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Upland surface water and genotoxicity assessment of watershed areas of Cavite, Luzon Island, Philippines

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ABSTRACT

Like estuaries, rivers are areas in which anthropogenic effects have its most direct influence due to increased pollution loads which triggers an increase hazard of adverse impacts. Due to the rapid changes happening in the Province of Cavite because of urbanization and unplanned land use, the river systems of Cavite becomes a dumping ground of pollutants coming from domestic, industrial and commercial sources. One of these pollutants that pose a great health hazard is the heavy metals. This paper determined the presence of heavy metal pollution in Cavite's upland waters and its tributaries through genotoxicity assessment. The water sampling results have shown that heavy metals such as Cd, Cu, Pb, Hg and Cr are less than the method of detection limit (mdl) or above the criteria set by national government standards. This may suggest that surface waters in upland Cavite watershed are still free from heavy metal pollution. However, the detection of nuclear alterations and cellular death through necrosis and apoptosis even in low frequency in Perna viridis, a known bioindicator, demonstrated the presence of genotoxic agents possible coming from lowland watershed areas and likely manifested a highly polluted environment. It is recommended that investigation of point and non-point sources of heavy metal pollution be determined in the province's midland and lowland watersheds.

Keywords: Heavy metal pollution, watershed management, surface water, Cavite, genotoxicity

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INTRODUCTION

The National Water Resources Board (NWRB) of the Philippines reported that the Philippines acquires its water supply from various sources which include rainfall, surface water resources like rivers and lakes and groundwater resources [1]. Though freshwater sources are abundant in the Philippines, the availability of these freshwater that can be utilized is the second lowest in Southeast Asia [2]. This condition on water availability, according to experts, would continue to be deficits in several river basins and would further aggravate for the coming decade due to water pollution, climate change, and inadequate management [3]. One of the provinces in the Philippines that has rich in freshwater sources but threatened in water availability for the coming years is Cavite. Cavite's surface freshwater resources are composed of six major rivers and its tributaries. These major rivers generally start from the upland mountainous areas of the province (i.e. Tagaytay City, Municipalities of Mendez, Alfonso, Magallanes and Amadeo) and which drains into the Manila Bay [4].

Like estuaries, rivers are areas in which anthropogenic effects have its most direct influence due to increased pollution loads which triggers an increase hazard of adverse impacts [5]. Due to the rapid changes happening in the province because of urbanization and unplanned land use, the river systems of Cavite becomes a dumping ground of pollutants coming from domestic, industrial and commercial sources. One of these pollutants that pose a great health hazard is the heavy metals. Heavy metals come from the discharge of sewage, agricultural and industrial effluents and these are carried to the rivers, lakes or reservoirs [6]. Some heavy metals, such as Cd, Hg and Pb, are toxic to living organisms in low concentrations while some such as Cu are needed biologically by aquatic organisms but become generally toxic in high concentrations [7]. Like many other pollutants, heavy metals are non-biodegradable and can therefore undergo bioaccumulation through the food chain.

Analytical measurement of water quality and use of aquatic organisms as indicators have been widely used in monitoring and assessment of safe environmental levels of heavy metals. This paper measured the water quality of upland water sources of Cavite and the lowland water quality using biological indicators. The aim is to have a preliminary assessment of water quality from these watershed areas and cite potential heavy metal pollution sources and causes, if any. The study presented a reliable indicator on the state of the aquatic ecosystem of Cavite and the potential impact of xenobiotics like heavy metals on the organisms and the general environment.

MATERIALS AND METHODS

Collection of samples

The water samples were collected from the different surface water sources in the upland mountainous area of Cavite specifically the municipalities of Magallanes, Maragondon, Indang, and Alfonso. They are situated at a very high elevation above 400 meters (1,300 ft) with slopes of more than 2%. Biological samples were collected from three (3) sites in Bacoor Bay (Figures 1 and 2) with coordinates 14° 28.581'N and 120°54.413 E for site 1, 14° 28.683' N and 120° 54.975 E for site 2 and 14°29.076' N and 120°56.566' E for site 3.

Figures 1 and 2. Sampling sites where water and biological samples were collected

Water samples taken from upland sources were tested for presence and concentration of heavy metals Pb, Cr, Hg and Cu following procedures recommended by APHA. Dissolved oxygen, conductivity and pH were also taken. Samples were gathered during dry (April) and wet (July) seasons and compared to Philippine acceptable standards. For the biological samples, the Mussel micronucleus cytome (MUMNcyt) assay were employed using the macroinvertebrate *Perna viridis*, which is a common organism in the Manila Bay area and has economic importance to the community where the samples were taken. The organism has been selected as sentinel organisms in the international monitoring program of genotoxicity because of their ability to accumulate both inorganic and organic contaminants allowing them to survive under stressed and polluted environment [8].

MUMNCYT Assay Test

The three (3) sampling sites were conducted in Bacoor Bay such that the first site is 200 meters away from the residential area and is enclosed within the Cavite Expressway (CavitEx). Site 2 was 2000 meters away from the residential area and is located where the outlet of Imus river and Bacoor bay meet; and Site 3 was 1500 meters away from the residential area and is almost in between the two sites. A total of sixty (60) *Perna viridis* samples were taken randomly with the assistance of the local fisherfolks.

Tests were conducted to the organisms to determine genotoxicity by measuring frequency of the following abnormalities in cells: (a). granular cells, (b). agranular cells, (c) apoptotic cells and (d). necrotic cells following the procedures employed by other scholars [9]. The criteria for identifying the different abnormalities were based on the following characteristics:

a. Granular hemocytes are the largest hemocytes (8–12 μm in diameter) with a round nucleus and low nucleus/cytoplasm ratio and are characterized by large numbers of granules (Fig. 3a).
b. Agranular hemocytes are primarily characterized by their small size (3–4 μm), high nucleus/cytoplasm ratio and a lack or low abundance of cytoplasmic granules and organelles (Fig. 3b).
c. Apoptotic agranular hemocytes are cells undergoing the process of programmed cell death. Different nuclear changes characterize the apoptosis process. At the nuclear level, an early apoptotic cell shows chromatin condensation in the nucleus. Late apoptotic cells show nuclear fragmentation and the appearance of small nuclear bodies within the cytoplasm. The cytoplasm in an apoptotic cell may have a lower staining intensity compared with normal cells (Fig. 3c).
d. Necrotic agranular hemocytes are cells undergoing a premature death caused by factors external to the cell, such as infection or exposure to cytotoxic agents. This process begins with damage to cellular membranes or organelles. Necrotic hemocytes can be identified by their pale cytoplasm and a large number of vacuoles, mainly in the cytoplasm and sometimes in the nucleus. The nucleus generally maintains a fairly intact structure with a stain intensity that is less than that observed in a normal viable cell (Fig. 3d).
e. Binucleated agranular hemocytes have a larger cytoplasm compared with the normal cells and contain two main nuclei instead of a single nucleus. The nuclei have the same morphology as that observed in normal cells. They may be very close and may sometimes touch each other. These cells are indicative of failed cytokinesis, which may be related to the exposure to toxic pollutants (Fig. 3e).
f. Agranular hemocytes with Micronuclei (MNi) are characterized by the presence of both a main nucleus and one or more smaller nuclei (MNi) that arise from acentric chromosome fragments or a whole chromosome lagging behind at the anaphase stage of cell division. MNi are round or oval in shape and their diameter should range between 1/3 and 1/16 of the main nucleus. MNi have the same morphology, and texture as the main nucleus. MNi should be in the same focal plane of the main nucleus and their boundary should be distinguishable from the nuclear boundary. The staining intensity of MNi is typically the same or less than that of main nucleus. MNi are nonrefractile and can be readily distinguished from any cytoplasmic inclusion (granules) or technical artifact such as staining particles. Most cells with MNi usually contain only one MN but it is possible to find cells with two or more MNi. Baseline frequencies for micronucleated mussel hemocytes are usually within the range of 0.5–5.0 MNi per 1,000 cells (Fig. 3f,g).

g. Agranular hemocytes with nuclear buds are cells containing small nuclear structures with similar morphology to MNi but with the difference that they are connected with the main nucleus by a bridge that is typically narrower than the diameter of the nuclear bud. Buds have the same texture and staining intensity as the MNi. The mechanism leading to nuclear bud formation is not known but it may be related to the elimination of amplified DNA or DNA repair complexes61 (Fig. 3h,i).

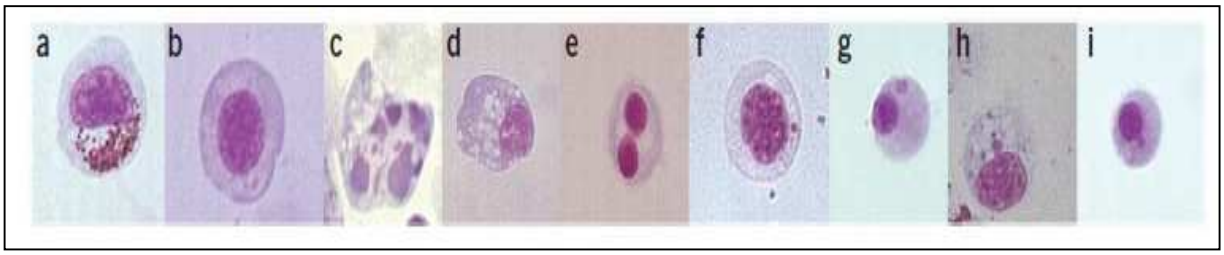

Figure 3. MUMNcyt assay in hemocytes: images of different cell types stained using 3% (vol/vol)Giemsa. (a) Granular hemocyte. (b) Agranular hemocyte. (c) Apoptotic agranular hemocyte. (d) Necrotic agranular hemocyte. (e) Binucleated agranular hemocyte. (f,g) Agranular hemocytes with MNi. (h,i) Agranular hemocytes with buds [9].

Analysis of Results

For the upland water samples, the test results were compared with Philippine water standards of surface water quality. For the MUMNct assay, the tissues slides were observed using transmitted light microscopy at ~1,000 magnification and abnormalities were scored using at least 1,000 cells per slide. The presence and frequency of granulated, agranulated, necrotic and apoptotic cells were determined and tallied.

RESULTS AND DISCUSSION

Heavy Metals in Cavite Upland Freshwater

Table 1 shows the average concentrations (mg/L) of heavy metals Pb, Cr, Cu and Hg taken during wet and dry seasons from various freshwater sites in the municipalities of Maragondon, Indang, Aguinaldo and Alfonso in the province of Cavite.

Table 1. Heavy metal concentrations in the four sampling sites

Municipality	Lead (in mg/L)	Chromium (in mg/L)	Copper (in mg/L)	Mercury (in mg/L)
Maragondon	<0.005	<0.010	<0.02	<0.0001
Aguinaldo	0.05	<0.010	<0.02	<0.0001
Alfonso	<0.005	<0.010	<0.02	<0.0001
Indang	<0.005	<0.010	<0.02	<0.0001

Following national government standards [10], all freshwater samples' heavy metal concentrations are within acceptable standards for drinking water. This means that upland water sources are still free from possible sources of contaminations of heavy metals. In terms of pH, the river water samples exhibited an alkaline pH with a range between 6.87 to 7.64 and an overall mean value of 7.20. It was observed that lower pH is measured during the wet season this may be due to the erosion, runoff or possibly the pH of the rainwater itself. The values conform to within safe limit for drinking water [11]. and for crop production [12]. The values of conductivity ranged from 250 to 304 with an overall mean of 266 uS/cm. Having this conductivity make the freshwater systems ideal to support a diverse aquatic life. In terms of dissolved oxygen, the freshwater samples DO readings range from 7.64 to 7.75 with a mean reading of 7.7 and conforming to national standards for safe drinking water [10].

Heavy metals impacts on Perna viridis

Table 2 shows the average number of nuclear anomaly in *Perna viridis* cells taken from Bacoor Bay.

Table 2. Average number of observed anomaly in *Perna viridis* cells taken from Bacoor Bay

	Necrotic	Binucleated	Micronuclei	Apoptotic
Average number of observed anomaly (per 1000 cells of *Perna viridis)*	86	88	12	220
Percentage	**8.6%**	**8.8%**	**1.2%**	**22%**

The study showed that there is epithelial micronuclei occurrence among mussels collected in Cavite Bay. This result showed 1.2 percent of micronucleus occurrence indicating exposure of aquatic environment from multiple contamination sources including highly persistent pollutants, and some emergent organic contaminants that are still unregulated such as industrial products. Particularly, these pollutants originated from waste discharges of

surrounding residential and industrial areas. Bacoor and Zapote Rivers and Imus watershed lead to Bacoor Bay. This elevates the number of pollutants in the area that eventually accumulated by the mussels which are known to be filter feeders. In addition, the genotoxic effects among sentinel organisms such as mussels could be attributed also by traffic congestion in the City of Bacoor and Manila-Cavite Express way. The vehicle emissions might add up to the surrounding water due to escalating growth rate in vehicle density brought about by increasing demand of population growth. This study only reflects that aquatic inhabitants had been exposed from heavy metals.

It was also evident in the study the presence of binocleated hemocytes. The result showed that there is 8.8 percent were found among mussels in Cavite Bay. The presence of larger and two main nuclei in a cell indicates failed cytokinesis. This clearly reflects that Cavite Bay has been contaminated by heavy metals and the aquatic animals as demonstrated by mussels has also been exposed to toxic pollutants. Hemocytes is known to have higher sensitivity as target cells among mussels [9]. Hemolymph is a major component of a mussel's soft tissue. The hemocytes, as circulating cells of an open vascular system, are involved in transport and digestion of nutrients, and in processes such as the elimination of toxic substances and small particles and the repair of tissue lesions. These cells are constantly exposed to waterborne pollutants and represent the key cells in the mussel's response to toxicants, with lysosomal enzymes and heavy metals accumulating in hemocytes as part of the cellular detoxification process. The hemolymph has the advantage of providing an easily collectable single-cell suspension as well as their functions and respective involvement in defense responses [13].

Heavy metals induce a plethora of toxicological effects to living systems. As trace elements, they are metabolically-significant in small concentrations but may be poisonous in higher amounts. In relation to their toxicity, heavy metals bioaccumulate, making them effective interferences in the normal physiology and balance of ecosystems and living organisms. Aquatic vertebrates such as mussels serve the functional purpose of being bioindicators for heavy metal pollution of oceans and large bodies of water because they accumulate heavy metals in their living tissues yet they continue to subsist. Often, these metals are transported inside living tissues of marine invertebrates and they stay there irreversibly [14].

From the results of the current study, apoptotic mussel hemocytes were found to be at 5.5% parallel to the concentration of heavy metals established from water samples that were analyzed. This can be supported by similar findings of Sokolava and colleagues using oyster hemocytes and the cells' immunotoxicity parameters [15]. Sokolava and colleagues found that in oyster hemocytes, a related invertebrate to mussels, exposure to Cadmium induced apoptosis in a dose-dependent manner. More so the exposure of the same cells in higher concentrations of the same heavy metal (200-1000 micromol) showcased a significant increase in the necrosis of the cells. These toxicities resulting to cellular death can be attributed to disruption of energy economics in hemocyte cells that consequently activates apoptotic pathways dependent to mitochondria.

CONCLUSION

The water sampling results have shown that heavy metals such as Cadmium, copper, Lead, Mercury and Chromium are less than the method of detection limit (mdl) or above the criteria set by national government standards. This may suggest that surface waters in upland Cavite watershed are still free from heavy metal pollution. However, the detection of nuclear alterations and cellular death through necrosis and apoptosis even in low frequency in *Perna viridis*, a known bioindicator, demonstrated the presence of genotoxic agents possible coming from lowland watershed areas and likely manifested a highly polluted environment.

This recommends that further investigation be conducted on the water quality of Cavite's midland and lowland surface waters and determine possible point and non-point sources of heavy metal pollution in water tributaries.

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