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Filter Media Layers Contribution in Removal of Pollutants from Concentrated Greywater by Vermifiltration.

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Abstract
The contribution from each filter medium layer in the removal of pollutants from the concentrated greywater treatment by vermifiltration was studied for 7 months. The two filters were made up of cylindrical DN200-PVC pipes and were filled with 10 cm gravel, 20 cm sand and 30 cm fine sawdust from bottom to top. Two hundred *Eudrilus eugeniae* earthworms were inoculated to one of the filters and the other was used as a control unit. The sample, first, at the outlet was collected, then the outlet was closed to collect samples from the other sampling ports, which were created at the bottom of each layer. Analysis were done for ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}), Chemical Oxygen Demand (COD), Total suspended solids (TSS), Dissolved Oxygen (DO), pH, and Temperature. The results showed that there were significant differences (<0.05) for removal efficiencies of all parameters among the different layers of the vermifilter and the control unit. However, for concentrations, there were significant differences only for COD, TSS, PO_4^{3-}, and DO among the layers. When the performances of the respective layers of the vermifilter and control unit were compared, there was no significant differences (>0.05) except for COD and DO concentrations but with a slightly less average effluent concentration of nitrate and orthophosphate in the control unit. To conclude, major removal of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, COD and TSS were occurred at the fine sawdust layer.
Keywords: Concentrated greywater; *Eudrilus eugeniae*; Filter materials; Vermifiltration

1.1 Introduction

The concentrated greywater, generated from the poor urban households of the developing countries, is a threat to the environment and a risk to public health. It is uncommon to see greywater treatment practice in the urban poor areas and became a custom to dispose the greywater to street and open spaces. However, due to the ponding on the streets, bad smell develops and becomes an ideal place for mosquito breeding. Several studies confirmed that, in urban poor areas, the soil and water sources are contaminated with pathogens, nutrients and micro-pollutants (Katukiza *et al*., 2013; Nyenje *et al*., 2013).

Vermifiltration is a recently developed technology which can be practiced in the hot climatic areas for treating concentrated greywater generated from the poor urban households of the developing countries (Adugna *et al.,* 2014) as it can remove both chemical and biological pollutants by a single facility (Arora *et al*., 2014). In the vermifiltration process, the pollutants were removed with the contribution of bedding materials, filter media, earthworms and microbial communities (Taylor *et al.,* 2003; Li *et al.,* 2009; Zhao *et al.,* 2010; Wang *et al.,* 2014).

Taylor *et al.* (2003) studied the removal of selected parameters along the filter depth with 10 cm interval using organic solid waste as a medium and concluded the importance of filter bed depth to further removal some nutrients and the additional oxygen demand. *Wang et al.* (2014) also reported that VF height had a significant effect on COD and total phosphorous removal rates, earthworm population, and actinomycetes number but didn't affect ammonia and total nitrogen removal rates, bacteria and fungi number. Moreover, Zhao *et al*. (2009), reported that pollutant removal efficiency was highest when the vermifiltration height is between 30 and 70 cm. However, there are no studies on the contribution of filter media layers for concentrated greywater treatment using vermifiltration. This study aims on quantifying the contributions from the fine sawdust layer, sand layer and gravel layer of the vermifilter and control unit in the removal of selected physico-chemical parameters and nutrients while treating the concentrated greywater by vermifiltration.

1.2 Materials and Methods

1.2.1 Experimental Set up

The research was conducted between June 10, 2014 and January 10, 2015 in International Institute for Water, Environment and Energy (2iE), Ouagadougou, Burkina Faso and the experiment was protected from direct sunlight and rainfall by constructing a shade. The two filters were filled with 10 cm gravel, 20 cm sand and 30 cm fine sawdust from bottom to top and one of the filters was inoculated with 200 *Eudrilus Eugeniae* (Figure 1). Sampling ports were installed immediately at the bottom of the filter medium layer to collect out samples using the extended ports passing the 200 mm diameter PVC wall.

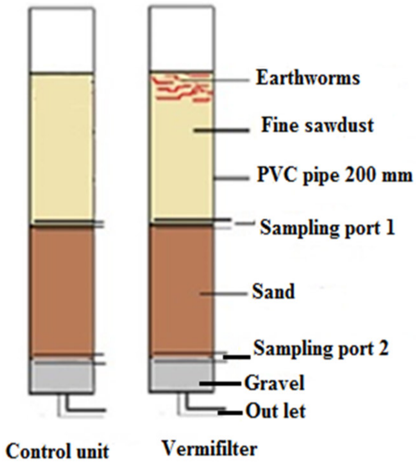

Figure 1. The crossectional view of the vermifilter and the control unit.

The vermifilter and a control unit were filled with 10 cm of gravel at the bottom which was composed of a layer of medium size gravel (5 cm thickness, aggregate size 20-40 mm) at the bottom and a layer of coarse gravel (5 cm thickness, aggregate size 10-20 mm) at the top. The sand has a uniformity coefficient of 1.36, effective size of 0.118 mm and density of 1517.6 kg/m^3. The fine sawdust was composed of *Khaya Ivorensis, Mansonia altissima, Milicia excelsa* tree species and collected from a nearby woodwork shop with the average pH and density of 6.47 and 96 kg/m^3 respectively. The filter media and bedding material were washed with tap water to remove the dust and other impurities.

After homogenizing the concentrated greywater collected from a poor urban household of Ouagadougou, it was supplied on the two filters three times per day at 8:00 am, 12:00 am and 4:00 pm from Monday to Friday at HLR of 95 L.m^{-2}.d^{-1} (1 L/batch*3 batch/day).

1.2.2 Characteristics of Greywater

Table 1 presents the average concentrations of the greywater with the standard deviation (SD). As shown on Table 1, the concentrated greywater is not only rich with organic pollutants and pathogens, but also with nutrients. The greywater was composed of high concentration of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}. The main causes for being concentrated are (1) the shower water was drained out with the drainage line constructed by the householder and (2) the other sources, the kitchen and the laundry wastewater, were used again and again due to water supply shortage.

Table 1. The characterstics of the greywater.

Parameters	Minimum	Maximum	Average	SD	NS
TSS (mg/L)	1270	3014	2079	605	12
COD (mg/L)	1910	4700	3551	623	9
PO_4^{-3} (mg/L)	2.6	796	86	200	14
NH_4^+ (mg/L)	3	95	25	25	14
NO_3^- (mg/L)	4	113	28	29	14
NO_2^- (mg/L)	0.12	100	37	28	14
pH	4.37	8.34	6.81	1.3	14
Temperature (^{o}C)	26.20	29.9	27.7	1.2	14
DO (mg/L)	0.2	2.6	1.6	0.8	14

NS = Number of samples

1.2.3 Water Quality, Earthworm and Bacterial analysis

The greywater supply and analysis were similar to the previous chapters for physico-chemical parameters and the effluents were sampled every 7 days from the sampling ports and the outlet. First, a sample was collected at the bottom outlet, then the outlet was closed to get samples from the upper sampling ports. Usually, the samples were analysed within the same day of sample collection. When it was not possible, the samples were stored at 4^{o}C for less than 24h before analysis.

Ammonium, nitrate, nitrite and phosphate were analysed using HACH DR/2000 direct reading spectrophotometer with the absorption wavelengths of 425 nm, 500 nm, 585 nm and 880 nm after adding Nessler, Nitra Ver, Niter Ver and Phos Ver reagents in the samples, respectively. The samples were filtered using a GF/C 0.45μm filter and diluted depending on the readability by the spectrophotometer.

Two hundred adult *Eudrilus eugeniae* were inoculated to the vermifilter and the one without earthworms was used as a control unit. To understand the earthworm dynamics, the earthworms and cocoons were counted after sorting with hand at the end of the experiment. Besides, the earthworms were weighed after washing with distilled water and drying with towel paper.

Quantification of bacterial population from the filter bed material (fine sawdust) was determined by spread plate method using nutritive agar medium after 10 g of sample was collected on the top layer (10 cm thickness) from each filter using sterilised glass bottles. A gram of sample was taken from the already collected sample and diluted with 9 ml sterile water and mixed with vortex then diluted upto 15 dilutions. Sample of 100 µl was taken from each dilution and spread on the autoclaved petri-dish to be incubated at 37^{o}C for 18-24h.

1.2.4 Sawdust Components and Biosolids Analysis

The degradation of the sawdust was analysed by quantifying ash, extractives and lignin after collecting sample from 0-10 cm depth of the top layer. The ash was determined using standard methods (APHA, 1998). The extractives were determined after boiling with acetone and distilled water for 6 and 2h. The samples were dried at 105^{o}C to calculate the weight difference from the initial weight. The lignin was determined by mixing the extracted sample with 72% sulfuric acid and let in the refrigerator at 10^{o}C for 2h then it was mixed with 300 ml distilled water and boiled for 1h. It was washed with 150 ml distilled water three times while filtered. From the mass balance, it was possible to determine the helocellulose. Moreover, the fine sawdust with the adsorbed solids from the greywater was analysed for VS using standard methods (APHA, 1998). Porosity was determined by volumetric method and the bedding material depth variation was measured by ruler.

1.2.5 Statistical Analysis

Microsoft Excel 2013 was used to carry out statistical analyses, develop bars and figures. The results were expressed as mean ± standard deviation and one way analysis of variance (ANOVA) was performed to determine significant difference ($P < 0.05$) between the vermifilter and the control unit layers and among the layers in the same filter.

1.3 Results and Discussion

1.3.1 Performance Evaluation for the Vermifilter and Control unit

Both the vermifilter and the control unit were able to remove pollutants mainly at the top (active) layer where microbial communities and earthworms dominate. There were significant differences (<0.05) for all removal efficiencies among the different layers of the vermifilter and the control unit. However, there were significant differences only for COD, TSS, PO_4^{3-} and DO concentrations among the layers of the filters (Table 2). When the performances of the two filters compared at each layer, there was no significant differences (<0.05) except for COD and DO concentrations. However, the effluent concentrations of NO_3^- and PO_4^{3-} were less in the control unit (Table 3).

Table 2. The significant differences of removal efficiencies among the layers in the vermifilter, control unit, and between respective layers of the vermifilter and control unit.

Parameters	Among layers in the filter		Between vermifilter and control unit		
	Vermifilter	Control unit	Sawdust layer X Sawdust layer	Sand layer X Sand layer	Gravel layer X Gravel layer
COD	4.85E-23(*)	4.5477E-22(*)	0.9277792	0.82296065	0.44508741
TSS	0.00266(*)	0.011604(*)	0.08094969	0.06730215	0.40256524
NH_4^+	0.018147(*)	0.02430835(*)	0.52390845	0.17442699	0.44315075
NO_3^-	0.00113(*)	0.02585607(*)	0.96759625	0.88247902	0.87013604
NO_2^-	6.01E-06(*)	3.5024E-05(*)	0.78565861	0.78565861	0.54061281
PO_4^{3-}	529E-07(*)	3.07E-06(*)	0.52778289	0.29012492	0.87694397

Table 3. The significant differences of concentrations among the layers in the vermifilter, control unit, and between respective layers of the vermifilter and control unit.

Parameters	Among layers in the filter		Between vermifilter and control unit		
	Vermifilter	Control unit	Sawdust layer X Sawdust layer	Sand layer X Sand layer	Gravel layer X Gravel layer
COD	1.05E-06(*)	0.00012326(*)	0.00058653(*)	0.00138133(*)	0.33394765
TSS	2.53E-39(*)	3.6614E-32(*)	0.65946822	0.96798988	0.40256524
NH_4^+	0.4239314	0.92278751	0.15741586	0.14644249	0.44315075
NO_3^-	0.81004787	0.78291139	0.74452331	0.93255737	0.61442587
NO_2^-	0.5970744	0.78636575	0.78565861	0.78565861	0.54061281
PO_4^{3-}	5.29E-07(*)	3.07E-06(*)	0.52778289	0.29012492	0.87694397
DO	0.002899(*)	0.02405979(*)	0.23399975(*)	0.04872426(*)	0.03564199(*)
pH	0.97878042	0.49478114	0.22566175	0.98943914	0.8964509

1.3.1.1 Physico-chemical

Table 4 presents the average effluent concentrations of the physico-chemical parameters and Figure 2 presents the TSS and COD removal efficiencies. The top layer removed more than 90% of TSS and 75% of COD both in the vermifilter and the control unit (Figure 2). However, the vermifilter was slightly better than the control. The concentration of pH and DO increased along the depth while temperature decreased for both filters (Table 4). In the vermifilter, the pH from the active (top) layer was 7.6 while at the bottom (out let) was 8.75. The observed pH increment might be due to the accumulated (precipitated) carbonates, bicarbonates and chemicals in the filter material at the bottom which were facilitated by the earthworms' activities. In the control unit, the top layer had 7.6 and the bottom layer had 7.89 which don't have much difference. More DO was on the top layer and decreased along the depth and the vermifilter had more DO compared to the control as aerobic condition was created by the existence of earthworms and the resting period due to the batch supply system.

Table 4. The effluent concentration of physico-chemical parameters.

Parameters		**Concentration of effluents at the sampling ports and the outlet**					
		Vermifilter			**Control unit**		
		60 cm	**50 cm**	**30 cm**	**60 cm**	**50 cm**	**30 cm**
COD (mg/L)	Average	**425**	**470**	**802**	**524**	**570**	**798**
	SD	42	57	209	42	29	144
	Maximum	614	686	1221	4942	4476	5700
	Minimum	414	492	593	454	492	593
TSS (mg/L)	Average	**49**	**64**	**105**	**80**	**106**	**146**
	SD	21	23	56	49	61	95
	Maximum	79	106	220	157	188	389
	Minimum	7	32	46	17	32	42
DO (mg/L)	Average	**4.7**	**3.4**	**2.1**	**3.6**	**2.7**	**2.1**
	SD	1.3	1.3	1.4	2.3	2.0	1.7
	Maximum	6.2	5.4	5.1	6.1	5.7	5.3
	Minimum	2.5	1.8	0.4	0.5	0.7	0.3
Temperature (^oC)	Average	**29**	**29**	**29**	**29**	**30**	**30**
	SD	2	3	3	2	3	3
	Maximum	33	34	35	33	34	34
	Minimum	26	26	26	26	26	25
pH	Average	**7.6**	**7.6**	**7.6**	**7.9**	**7.6**	**7.6**
	SD	0.5	0.4	0.8	0.5	0.5	0.5
	Maximum	8.5	8.3	8.5	8.8	8.7	8.4
	Minimum	6.9	7.0	5.4	7.0	6.9	6.9

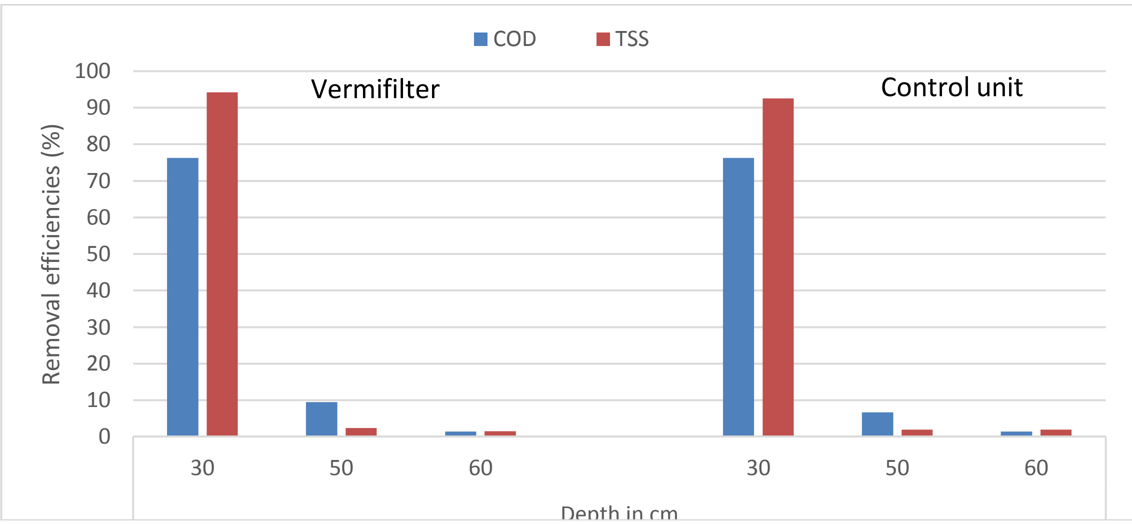

Figure 2. TSS and COD removal efficiencies at different layers.

Generally, more removal was achieved above 30 cm (fine sawdust) for most parameters and it was in line with the finding of Zhao *et al*. (2009) that found pollutant removal efficiency was highest when the VF height was between 30 and 70 cm.

1.3.2.2 Nutrient removal

The effluent concentration of nutrients and performance for nutrients removal are presented on Table 5 and

Figure 3. The top layer removed 45% and 39% of ammonium concentration by the vermifilter and the control unit respectively (Figure 3). The good removal of ammonium might be due to the aerobic condition created by earthworms. Taylor *et al*. (2003) found out that earthworm cast production oxygenated the influent and facilitate ammonia nitrification by microbes. Due to ammonium nitrification, additional nitrate was produced which might hinder the better removal of nitrate. The good removal at the top might be due to adsorption by the sawdust and utilization by microbes and earthworms as reported by Wang *et al*. (2011) and Wang *et al*. (2013), and organic matter degradation and nitrification occurred mainly in the top 20 cm of the bark filter (Dalahmeh *et al.,* 2011).
As shown on the Figure 3, the total removal of nitrite and orthophosphate was higher in the control unit than the vermifilter. However nitrite removal was better on the top layer for the vermifilter which might be due to the contribution of microbial community and earthworms, in addition to the adsorption by the sawdust. Fang *et al.* (2010) found that fixation, adsorption and co-precipitation in earthworm packing beds, was the main mechanism for P removal. The activities of microbial community and earthworms may have increased the conversion of the particulate phosphorus into soluble (orthophosphate). For instance, Parthasarathi *et al.* (2007) identified the phosphate solubilizing and nitrifying bacteria significantly in the guts and casts of the earthworms.

Table 5. The effluent concentration of nutrients.

Parameters (mg/L)		Concentration of effluents at the sampling ports and the outlet					
		Vermifilter			Control unit		
		60 cm	50 cm	30 cm	60 cm	50 cm	30 cm
NH_4^+	Average	**8**	**8**	**13**	**14**	**17**	**18**
	SD	14	12	11	16	18	19
	Maximum	57	47	36	43	51	57
	Minimum	0.5	0.5	1.3	0.7	0.9	1
NO_3^-	Average	**11**	**10**	**9**	**10**	**10**	**8**
	SD	10	9	8	12	11	6
	Maximum	40	36	24	48	42	18
	Minimum	1.5	0.5	0	0	0.5	0
NO_2^-	Average	**11**	**12**	**17**	**12**	**14**	**20**
	SD	7	11	26	15	13	26
	Maximum	25	35	100	60	40	100
	Minimum	0.1	0.5	0.8	0.1	0.4	0.1
PO_4^{-3}	Average	**22**	**25**	**26**	**16**	**18**	**25**
	SD	32	36	42	22	22	38
	Maximum	116	130	166	86	86	150
	Minimum	1.0	1.3	3.1	0.6	0.8	0.8

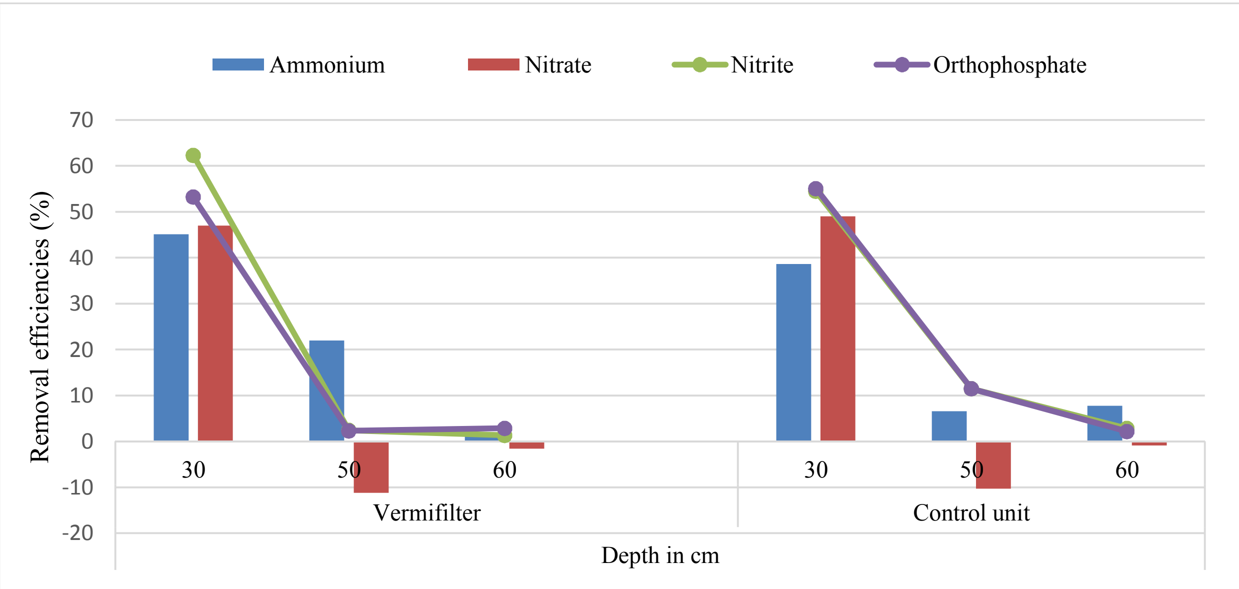

Figure 3. Ammonium, Nitrate, Nitrite and Orthophosphate removal efficiencies at different layers.

The removal of nitrate and nitrite in the same filter indicated that both aerobic and anaerobic/anoxic conditions were inside the filters (Taylor *et al*., 2003).

1.3.2 Earthworm and Bacterial Community Analysis

Eudrilus eugeniae showed significant growth and reproduction performance after acclimatized the new environment. The change in earthworm weight, growth rate, number of cocoons and reproduction rate at the end of the experimental period are shown in Table 5-6. The condition in the vermifilter favours the increment of earthworm population which is important for the better removal of pollutants. Bajsa *et al*. (2003) found that earthworm population plays an important role in the adsorption and stabilization of dissolved and suspended organic matter and nutrients through complex biodegradation processes.

As shown on Table 6, more bacterial communities were observed in the vermifilter than the control unit. The organic matter in the greywater and the hot temperature might have contributed besides the earthworms' activities which favored some microbial communities to develop as found by (Canellas *et al*., 2002).

Table 6. The earthworm development and bacteria community enumeration.

	Earthworms	(After 6 months)		
		Mature	Immature	Cocoons
Number	200	278	535	350
Total wt. (gram)	120.3	158.2	37	-
		Microbial community enumeration (ufc/g)		
Dates	24/09/2014	21/10/2014	14/11/2014	18/12/2014
Vermifilter	3.65*10^11	4.84*10^12	1.7*10^14	5*10^15
Control unit	2.33*10^ 8	4.18*10^10	1.5*10^11	1*10^12

1.3.3 Effect on Bedding Materials and Filter Media

1.3.3.1 Volatile solids change in the filtres

The volatile solids are indicator for organic carbon. In this experiment, after 6 months, the volatile solids decreased from 92% to 73% for the vermifilter and 84% for the control unit. It showed that earthworms' availability promoted the degradation of the volatile solids. Similarly, Li *et al*. (2013) found out earthworms promote organic matter degradation in the biofilms.

1.3.3.2 Depth of the bedding material shrinkage

The vermifilter decreased by 12 cm and the control unit increased by 1 cm. Hence there was 40% bedding material reduction in the vermifilter and about 1.5% increment in the control unit. The decrease in the vermifilter is due to the earthworm and microbial activity seeking for energy and the increment in the control unit might be from the accumulation of inorganic and slowly degradeable organic solids from the greywater.

1.3.3.3 Fine sawdust component degradation

There was a significant reduction of the cellulose from the bedding material in vermifilter than the control. After 6 months, 57% of the helocellulose in the sawdust was reduced to 31% in the vermifilter and to 47% in the control unit. There was degradation of helocellulose in the control unit which is mainly done by microorganisms. It is similar to Morgan and Burrows (1982) finding which stated that the earthworms and the microbes act symbiotically and synergistically to accelerate the decomposition of organic matter and it is the microorganisms that break the cellulose.

1.3.3.4 Porosity of the bedding material

Porosity decreases in all filters, but at a slower rate for vermifilters than the control unit. The decrease in porosity may be due to the size reduction of the sawdust, accumulation of slowly degraded organic and inorganic solids from the greywater and the biomat formation. From this experiment, the initial the porosity was reduced from 85% to 78% for the vermifilter and to 72% for the control unit, at the end of the experiment.

1.4 Conclusions

In this study, major removal of pollutants from the concentrated greywater was achieved by the top (active) layer. However, the vermifilter performance was slightly better in most aspect except nitrite, orthophosphate and TSS. There were also fluctuations from time to time for nitrate and orthophosphate. Besides, there was increment for dissolved oxygen and average temperature along the depth which might be due to earthworms' activity and additional depth may increase the removal of nitrate and the remaining oxygen demand.

The performance of the control unit was deteriorating due to the accumulation of solids in the filter media spaces and it was not possible to collect more samples. Besides, more number of bacteria was found in the vermifilter compared to the control unit which may be associated with presence of earthworms. This may indicate that the earthworms favoured some group of bacteria which helped to increase the removal efficiency and degradation of the sawdust.

Finally, there was a decrease in volatile solids from 92% to 73% for the vermifilter and to 84% for the control unit. And 40% reduction in bedding material was observed in the vermifilter and about 1.5% increment in the control unit. Moreover, the helocellulose in the sawdust was reduced from 57% to 31% in the vermifilter and to 47% in the control unit. The porosity decreased in both filters, but at a slower rate for the vermifilter. All changes might be due to the activities of earthworms and bacterial community that used the sawdust and the accumulated organic matter as energy source.

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