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Bacterial Load Reduction after Primary and Secondary Treatment in Guheswori Sewage Treatment Plant, Kathmandu, Nepal

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ABSTRACT

Background: Water sources such as lakes, ponds, river etc. have been continuously contaminated by the micro organisms and chemicals. The former can pose a significant threat to human health. This work aims at detecting the bacterial load before and after the sewage treatment and hence isolating pathogens from the sewage before primary treatment and secondary treated effluent. **Methods:** Grab sampling (50mL sewage before primary treatment and secondary treated effluent) was performed for 20 days in the Guheswori sewage treatment plant. The reduction in microbial load was determined through heterotrophic plate count. Pathogens were screened from the effluent obtained from the secondary treatment plant. **Results:** Bacterial load reduction was found to be about 48.02% on average. The observed bacterial load reduction might have been caused by bacteriophage flocculation and sedimentation. Pathogens isolated from the treated effluent were *Escherichia coli, Salmonella Typhi, Enterococcus faecalis, Staphylococcus aureus*, *Coagulase negative Staphylococcus (CONS), Citrobacter fruendii, Enterobacter aerogenes, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa*. **Conclusions:** It has been found that the sewage treatment plant helps to reduce the bacterial load which is, however, not capable of effluent polishing where all pathogens are killed.

Keywords: bacterial load; pathogens; sewage treatment.

INTRODUCTION

Guheswori sewage treatment plant is located in the north-eastern part of Kathmandu valley in the bank of Bagmati river. Guheswori sewage treatment plant is a municipal wastewater treatment plant which utilizes extended aeration, activated sludge, deep oxidation ditch of Carrousal type for treatment (C1, E, C2; Figure 1). The drainage system of Gokarna, Boudha, Jorpati, Chabahil, Gaurighat and Pasuphati leads to the inlet of this plant (A1, A2; Figure 1).[1] The main aim of this plant is remove grits and plastics, to reduce microbial load, turbidity, chemical oxygen demand, biological oxygen demand, available nutrients, direct the effluent to a safe location (B, C, D; Figure 1); as river pollution is one of the oldest existing problem in Nepal.

Sewage production in developing countries is directly proportional to population growth; as there is an increment in demand of freshwater in domestic, commercial, and industrial sectors.[2, 3] Lack of education, financial and technical resources in many developing countries have led to irrigation.[4] Human health risks from wastewater irrigation include firstly people (majority female) involved in agriculture and farming followed by consumers' exposure to pathogens with the inclusion of helminthic infections,[3, 5] and chemicals.[6] Furthermore, if the sewage is not treated then the pathogens might lead to outbreaks.[7] If sewage is treated then microbial load, turbidity, biological oxygen demand, the risk of outbreaks, organic compound's etc will be reduced.[8, 9] The treatment process can be optimized further with the addition of activated sludge.[8] If the sewage is subjected to tertiary treatment then potable water can be recovered, lost beauty of the aquatic body can be regained, aquatic life can flourish once again in the water.[8-10]

Microorganisms and chemicals are generally introduced into water bodies through various routes (such as industrial effluents, raw as well as treated sewage, storm-water, and animal manure runoff).[11] The release of waste water (both black and gray,

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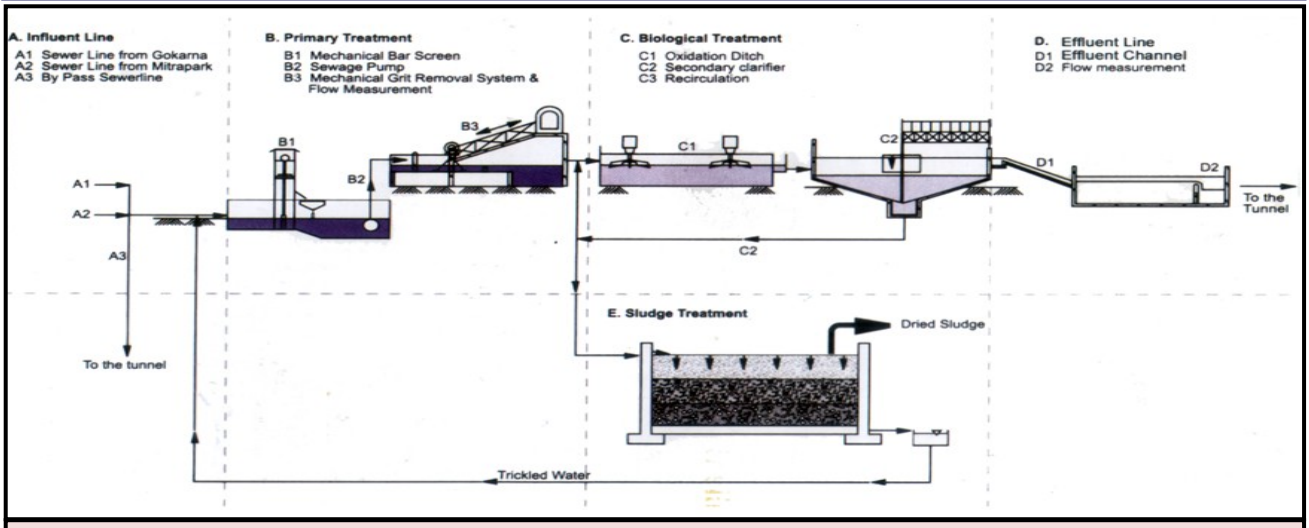

Figure 1. Flow diagram of wastewater treatment plant (Picture courtesy: HPCIDBC, Ministry of Urban Development).

has increased with the increase in population and urbanization.[9] It has been reported that blackwater consists of the discharges from toilets which contains intestinal flora, hormones, traces of pharmaceutical compounds, nitrogen and phosphorous in high concentrations.[10,12] In contrast, grey water may contain detergents, pesticides, fat, oil and grease, dyes, solvents, phenols, cyanide, nitrogen, phosphorus, ammonium, xenobiotic organic compounds, metal ions (such as Hg, Pb, Cd, Cr, Cu, Ni etc.).[9] Wastewater contains some chemicals which are required for their metabolism and/or to maintain their osmotic pressure (such as Cl-, K+, Na+, Fe++, Zn++, Ca++ etc.). [10]

Wastewater treatment processes do not remove or inactivate all pathogenic microorganisms and the heavy metal ions but then microbes and metal ions may get attached to the organic sludge.[13,14] The microbes tend to survive better when attached to solids than being suspended in water.[13] Depending on the environmental factors (such as temperature, moisture content, sunlight etc.) pathogens most commonly present in sewage sludge are bacteria (such as *Salmonella, coliform(s), Staphylococcus spp, Vibrio* etc.), viruses (such as *adenoviruses, hepatitis, enteroviruses*), protozoa (*Cryptosporidium, Giardia* etc.) and helminths (such as *Ascaris, Taenia* etc.).[7, 8, 15]

Government all around the globe have been struggling to remove the pathogens, chemicals etc from wastewater. The major sources of these pollutants are unplanned urbanization, lack of education, increase in industries, introducing the sewage directly into the river without any prior treatment etc. Several studies relating to microbiological load have conducted across the globe but optimization of the sewage treatment plant has rarely been done. This study focuses on bacterial load reduction, isolation of pathogens and their susceptibility to bacteriophage. This research will illuminate on the importance of sewage treatment and on its role in lowering bacterial load.
In the present study, we determined the bacterial load in wastewater (before and after treatment), determined the reduction in bacterial load after treatment and isolated pathogens from treated wastewater.

METHODS

Sample collection and investigation

The study was conducted at Microbiology laboratory, Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal during the period of April 16 to June 16, 2017.
For the study, grab sampling was performed (50 mL of sewage before primary treatment and secondary treated effluent) in 2 sterile plastic bottles at 9:30 AM. The process was repeated for 20 days. The sample was kept in a mini cooler full with ice-pack and was transported to the laboratory.

Microbial load count

The sample was diluted in sterile distilled water (containing 0.85% NaCl solution) for enumeration of bacteria. Pour plating was performed from 108 for primary inlet and 108 for secondary outlet effluent using 1 mL sample 16. The plates were incubated at 37 ℃ for 24 hours. Following day, colony count was performed and colony forming unit per milliliter (cfu/mL) was calculated as:
Cfu/ml=C*D/V where, C=total colonies counted, D=dilution fold, V volume of sample dilution.

Consequently, themicrobial load reduction percentage was determined using following equation [17]:

Cfu/ml=(B-A)*100%/B

where, B= microbial load before treatment, A= microbial load after treatment.

Isolation, characterization of the pathogens

With the help of sterile micropipette and sterile glass spreader, 100 µl sample (after treatment) was spread on cetrimide agar, mannitol salt agar and mac conkey agar and were incubated at 42℃, 37℃ and 44.5℃ for 24 hours respectively 16-20. 10ml sample was first enriched in Selenite F broth for 5 hours at 42℃; and then was spread on salmonella-shigella agar were incubated at 42℃ for 24 hours respectively. 5 mL sample was added in 10ml azide dextrose broth containing tube and were incubated at 37℃ for 24 hours. On the following day, colonial morphology was noted for the isolated colonies and then was subjected to biochemical tests 17.

Quality control and statistical analysis

A sample was triplicated and was repeated 2 times in an interval of a week. Purity plating was performed for the media plates and equipment were calibrated. Statistical analysis was done on SPSS version 19.

RESULTS

Total plate count of the sewage before treatment ranged from 150 X 10^8 cfu/ml – 178 X 10^8 cfu/ml (M=164 X 10^8, SD=8.41 X 10^8). Total plate count of the sewage after treatment ranged from 74 X 10^8 cfu/ml – 95 X 10^8 cfu/ml (M= 85 X 10^8, SD=4.80 X 10^8). bacterial load reduction after treatment ranged from 38.71% – 53.75% (M=48.02, SD=4.28). This result is presented in Table 1 and Figure 1. The results of t-tests indicated that the bacterial load determined on the wastewater sample before and after treatment, demonstrated a statistically significant difference ($p < 0.001$ at 95% confidence interval). Shapiro-Wilk test indicated that the bacterial load before and after treatment, demonstrated a statistically significant distributed ($p= 0.739$ at 95% confidence interval) (Figure 2). Paired t-test also indicated that the bacterial load determined on the wastewater sample before treatment and after treatment, demonstrated a statistically significant difference between the means ($p < 0.001$ at 95% confidence interval).

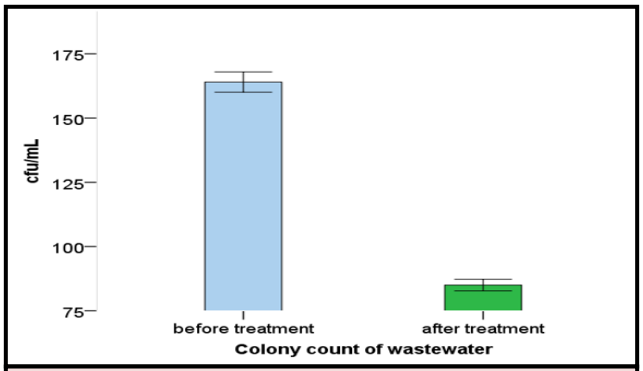

Figure 2. Bacteria load before and after treatment; implying decrease in microbial load after treatment.

With the help of biochemical tests of the sewage samples obtained after treatment; *S. aureus, CONS*,

Table 1. Enumeration of bacterial load in sewage before and after treatment.

S. N.	Sewage sample before treatment			Sewage sample after treatment			Bacterial load reduction (%)
	Dilution fold	Observed colonies (cfu)	Bacterial load (cfu/mL)	Dilution fold	Observed colonies (cfu)	Bacterial load (cfu/mL)	
1		165	165 X 10^8		86	86 X 10^8	47.8788
2		160	160 X 10^8		85	85 X 10^8	46.875
3		158	158 X 10^8		83	83 X 10^8	47.4684
4		172	172 X 10^8		88	88 X 10^8	48.8372
5		170	170 X 10^8		88	88 X 10^8	48.2353
6		178	178 X 10^8		88	88 X 10^8	50.5618
7		168	168 X 10^8		79	79 X 10^8	52.9762
8		160	160 X 10^8		74	74 X 10^8	53.75
9		153	153 X 10^8		83	83 X 10^8	45.7516
10	10^8	166	166 X 10^8	10^8	86	86 X 10^8	48.1928
11		170	170 X 10^8		91	91 X 10^8	46.4706
12		177	177 X 10^8		82	82 X 10^8	53.6723
13		150	150 X 10^8		89	89 X 10^8	40.6667
14		163	163 X 10^8		88	88 X 10^8	46.0123
15		155	155 X 10^8		95	95 X 10^8	38.7097
16		175	175 X 10^8		83	83 X 10^8	52.5714
17		169	169 X 10^8		79	79 X 10^8	53.2544
18		162	162 X 10^8		80	80 X 10^8	50.6173
19		151	151 X 10^8		88	88 X 10^8	41.7219
20		158	158 X 10^8		85	85 X 10^8	46.2025

E. faecalis, C. freundii, E. coli, E. aerogenes, P. mirabilis, P. vulgaris, Salmonella Typhi, P. aeruginosa were isolated. The highest frequency of bacteria being isolated were *Staphylococcus spp (100%), E. fecalis (70%), C. freundii (100%), E. aerogenes (100%), Proteus spp (100%), Salmonella Typhi (85%), P. aeruginosa (100%)*. The data are presented in Table 2.

Table 2. The list of bacteria isolated from the treated sewage with their frequency of isolates.

Isolates from sewage (after treatment)		Frequency of isolates (n=20)
Staphylococcus	***S. aureus***	20
	CONS	11
E. faecalis		14
C. freundii		20
E. coli		20
E. aerogenes		20
Proteus	***P. mirabilis***	12
	P. vulgaris	8
***Salmonella* Typhi**		17
P. aeruginosa		20

n= total sample collected days

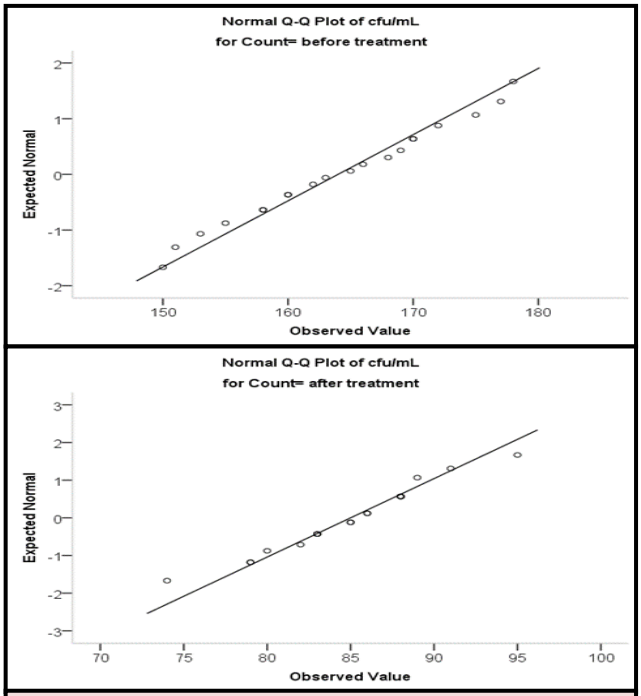

Figure 3. Normal Q-Q plot showing that the data significantly distributed in bacterial load before and after treatment of wastewater respectively.

DISCUSSION

The isolated bacteria from the effluent after treatment were *S. aureus, CONS, E. faecalis, C. freundii, E. coli, E. aerogenes, P. mirabilis, P. vulgaris, Salmonella Typhi, P. aeruginosa*. These bacteria might have been infected with bacteriophage but instead of the lytic cycle, the lysogenic cycle could have been initiated,[8] antibiotic resistance could also have been gained by these bacteria.[14] In Guheswori treatment plant, the contents of the sewage are first separated with the help of bar screening followed by fine screening. The sewage is then subjected to secondary treatment followed by sedimentation and flocculation which helped to reduce the bacterial load by 48.02% on average. The inconsistency of bacterial load reduction per day might be due to dilution caused by rainfall and runoffs.[1] Another investigation conducted in South Africa by Buthelezi et al (2009), reached the same conclusion where bacterial load decreased after flocculation process. The result agrees with the study conducted in the USA by Munir et al (2011) where bacterial load decreased after treatment. This could be because of the lytic cycle of the bacteriophage, increment of the microbial mass due presence of ample amount nutrition and getting attached to/in sludge.[8,14,21] In an investigation conducted by Tyrrel and Quinton (2003), wastewater containing pathogens of fecal origin were used in irrigation increasing the threat to the public masses consuming agricultural goods.

The main reason for collecting the sample at a specific time was due to the sunlight inactivation of microorganisms present in wastewater.[22]

CONCLUSIONS

It has been found that the treatment of the sewage water caused a significant (up to 48%) drop in the bacterial load. Even after treatment, pathogens are still viable in the sewage. If the treated sewage is used in irrigation then it might lead to outbreaks through agricultural products.

Recommendations

1. In further studies, biofilm forming capacity of certain bacteria can be determined.
2.In further studies, secondary metabolite producing microorganisms can be determined.
3.Continuous monitoring and surveillance of sewage should be done to know reduction in microbial load.

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