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Point source effluents and its effect on the microbiological assessment of its effluent-receiving Brackish water

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Abstract

Two identified point sources of effluents; and the water-effluent receiving brackishwater of Bulua, Cagayan de Oro City Philippines were assessed during low tide and high tide. In-situ parameters were done at the sampling sites and collected algal samples were brought to the laboratory for phytoplankton density and identification. Majority of the effluent parameters, temperature, total dissolved solids (TDS) for the two samplings sites; total suspended solid (TSS) & dissolved oxygen (DO) of the market effluent exceeded the prescribed DENR allowable values. The condition of the brackishwater was supported by its water quality variables that exceeded the tolerable limits. Microbiological examination recorded a high level of total coliform count at both tempo-spatial variations and has exceeded the water-effluent quality standards. The phytoplankton density varies significantly in terms of sampling period and the sampling areas. The highest recorded cell density was observed during high tide for both sampling areas. Blue-green algae obtained the highest planktonic cell density with reference to temporal variations and the presence of *Oscillatoria* sp., a well-documented bloom-forming species, with *Nitzschia* sp. and *Navicula* sp., which are pollution-sensitive species were identified in the area. A positive correlation coefficient, (r) of 0.875 were identified between phytoplankton density and the nitrates & phosphates; and r of 0.615 between phytoplankton density and the amount of lead (Pb). Regression Analysis significantly identified phytoplankton density in the brackishwater as caused by the physico-chemical parameters; nitrates ($p \leq .01$), phosphates ($p \leq .05$), lead ($p \leq .05$) and salinity ($p \leq .01$).

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Introduction

Brackishwater is considered as an important ecosystems due to the land-derived materials to the coastal waters that serve as conveyors of effluents and wastewater. This is applicable especially in situation where brackishwater in barangay Bulua, Cagayan de Oro City Philippines is being used as discharge areas of effluents and a waterway down to coastal area.

Recently, there has been a public outcry at the perceived impacts of these murky, stinky and continuous flow of wastewater going out from the drainpipe of the said public market and currently, residents were complaining of these unbearable smells and the aesthetics of the surface water has been altered significantly. The addition of wastewater coming from the identified sources into the brackishwater must be clearly understood if such contributions of the actual effluents to the water have a significant impact. Thus, it is for these reasons that the effluent quality was characterized and was determined to compare with the tolerable limit set by the enforcement agency that monitors and regulate the activity. The purpose of the study was to assess the existing brackish water conditions in Bulua, Cagayan de Oro City Philippines in terms of physico-chemical and microbiological variables as impacted by the intensive activities in the area and to provide scientific evidence that will lead to call for environmental care and action as deemed necessary and urgent.

Materials and methods

Study Area

The study area is shown in Fig. 1, which is located five (5) kilometers away from the city proper, in the North western part of Cagayan de Oro City, Philippines.

Geographically located at 08°30’.276” and 124°36”.749’. The brackish water in barangay Bulua is bounded with three bodies of water; these are the Iponan River, the Sapong Creek and the marine water of Macajalar bay. Approximately the length of the brackishwater is about 3 kilometers. It is being used by the locals for fishing, fishpond farming; and sometimes as dumpsites of household activities of the residences as well as dumpsite of wastewater from commercial establishments & industries.

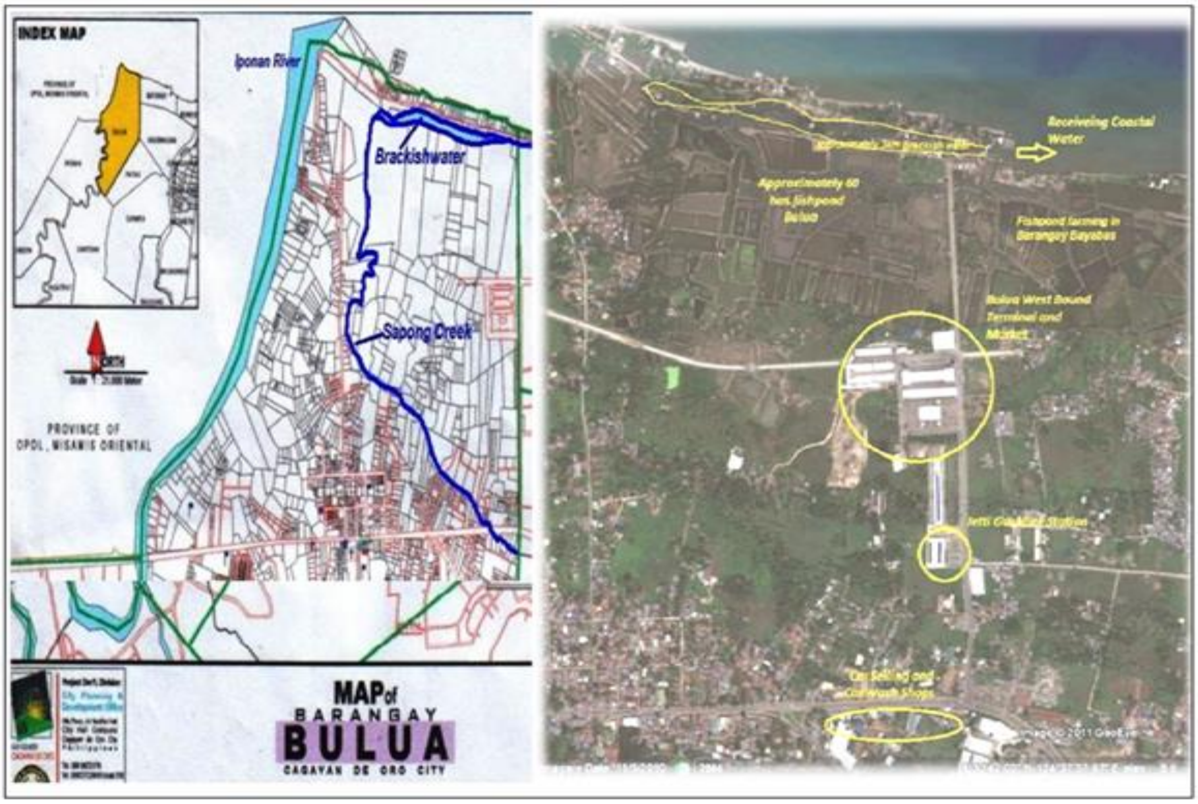

Fig. 1. Aerial view of the research area and the sampling sites.

Collection of Effluent and Surface water and Algal Samples

Two sampling areas were identified, established and located approximately 800 meters away from each other. Each sampling area comprised three sampling stations approximately 30 meters away from each other. Sampling area 1 was established near the Westbound Bus Terminal, Wet Market and Monte Carlo Fish Vendor. Sampling area 2 was established near the fishponds of approximately 60 hectares semi-intensive fish farming activity. Surface water, microbiological and algal samples were collected at each sampling stations. For effluents and algal samples, a composite sample per site was performed. Sampling was done twice during low tide and high tide and an average of triplicate measurements were tabulated. The effluent samples were collected at the point source or at the end-of-pipe effluents. For the collection of phytoplankton, a total of 30L of water samples from each sampling area were collected at 0.5m from the surface water and filtered using a plankton net # 20, mesh openings of 76μm. A 10mL filtered concentrates were stored in an opaque bottle, preserved with Lugol's solution, so as not to alter chlorophyll values and to avoid pigment degradation.

Laboratory Determination of Samples

Water and Effluent samples were brought to a laboratory for the analysis of total suspended solids (TSS), nitrates, phosphates, lead and total coliform following the prescribed analytical methods by the Department of Environment and Natural Resources. *In –situ* parameter test included pH, temperature, salinity, dissolved oxygen (DO), total dissolved solids (TDS), surface current and turbidity.

Identification and Counting of Phytoplankton Specimens

Using a graduated cylinder, the volume of each samples were measured. During the sub-sampling, each bottle was shaken vigorously to obtain 1mL using a pipette. This process was repeated for three times for each of the other bottles. The obtained 1mL subsample was then transferred to a sedge wick rafter counting chamber for identification and counting.

Under a compound microscope, specimens were counted and photo-documented and identification was made possible using various reference guides (Bellinger and Sigee, 2010). The process of identification was done in triplicates for each bottles.

Computations of Phytoplankton Density

Cell density of phytoplankton was calculated using the given formula:

$$\text{Cell Density} = \frac{(a)(b)(c)}{d}$$

Where:

a: *refers to the subsample cell count, expressed in cells/mL*
b: *Raise Factor = Total number of fields in the sedgewick number of fields viewed the Raise Factor used during the counting was 5*
c: *the volume of the sample in each bottle*
d: *Original volume of filtered water = 30L*

Results and discussions

Characterization of Point Source Effluents

The two identified effluents, market effluent and fishpond effluent showed an overwhelming result in some of the physico-chemical characteristics, nutrients, microbial and lead content as shown in Table 1. Both effluents showed exceeding amounts of total dissolved solids (TDS), nitrates, phosphates and the total coliform bacteria count as compared to the values set by the Department of Environment and Natural Resources (DENR) standards.

Table 1. Physico-chemical and Microbiological Parameters of the Effluents.

Physico-chemical Microbiological Parameter	Point source Effluents		DAO 2008
	Market	Fishpond	DENR Standard for Effluent
pH	7.94	7.65	6.50-8.50
Temperature, ^{o}C	30	30	25-31^{o}C
Salinity, g/L	2.0	1.8	-
Surface current, m/s	0.21	0.12	-
Total suspended solids, mg/L	114	39	<80
Total dissolved solids, ppm	3,680	11,030	Max of 1,000
Dissolved oxygen, mg/L	4.0	10.45	Min of 5.00
Nitrates, mg/L	23.2	9.00	7.00
Phosphates, mg/L	67.0	1.0	0.5

Physico-chemical Microbiological Parameter	Point source Effluents		DAO 2008
	Market	Fishpond	DENR Standard for Effluent
Lead, ppm	0.02	0.03	0.5
Turbidity, NTU	123.00	22.68	-
Total Coliform, MPN/100mL	>16 x 10^4	33 x 10^2	200

The higher values of pH, salinity, TSS, nitrates, phosphates, and turbidity generally common to market effluents were expected because the public market is actively managed, with its total daily loading of wastewater for both dry and wet products like fruits, vegetables, fish and meat. Though the parameters like pH, temperature and lead for both point sources, TSS in the fishpond effluent, were within the allowable values but the value of dissolved oxygen of the market effluent which is below minimum for marine organisms to survive was apparently alarming. Its wastewater effluents is composed of different sources of waste generation, an indication that wastewater treatment was not strictly implemented.

Physico-chemical and Microbiological Conditions of the Brackishwater in Bulua.

The water quality conditions of the brackish water in Bulua, Cagayan de Oro City Philippines during low tide and high tide is shown in Table 2.

The results from both sampling areas exceeded the total dissolved solids (TDS), turbidity and phosphates concentration with reference to the DAO-34, 2008 DENR standards. The other physico-chemical parameters like pH, total suspended solids, dissolved oxygen and lead content has obtained values that were within acceptable range. These normal values obtained can be due to some actions of physical processes like dilution capacity of the receiving water, tidal processes and stratification, thus, reducing its concentrations.

Table 3 showed the bacterial counts during low tide and high tide for both market and fishpond area. US EPA (2002) identified total coliform as a useful indicator of pathogens.

The obtained total coliform bacteria counts were very high for both sampling areas and sampling period. All the sampling areas exceeded the maximum values of total coliform count of 15,000 MPN/100mL (DENR - DAO 34, 2008).

Table 2. Physico-chemical parameters of the effluent -receiving brackishwater.

Physico-chemical Parameters	Brackishwater				DAO 2008 DENR Standard for Class A-C
	Market Area		Fishpond Area		
	Low Tide	High Tide	Low Tide	High Tide	
pH	7.60	7.63	7.70	7.68	6.50-8.50
Temperature, ^{O}C	31.70	31.78	32.19	32.22	26.31^{o}C
Salinity, g/L	2.60	2.89	2.39	1.08	-
Surface current, m/s	0.20	0.17	0.25	0.23	>0.1(fast)
Total suspended solids, mg/L	50.20	51.00	41.78	54.56	<110.00
Total dissolved solids, ppm	25,922	11,688	28,355	12,387	Max of 1000
Dissolved oxygen, mg/L	7.00	15.15	5.49	5.79	Min of 5.00
Nitrates, mg/L	6.60	7.18	1.37	2.90	7.00
Phosphates, mg/L	2.60	2.44	1.42	2.17	0.50
Lead, ppm	0	0.03	0.03	0.03	0.05
Turbidity, NTU	22.50	23.29	33.76	33.14	≤ 10 Excellent*

*NAMOI Catchment Management Authority, Water Quality Parameters and Indicators.

Table 3. Microbiological parameter of the effluent-receiving brackishwater during lowtide and high tide.

Microbiological Parameter	Brackishwater			
	Market Area		Fishpond Area	
	Low tide	High Tide	Low Tide	High Tide
Total Coliform,MPN/100mL	>16 x 10^4	>16 x 10^4	70 x 10^3	>16 x 10^4
DENR Standards, MPN/100mL	15 x 10^3			

The data suggests that total coliform levels in both areas indicate severe water pollution. The contamination of the brackishwater with coliform bacteria indicates an inadequate treatment of wastewater and requires an immediate investigation. This can be serious especially when coliform growth rate can be elevated by the favorable values in the brackishwater of barangay Bulua in terms of its temperature, nutrients, total dissolved solids and low dissolved oxygen.

Phytoplankton Density of the Brackishwater Environment in Bulua

A change in the phytoplankton density brought by a heavy nutrient load can have a serious implications in coastal communities especially those sectors that depended on this resource for livelihood (Palmer, 2012).

Table 4 presents the mean cell density of different phytoplankton groups and the species composition in the two sampling areas during low tide and high tide. *Oscillatoria sp.* an algal bloom forming species is the most notable organisms identified next to *Scenedesmus sp.* during high tide in the market area. The presence and dominance of *Scenedesmus sp.* in the market during low tide and high tide confirm the polluted nature of brackishwater in barangay Bulua as revealed by a similar findings of other researchers (Davies and Ugwumba, 2013). The accumulation of phytoplankton in the brackishwater environs caused by nutrient influx can be identified by the anthropogenic activities of its sources (Palmer, 2012). In the data presented, the possibility of algal bloom is likely to occur due to the different phytoplankton identified from the sampling sites.

Table 4. Mean Cell Density (cell/mL) of phytoplankton groups in the two sampling areas.

Phytoplankton Groups		Cell Density in the Brackishwater (cell/mL)			
		Market Area		Fishpond Area	
		Low Tide	High Tide	Low Tide	High tide
Green Algae					
Chlamydomonas sp.		0	1,615	350	3,267
Scenedesmus sp.		94	0	152,851	0
	Total	94	1,615	153,646	3,267
Blue-green Algae					
Lyngbya sp		23	0	0	80
Oscillatoria sp.		31,639	11,673	81,828	72,853
	Total	31,662	11,673	81,828	72,933
Diatoms					
Chaetocerus sp.		165	0	0	760
Coscinodiscus sp.		0	0	32	13
Cymbella sp.		24	68	32	120
Diploneis sp.		24	0	0	67
Fragilariaflorma sp		0	0	0	267
Gyrosgima sp		33	0	0	360
Navicula sp.1		0	295	0	147
Navicula sp.2		57	0	0	1387
Nitzschia sp.		411	3,179	445	9200
Pleurosigma sp.		19	0	0	933
Rhizosolenia sp		38	0	0	0
Total		771	3,542	509	13,254

As shown in the table (4), the observed *Navicula* sp. 1, *Navicula* sp. 2, and *Nitzschia* sp. and *Cymbella sp.* were important bioindicator of aquatic pollution (Brunn, 2012). These species were confirmed by the study of Davis and Ugwumba (2013) as organic pollution indicator species. These species preluded that the brackishwater in barangay Bulua is environmentally-stressed from inputs of market and fishpond activities.

A similar study identified the presence of *Navicula* sp. and *Nitzschia* sp. to be dominating in a contaminated freshwater of Bitan-ag Creek in Cagayan de Oro City (Canencia, *et. al.,* 2009). As indicated in Table 4. The blue-green algae group obtained the highest cell density (cells/mL) in both sampling areas and sampling period.

The presence of these species further highlighted the inefficient disposal of domestic waste to the receiving brackishwater. Moreover, table 4 showed the dominance of diatoms in terms of species composition indicating pollution (Krumme and Liang, 2004). Diatoms are sensitive to a wide range of environmental variables. This might be the possible reason for the dominance of diatoms species in barangay Bulua brackishwater. They were resilient to the increased anthropogenic inputs.

Fig. 2 shows the identified structures and forms of the phytoplankton as seen under the microscope. (Note: *Due to inadequate light amplification during photo documentation, some pictures were taken from internet for phytoplankton of similar identity).

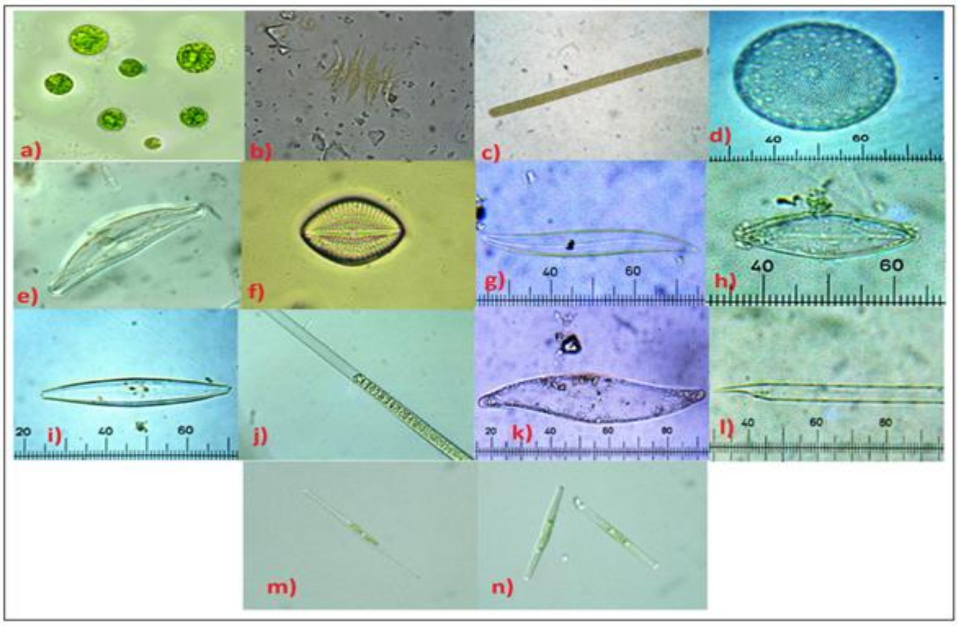

Fig. 2. Phytoplankton species* in the sampling areas, green algae (a) *Chlamydomonas* sp. (b) *Scenedesmus* sp., blue-green algae (c) *Oscillatoria* sp.,diatoms (d) *Coscinodiscus* sp. (e) *Cymbella* sp. (f) *Diploneis* sp., diatoms (g) *Gyrosigma* sp (h) *Navicula* sp.1. (i) *Navicula* sp.2, blue-green algae (j) *Lyngbya* sp., diatoms (k) *Pleurosigma* sp. (l) *Rhizolenia* sp (m) *Nitzschia* sp. (n) *Fragilaria* sp.

The variety of the phytoplankton identified and the high cell density abundance of the sampling sites may have an eventual Harmful Algal Bloom (HAB). This can be confirmed in the critical value of its low-dissolved oxygen parameter in table 2 in the brackishwater of the area and the effluents from the market area (Kudela, 2015).

The physico-chemical conditions in the brackishwater and the influence of effluents from point sources

To determine the effects of effluents from two point sources relative to the water quality environment in the brackishwater, a nonparametric Mann-Whitney U test was determined. Table 5 and 6 shows the summary of Mann-Whitney U test of the physico-chemical parameters of the effluents in the market and fishpond.

Table 5. Summary of Mann-Whitney U Test of Effluents in the Market.

Predictor	Mann-Whitney U	p-value	Remarks
pH	<.001*	.012*	Significant
Salinity	<.001*	.011*	Significant
Total suspended solids	<.001*	.013*	Significant
Total dissolved solids	<.001*	.013*	Significant
Dissolved oxygen	<.001*	.012*	Significant
Nitrate	<.001*	.012*	Significant
Phosphate	<.001*	.012*	Significant
Lead	<.001*	.011*	Significant
Turbidity	<.001*	.009	Significant

*$p \leq 0.05$

Table 6. Summary of Mann-Whitney Test of Effluents in the Fishpond.

Predictor	Mann-Whitney U	p-value	Remarks
Salinity	<.0001*	.009*	Significant
Surface Current	.500	.015*	Significant
Total suspended solids	1.000	.020*	Significant
Dissolved oxygen	2.000	.033*	Significant
Nitrate	<.0001*	.013*	Significant
Phosphate	<.0001	.012*	Significant

*$p \leq 0.05$

The data shows majority of the effluent quality parameters significantly contributed to the conditions of the brackishwater particularly the turbidity of water, the presence of lead and salinity of the water at $p \leq 0.01$. Similarly, DO, nitrates, phosphates, pH, total suspended solids and dissolved solids had an influence on the brackishwater conditions significant at $p \leq 0.05$. On the other hand, there were six water quality variables found to be significantly influencing the aquatic environment of the brackishwater from the effluents of fishpond as presented in Table 6. The values for salinity, total suspended solids, surface current, nitrates and phosphates significantly affect the water condition of the brackish water at $p \leq 0.05$.

Correlation among Nitrates, Phosphates, Lead and Phytoplankton Density

Results from regression analysis as shown in Table 7, revealed two significant predictors for the presence of phytoplankton. The most significant predictor is the nitrate concentration followed by phosphate concentration. The F-value of 53.849 significant at $p \leq .05$ suggests that these two predictors formed a very significant set of predictors for the presence of planktons. Likewise, 76.5% (R^2=.765) of the variance in the observed presence of planktons could be explained by the two significant predictors. In order to determine if the presence of Lead (Pb) in the sampling area could be another predictor for the observed presence of planktons, a similar test was performed. In the light of the findings, results pointed out that the phosphorus and nitrogen from effluent are utilized by blue-green algae, and ultimately result in the development of large blooms and mats (Chorus and Bartram, 1990; Kudela RM, *et al.* 2015).

Table 7. Regression Coefficient values of Nitrate, Phosphate and Lead concentration with plankton Density.

Predictor	Regression Coefficient	Standard Error	t	p-value	F-value
Nitrate	-22007.678	2815.235	-7.817	<.005	
Phosphate	-38218.949	10198.703	-3.747	<.01	
Lead	-10659879	2252638.0	-4.732	<.005	
constant	275440.69	22471.482	12.257	<.005	53.849*

Relationships between Physico-chemical Parameters and Variations of Phytoplankton Density

To determine the extent of the physico-chemical variables in the brackishwater in terms of variability of the phytoplankton, a correlation analysis test was performed. Data in Table 8 pointed out four out of eleven predictors that influenced the variations of phytoplankton. Results showed that phytoplankton density was significantly influenced by the presence of salinity ($p \leq 0.01$*), nitrates ($p \leq 0.01$*), phosphates ($p \leq 0.05$*) and lead ($p \leq 0.05$*). The most significant predictor is nitrate, followed by salinity, lead and phosphate. The results of the study confirmed with the findings of Bonilla, Pick, 2017 who reported that nutrient loading and some physico-chemical changes will favorably produce a severe cyanobacterial blooms and these freshwater blooms will have a great impact on the environmental ecosystem. The formation of bloom depends largely on the availability of nitrogen and phosphorus and other environmental factors like salinity, temperature, surface current, total dissolved solids, dissolved oxygen and turbidity. Though Table 8 showed the significant effects of four variables but table 2 showed the range of minimum values of the other environmental factors such as temperature, TDS, DO and turbidity that were in a critical values as compared to the allowable range set by DENR.

Table 8. Correlation Analysis output of Physico-chemical parameters and Planktons.

Predictor	Regression Coefficient	Standard error	t	p-value
Salinity	32476.272	11013.203	2.949	.007*
Nitrate	-17555.817	4117.523	-4.264	<.005*
Phosphate	-28853.647	13332.801	-2.164	.041*
Lead	-5370616	2424393.1	-2.215	.036*
constant	722753.52	817718.33	.884	.386

*$p \leq 0.05$

Conclusions and recommendations

Based on the findings of the study, there was a pollution causing-effluent quality of market and fishpond sources being dumped in the brackishwater in barangay Bulua, Cagayan de Oro City. The findings clearly showed that the physico-chemical conditions and the biological integrity of the water is impacted, causing a detrimental effect to the aquatic environment of the site as reflected by the recorded values that has exceeded the limits set by DENR DAO-34, 2008. Tidal influences have varied effects on the physico-chemical parameters, nutrients status, coliform counts, phytoplankton density and composition of species. High tides greatly influenced the phytoplankton density since higher values were recorded in all plankton groups. Blue-green algae (a bloom-forming species) dominated the phytoplankton groups in both low tide and high tide. The current concentration of nutrients present in the brackishwater were favorable for harmful algal blooms to occur. The occurrence of *Navicula* sp.1, *Navicula* sp. 2, *Nitzschia* sp., *Oscillatoria* sp., *Scenedesmus* sp. and *Cymbella* sp. showed that the brackishwater is under stress from inputs of market and fishpond activities. The dominance of diatoms in terms of species composition indicated that the brackishwater in barangay Bulua is polluted. Nitrates, phosphates and the presence of lead in the study areas were a significant contributor to the presence of phytoplankton. Its presence in the effluent can be utilized by blue-green algae and ultimately may result the development of large blooms and mats (Chorus and Bartram, 1990). Interestingly, the most significant environmental factors among all the parameters studied which influenced phytoplankton were salinity, nitrates, phosphates and lead.

In the light of the conclusions, there is a need for the following to be done: a concerted environmental surveillance must be advocated to reduce inflow of pollutants from market and fishpond into this effluent-receiving water; a greater involvement of the local government unit, enforcement agency, the locals, and administrators of the public market and fishpond owners to address multi-sectoral participation in the restoration and clean-up of its water environment in the area.

For future researchers, a long time series of data is a warrant and that includes, sampling period coverage, to take into consideration the diurnal variations for both water and effluent quality and biological components. It is further suggested to consider event-scale variability like rainfall and surface-runoff as it can increase loading or decrease indicators due to dilution.

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