

**OCCURRENCE OF GIARDIA IN THE EFFLUENTS OF A WASTE WATER TREATMENT PLANT IN DHAKA**

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**Abstract:** The present study was designed for the qualitative and quantitative detection of *Giardia* in the Pagla Sewage Treatment Plant (PSTP) in Dhaka. The physico-chemical parameters of the sewage water were also recorded. Total 72 raw and treated samples were collected from PSTP throughout the year 2008 and these were processed with the Formol-Ether Concentration method and IMS followed by microscopy to identify *Giardia* in the sewage water. The protozoan parasite *Giardia* was abundant (2.23±1.44 × 10<sup>5</sup> cyst/l) in the sewage water dominating the sampling sites-Grit chamber (44%), Measuring chamber (34%) and Outlet lagoon (38%). A low abundance of *Giardia* in the PSTP starting from the first point to the last one indicates the waste water treatment efficiency for removal of the pathogen. A significant correlation was found for log (number of *Giardia* +1) with turbidity (r= 0.729) and TDS (r= 0.536) at 0.01level.

mvj -mstf|ct eZ@vb Mtel Yvq XvKivq Aew`Z cvMjv cqteR© cwi tkvab c`#Uj cwbZ *Giardia* Gi \_bMZ l cwi gvbMZ Dcw`wZ wbyq Kiv ntqtQ| eZ@vb Abym`ntb tgvU 72 wJ bgbv mslMh Kiv nq PSTP t\_tK Ges G\_s tj v ci`f|v Kiv nq di`gij B\_vj tK`xfZKiY cU`qvq Ges IMS Ovi v| *Giardia* cqteRqZ tenk wQj (2.23 ± 1.44 × 10<sup>5</sup> mv ÷ /vj .) hv Ab`vb` bgbv mslMhni `vtbl wQj | h\_v, 44% cv`ji cKvtó, 34% cwi gvcK cKvtó Ges 38% wM@b Rj vktq| cUg t\_tK tk| cKvtó chS-*Giardia* mv`÷i cPthP Aatµg cqteR© cwi tkvab Kti Rxeby Kgvbvi h\_v`Zv wbt`R Kti | Turbidity (r= 0.729) Ges TDS (r= 0.536) Gi mv` 0.01 ch`q *Giardia* mv`÷i cwi gvtbi log (*Giardia* msl`v + 1) Gi GKwU \_i`ZcY`m`úK`cvl qv wMtqtQ|

**Key words:** Giardiasis, Formol-Ether Concentration Method, IMS, Abundance, Correlation, Turbidity, TDS.

**INTRODUCTION**

Waterborne parasitic infections are common worldwide due to the shortage of drinking water, and unhygienic conditions of storage and manipulation of contaminated water in different daily purposes (WHO 1991). At least 325 reported water related outbreaks of protozoan parasites worldwide where *Cryptosporidium* and *Giardia* have been reported to account for a majority of these outbreaks affecting millions of people ( Karanis *et al.* 2007).

About 200 million people have symptomatic Giardiasis worldwide, with prevalences of 2-5% in industrialized countries and 20–30% in developing regions of Asia, Africa and Latin America (Carmena 2010). Transmission of

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*Giardia* occurs by faecal-oral route of exposure and is sustained by zoonotic and anthroponotic cycles where several species and genotypes are enrolled (Almeida *et al.* 2010). Besides adults, children are highly susceptible whereas breast-fed infants (less than 6 months of age) are not likely to be infected (EPA 1998). The infected hosts shed a large number of transmissible and infective cysts in the environment, contributing to an increase of environmental contamination, in particular water courses. The cysts remain infective for long periods in natural environment, resistant to the conventional water treatment processes and small infectious dose, representing a serious problem of public health (Nikaeen *et al.* 2003).

Increased human consumption and pollution have caused a shortage of fresh water resources worldwide forced to use of recycled waste water with a low level of physico-chemical and biological contaminants (virus, bacteria and other parasites) is a common practice because absolute purification of waste water is not always possible but some type of recycling plants can be considered to reduce the contaminations (Caccio *et al.* 2003, Parvez *et al.* 2007). The occurrence of *Giardia* in the sewage treatment plants varies due to the differences in the standard and treatment technology in the respective sewage treatment plant (Roach *et al.* 1993, Wallis *et al.* 1995, Amahmid *et al.* 2002, Farias *et al.* 2002, Scott *et al.* 2003). *Giardia* cysts more readily (40%-100%) penetrate through conventional waste water treatment processes depending upon the degree of treatment (Sykora *et al.* 1987). Sewage treatment plants have the potential to become a source of contamination if malfunctions or inadequately treat the effluents before discharging into nearby water body.

To check the efficiency of any treatment plant most frequently used system is biological or physico-chemical indicator alone or simultaneously. Public health is not adequately protected by simply monitoring schemes based on detection of a single indicator. It is necessary to have a look on both physico-chemical and biological indicators simultaneously. Different organisms such as algae, fish, microorganisms and parasites are frequently used as biological indicators. According to the literature, to evaluate the system performance an inexpensive method is the microscopic observation of different protozoal species, combined with personal judgement of evaluators (Luna-Pabello *et al.* 1990). Physico-chemical parameters such as temperature, pH, Total Dissolved Solids (TDS), conductivity and Total Suspended Solids (TSS) are generally used for evaluating effluent quality. These criteria are important for monitoring and controlling contaminations of recycled effluents repeatedly emphasized (Parvez *et al.* 2007). The efficiency of sewage treatment plants to reducing the concentration of *Giardia* cysts along with physico-chemical parameters has not been evaluated in

Bangladesh so far. Probably unavailability of satisfactory methodology for concentrating and detecting *Giardia* in wastewater samples is responsible. Recent studies included microscopic evaluation coupled with IMS (Immunomagnetic Separation).

Domestic and industrial wastes of Dhaka city primarily discharged into the river Buriganga by different pathways where Pagla Sewage Treatment Plant (PSTP) outfall is one of them that utilizes waste stabilization method, operated by Dhaka Water Supply and Sewerage Authority (DWASA) situated in the eastern part of Dhaka, Bangladesh. The PSTP covers about 18% of the city population. The capacity of this treatment plant is only 0.12 million m<sup>3</sup>/day while the total sewage generated by the people of Dhaka city as estimated by DWASA is about 1.3 million m<sup>3</sup>/day (Hasan *et al.* 2006). No previous record concerning sewage treatment plant with parasites and physico-chemical parameters was found; hence the present study is very significant to assess the health hazard of the people of Dhaka city. The qualitative and quantitative detection of *Giardia* for assessing physico-chemical parameters will provide key informations for water utilities and improve water resource management for environmental health protection. Besides, the detection of *Giardia* in waste water may provide important clues for the environmental epidemiology of the parasites in this region.

### OBJECTIVES

The present study was aimed to monitor treatment efficiency of the effluents of Pagla Sewage Treatment Plant using selected novel and classical physico-chemical and biological indicators. The qualitative and quantitative detection of *Giardia* for assessing physico-chemical parameters were also studied.

### MATERIAL AND METHODS

In the Pagla Sewage Treatment Project (PSTP) in Dhaka, 72 effluent samples were collected fortnightly in 24 successful rounds. The samples were collected from three separate points- raw sample from Grit Chamber, reservoir sample from Measuring Chamber and treated sample from Outlet Lagoon of the PSTP. From each sampling site 2L effluent samples were collected from 13<sup>th</sup> February to 3<sup>rd</sup> March 2008. All the samples were immediately transferred to the laboratory for parasitological examination and detection of chemical parameters.

*Parasitological examination:* Samples were processed by Formol-Ether Concentration Method following a light microscopy to observe the parasites. Due to minute size, attachment to particulate matter in the sample and relatively few in number in external environment make *Giardia* cysts very difficult to detect. To overcome this problem a recent method IMS was performed. *Giardia* cysts were

isolated by the method using the Dynabeads® GC-Combo IMS kit (Invitrogen Dynal AS, Oslo, Norway) according to the manufacturer's instruction.

*Physico-Chemical parameters detection:* A portion of each collected sample was used to test different physico-chemical parameters by laboratory expertise using standard electronic meters.

*Data analysis:* Data were analyzed by using Microsoft Excel and SPSS software.

## RESULTS AND DISCUSSION

The present study was designed for qualitative and quantitative detection of *Giardia* spp. to insight into the physico-chemical parameters of the treatment plant. Total 72 raw and treated samples were collected from PSTP throughout the year (2008) and processed with Formol-Ether Concentration method and IMS followed by microscopy to identify *Giardia* in sewage. Microscopic observations revealed the presence of protozoan parasite *Giardia* in sewage water. Also, *Entamoeba coli*, *Endolymax nana*, *Iodoamoeba butschlii* and *Balantidium coli* were found (Khanum et al. 2011).

The data (Table 1) was manipulated to apply it in the population concept of parasites according to the Crofton Model which showed the variance being much greater than the mean value indicates that the distribution was aggregated or clustered. The variance in case of Measuring Chamber was greater than the mean value (Table 1). So the distribution of *Giardia* is also aggregated or over dispersed in measuring chamber.

Although, the interquartile range in outlet lagoon is zero, the relation between variance and mean remains the persistent as previous two sites due to a few extreme values (Table 1). From the analysis of the distribution data, it is obvious that the positive skewness increase as the treatment progresses in PSTP.

*Physico-chemical parameters:* The number of *Giardia* increased proportionally with turbidity. The lowest value of turbidity was associated with the lowest occurrence of *Giardia* while the highest turbidity value showed the region for the highest occurrence of *Giardia* in Grit Chamber. The decrease in turbidity was related to the concentration of water content. The pattern of linear relationship between turbidity and number of *Giardia* can be explained with scatter plot. As the other two sampling sites contained water after a series of treatment process, there might be a range of physico-chemical parameters and the occurrence of *Giardia*. On the contrary, the first sampling site containing the raw sewage before any treatment was done (Table 2).

**Table 1. Distribution of *Giardia* in Grit chamber, Measuring chamber and Outlet lagoon of the Pagla Sewerage Treatment Project in Dhaka in 2008.**

Statistics	Grit chamber		Measuring chamber		Outlet lagoon	
	Value	Std. Error	Value	Std. Error	Value	Std. Error
Mean	227083.33	28548.707	85416.67	17152.124	14583.33	6369.628
95% confidence interval of mean						
Lower bound	168025.83		49934.80		1406.75	
Upper bound	286140.83		120898.54		27759.91	
5% Trimmed Mean	219444.44		78703.70		10648.15	
Median	200000.00		50000.00		0.00	
Mode	200000.00		50000.00		0.00	
Variance	19560688405.797		7060688405.797		973731884.058	
Std. Deviation	139859.531		84027.903		31204.677	
Minimum	50000		0.00		0.00	
Maximum	550000		300000.00		100000.00	
Range	500000		300000.00		100000.00	
Interquartile Range	225000.00		125000.00		0.00	
Skewness	0.921	0.472	1.104	0.472	2.062	0.472
Kurtosis	0.022	0.918	0.604	0.918	3.237	0.918
Percentiles-						
25	112500.00		12500.00		0.00	
50	200000.00		50000.00		0.00	
75	337500.00		137500.00		0.00	

Table 2. Different chemical parameters of the sewage effluents of wastewater treatment plant.

Rounds	Grit Chamber			Measuring Chamber			Outlet Lagoon					
	Conduc-tivity ( $\mu$ S/cm)	pH	TDS (mg/l)	Turbidity (NTU)	Conduc-tivity ( $\mu$ S/cm)	pH	TDS (mg/l)	Turbidity (NTU)	Conduc-tivity ( $\mu$ S/cm)	pH	TDS (mg/l)	Turbidity (NTU)
1	1166	7.18	575	139	1124	8.28	553	59	872	8.11	426	55
2	1055	7.44	519	81	889	8.11	435	50	1062	7.94	522	44.43
3	1070	7.19	527	68.6	1065	7.11	524	56.6	907	8.17	444	53.3
4	692	7.27	337	34.7	967	7.8	574	25.5	780	8.47	381	75.3
5	895	7.6	439	71.23	783	8.34	382	41.24	831	7.56	406	47.7
6	963	7.67	472	70	888	8.04	435	21.6	766	8.21	373	96
7	535	7.15	259	66.1	821	7	399	26.5	760	7.99	371	66.8
8	643	6.68	314	88	691	6.72	337	67	599	8.06	299	23.64
9	755	7.4	369	21.6	918	7.14	450	92.2	629	8.44	306	38.3
10	755	7.4	369	21.7	808	7.43	395	108	525	7.52	255	61.1
11	662	6.68	322	48.6	635	6.52	308	42.1	282	8.63	135	97.5
12	789	7.7	375	21.67	985	7.1	425	38.89	799	7.98	357	42.1
13	791	7.25	385	39.2	701	7.32	329	22.5	567	7.88	275	113
14	674	7.12	325	64.8	669	7.15	325	43.7	897	8.94	439	50.4
15	821	6.98	401	86.9	831	7.05	406	53.9	793	9.14	387	30.9
16	75	6.79	353	76.5	283	6.82	136	82	490	7.18	238	66.5
17	806	7.22	393	58.1	749	7.13	365	57.7	496	7.08	241	81
18	851	7	417	60.5	823	6.85	403	58.2	501	5.79	242	43.7
19	831	8.95	405	58.1	667	6.96	324	40.9	452	7.43	217	70.3
20	674	7.12	325	64.8	669	7.15	235	43.7	897	8.95	439	50.4
21	913	7.2	447	34.81	911	7.26	446	36.41	460	8.6	222	40.01
22	821	6.89	405	84.8	723	7	288	29.89	578	7.29	335	68
23	1015	6.7	527	135	925	7.09	476	43.2	598	7.26	317	106
24	861	7.07	454	133	938	7.2	507	102	641	7.63	341	86.2

Pearson's co-efficient of correlation was used to determine the correlation between the occurrence of *Giardia* and the physico-chemical parameters. By Pearson's correlation analysis a distinct linear relation between the *Giardia* with Total Dissolved Solids (TDS) and turbidity was found in the present study (Table 3). By Pearson's bivariate correlation analysis, an effective linear correlation was found between the number of *Giardia* cysts in raw sewage water with turbidity ( $r = 0.729$ ) and TDS ( $r = 0.597$ ), significant at 0.01 level. The linear relationship pointed out the fact that increasing turbidity and TDS influenced the elevation of *Giardia* occurrence. Although elevated turbidity may indicate an increased likelihood of parasites, water with low turbidity should not be considered without risk (Robertson *et al.* 2001).

**Table 3. Pearson correlation between *Giardia* and different chemical parameters.**

	Log ( <i>Giardia</i> +1)	Temperature	pH	Conductivity	TDS	Turbidity
Log ( <i>Giardia</i> +1)	1	-0.456(*)	-0.052	0.441(*)	0.597(**)	0.729(**)
Temperature	-0.456(*)	1	0.241	-0.078	-0.186	-0.218
pH	-0.052	0.241	1	0.234	0.114	-0.316
Conductivity	0.441(*)	-0.078	0.234	1	0.788(**)	0.293
TDS	0.597(**)	-0.186	0.114	0.788(**)	1	0.526(**)
Turbidity	0.729(**)	-0.218	-0.316	0.293	0.526(**)	1

\*Correlation is significant at the 0.05 level (2-tailed) \*\*Correlation is significant at the 0.01 level (2-tailed)

*Assessment on treatment efficiency for removal of Giardia:* To assess the efficiency of treatment procedure in sewage treatment plant, data were analysed using box plot. As the data covered a large range of values, a logarithmic function was used to concise the data into a more manageable range and also to overcome the biases due to the variable number of cysts and the larger differences between them as recommended by Caccio *et al.* (2003).

The box-plot represents a noticeable difference in the distribution of *Giardia* in the sampling sites. The length of the box (interquartile range) increased from the first sampling point to the second one. On the contrary, the median log (number of *Giardia* spp. +1) decreased from the first to the second site and became the least (zero) in the third site with a few extreme values. A decrease in median indicated the efficiency of treatment procedure in the removal of *Giardia* (Fig. 1).

The change in box length in second site might be associated with the increasing anomaly of distribution. The box extended towards the lower quartile, which pointed out the fact that most of the observations were present in the lower quartile. The overall pattern of the box-plot revealed the tendency to lower the number of *Giardia* as the treatment process advances. Even though the

median log (number of *Giardia* +1) in outlet lagoon is zero, a couple of extreme values were also found which might be the result of partial infancy of treatment procedure or secondary contamination.

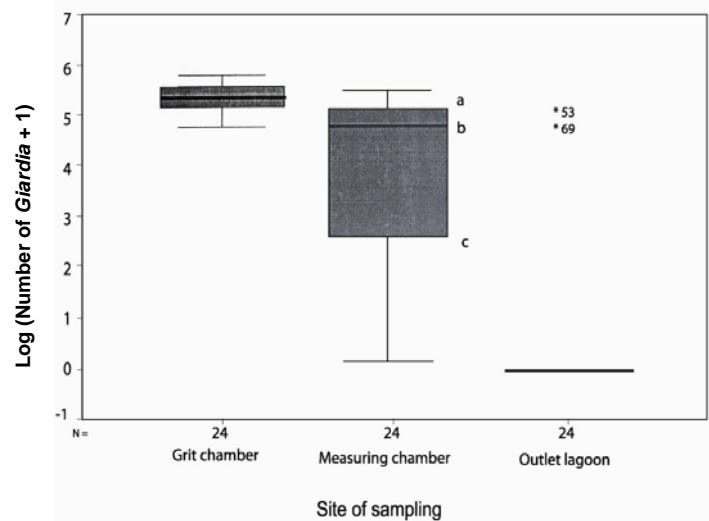


Fig. 1. Box-plot of log (Number of *Giardia* +1) by site of sampling (a→ Upper quartile, b→ Median quartile, c→ Lower quartile, a-b→ Interquartile range and \*→ Extreme values)

An array of parasites were detected, *Giardia* and *Entamoeba* were found most frequently than others; especially *B. coli* which was found seldomly. Similar parasites were found by Lim *et al.* (2007) in Malaysia. Other studies conducted in Sweden, Norway and Canada also reported of constant detection of *Giardia* in sewage (Ottoson *et al.* 2006). The prevalence of *Giardia* was similar to Lim *et al.* (2007). Mean intensity, abundance and dominance were also gradually decreasing which was an indirect indication of treatment efficiency though the effluent of outlet lagoon which will be discharged into nearby waterbody does not meet the WHO 1989 criteria (Gerba and Rose 2003).

Among many other physico-chemical parameters, a very good relationship between *Giardia* cyst occurrence and raw water quality parameters was found in Canadian province by LeChevallier *et al.* (1991). The pH level of the water was kept for avoiding corrosion in the water reutilization system. The turbidity values contributed to assuring the removal of *Giardia* cysts (Bello *et al.* 2008). There was a positive correlation between TDS and the number of *Giardia* cysts found. The positive correlation between conductivity and the number of *Giardia* cysts in the wastewater effluents; and negative correlation with temperature in the present study was significant at 0.05 level. TDS and conductivity values



indicate the presence of organic and inorganic nutrients for the organisms which might help *Giardia* cysts to survive.

The results of the present study suggest that the treatment of waste water promoted a reduction of infectious cysts of parasites when different samples from different treatment stages were compared but it could not remove those protozoan parasites properly, reflecting a constant risk of infection for the environment and the community.

### CONCLUSION

Environmental protection is the original motivation for water reuse, with technologies developed for the safe reintroduction of treated waste water into the environment. To protect the surface water from pollution and contamination effective treatment and management of wastewater is a must. The reuse of recycled waste water could become an important water management option both to shore up conventional resources and to reduce the environmental impact of discharges. From the present study a better understanding of the influence of physico-chemical parameters on the treatment efficiency of PSTP was obtained. As the treated water from PSTP is directly discharged into Buriganga River which embanks Dhaka city, it's very important.

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(Manuscript received on December 11, 2011; revised on February 4, 2012)