Development of an acute sediment toxicity test using an endemic benthic macroinvertebrate, *Chironomus species* to assess the toxicity of Philippines' Pasig River sediments

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Abstract. In the absence of a sediment quality guideline, mere characterization regarding the identity and levels of the constituents present in sediments, will not contribute much to an effort of establishing the impact of sediment quality to the aquatic environment. Available standard methods for sediment toxicity tests require the use of species that may not be accustomed to a tropical climate such as in the Philippines. In this regard, area of this study was to assess toxicity of the sediment from Pasig River through the use of an endemic midge (order *Diptera*, family *Chironomidae*) collected from Los Baños, Laguna, Philippines as test species. Pasig River is one of the most important waterways in the Philippines, traversing six major cities of Metro Manila. Such strategic location of the River, also explains why it is heavily impacted by both point and nonpoint sources of toxicants. Sediment samples were collected in April 2007, month of dry season and November 2007, month of wet season at five sampling stations along the Pasig River—Delpan, Nagtahan, Pandacan, Guadalupe, and Bambang. A modified 96-hour acute toxicity test was adopted in this study in which sediment samples were mixed with "control" sediment, also obtained locally and subsequently and exposed to the test species. Results suggested that the most polluted and most toxic was the Delpan station which yielded the lowest LC50. The LC50 was experimentally determined via probit analysis. Moreover, it was determined that sediment samples collected during the dry season exhibited lower toxicity towards these organisms than those samples collected during the wet season possibly due the effect of runoff. In view of the results obtained, the locally developed ecotoxicological assay performed on sediments of Pasig River can be used to assess and to distinguish sites along Pasig River that could be potentially toxic based upon its response to the locally grown *Chironomids* species.

1. Introduction

Pasig River stretches for 27 kilometers from Laguna de Bay to Manila Bay in the Philippines. It serves as a major transport route, lifeline of Laguna de Bay, which is one of the biggest freshwater lakes in the world [1]. The Pasig River system includes Pasig, San Juan and Marikina Rrivers in greater Manila. Pasig River traverses six major cities of Metro Manila (Manila, Mandaluyong, Makati, Pasig, Quezon City and Marikina) and has served as a habitat for 25 varieties of fish and 13 different types of aquatic plant. Today, there are only six species of fish and two types of plants left that can tolerate the polluted water. It is heavily polluted with industrial, municipal and domestic wastes [2].

Various chemical contaminants have been detected in Pasig River as a result of the various human activities along the river banks as well as operations of major industries also located along the stretch of Pasig River that include past waste disposal practices and accidental oil spills from barges plying the area. For instance, heavy metals enter aquatic ecosystems from various sources such as municipal wastewater, industrial waste discharges, and river runoff. Cu, Pb, Mn, Ti, and Fe were among the metals identified to be present in Pasig River sediments [1]. Over time, these heavy metals found in the water column tend to settle on the sediment bed [3]. Sediments can serve both as reservoirs and as potential sources of contaminants to the water column [4]. Contaminants associated with sediments can adversely affect resident sediment-dwelling organisms by causing direct toxicity or by altering benthic invertebrate community structure [5]. Furthermore, these contaminants can also harmfully affect fish and wildlife species, either by direct exposure or through bioaccumulation in the food web [6].

The contribution of contaminated sediments on sediment-dwelling organisms (including plants and invertebrates), aquatic-dependent wildlife (amphibians, reptiles, fish, birds, and mammals), and human health has become more apparent in recent years [7-10]. Quantities of known toxicants such as metals, polyaromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), ammonia, etc. are typically determined during environmental monitoring [11]. However, these data would not specifically indicate the magnitude of the impact in terms of the potential toxicity to concerned aquatic living organisms. Short term toxicity test such as the 96-hour test can be employed to assess acute toxicity [12,13], usually expressed as LC50 or the lethal concentration or lethal dose at which 50% of the population of the test species have died, apparently due to the toxic effect of pollutants present in the sample [14]. LC50 when validated could be an indicator of the presence of cause-effect relationship between the test organisms and that of the toxicant/s present in the sample.

In the absence of a sediment quality guideline, comparing those sediment chemistries obtained from routine monitoring against sediment quality guidelines of developed countries, [15] will not be ideally reliable to assess sediment contamination, particularly in the Philippine setting. Likewise, standard toxicity methods using organisms that are not endemic in the Philippines or in countries with tropical climate, may also give unreliable responses due to extreme sensitivity to prevailing climatological and meteorological conditions. Hence, this study attempted to come up with an acute 96-hour sediment toxicity test using a local *chironomidae* species as the test organisms. For this purpose, the non-biting midges (order *Diptera*, family *Chironomidae*) were collected from the fields and were cultivated in the laboratory under controlled conditions to be used in evaluating the relative toxicity of Pasig River sediments [16]. The response (mortality) of the test organisms at the end of the 96-hour sediment toxicity test was then statistically treated to derive LC50. In the absence of sediment quality guidelines for tropical climate countries, the results of this study can serve as platform for assessing the environmental conditions of river sediments in the Philippines [17]. Accordingly, efforts are systematically organized leading to possible design of rehabilitation schemes for areas which shall be found to manifest toxic effect and therefore, will demand urgent mitigating measures.

2. Materials and methods

Reagents used in this study were all of spectroscopic grade such as Spectrosol Copper (II) Nitrate AAS Standard, Spectrosol Lead (II) Nitrate AAS Standard, Spectrosol Cadmium Nitrate AAS Standard, and Univar Nitric Acid (70%). HACH HQ30D pH/Conductivity/LDO meter and probes was used for in-situ monitoring of pH, conductivity, dissolved oxygen, hardness and salinity. GARMIN e TREX H Global Positioning System was used to generate the locations of the samples collected. Surface sampling through the use of Eckman Dredge was employed to collect the sediments. A diver was necessary to assist in the collection of sediment samples in areas in which the sediment bed was found to be covered with solid wastes (garbage). U.S.A Standard Testing Sieve, A.S.T.M. Mesh No.

170 was used to collect uniformly-sized sediments. Perkin Elmer AAnalyst 100 Spectrometer was utilized to measure the metal concentration.

2.1. Study area and sampling sites

Pasig River flows through Manila, the capital of the Philippines, and highly impacted by anthropogenic activities. The river is only 27 kilometers long but plays a crucial role in linking Laguna de Bay with Manila Bay as shown in figure 1. Its banks provide a small space to live for about 70,000 squatters. Its average depth is 91.20 m.

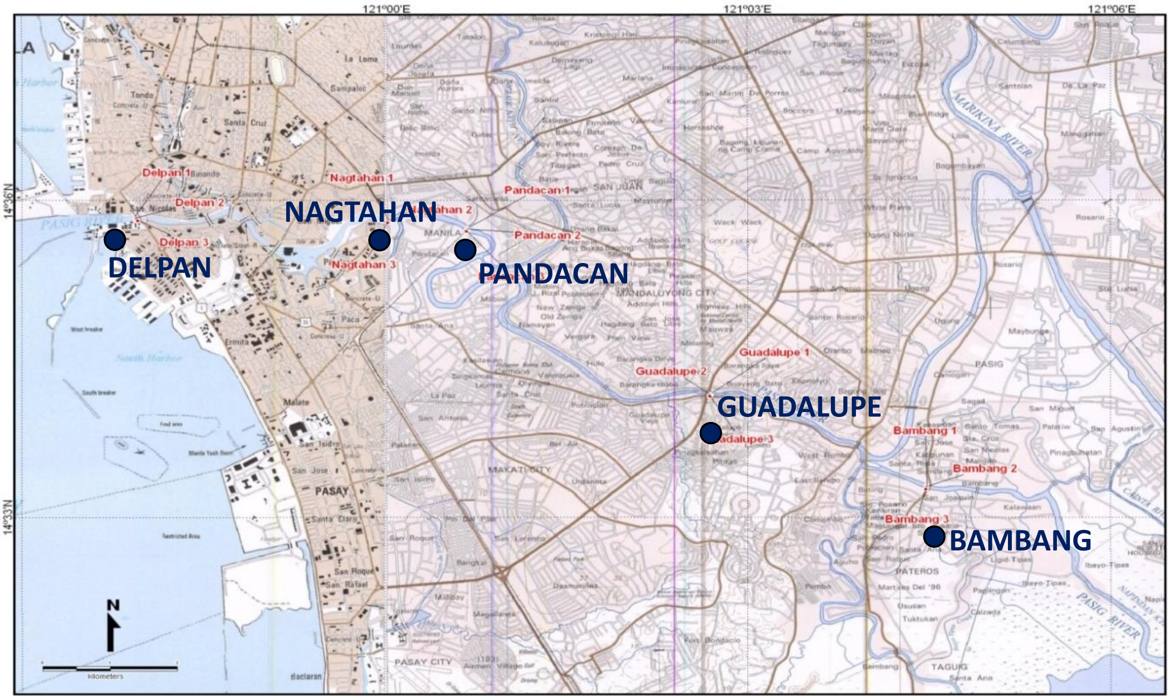

Figure 1. Site map of Pasig River and location of sediment sampling points.

Pasig River is crossed by a number of bridges. Sediment samples were taken from underneath these bridges, starting from (1) Delpan Bridge which is located downstream of Pasig River, near Manila Bay, characterized by the presence of many fishing and cargo boats; (2) Nagtahan Bridge, where several houses are built along the banks (3) Pandacan Bridge, where in its vicinity are located the oil depots of the three major companies; (4) Guadalupe Bridge where water merges with that of coming from Marikina River; and (5) Bambang Bridge, located near the Laguna Lake, which has become a dwelling site for humans. Each site had been sectioned into three and samples were taken from the two sides as well as in the middle part of the river.

2.2. Sample collection and storage

A pumpboat lent by the Philippine Coastguard was used to navigate the stretch of Pasig River. Three independent grab samples were taken at each station using an Eckman-Dredge Grab Sampler; one from the middle part of the river and one from each side of the river. A grab was considered adequate if it was filled with sediment and both the grab and access doors on top of the grab were closed tightly. The depth at which each sample was collected had been noted. The top 10-cm layer of the sediment, not in contact with the sidewalls of the grab, was collected with a plastic scoop and placed inside a zipped-lock polyethylene bags and stored in ice chests for subsequent analysis. The samples were refrigerated in the laboratory at 4°C prior to sample treatment procedure. Exact location of the sampling point was recorded using GARMIN e TREX H Global Positiong System (GPS).

In situ measurements of pH, temperature, electrical conductivity (EC), total dissolved solids (TDS) and salinity, using conductivity meter, and dissolved oxygen (DO) were performed at each sampling site. Water samples were also collected (triplicate samples for each site). Water samples were placed in acid-washed Nalgene bottles and immediately after collection, samples were placed in ice chests containing Coleman® blue ice packs and transported to the laboratory. Acidifying water samples with concentrated nitric acid (Merck, analytical grade) ensured the preservation of the water samples collected by preventing possible loss of target analytes (metals).

2.3. Characterization of the sediments for total metal and polyaromatic hydrocarbons (PAHs)

Sediment samples were air dried, pulverized and sieved to 170 mesh (U.S.A Standard Testing Sieve) prior to digestion with HF-HCl-HNO_3, three-acid system. Copper, lead, and cadmium concentrations were measured using AAS (Perkin Elmer AAnalyst 100 Spectrometer). To ensure that no metal contamination occurred during sample collection and preparation, all sampling bottles and glasswares were soaked in 14% nitric acid solution and washed with distilled water.

The presence of PAHs in the sediment samples was also determined. Sediment samples from each sampling site were submitted to a third-party laboratory, CRL Laboratories, for PAHs analysis. Standard US EPA method was adopted by the said third party analytical laboratory to determine the concentration of PAHs in the sediment samples [18].

No attempt was made to quantify the organic carbon. Neither the acid volatile sulfide in the sediments was measured as sediment samples were not stored in nitrogen. Further, sediment samples were pre-treated (air drying, sieving, etc.) prior to toxicity testing.

2.4. Cultivation of test organisms

The advantage of laboratory-cultured test organism over field collected animals is that, age, life history and existing conditions are documented and that, responses of these organisms are more consistent. Midge larvae are preferred over other benthic insects because of ease of culture and abundance in any freshwater systems.

Midge larvae used were previously collected from UP Los Baños and had been identified by a biologist-collaborator. The larvae were then maintained in an aquarium at the National Institute of Geological Sciences in UP Diliman. Standard protocols for cultivating benthic macroinvertebrates were strictly followed to ensure that *Chironomid* species used in this study were healthy and of the same age. *Chironomid* species were cultured in a 2 L glass of 12 in x 8 in x 5 in aquarium and covered with net to trap emerging adults. Overlying water was replaced every three days. Each egg mass was collected and placed in a 250 mL beaker containing 100mL of aerated water. Each beaker was then marked with the date when each egg mass had been collected. After two days, when all the eggs hatched, larvae of the same sizes were then transferred onto clean, separate beakers. These were given powdered fish flakes for food and shredded tissue paper. *Chironomid* species that reached the length of 7mm or 3rd instar were the source of all test organisms (ASTM, 1992a). Table 1 summarizes the conditions employed for the cultivation of the test organisms.

Table 1. Conditions employed in culturing the test organisms, *chironomids* species.

Parameter	Specifications
pH (pH meter)	7-8
temperature (thermometer)	27^{o}C
Dissolved oxygen	Not determined
Photoperiod	7 ppm
Aeration	16:8 hours light:dark
Dilution water	low intensity
Feeding regime	aerated water
	0.025g fish flakes/day

2.5. Toxicity test for sediments

An initial range finding test was conducted to establish the optimum concentration that will exhibit 0-100% mortality to C*hironomids*. Control sediment was mixed with each sediment sample in various ratios for a combined weight of 10g. Mixtures containing 5%, 10%, 15%, 20%, 30%, 40%, and 50% of sediment samples were used in the 96-hour static toxicity test. Diliman Tuff sediment was used as the control sediment or the one that represent the background conditions arising from localized pollutant influx (ASTM, 1995b). Exposures of the Diliman Tuff are confined to the western block of the Western Marikina Valley Fault. The Diliman Tuff was interpreted to be the product of various sedimentary and volcanic processes like sub-aerial debris flows, fluviatile with associated overbank and floodplain facies, pyroclastic flows and airfalls.

Mixtures of sediment samples and Diliman Tuff of varying percentages were placed in different plastic beakers. The river sediments and control sediment were mixed manually until both are homogeneously distributed throughout the mixture. To each container, twenty (20) 10-d old *Chironomid* species were transferred. Aerated water was then added up to 200-mL mark of the beaker. *Chironomids* cannot tolerate high levels of chlorine and hence aerated water was used. The organisms were allowed to come in contact with the sediment for 96 hours.

After 96 hours, test organisms that survived were determined and the mortality percentage was recorded. All tests were performed in three trials. The criterion for death was immobility and/or lack of reaction to mechanical stimulus. Mortality percentage was calculated using Abbot's formula as shown in equation (1):

$$\%P = \frac{p' - C}{100 - C} \quad (1)$$

where: P = adjusted mortality, p' = observed mortality, C = mortality in the control (organisms exposed in distilled water) (all values are expressed in percentages).

Surviving *Chironomid* species exposed to the different sediment samples only (i.e. not mixed with Diliman Tuff) were acid digested and analyzed using AAS (Perkin Elmer AAnalyst 100 Spectrometer).

2.5.1. Calculation of LC50. The median lethal concentration (LC50) was calculated using semi-graphical method called Litchfield-Wilcoxon method [19]. The values of % mortalities were transformed to probit values obtained from appendix of Newman, 1995. Logarithm of concentration was plotted against these probit values. The LC50, LC16 and LC84 can be extracted from this graph by taking the antilogarithm of the logarithmic concentration corresponding to the appropriate probit values for this percentage. In order to estimate the 95% confidence interval of LC50, the slope function was calculated through the following equation (2)

$$S = \frac{\frac{LC84}{LC50} - \frac{LC50}{LC16}}{2} \quad (2)$$

The total number of individuals tested (N') between the 16% and 84% response as predicted by the line was determined. The 95%confidence interval was calculated as follows [20]:

$$fF_{LC50} = \frac{S^{2.77}}{\sqrt{N'}} \quad (3)$$

Upper limit of 95% CI:

$$Upper = LC50\,(fF_{LC50}) \quad (4)$$

Lower limit of 95% CI:

$$Lower = \frac{LC50}{fF_{LC50}} \quad (5)$$

3. Results and discussion

3.1. Determination of sediment toxicity at each sampling stations

This study employed the use of a 96-hour toxicity test using laboratory-grown *Chironomid* species to assess the toxicity of sediments collected from various points along the Pasig River. *Chironomids* were exposed to different ratios of combined Pasig River sediments and controlled sediment (Diliman Tuff). Figures 2 and 3 show the results of the toxicity test conducted for sediments collected during the dry and wet seasons. The control sediment proved to be not toxic towards the test species as shown in the plots (0% mortality). Moreover, it can be gleaned from figure 2 that mortality tends to increase with an increase in the ratio of sediment samples which was pronounced in the case of sediments collected from Guadalupe. Also, it was found that the range of average mortality for *Chironomids sp.* upon exposure to sediments obtained from Pasig River may be approximated to be between 55% and 75%. This implies that the test organism *Chironomids sp.* could possibly be tolerant and was not greatly affected by exposure to Pasig River sediments.

Another significant observation from the plots is that sediments in Pasig River tend to be more toxic to *Chironomids* during the dry season as suggested by the higher percentage mortality observed compared with sediments collected during the rainy season. During dry season, the flow in the river is laminar causing the wastes to accumulate more in the sediments unlike during wet season wherein the rainfall causes the river to flow more turbulently consequently washing away pollutants. Figure 3 gives the average mortality observed as a function of varying ratios of sediments collected during the wet season.

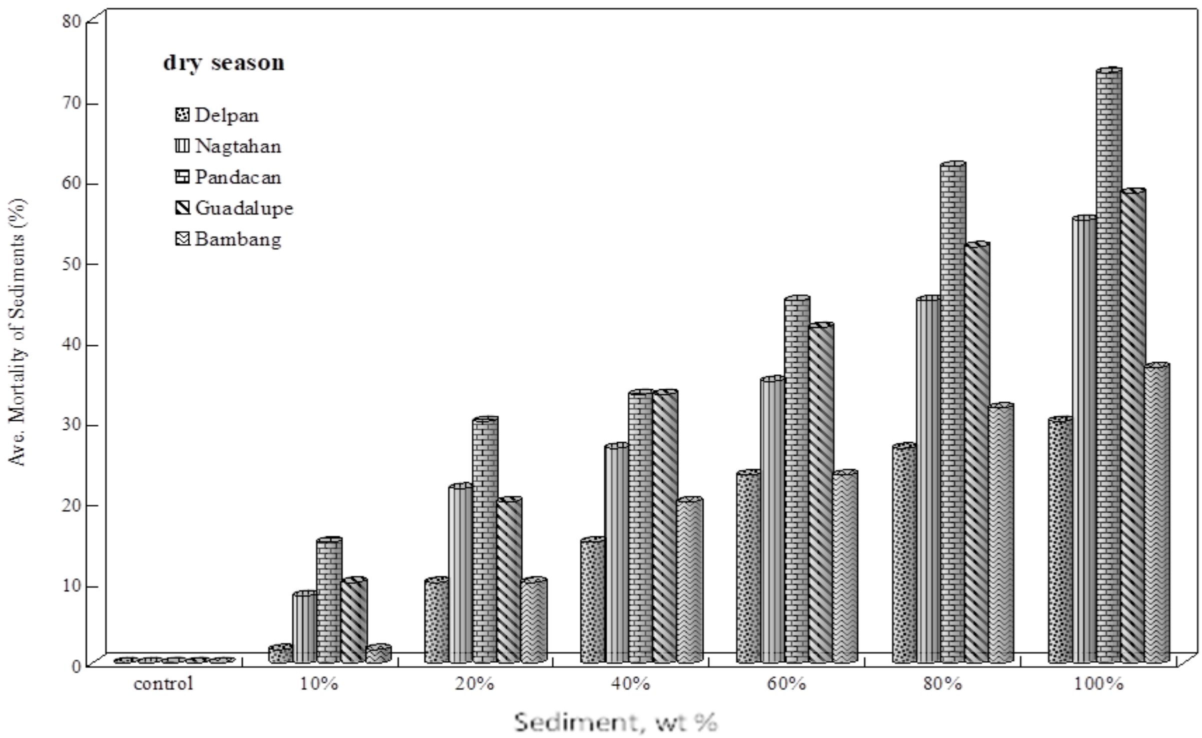

Figure 2. Average mortality as a function varying ratios of Pasig River sediments (collected from five sampling stations) and control sediment during the dry season.

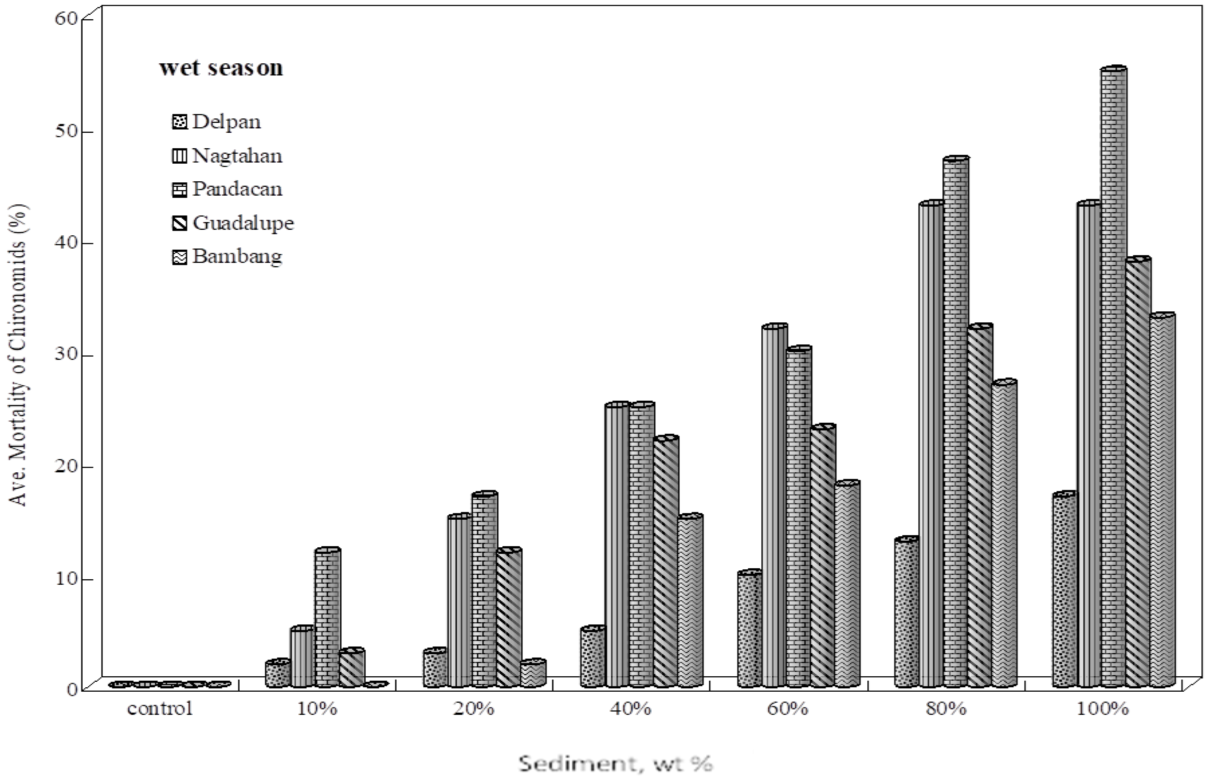

Figure 3. Average mortality as a function varying ratios of Pasig River sediments (collected from five sampling stations) and control sediment during the wet season.

Mathematical transformations were performed to normalize the distribution of tolerances. % mortality of organisms were transformed to Probit values. The LC50, LC16 and LC84 can be extracted from the Probit plots by taking the antilogarithm of log concentration corresponding to the appropriate Probit values of these percentages. The 95% CI is calculated using the Litchfield-Wilcoxon method.

Table 2 shows the summary of computed values of LC50, 95% CI and other variables. The data given on the table supports earlier observation that *Chironomid* species exhibit higher mortality when exposed to mixtures of Pasig River sediment: control sediment (Diliman Tuff) with very high ratio for the Pasig River sediments and low mortality when only about 5% of the sediment sample was mixed with the control sediment (Diliman Tuff). The control sediment may have diluted the concentration of the pollutants in the sediment samples thus reducing the toxicity towards the test organisms.

Table 2. Calculated LC50, 95% CI and other variables obtained from the Probit plots.

Stations	**Delpan**		**Nagtahan**		**Pandacan**		**Guadalupe**		**Bambang**	
	dry	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**
LC16, w/w%	3.38	3.05	3.98	3.72	4.28	3.942	3.99	3.55	3.38	1.75
LC50, w/w%	4.12	3.715	4.5	4.425	4.73	4.405	4.68	4.25	4.23	4.12
LC84, w/w%	4.41	3.918	4.93	4.83	5.37	4.962	5.09	4.56	4.56	4.42
N'	60	60	60	60	60	60	60	60	60	60
S	1.145	1.136	1.039	1.141	1.12	1.122	1.13	1.13	1.17	1.72
*f*FLC50	1.05	1.047	1.056	1.048	1.041	1.042	1.045	1.05	1.06	1.21
upper 95% CI	4.326	3.89	4.752	4.637	4.924	4.59	4.891	4.44	4.47	4.99
lower 95% CI	3.924	3.548	4.261	4.222	4.544	4.227	4.478	4.06	4.01	3.39

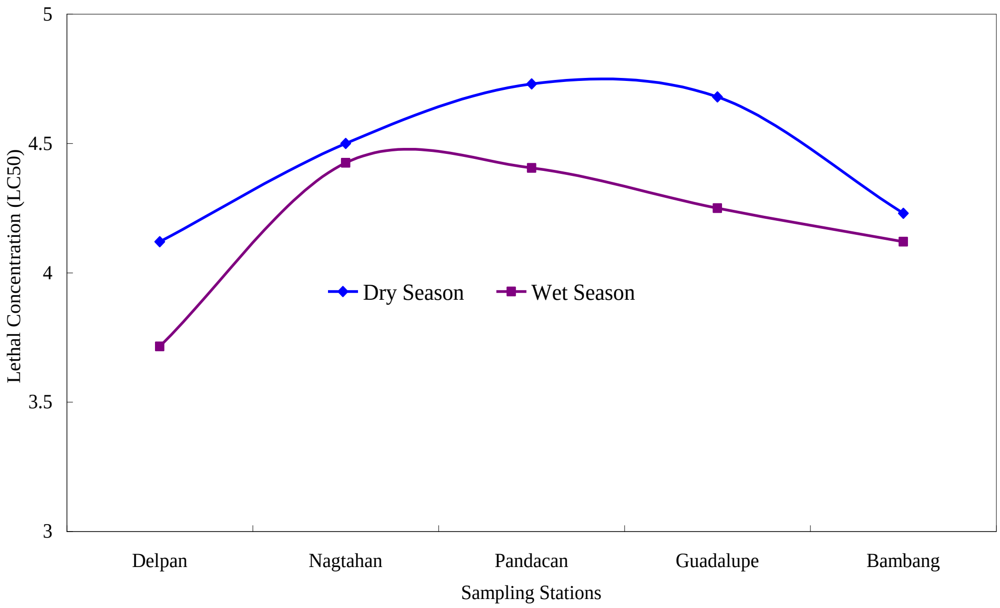

Figure 4. Correlation plot relating lethal concentration of Pasig River sediments with different sampling stations (both for dry and wet seasons).

Figure 4 compares the statistically determined relative acute toxicity of sediments (in terms of lethal concentration) at each sampling site along the Pasig River. As LC50 is indicative of toxic effect, hence lower lethal concentration value suggests higher potential toxicity of the sediment to *Chironomids* over a 96 hour period. Accordingly, this study assumed that sediment yielding lower LC50 was the most contaminated or polluted. It is worth-noting that among the sites investigated, sediments located along the Delpan site were found to be potentially harmful to the test organisms especially during the dry season. The Delpan site is characterized by the presence of several potential pollution contributors among which include the presence of large human communities, several barges dock along the sites and some industrial companies. Interestingly, sediments collected from that same station, was also found to be potentially hazardous for the *Chironomids* during the rainy season, as suggested by very low lethal concentration value. It could be due to changes in the water and sediment qualities attributable to the mixing of freshwater and salt water coming from the Manila Bay, especially during high water run-off. *Chironomids* species are known to thrive in the freshwater environment. Therefore, exposure to elevated level of salt content could also be a factor which would explain the observed toxicity of the Delpan sediment to the test organisms.

Further, as shown in figure 4, the sediments along the Pasig River can be more toxic to *Chironomids sp.* during the dry season than during the rainy season. The values given indicate the lethal concentration tolerable by *Chironomid* species during the dry season is relatively lower compared with those obtained during the wet season. Beyond these concentrations (LC50), increased toxicity may be expected and therefore may result to instantaneous death for the *Chironomid* species. The potential toxicity of the sediments follows the trend: Delpan > Bambang > Nagtahan > Guadalupe > Pandacan. On the other hand, the trend for toxicity of sediments during rainy season follows this order: Delpan > Bambang > Guadalupe > Pandacan > Nagtahan.

Results obtained from these experiments suggest that it is possible to identify areas along the Pasig River which may potentially exhibit toxic effect as implied by high percentage mortality for *Chironomids* after 96-hour exposure to contaminated sediments. As this study is focused on the determination of the potential impact brought about by exposure of *Chironomids* species to sediments taken from Pasig River only, it is acknowledged, that there is a need to validate the results to clearly pinpoint the contaminants responsible for the observed toxic effect. The sediments may contain mixture of potentially toxic components such as PAH and other organic components in addition to metal ions and may have contributed to lethality of the sediments to the test species. It would be extremely useful to be able to establish the response of this local *Chironomids* species to individual toxicant using the 96-hour acute toxicity test to verify which actually among those toxicants that could be present in Pasig River sediments was responsible for the observed toxicity. With the aid of this locally developed sediment toxicity test, areas along Pasig River which would require high priority in terms of rehabilitation and/or remediation can be established [12].

Nonetheless, this study was able to manifest the variations in terms of sediment toxicity along Pasig River. This suggests that point sources may contribute to the quality of the sediment for a particular site along Pasig River. Moreover, identification of the toxicant responsible for such toxicity can be zeroed in from those potential point sources. Further, to confirm such response by the local test species, other test organisms in addition to those standard test organisms need to be used in future investigations. Doing so shall enable the standardization of this proposed 96-hour acute sediment toxicity test with endemic benthic, *Chironomids sp*. as test organisms.

3.2. Comparison of the total metal concentrations in sediments collected during dry season versus the total metal concentrations in sediments collected during the wet season

Total metal concentrations measured in Pasig River sediments collected during dry and wet seasons are shown in table 3. These values were also plotted against sample location to provide a glimpse of the existing trend in terms of metal concentration in each sample site. It can be gleaned from figure 5 that all sediment samples collected during the dry season yielded very high concentrations of Cu. Average concentration of Cu in the sediment ranged from 78.23 ± 0.005 to 157.3 ± 0.231 µg/g during dry season while Pb and Cd concentration ranges varied from 24.51 ± 0.012 to 55.19 ± 0.021 µg/g and from 0.23 ± 0.002 to 5.780 ± 0.007 µg/g, respectively. It was noticeable that the Cu level decreased during the wet season, compared to dry season. Values ranged from 22.85 ± 0.032 to 30.92 ± 0.035 µg/g during wet season. On the other hand, there is only a slight decrease in the concentration of Pb measured in sediments collected during the wet season, varying between 22.93 ± 0.06 and 48.14 ± 0.038 µg/g. No significant changes in the total Cd concentrations were noted (0.11 ± 0.007 to 3.69 ± 0.044 µg/g) as shown in figure 5.

Table 3. Total metal concentration of Pasig River sediments in dry season.

Sampling Stations	**Cu** dry (µg/g)	wet (µg/g)	**Pb** dry (µg/g)	wet (µg/g)	**Cd** dry (µg/g)	wet (µg/g)
Delpan	78.23 ±.005	22.85 ±.032	24.51 ±.012	22.93 ±.06	0.23 ±.002	0.11 ±.007
Nagtahan	94.73 ±.003	24.97 ±.014	41.31 ±.013	35.46 ±.021	2.29 ±.006	1.02 ±.031
Pandacan	157.26 ±.231	26.30 ±.046	55.19 ±.021	48.14 ±.038	5.78 ±.007	3.69 ±.044
Guadalupe	127.87 ±.005	30.92 ±.035	53.52 ±.012	31.22 ±.051	3.39 ±.008	1.94 ±.009
Bambang	97.13 ±.005	30.67 ±.038	40.23 ±.010	33.89 ±.048	1.44 ±.002	0.37 ±.013

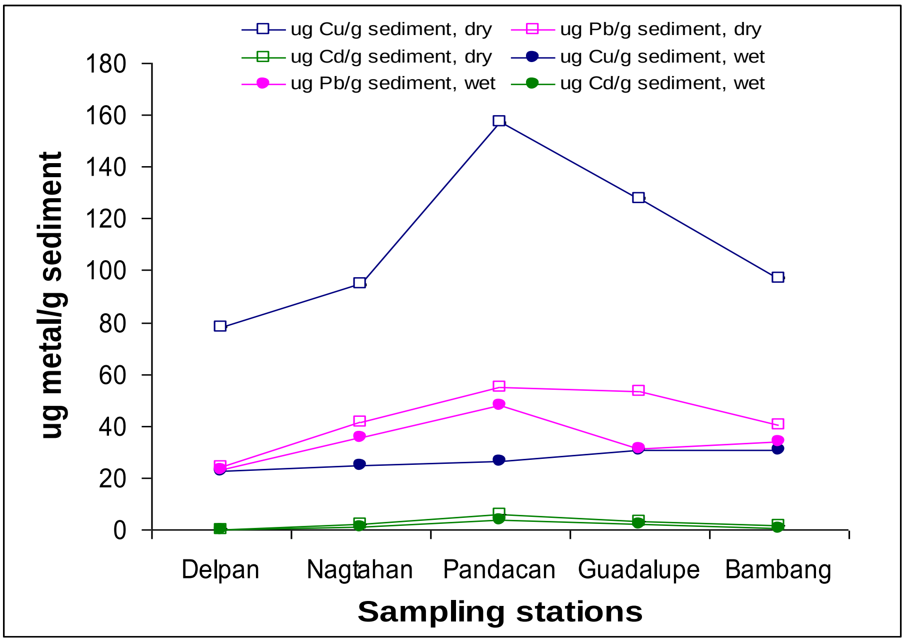

Figure 5. Total metal concentrations in sediments collected at different sampling stations during dry and wet season.

It is notable that the sediment collected in Pandacan exhibited high levels of Cu, Pb and Cd. This location is characterized by the presence of petrochemical facilities and several housing communities which may suggest potential sources of these pollutants. Moreover, San Juan River which intersects the Pasig River at this station carries loads of domestic and industrial wastes.

Laguna Lake is found on the upstream of Pasig River while Manila Bay is situated on the downstream. During the dry season, the flow in the river is minimal causing accumulation of pollutants at this sampling site. On the other hand, during the wet season, the turbulent flow caused by continuous rainfall contributes to the reduction in the heavy metal concentrations. Sediments obtained from Delpan station gave the lowest total metal concentration among the five stations due to possible dilution/mixing happening between water coming from Pasig River and salt water of Manila Bay. The same explanation may also account for the low levels of metals in sediments collected along the Bambang Station due to its proximity to the Laguna de Bay.

In terms of the presence of polyaromatic hydrocarbons (PAHs), majority of the samples analyzed were below the method's detection limit. Only sample taken from Nagtahan was positive for the presence of PAHs.

4. Conclusions and recommendations

This study has demonstrated the potential use of *Chironomids* species as bioindicator of sediment toxicity in Pasig River. The potential environmental risk that pollutants present in sediments of Pasig River posed to aquatic organisms has been successfully established. Results of proposed 96-hour acute ecotoxicological assay of the sediments obtained from five sampling stations along the Pasig River showed varying levels of toxicity toward *Chironomids* species. Sediments obtained from Delpan station were found to be the most toxic to these test organisms during the summer and wet seasons, as suggested by relatively lower LC50. Industrial wastes and barges dock along the Delpan station as well as domestic wastes may be potential sources of pollutants. The trend established in the order of decreasing toxicity is as follows: Delpan > Bambang > Nagtahan > Guadalupe > Pandacan. The same trend can be used to rank sites along the Pasig River considered to be highly contaminated/polluted. In the absence of a sediment quality guideline in the Philippines, this proposed 96-hour acute sediment toxicity test may be a practical technique to assess the potential risk of sediments. Moreover, with the use of this technique, the site which requires further and continuous monitoring has been zeroed in. In this case, it was the Delpan site that must be given much attention and studies relating to appropriate remedial action must soon be outlined. However, it should be emphasized that the actual conduct of the 96-hour acute toxicity testing especially for routine sediment monitoring must be further validated using standard acute sediment toxicity tests. This is also to ensure that the control sediment to be used in this proposed method will not tend to influence the test results.

This study successfully gave an assessment of the toxicity of Pasig River sediments using *Chironomid* species but other factors that may affect the health of *Chironomid* species during the 96-hour exposure period should be further studied. Further study on other benthic organisms that can be used as bioindicator aside from *Chironomid* species should also be conducted. A survey of aquatic organisms residing in Pasig River sediment bed would eventually be pursued to be able to determine other potential bioindicators. Other tests should also be performed to determine total and dissolved organic carbon, acid volatile sulfides, ammonia (NH_3-N) and varying pH, factors which may potentially affect the observed toxicity of sediments. Sediment samples that have been found to be toxic and heavily polluted must be further analyzed intensively. This is to determine the contaminant or group of contaminants that are actually causing the toxicity to *Chironomids*. In this manner, remedial action may be eventually undertaken to reduce the toxicity at a particular site along the Pasig River.

It was also observed that pollutants present in sediments of Pasig River can be potentially toxic during the dry season. Moreover, results show that sediments exhibit higher total metal concentrations during the summer season than during the wet season due to fewer disturbances in the flow along the Pasig River. Elevated levels of Cu and Pb were found in sediments collected and follow the trend: Pandacan > Guadalupe > Bambang > Nagtahan > Delpan.

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Declaration of no competing interests
We declare we have no competing interests and/or no non-financial competing interests, or other interests that might be perceived to influence the interpretation of this article.

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