Original Article

Life Science

Comparative Parasitological Evaluation of Wastewater using Biosand Filter and Waste Stabilisation Ponds

Ocheme J OKOJOKWU, Helen I INABO

ABSTRACT [ENGLISH/ANGLAIS]

Parasitological status of wastewater and the efficacy of biosand filter in the elimination of *Giardia lamblia* cysts and oocysts of *Cryptosporidium parvum* were evaluated using the sucrose floatation and Kinyoun modified Acid-Fast (modified Ziehl-Neelsen) staining techniques. The parasite removal efficiency of biosand filter was tested in comparison with waste stabilisation ponds. A total of 960 L of wastewater was examined and a significant level of parasite eggs, cysts and oocysts were detected. In all, 874 parasites eggs, cysts and oocysts per litre were counted in sewage from waste stabilization ponds. *Ascaris lumbricoides* accounted for the highest eggs/litre (n = 198; 22.65%) followed by *Taenia* spp (n = 155; 17.73%) and *Ancylostoma duodenale* (n = 123; 14.07%). Cysts of *Giardia lamblia* had the least count/litre (n = 3; 0.34%). The mean egg or (oo)cyst/litre of sewage from the waste stabilization ponds revealed that the raw sewage had 12.38 ± 0.93 *Ascaris lumbricoides* egg/litre. Wilcoxon's Signed Ranks Test indicated a significant difference ($p < 0.05$) with biosand filter having lower counts per litre. The results obtained therefore demonstrated that the raw wastewater was laden with parasite eggs, cysts and oocysts and hence pose public health threat to the users of the effluent downstream. The biosand filter was more efficient than the waste stabilization ponds; its effluent contained insufficient level of the ova, cysts and oocysts of parasites well below the less than one (<1) helminth ova/protozoa cysts as recommended by World Health Organisation.

Keywords: Wastewater, biosand filter, waste stabilization ponds, parasites, helminths, oocysts

RÉSUMÉ [FRANÇAIS/FRENCH]

Statut parasitologique des eaux usées et l'efficacité du filtre biosable à l'élimination des kystes de Giardia lamblia et les oocystes de Cryptosporidium parvum ont été évaluées en utilisant la flottation du saccharose et Kinyoun modifiés acido-techniques de coloration (Ziehl-Neelsen modifiée). L'efficacité d'élimination des parasites du filtre biosable a été testé en comparaison avec les bassins de stabilisation des déchets. Un total de 960 L d'eaux usées a été examinée et un niveau significatif d'œufs de parasites, des kystes et des oocystes ont été détectés. En tout, 874 œufs de parasites, des kystes et des oocystes par litre ont été comptés dans les eaux usées des bassins de stabilisation des déchets. Ascaris lumbricoides représentaient plus les oeufs / litre (n = 198; 22,65%) suivie par Taenia spp (n = 155; 17,73%) et Ancylostoma duodenale (n = 123; 14,07%). Les kystes de Giardia lamblia a le moins compter / litre (n = 3; 0,34%). L'oeuf ou de dire (oo) kystes / litre d'eaux usées provenant des bassins de stabilisation des déchets a révélé que les eaux d'égout brutes a 12,38 ± 0,93 Ascaris lumbricoides oeuf / litre. Test de Wilcoxon Signed Classé indiqué une différence significative (p <0,05) avec filtre biosable ayant de faibles impulsions par litre. Les résultats obtenus donc démontré que les eaux usées brutes a été chargé d'œufs de parasites, des kystes et des oocystes et donc constituer une menace de santé publique pour les utilisateurs de l'aval des effluents. Le filtre biosable a été plus efficace que les étangs de stabilisation des déchets; ses effluents au niveau contenu insuffisante des ovules, des kystes et des oocystes des parasites bien en dessous du moins d'un (<1) helminthes ovules / protozoaires kystes tel que recommandé par l'Organisation mondiale de la Santé.

Mots-clés: Des eaux usées, filtre biosable, les étangs de stabilisation, les parasites, les helminthes, les oocystes

Affiliations:

Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, NIGERIA

* Email Address for Correspondence/ Adresse de courriel pour la correspondance: okojokwuoj@yahoo.com

Accepted/Accepté : December, 2011

Full Citation: Okojokwu OJ, Inabo HI. Comparative Parasitological Evaluation of Wastewater using Biosand Filter and Waste Stabilisation Ponds. World Journal of Life Sciences and Medical Research 2012;2:8-15.

INTRODUCTION

Wastewater refers to any water whose quality has been adversely affected by anthropogenic influence. This includes liquid waste discharged from domestic home, industries, agricultural and commercial sectors. Wastewater can be a vehicle of various diseases and high concentrations of pathogens are often reported in raw sewage worldwide [1]. The practice of discharging wastewater into water bodies causes considerable damage to the ecosystem, waterfront inhabitants, swimmers, and fishermen [2, 3]. When wastewater is discharged directly into water bodies it causes the problem of contamination of such water bodies as evident in intoxications, skin problems and intestinal parasitiasis [4, 5]. This problem is compounded when these discharges are deposited in the

vicinity of low-income neighbourhoods where basic hygiene practices are totally absent [6].

Globally, millions of people suffer from parasitic infections such as Ascariasis (1.2 billion), Trichuriasis (795 million), hookworm infections (740 million) [7], Amoebic dysentery (50 million) [8] and Giardiasis (2.8 million) [9]. In humans, these parasites are significantly associated with diarrhoea [10]. Faecal oral route is important in the transmission of parasitic infections to humans via poor personal hygiene, environmental conditions like contamination of soil and water sources with human faeces [11] and poor wastewater disposal such as use of night soil for fertiliser [12].

This work was therefore aimed at the comparative parasitological evaluation of wastewater using biosand filter and waste stabilisation ponds in the removal parasites ova and (oo)cysts from wastewater.

MATERIALS AND METHODS

The Ahmadu Bello University (A.B.U.) community served as the study area. Liquid wastes from the halls of residence and staff quarters are collected in a sewer and channelled to the waste stabilisation pond (WSP). There the sewage is treated biologically, before being discharged into the Kubanni dam (reservoir) which is abstracted for the water needs of the entire university community. The Kubanni dam located within latitude 11°11' N and longitude 7°38' E in Samaru Zaria, Kaduna State [13].

Construction of Biosand Filter

Concrete Filter Body

To prepare the concrete filter body, 4 parts of Portland cement (approximately 100 kg (220 lbs), 6 parts of clean pea size gravel and 2 parts clean sand (freshly crushed from the quarry) were mixed and clean water was added to make concrete slurry. The slurry was then poured into an already constructed metal mould and allowed to set.

Fine Sand Filter Media

Clean, freshly crushed rock (sand dust) from quarry was used. The sand dust was screened through metal mesh screen into three different sizes using mesh screen of 0.5 mm, 1 mm, and 12 mm pore sizes.

Diffuser

One hundred (100) holes, no larger than 3/16" in diameter, were drilled or punched in a plastic (HDPE) material on a 1" x 1" grid and then used as the diffuser.

Sample Collection

Raw wastewater and effluents from anaerobic, facultative and maturation ponds of the waste stabilisation pond of the Ahmadu Bello University, Zaria were sampled. A total of 960 L of sample was collected consisting of 320 L each of raw wastewater, effluents from anaerobic and facultative, and maturation ponds respectively. Each sample, in volume of 20 L, was divided into two portions and one portion (10 L) filtered through the biosand filter; the filter effluent (filtrate) was collected and analysed. The other portion (10 L) of raw wastewater was analysed for parasite ova, cysts and oocysts without filtering through the biosand filter.

The method of concentration which seems the most successful in the parasitological analysis of the samples was selected based on previous studies. The method of USEPA [14] as modified by Lim *et al.* [15] was adopted with slight modification.

Sample Processing

Biosand Filtration

Ten (10 L) litres of the wastewater sample was filtered through the biosand filter. The effluent (filtrate) was collected in clean 20 L plastic jerry can and then tested as outlined in the next procedure.

Sample Concentration (Cold Sucrose Floatation Technique)

Raw Wastewater (from Waste Stabilization Ponds)

Each 10 L sample was concentrated by repeated centrifugation at 1500 × g for 10 minutes in 250 ml conical centrifuge tubes before being concentrated to 10 ml. Sample was slowly under-layered with 10 ml of cold sucrose solution (specific gravity of 1.18) and centrifuged at 1000 × g for 5 minutes. Without disturbing the pellet, the entire supernatant including the interface was gently decanted into a clean centrifuge tube. Residual sucrose was removed by washing the pellet three times in pH 7.2 phosphate buffered saline (PBS) (Oxoid, Hampshire, UK). The final concentrated sample was then reduced to a final volume of 1 to 5 ml depending on the turbidity of the sample. Using a plastic Pasteur pipette, the well of McMaster slide was gently filled with the concentrate and observed microscopically for helminth ova.

Effluent from Biosand Filter and Waste Stabilisation Ponds

Cfiltrates (effluents) from the both biosand filter waste stabilisation ponds were concentrated as described above for raw sewage from the waste stabilization ponds.

Kinyoun Modified Acid-Fast (Ziehl-Neelsen) Staining

A smear was prepared from the sediment obtained above, air-dried and fixed in methanol for 3 minutes. The methanol-fixed smear was immersed in cold strong carbol-fuchsin and stained for 15 minutes. It was then thoroughly rinsed in distilled water and decolourised in 1% acid-methanol for 10–15 seconds. The slide was rinsed in distilled water and counterstained with 0.4% malachite green for 30 seconds, after which it was rinsed in distilled water. Duplicate slides were made from each sample.

Microscopic Examination and Enumeration of Oocysts of *Cryptosporidium parvum* and *Giardia lamblia* Cysts

The slides were air-dried and examined for oocysts of *Cryptosporidium parvum* and cysts of *Giardia lamblia* using the ×40 objective lens of a bright-field microscope (Olympus Microscope XSZ – 107BN No. 001677, Japan). The presence of oocysts and cysts were confirmed under the oil immersion objective lens. The shapes of the red-stained bodies were noted and their sizes were measured using micrometre mounted on the eyepiece of the microscope.

The ova, cysts and oocysts were enumerated according to the method outlined by Mahvi and Kia [16]:

$$\text{No. of (oo)cysts per litre} = \frac{N \times C}{A \times \frac{1}{2} \times F}$$

N = number of (oo)cysts observed on the slide

A = volume of the McMaster slide = 0.15 ml or volume smeared on microslide (ml) for modified Ziehl-Neelsen staining

C = concentrated volume (ml)

F = original sample volume (l)

It was assumed that a drop of sample was placed on the slide so the volume analysed "A" was 0.05 ml [17].

Statistical Analysis

The percentage removal of the parasites was calculated according to the formula used by Falabi *et al.* [18]:

$\%\ \text{removal} = (N_{influent} - N_{effluent}) \times 100 / N_{influent}$

Where $N_{influent}$ = number of parasites eggs in the influent wastewater,

$N_{effluent}$ = number of parasites eggs in the effluent wastewater.

The computer statistical software, Statistical Programme for Social Science version 17.0 (SPSS v 17.0) for Windows® (SPSS Inc., Chicago, IL, USA), was used for the statistical analysis of the data. Wilcoxon's Signed Ranks Test was used to compare the two wastewater treatment types. The confidence interval in 95% was used to calculate and compare the mean of parasite egg, cyst or oocyst concentration in the samples.

RESULTS

A total of 320 L of raw sewage (influent) was collected from the Ahmadu Bello University waste stabilisation ponds (ABU-WSP) on the Main Campus and analysed for parasite eggs, cysts and oocysts. Table 1 shows that a total of eight hundred and seventy-four (874) eggs, cysts and oocysts per litre were counted. *Ascaris lumbricoides* accounted for the highest eggs/litre (n = 198; 22.65%) followed by *Taenia* spp (n = 155; 17.73%) and *Ancylostoma duodenale* (n = 123; 14.07%). Cysts of *Giardia lamblia* had the least count/litre (n = 3; 0.34%). The mean egg or (oo)cyst/litre of sewage from the ABU-WSP revealed that the raw sewage had 12.38 ± 0.93 *Ascaris lumbricoides* eggs/L (Table 2).

A cursory look at Table 3 revealed that biosand filter was able to remove 98.89% of *Enterobius vermicularis* and 98.51% of *Trichuris trichiura* as opposed to the 93.33% and 92.54% removal by waste stabilisation pond respectively. There was 100.00% removal or eggs and cysts of *Schistosoma*, *Taenia*, *Toxocara* and *Giardia* species by both biosand filter and waste stabilisation pond. While there was 100.00% removal of oocysts and eggs of *Cryptosporidium parvum*, *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Enterobius vermicularis* by biosand filter, waste stabilisation pond removed 92.50%, 97.56%, 98.48%, 92.54% and 93.33% respectively. Figure 1 compares the removal efficiency of biosand filter, waste stabilisation pond and sewage treatment plant in the removal of parasite eggs and (oo)cysts.

A comparison of the mean parasite eggs/litre obtained from the Ahmadu Bello University biosand filter treated effluent and the effluent of the Ahmadu Bello University waste stabilisation ponds using Wilcoxon's Signed Ranks Test at significant level of 0.05 indicated that both treatment methods removed 100.00% of *Schistosoma*, *Taenia*, *Toxocara* species and *Giardialamblia* (Table 4). Though biosand filter was ranked higher in efficiency than Ahmadu Bello University waste stabilisation ponds in the removal of *Cryptosporidium parvum* oocysts and ova of *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Hymenolepis* species, and *Enterobius vermicularis* from raw sewage, only the removal of *Cryptosporidium parvum* was statistically significant ($z = -2.121$; $p = 0.034$).

Table 1: This table shows the occurrence and mean of ova or (oo)cysts/litre of raw sewage (influent)

Parasite	Size (µm)	Eggs or (oo)cysts/litre of Influent	Percentage
Cryptosporidium parvum	4 – 6	80	9.15
Schistosoma spp	83 – 187 x 45 – 70	5	0.57
Taenia spp	30 – 43 x 29 – 38	155	17.73
Ancylostoma duodenale	60 – 75 x 36 – 40	123	14.07
Ascaris lumbricoides	55 – 75 x 35 – 50	198	22.65
Toxocara spp	65 – 70 x 75 – 90	111	12.70
Trichuris trichiura	50 – 55 x 22 – 24	67	7.67
Hymenolepis spp	44 – 62 x 30 – 32	42	4.81
Enterobius vermicularis	50 – 60 x 20 – 32	90	10.30
Giardia lamblia	8 – 12 x 7 – 10	3	0.34
Total		**874**	**100.00**

Table 2: This table shows the occurrence and mean of ova or (oo)cysts/litre of sewage (influent and effluents) from ABU waste stabilisation ponds

Parasites	Eggs or (oo)cysts/litre	Mean ± SEM		
		Raw Sewage (WSP Influent)	WSP Effluent	Biosand Filter Effluent
Cryptosporidium parvum	80	5.00 ± 0.41	0.38 ± 0.16	0.00 ± 0.00
Schistosoma spp	5	0.31 ± 0.12	0.00 ± 0.00	0.00 ± 0.00
Taenia spp	155	9.69 ± 0.65	0.00 ± 0.00	0.00 ± 0.00
Ancylostoma duodenale	123	7.69 ± 0.30	0.19 ± 0.10	0.00 ± 0.00
Ascaris lumbricoides	198	12.38 ± 0.93	0.19 ± 0.10	0.00 ± 0.00
Toxocara spp	111	6.94 ± 0.34	0.00 ± 0.00	0.00 ± 0.00
Trichuris trichiura	67	4.12 ± 0.53	0.31 ± 0.51	0.12 ± 0.06
Hymenolepis spp	42	2.62 ± 0.46	0.06 ± 0.06	0.00 ± 0.00
Enterobius vermicularis	90	5.62 ± 0.36	0.38 ± 0.13	0.12 ± 0.06
Giardia lamblia	3	0.19 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
Total	**874**			

SEM = Standard error of mean; WSP = waste stabilisation pond; ABU= Ahmadu Bello University, Zaria, Nigeria

DISCUSSION

The parasitological profile of the wastewater revealed that two protozoa (*Cryptosporidium parvum* and *Giardia lamblia*) and eight (8) helminths were found among which are *Ascaris lumbricoides, Taenia* spp, *Ancylostoma duodenale, Hymenolepis* spp and *Enterobius vermicularis.* The WHO limit for helminth ova in wastewater for use in crop irrigation is less than one (≤ 1) Helminth Ova (HO) per litre [19]. Mean egg counts of helminths such as *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Toxocara* spp in the untreated wastewater (influent) were higher than the effluent from the sewage treatment plant. There is a great public health risk of reuse of these waters for crop irrigation without prior adequate treatment. However, the effluent from the biosand filter contained less than one mean egg per litre. Previous studies by Feachem *et al*. [20] and Shanthala *et al*. [21] have revealed that helminth ova can survive in water, soil, and crops for several months or years.

Cryptosporidium parvum oocysts and *Giardia lamblia* cysts were detected frequently and at maximum concentration of 130 oocysts/litre and 58 cysts/litre respectively. *Cryptosporidium parvum* oocysts persistently outnumbered *Giardia lamblia* cysts in both raw and treated sewage samples. In contrast to this study, other studies in Sweden, Norway and Canada reported constant detection

of *Giardia lamblia* cysts in sewage [22, 23] and a rather erratic occurrence of *Cryptosporidium parvum* oocysts [24] as opposed to the significantly greater number of *Cryptosporidium parvum* oocysts compared to *Giardia lamblia* cysts observed in this study.

The type of treatment processes play an important role in determining the reduction of parasites such as *Cryptosporidium parvum* and *Giardia lamblia* as indicated in this study. The (oo)cysts of both protozoan parasites were efficiently removed by the biosand filter. It is of utmost importance that pollution of water and vegetables is prevented by substituting the rapid sand filter used in the sewage plant with the biosand filter.

Table 3: This table shows the average of reduction of eggs or (oo)cysts/litre of influent and effluents sewage from Ahmadu Bello University waste stabilisation pond

Parasite group	No. of eggs or (oo)cysts/l of influent	Biosand filter Effluent		WSP Effluent	
		Number of eggs or (oo)cysts/litre	Percentage reduction (%)	Number of eggs or (oo)cysts/litre	Percentage reduction (%)
Cryptosporidium parvum	80	0	100.00	6	92.5
Schistosoma spp	5	0	100.00	0	100.00
Taenia spp	155	0	100.00	0	100.00
Ancylostoma duodenale	123	0	100.00	3	97.56
Ascaris lumbricoides	198	0	100.00	3	98.48
Toxocara spp	111	0	100.00	0	100.00
Trichuris trichiura	67	1	98.51	5	92.54
Hymenolepis spp	42	0	100.00	1	97.62
Enterobius vermicularis	90	1	98.89	6	93.33
Giardia lamblia	3	0	100.00	0	100.00
Total	**874**	**2**	**99.77**	**24**	**97.25**

WSP = waste stabilisation pond

Figure 1: This figure shows comparison of parasite removal efficiencies of biosand filter and waste stabilisation pond.

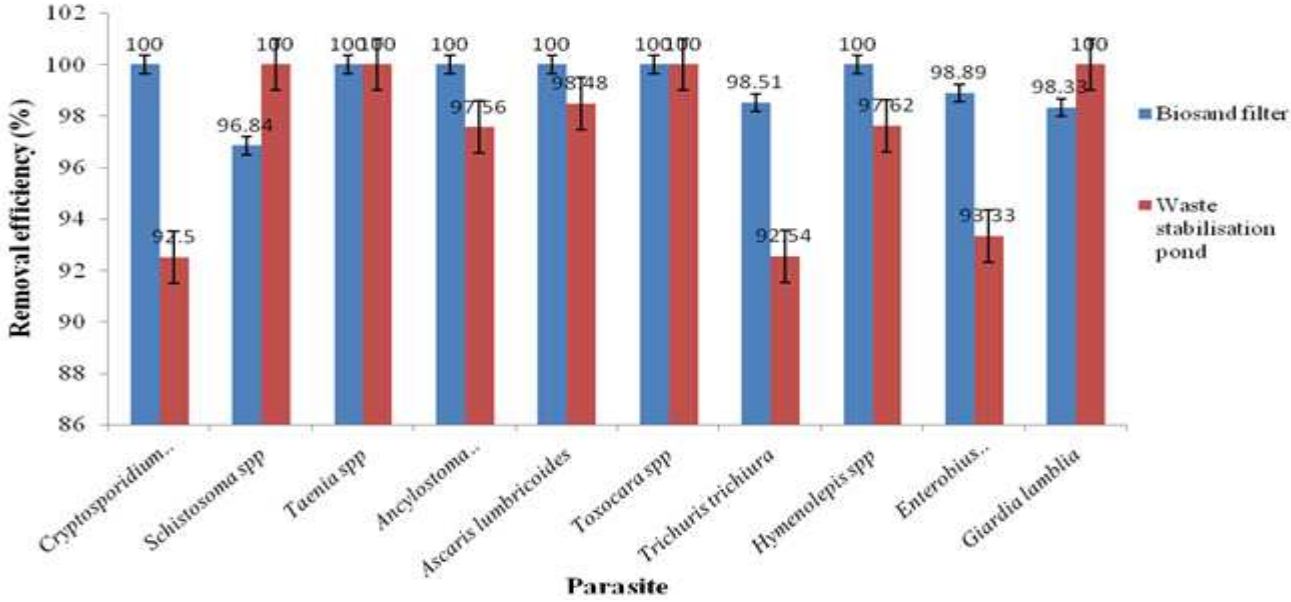

Table 4: This table shows the Wilcoxon's Signed Ranks Test of mean differences between effluents of biosand filter and waste stabilisation pond.

Biosand filter	Waste Stabilisation Pond	*z*	*p*	Decision
Cryptosporidium parvum	– *Cryptosporidium parvum*	– 2.121	0.034*	BSF < WSP
Schistosoma spp	– *Schistosoma* spp	0.000	1.000	BSF = WSP
Taenia spp	– *Taenia* spp	0.000	1.000	BSF = WSP
Ancylostoma duodenale	– *Ancylostoma duodenale*	– 1.732	0.083	BSF < WSP
Ascaris lumbricoides	– *Ascaris lumbricoides*	– 1.732	0.083	BSF < WSP
Toxocara spp	– *Toxocara* spp	0.000	1.000	BSF = WSP
Trichuris trichiura	– *Trichuris trichiura*	– 1.414	0.157	BSF < WSP
Hymenolepis spp	– *Hymenolepis* spp	– 1.000	0.317	BSF < WSP
Enterobius vermicularis	– *Enterobius vermicularis*	– 1.633	0.102	BSF < WSP
Giardia lamblia	– *Giardia lamblia*	0.000	1.000	BSF = WSP

*BSF = Biosand filter; WSP = waste stabilisation pond; BSF < WSP = the number of eggs or (oo)cysts in effluent of BSF is less than that in WSP i.e. BSF is more efficient than WSP at p< 0; BSF = WSP means both treatment types exhibited equal efficiency in egg removal at the p –values indicated; * = statistically significant difference exists between the means of BSF and WSP at the p-values indicated*

The rate of ova removal by biosand filter and waste stabilisation ponds for *Schistosoma, Taenia, Toxocara* species and *Giardia lamblia* was 100.00% while for *Cryptosporidium parvum* it was 98.89% and 92.5% for biosand filter and waste stabilisation ponds respectively. Experimental data suggest that waste stabilisation ponds may remove practically all the protozoan and helminth eggs producing a final effluent that meets the WHO guidelines and recent revisions for use of treated wastewater in agricultural unrestricted irrigation [25]. The results of this study are in consonance with such studies and confirm the suitability of the biosand filter and waste stabilisation ponds for removing pathogens from wastewater. These performances were higher than those observed in conventional sewage treatment processes such as activated sludge or primary treatments where reductions of 70 – 99% of protozoan and helminth eggs were reported [22, 26, 27, 28]. This shows that the biosand filter is more efficient in the removal of helminth ova and oocysts of *Cryptosporidium parvum* and *Giardia lamblia* as indicated by the statistically significant difference when compared with the waste stabilisation pond and the sewage treatment plant. The analysis revealed that 52.61% of the parasite eggs, cysts and oocysts were removed by the sewage treatment plant, 97.25% was removed by waste stabilisation ponds while egg removal efficiency of the biosand filter was between 97.45 – 99.7%.

CONCLUSION

The study has shown that biosand filtration (slow sand filtration) of wastewater is a better alternative to rapid sand filter which is used in the conventional sewage treatment plant as it ensures removal or reduction of helminth ova and protozoan (oo)cysts. The use of biosand filtered effluent for irrigation farming in Zaria metropolis will prevent the parasitological pollution of vegetables that may be eaten raw. Statistical analysis showed that waste stabilisation ponds performed better than the conventional wastewater treatment plant; while the efficiency of biosand filter was not statistically different from that of waste stabilisation ponds in the removal of parasites eggs and (oo)cysts.

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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

No conflict of interests was declared by the authors