: medicinal herbal tea effects on vegetal and test systems. *ActaScientiarum*, 23: 593-598.
40. White, P.A. and Claxton, L.D. (2004): Mutagens in contaminated soil: a review. *Mutation Res.* 567:227-345.
41. Yoshimura C., Yamanaka C., Fuujii M., Leungprasert S. and Tanchuling, M. (2015): Heavy metals in suspended sediments in rivers flowing through megacities in South East Asia. *ASEAN Engineering Journal*, 4: 63-7.
42. Zhuang, P., Zou, B., Li, N.Y., and Li, Z.A. (2009): Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health. *Environ Geochem Health*, 31:707-715.