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RESEARCH ARTICLE

An assessment of the effects of hospital wastes released to Nilwala river, Matara

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Abstract: Wastewater from hospitals either treated or partially treated can alter water quality and biological diversity in adjacent water. This study focused to assess effects of hospital wastes discharged from the General Hospital Matara to the Nilwala River. Two sampling sites were located upstream of the entrance of wastewater while two sites were located downstream of the entrance of wastewater discharge and one site was selected at the entrance of the wastewater channel. Water, sediment and plankton samples were collected biweekly during February to April, 2012. Nitrate, phosphate, pH and Cu concentrations in water sampled from the hospital wastewater discharging canal were significantly (One-way ANOVA, $p<0.05$) different from the other sampling sites. However, Cu concentration in surface water did not exceed the EU limits of 0.0005-0.112mg/l for fisheries and aquatic life and similarly water pH did not exceed the maximum allowable limit (6-8.5) for fisheries and aquatic life in Sri Lanka. Mean concentration of Cu in sediment collected at the entrance of the wastewater channel was 0.75±0.05 mg/g dw. Although total coliform concentration (MPN/100ml) did not vary significantly among the sampling sites, it was relatively low (39.66±17.7) at the waste discharging canal due to the presence of disinfectants in hospital wastewater. *Pediastrum simplex* was the most abundant phytoplankton species at all the five sampling sites. Cladocera was the most abundant group of zooplankton in all the sites except at the entrance of wastewater discharging canal and Protozoa was the most abundant group present at the wastewater discharging canal. According to rotated matrix of Principle Component Analysis (PCA) water pH, PO_4^{-3}, NO_3^-, TDS, Salinity and Zooplankton abundance can be considered as important parameters at the study site of wastewater entrance (site 3). The toxicity (72h EC_{50}) of hospital wastes on *Pediastrum simplex* was 0.08 Toxic Units (TU) and it was not an acute toxic effect on the alga when compared with hazard classification systems for wastes discharge into the aquatic environment.

Keywords: Hospital wastes, Cladocera, Protozoa, hazard classification, toxicity

Introduction

Hospital wastes come mostly from the administrative and housekeeping functions of health-care establishments and may also include waste generated during maintenance of health-care premises. However, only 10-25% of the wastes generated by the hospitals are regarded as hazardous wastes and they can create variety of health risks (Chapmen and Kimstach, 1992). The most frequent contaminants in hospital wastewater are viruses and pathogenic bacteria, molecules from unused and excreted non-metabolized pharmaceuticals, organohalogen compounds, such as the halogenated organic compounds absorbable on activated carbon (AOX), and radioisotopes (Emmanuel et al., 2005). The pharmaceutical residuals consists a largest proportion of active pharmaceutical substances and metabolites excreted via urine, faeces and appear in bath and shower water in the case of externally applied products those have been considered as potential contaminants in the environment (Germer and Sinar, 2010).

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The quantity and composition of healthcare waste varies between and within the countries and this variation can be attributed to the size of establishments, proportion of in and outpatients, type of institution and specialization, available waste aggregation options, proportion and use of reusable items, wealth of user, and the prosperity of the country (Visvanathan and Adhikari, 2006). Even though the amount of wastewater discharged from hospitals depends on the capacity of the hospitals, it has been estimated as 400 to 1200/liters/day/bed (Ibeh and Omoruy, 2011) and the water consumption in hospitals of developing countries has been published as 500L/bed/day (Laber et al., 1999).Hospital wastewater quality and management have become critical issues as they possess potential health risks and damage to the environment, which has taken a key position in national health policies of many countries. The disposal of untreated hospital wastewater which contains antibiotic-resistant bacteria is also a matter of concern, since it constitutes a health risk to the population (Mithila et al., 2013). The risk level of wastewater varies and depending on the content of wastewater. If the characteristics of wastewater are not as per the accepted standards then the wastewater is more hazardous and could be dangerous to the ecological balance and public health. Furthermore, pathological, radioactive, chemical, infectious, and pharmaceutical wastes, if left untreated could lead to outbreaks of communicable diseases such as diarrhea, cholera, skin diseases and enteric illnesses. These facts emphasize that a pre-treatment is necessary for hospital wastewater to prevent the hazard being transferred to other water sources through cities sewerage. Toxic effect studies done by Emmanuel et al. (2005) have found that the existence of hazardous substances in the studied hospital in Southern France. The results of risk assessment using *Daphnia magna* has shown that the risk is acceptable and it is not completely far away from the red line in the same study. Objectives here is to study the variation of water quality at the downstream and upstream sites of wastewater discharging point from General Hospital Matara, on the basis of the statistical evaluation of PCA and also to carry out an initial trial of toxicological test for the hospital wastes to study the toxicity level based on the EPA probit analysis program (version 1.5) and the hazard classification.

Materials and methods

General Hospital Matara releases its wastewater via the wastewater treatment plant which includes the steps of grit removal, settling, aeration and chlorination. However, inefficiency of some steps can be observed during the study period of February to April 2012. Two sampling sites (site 1 and site 2) were selected at the upstream before reaching the wastewater to the river. The sampling site 4 and site 5 were located downstream of the entrance of wastewater to the river and the site 3 was located at the entrance of wastewater outlet to the river (Figure 1). The distance between each site was nearly 200m.

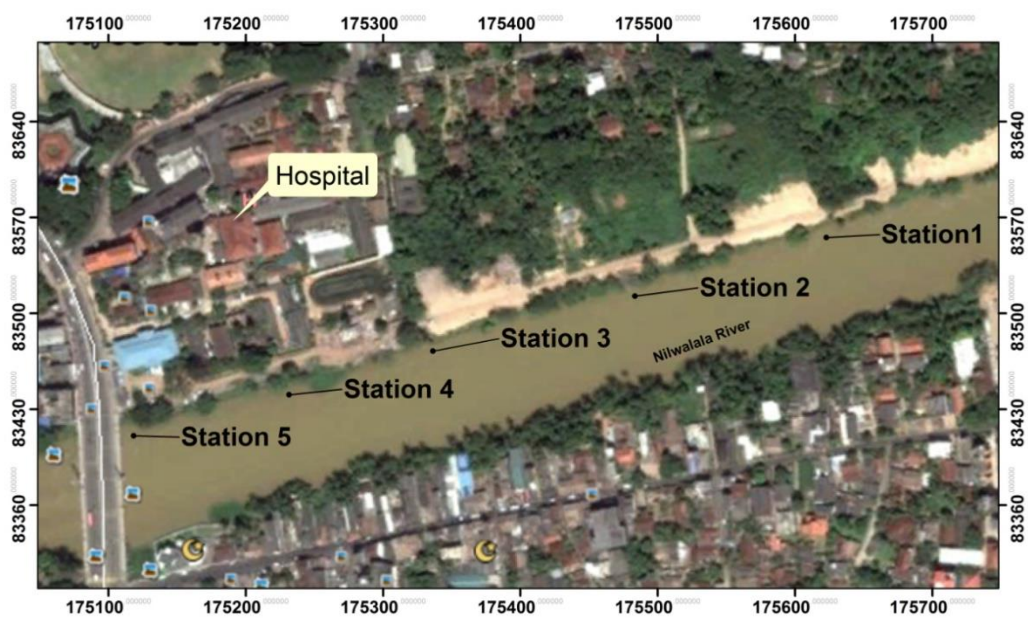

Figure 1. Sampling sites in Nilwala River

Water temperature, pH, depth, Secchi depth and flow rate of the river water were measured on site. All the other parameters of DO, BOD_3, salinity, PO_4^{-3}, NO_3^-, TDS, TSS, Cu, Clorinity, and total Coliform (MPN number) were measured according to the methods described in APHA (1998). Spectrophotometric methods were used for measuring PO_4^{-3} and NO_3^- concentrations using HATCH DR 2000, (at an λ accuracy ±2nm for 400-700nm and ±3nm for 700-900nm). Cu concentration in water and sediment were determined using AAS (Varian 220). Cu was selected to analyse because it is an essential metal for life forms and only the exceeded amount can be harmful (Liyanage, 2001). Acid digestion was done for dried sediment samples using 4ml $C.HNO_3$ and 1ml H_2O_2 for preparation of the sediment samples prior to analysis for Cu and the accuracy of the method was determined according to the standard addition method (Dauvalter, 2001). Phytoplankton and other biological samples (zooplankton) were taken from the water column by filtering 5L of water via the plankton nets of 30 µm and 80 µm mesh respectively. The concentrated sample volume was 5ml and those were preserved in Lugols Iodine and 40% formalin respectively for phytoplankton and zooplankton. The abundance (number of individuals/m^3) was estimated using the number of cells in 1ml on the Sedgwick rafter cell, according to the following equation in APHA methods (1998); $n = \frac{a \times b \times 1000 \times 10^6}{N \times L}$ where n = Number of plankton in m^3, a = Number of plankton in the counted cells, b = Volume of concentrate express in ml (5ml), N = Number of cells counted and L = Volume of water filtered (5L).

Ecotoxicological test: *Pediastrum simplex* was selected as the test organism for hospital waste, due to their high abundance at all sampling sites and their suitability to maintain under the laboratory conditions. It is non-motile, free floating coenobial green algae and the cells are disc shaped to stellate in form. Rarely the large coenobia have cells arranged in two layers in the center and the number of cells in each colony varies from 4 to 128 depending on the species (Vashithta, 2002). A monoculture of the *Pediastrum simplex* was maintained under the laboratory conditions using the B2 culture medium with major nutrients in sterilized water and artificial light was provided during day time of 7 days. Wastewater samples were collected from the outlet canal of the hospital wastewater treatment plant and filtered using 30µm meshes to remove the planktons. A series of hospital wastewater concentrations (0%, 2%, 4%, 8%, 20% and 40%) were prepared with three replicates in sterile plastic containers. Each replicate was included 50ml of the B2 culture medium and 10ml of the monoculture of *Pediastrum simplex and* the total volume was adjusted up to 100ml using sterilized water. The algae densities were determined in each series after 72 hours of the experiment using optical microscope and sedgewick rafter cell. 72-h EC_{50} value was calculated using EPA probit analysis program (version 1.5) and the result was converted into toxic unit ($1TU=100/EC_{50}$) and toxicity was assessed according to toxicity classification in Table 1.

Table 1. Hazard classification systems for wastes discharged into the aquatic environment (Persoone *et al.*, 2003)

TU	Class	Toxicity
<0.4	I	No acute toxicity
0.4<TU<1	II	Slight acute toxicity
1<TU<10	III	Acute toxicity
10<TU<100	IV	High acute toxicity
TU>100	V	Very high acute toxicity

Results and Discussion

Physicochemical parameters such as BOD, TDS, TSS and secchi depth in surface and bottom water, MPN value for coliform, zooplankton and phytoplankton density did not vary significantly among the sampling sites. However, water pH, depth, nitrate, phosphate and Cu concentrations in water and in sediment varied significantly among the sampling sites. Although BOD, TDS, TSS and secchi depth did not show significant spatial variation among the sampling sites, those parameters were relatively higher near the hospital waste discharging canal at the site 3. The MPN for coliform was relatively lower at the waste discharging canal to the river indicating the presence of disinfectants. The effect of disinfectants has been observed for bacterial flora in hospital wastewater in Southern France (Emmanuel et al., 2005). The mean values of physicochemical parameters which varied significantly among the sampling sites are given in the Table 2.

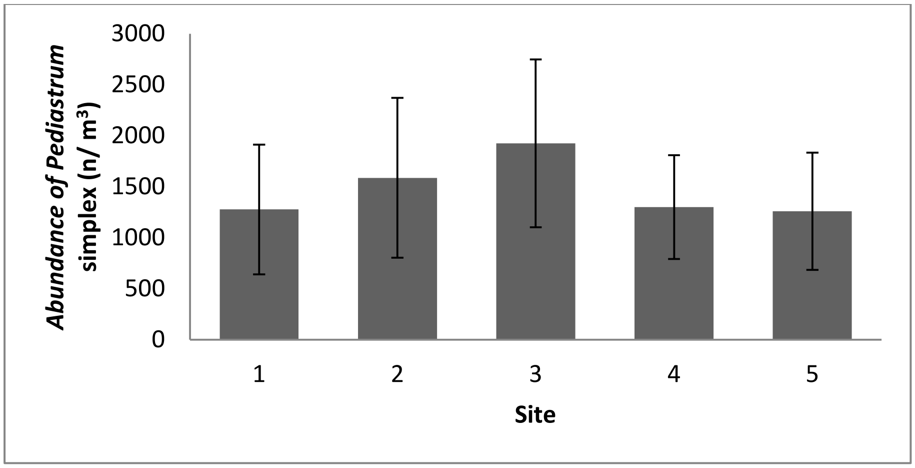

Figure 2. Abundance of *Pediastrum simplex* at the sampling sites

The ranges of minimum and maximum values of DO, BOD_3, Chlorinity, Salinity, TSS and TDS were 6.6 – 9.34 mg/l, 5.5 – 9.4 mg/l, 0.4 – 4.4 ppt, 0.7 – 8 mg/l, 7.7 – 25 mg/l and 177.4 – 672.6 mg/l respectively, during the period of study. DO concentration and water pH at the sampling sites were at the acceptable level according to proposed inland quality standards for fish and aquatic life in Sri Lanka (DO 6mg/l and pH 6-8.5, Azmy, 1998). However, BOD values at the sampling sites exceeded the above said quality criteria of 4mg/l. The TSS values and Cu concentrations at the sampling sites were at the considerable level according to maximum allowable concentration of TSS (25 mg/l) and Cu 0.0005-0.112 mg/l for fisheries and aquatic life (Chapmen and Kimstach., 1992). The contaminants cannot be effectively diluted from the waste discharging point towards the down stream sites due to not significant variation of water flow among the sites during the study period. Among the phytoplankton species *Pediastrum simplex* was the most dominant phytoplankton species and their abundance (1924±822/m^3) was also relatively higher at the entrance of the wastewater discharging canal (Figure 2). Copepods, Cladocerans, Protozoan, Rotifers and Shrimp larvae were the observed biological groups including some zooplanktons in the filtered water at the sites. Among the mentioned groups, Cladoceran abundance was relatively higher in all the sites except at the entrance of the wastewater discharging canal and that site was represented by the high abundance of Protozoan due to its water quality (Figure 3).

Table 2. Mean values of physicochemical parameters at selected sampling sites (mean±SE).

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5
pH	6.5 ± 0.0*	6.8 ± 0.0*	7.0 ± 0.1*	7.0 ± 0.1*	7.0 ± 0.1*
Secchi depth (m)	0.71 ± 0.01	0.75 ± 0.15	0.48 ± 0.24	0.72 ± 0.17	0.72 ± 0.13
Flow rate (m/s)	0.12 ± 0.04	0.17 ± 0.08	0.07 ± 0.01	0.08 ± 0.04	0.07 ± 0.03
Depth (m)	4.02 ± 0.54*	3.80 ± 0.4*	0.77 ± 0.20*	3.75 ± 0.12*	3.05 ± 0.66*
Nitrate (mg/l)	0.15 ±0.01**	0.13 ± 0.01**	0.32 ± 0.04**	0.28 ± 0.03**	0.13 ± 0.07**
Phosphate (mg/l)	0.027±0.004**	0.04 ± 0.01**	0.76 ± 0.17**	0.08 ± 0.01**	0.05 ± 0.01**
Cu in surface water (mg/l)	0.05 ± 0.01*	0.07 ± 0.001*	0.08 ± 0.002*	0.08 ±0 .001*	0.07 ± 0.002*
Cu in sediment (mg/gdw)	0.19 ± 0.04*	0.24 ± 0.07*	0.75±0.05*	0.32 ± 0.05*	0.39 ± 0.07*
Coliform count (MPN/100ml)	456 ± 321	413 ± 344	39 ± 17	220 ± 10	503 ± 302
Phytoplankton (n/m^3)	1772 ± 539	1948 ± 733	2519 ± 1017	1884 ± 649	1938 ± 557
Zooplankton (n/m^3)	453 ± 180	314 ± 235	373 ±249	332 ± 94	362 ± 98

Note: Values are significant at *p<0.01, **p<0.05 level among the sampling sites

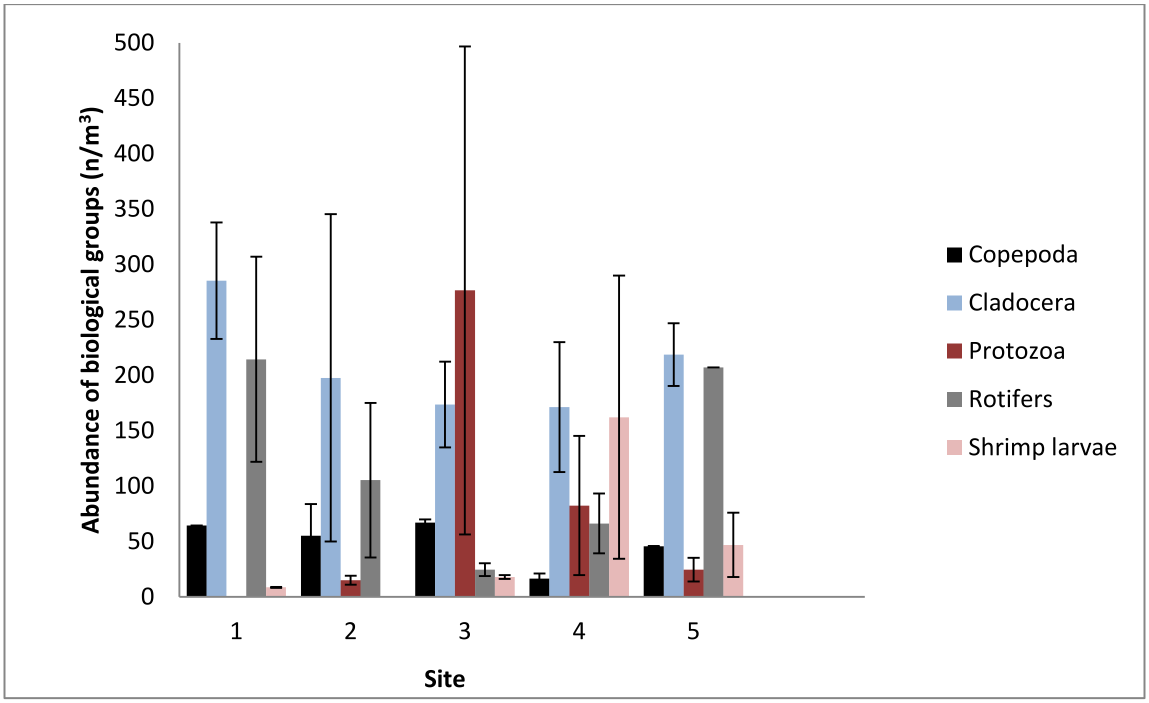

Figure 3. Abundance of biological groups at the selected sites in Nilwala River

Table 3: The rotated component matrix with the factor values under two components (C1, C2) for the five sites

Parameter	Site 1		Site 2		Site 3		Site 4		Site 5	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
pH	**-.899**	-.427	.628	-.264	**.955**	.230	-.014	**.975**	-.014	.148
Temperature	.707	.585	.665	.242	.404	-.881	-.866	.298	-.866	.089
DO	-.111	**.952**	**-.965**	.121	-.856	.513	.867	-.366	.867	-.100
BOD	.016	**.984**	**-.980**	.195	-.842	.537	.831	-.370	.831	-.219
TSS	-.366	**-.901**	-.396	.040	-.491	.224	**.976**	-.078	-.778	-.546
TDS	.316	.770	**.974**	-.170	**.912**	.160	-.358	.651	.114	-.144
Secchi_depth	**.986**	.098	**.972**	.090	.385	.242	-.431	.879	.297	.407
Depth	-.428	.231	.293	-.546	-.184	.170	.658	-.696	**-.992**	.010
Chlorinity	**.999**	.007	**.935**	-.026	**.925**	-.216	-.560	.824	-.293	.833
Salinity	**.999**	.007	**.935**	-.026	**.925**	-.216	-.560	.824	-.293	.833
Phosphate	-.048	.209	-.209	**.960**	**.964**	-.190	**.997**	-.057	.099	**.900**
Nitrate	-.745	-.262	-.583	.211	-.125	**.917**	**.918**	-.217	.009	-.712
Phytoplankton abundance	-.388	**-.910**	.451	-.263	-.099	**.972**	.332	-.091	-.616	-.695
Cu_in_water	.154	-.446	.189	.362	-.388	-.265	-.375	-.101	**.891**	-.128
Cu_in_sediment	-.083	.127	.242	.726	-.566	-.413	.197	.793	.870	-.373
Zooplankton abundance	−0.214	0.249	-.106	**.911**	.061	**.972**	-.153	-.039	**.891**	-.096
Eigen value	7.296	3.875	8.416	3.691	7.872	4.478	9.060	3.709	5.652	4.825
Cumulative %	45.59	69.81	52.60	75.67	49.20	77.18	56.62	79.80	35.32	65.48

The rotated component matrix with the factor values in two components (C1 and C2) for five sites are given in the Table 3 and the parameters with bolded values (factor values > 0.89) were considered as important parameters at the relevant sites. According to the factor values water pH, PO_4^{-3}, NO_3^{-}, TDS, Clorinity, Salinity and biological abundance can be considered as important parameters at the wastewater entrance (site 3). The factor values explain that DO, BOD, Secchi depth, clorinity and salinity were common important parameters for the water quality of up stream sites (site 1 and site 2). The common important water quality parameter for the two downstream sampling sites (site 4 and site 5) was phosphate concentration. However, the site located at the most downstream part (site 5) represented by the parameters of phosphate concentration, Cu concentration in water and zooplankton abundance.

In the present study positive correlations were observed for TDS and DO with zooplankton abundance. Sharma et al. (2008) have also revealed that DO concentration is positively correlated with Zooplankton abundance while total solid is negatively correlated with the zooplankton abundance in water contaminated with textile effluents in Rajasthan, India. According to Emmanuel et al. (2005) it is possible to carry out the ecotoxicological risk assessment of hospital effluents by the use of standardized bioassays, global physicochemical parameters and the analysis of some targeted pollutants. Therefore, the toxicity test was done in this study for *Pediastrum sp* because it was the most abundant species among the identified phytoplankton in the sampling sites and the results were compared with the toxicity classification system (Persoone et al., 2003). The 72-h EC_{50} value for *Pediastrum simplex* was 1196.3ml of the waste (0.08TU) (Table 4) and according to the toxicity classification the value belongs to the class I of no acute toxicity group (Persoone et al., 2003).

Table 4: Estimated LC or EC (LC/EC) values according to the EPA probit analysis program (version 1.5)

Point	Exposure volume of hospital wastes (ml)
LC/EC 1.00	0.456
LC/EC 5.00	4.573
LC/EC 10.00	15.637
LC/EC 15.00	35.854
LC/EC 50.00	**1196.301**
LC/EC 85.00	39915.094
LC/EC 90.00	91523.297
LC/EC 95.00	312973.938
LC/EC 99.00	3140713.750

Note: Bolded value represents the LC/EC_{50} of the study

Conclusion

According to the Principle Component Analysis water pH, PO_4^{-3}, NO_3^{-}, TDS, Salinity and biological abundance with zooplanktons can be considered as the most important parameters for explaining the water quality of hospital waste discharging point in the Nilwala river. The significant spatial variation of the water pH, NO_3^{-}, Cu concentration in water and sediment and phytoplankton abundance was observed to explain the water quality variation. The relatively low MPN of coliform at the wastewater discharging point is also important to conclude that the presence of disinfectants in the wastewater. The Protozoans and *Pediastrum simplex* can be considered as the biological indicators for hospital wastewater due to their high abundance at the wastewater discharging point. However, the toxicity test showed that the 72-h EC_{50} value for *Pediastrum simplex* is included under the category of no acute toxicity of the hospital wastes release to the Nilwala river via the existing wastewater treatment plant.

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