Removal of helminth eggs by surface filtration

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Abstract:
This study focuses on the removal of helminth eggs by micro screening. Woven filter cloths made of polyester and stainless steel with pore sizes of 17, 18, 20, 33, 37, and 54 µm were tested in laboratory scale. The removal of six relevant helminth species was examined. Experiments using tap water as well as wastewater samples seeded with helminth eggs were carried out to quantify the influence of suspended solids on the removal efficiency. Filter cloths with a pore size of 17 and 18 µm ensure the removal of all examined helminth eggs. Eggs of *Ascaris lumbricoides*, the most common parasitic helminth, were detected only in the filtrate of the 54 µm filter cloth. Solely *Trichuris trichiura* eggs passed the 20 µm filter cloth. Microscopic analysis of the used filter cloths led to the conclusion that in the presence of suspended solids the removal of helminth eggs might be increased by the attachment to particulate matter.

Keywords: helminth eggs removal; micro screening; water reclamation

Introduction:
For the safe use of wastewater for reclamation in agriculture the World Health Organization recommends a threshold of less than 1 helminth egg/L (WHO, 2006). It is a well-established fact that a safe inactivation of helminth eggs cannot be achieved by common disinfection processes applied for the treatment of domestic wastewater. Hence, mechanical separation, such as filtration or sedimentation, is a feasible technique to remove helminth eggs from wastewater (Jiménez, 2007; Jiménez and Maya, 2007). Previous laboratory- and pilot-scale studies indicate that micro screening provides a promising technology for the removal of helminth eggs from wastewater. Laboratory test showed that 10 µm polyester (PET) filter cloths ensure the removal of *Trichuris suis* eggs (Quinzaños *et al.*, 2008). These results were confirmed in pilot-scale tests using a disc filter equipped with 10 µm PET filter cloths and *Trichuris suis* eggs (Sanz *et al.*, 2009).

To predict the removal of helminth eggs by micro screening it is crucial to estimate the dominant filtration mechanism. In general, filtration processes can be classified according to the location of particle retention. The separation may take place within the pores of a filter media (depth filtration) or on the surface of the filter media (surface filtration) (Ives, 1975). Micro screening can be dedicated to surface filtration. In case of surface filtration there are two general models to describe the separation mechanism. *Complete blocking filtration* based upon the screening effect of the filter media, i.e. the retained particles are larger than the pore size of the filter media (Rushton *et al.*, 1996; Wakeman and Tarleton, 2005). In contrast, *bridging filtration* occurs if particles smaller than the pore size of the filter media form stables bridges resp. a *filter cake* (Rushton *et al.*, 1996; Wakeman and Tarleton, 2005). The latter

filtration model is also referred to as *cake filtration.* In this case, particles smaller than the pore size of the filter media, for instance helminth eggs, might be retained as well.

Regarding micro screening, it has been showed that *complete blocking filtration* is the predominant mechanism using woven filter cloths in common disc filters (continuous backwash and 250–300 mm differential pressure across the filter cloth) (Persson *et al*., 2006). Hence, particles smaller than the pores of the filter cloths are retained only to a minor extent. Consequently, the filter cloth, more precisely the pore size of the filter cloth, strongly influences the separation efficiency of helminth eggs. In practice, during the filtration process of wastewater, *bridging filtration* will occur as well and might lead to a removal of helminth eggs which are smaller than the pore size of the filter cloth. However, there is a strong indication that the pore size of the filter cloth is the critical factor regarding the removal of helminth eggs by micro screening. Therefore, this study focuses on the removal of eggs of six different helminth species by filter cloths with pore sizes of 17 to 54 µm, common pore sizes of filter cloths used in disc filters for tertiary treatment of domestic wastewater. Due to the fact that previous studies indicate that helminth eggs tend to attach to particulate matter (Sengupta *et al*., 2011), the influence of suspended solids on the removal efficiency was examined as well. It is supposed that the formed aggregates of particulate matter and helminth eggs are easier to remove caused by the larger particle size of the aggregates. Furthermore, the presence of suspended solids leads to the formation of a *filter cake* (*bridging filtration*) that might increase the removal efficiency.

Material and Methods:

The filter apparatus consisted of two cylindrical PVC tubes connected by a bolted flange connection. The filter cloths were fixed between the two tubes flanges and the apparatus was positioned vertically. The length of the upper and the lower tube was 294 mm resp. 39.5 mm. The inner diameter of the tube was 93 mm, thus results a filter area of 67.93 cm². The lower tube was closed by a conic bottom. The upper tube was filled with 1.65 L test solution. The initial differential pressure was about 245 mm hydrostatic head or rather 24 mbar. By opening a valve at the bottom of the lower tube the fluid flew through the filter cloth. After each experiment, the apparatus was carefully cleaned. Woven monofilament filter cloths of polyester (PET) and stainless steel as common in disc filters were used. It should be mentioned that cloths of PET and stainless steel differ fundamentally in their weave construction (Purchas and Sutherland, 2002). The fluid flowed vertically through the cloths of PET. In contrast, in case of stainless steel cloths the fluid streams in vertically and streams out horizontally. Hence, the fluid is redirected within the filter cloth. As a result of the different weave construction the pore shape differs as well. The pores of a PET cloth are quadratic whereas the pores of the stainless steel cloths are approximately triangular.

Test solutions were prepared by adding a defined amount of helminth eggs to tap water or wastewater. Therefor a highly concentrated solution of helminth eggs and tap water was prepared. The added helminth species and geometric characteristics of theirs eggs are listed in Table 2. The helminth eggs concentration of the concentrated solution was determined by optical counting of the eggs in 500 µL and 1000 µL samples. For enumeration a Doncaster Cell and a light microscope (Axio Lab A1, Carl Zeiss) were used. The values were averaged and subsequently extrapolated to the whole concentrated solution. For preparing test solutions, 500 µL of the concentrated solution were added to 1.65 L of tap water resp. wastewater from the secondary clarification effluent of the wastewater treatment plant Cerro de la Estrella of Mexico City, Mexico. The helminth egg concentration of the raw wastewater was about 0-5 eggs/L. The temperature of the test solutions ranged between 10 and 20 °C, the

Total Suspended Solids (TSS) concentration of the used wastewater test solutions was about 11 mg/L.

Table 1 Common shape and size of the helminth eggs used in the experiment.

Species	*Ascaris lumbricoides*	*Trichuris trichiura*	*Hymenolepis nana*	*Hymenolepis diminuta*	*Taenia solium*	*Toxocara canis*
Shape[1]	Ellipse-shaped to round[2]	Barrel-shaped	Spherical to nearly spherical	Spherical	Spherical	Nearly spherical
Size[1] **[µm]**	45-75 in length[2], 35-50 in width[2]	50-55 in length, 22-24 in width	30-47 in diameter	70-85 by 60-80 in diameter	31-43 in diameter	80-85 by 75 in diameter

[1]according to (Ash and Orihel, 2007)

[2]fertilised

For analysis of the filtered test solutions, the filtrates were collected in 5 L plastic containers. Following a manual agitation the sample was filtered through a 20 µm test sieve (3 inch diameter; test sieve according to ASTM standard No. 635). Based on the narrow pore size distribution, it is supposed that the used helminth eggs cannot pass this test sieve. The plastic containers were rinsed carefully with tap water. Retained helminth eggs on the test sieve were recovered in a conical centrifugation tube (50 ml) by rinsing with tap water. After centrifugation at 660 g for 15 min the supernatant was removed. The remaining volume was analyzed optically by using a Doncaster Cell and a light microscope with magnification of 100x, 400x and 630x. The analysis of filtrates with increased TSS concentrations (filter test using 33 µm up to 54 µm filter cloths and wastewater) included a flotation step with zinc sulfate as well. After centrifugation and removing the supernatant as described above approximately 50 mL of a zinc sulfate solution (relative density = 1.3) was added to the remaining volume of the sample. The samples were centrifuged at 660 g for 15 min and the supernatant was filtered through the test sieve once again. Subsequently, the above mentioned procedure, including centrifugation and removal of the supernatant, was repeated.

It should be mentioned that every test was carried out only one time. Due to the little quantity of realized filter tests a statistical evaluation of the results is not feasible.

Results and Discussion:

Table 2 shows the results of the filter tests using tap water. Filter cloths with pore sizes of 17 and 18 µm ensured the removal of all species. Figure 1 shows a *Trichuris trichiura* egg removed by a 17 µm filter cloth. It can be seen that screening seems to be the crucial separation mechanism. The eggs are apparently larger than the pores of the filter cloth. That applies as well for the 18 µm filter cloths, as illustrated in Figure 2. The microscopic shots lead to the conclusion that the smallest eggs used in the experiment (eggs of *Trichuris trichiura* and *Taenia solium*) can be separated by the 17 and 18 µm filter cloth. However, in case of the stainless steel cloths is it not possible to compare the size of the eggs and the pores by considering the microscopic shot.

In the filtrate of the 20 µm PET cloth solely one *Trichuris trichiura* egg was detected. That could be expected due to the fact that *Trichuris trichiura* is the species with the smallest eggs (22-24 µm width). Figure 2 shows a *Trichuris trichiura* egg removed by a 20 µm filter cloth. It is supposed that due to irregular pore sizes of the filter cloths eggs of *Trichuris trichiura* might pass the 20 µm filter cloth.

Table 2 Results of the filter tests using tap water. Concentrations are stated in helminth eggs/1.65 L.

Species		*Ascaris lumbricoides*	*Trichuris trichiura*	*Hymenolepis nana*	*Hymenolepis diminuta*	*Taenia solium*	*Toxocara canis*
Initial concentration		**76.6**	**120.3**	**124.6**	**16**	**6**	**5**
Filtrate concentration	17 µm (PET)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	18 µm (Steel)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	20 µm (PET)	N.D.	1	N.D.	N.D.	N.D.	N.D.
	33 µm (PET)	N.D.	30	4	N.D.	1	N.D.
	37 µm (Steel)	N.D.	35	13	N.D.	4	N.D.
	54 µm (PET)	69	123	77	N.D.	5	N.D.

N.D. = not detected

The PET filter cloth with a pore size of 33 µm ensured the removal of *Ascaris lumbricoides* *Hymenolepis diminuta* and *Toxocara canis* eggs. Figure 4 (on the left) shows a separated *Ascaris lumbricoides* egg; apparently screening was the crucial separation mechanism. However, the small- and medium-sized eggs of the species *Trichuris trichiura, Taenia solium* and *Hymenolepis diminuta* passed the filter cloths, corresponding to the size of the eggs. The comparative high removal of *Trichuris trichiura* eggs can be explained by Figure 4 (on the right). The microscopic shot shows a *Trichuris trichiura* egg removed by a 33 µm filter cloth. It seems as if the egg could have passed the filter cloth by changing its position. In this case, the elongate shape of the *Trichuris trichiura* egg significantly affects the separation. However, a conclusion regarding the stability of this blocking, especially in large-scale processes, cannot be made. The results of the filter test with stainless steel 37 µm cloths are roughly comparable to the filter test with the 33 µm PET filter cloth. However, as expected, a slightly lower removal for *Trichuris trichiura, Taenia solium* and *Hymenolepis nana* was observed. Figure 5 shows an egg of *Ascaris lumbricoides* (left image) and of Trichuris trichiura (right image) retained by the above mentioned filter cloth. Again, a comparative high removal of *Trichuris trichiura* eggs was noticed that supposable is caused by the elongate shape of the eggs.

As expected, the 54 µm PET filter cloth removed solely the eggs of *Toxocara canis* and *Hymenolepis diminuta*. Figure 6 shows eggs of *Hymenolepis diminuta* (left image) and *Toxocara canis* (right image) retained by a 54 µm PET filter cloth. Apparently, the eggs are trapped by the pores of the filter cloth. The eggs of *Ascaris lumbricoides* passed the filter cloths. Due to the size (35-50 µm in width) and the spherical shape of *Ascaris lumbricoides* eggs this result was expected. However, by using a 54 µm filter cloth a significant removal of *Hymenolepis nana* was observed, although the eggs diameter ranges between 30 and 47 µm (Ash and Orihel, 2007). Due to the fact that it was not possible to find a *Hymenolepis nana* egg by microscopic analysis of the used filter cloths the removal mechanisms cannot be explained adequately.

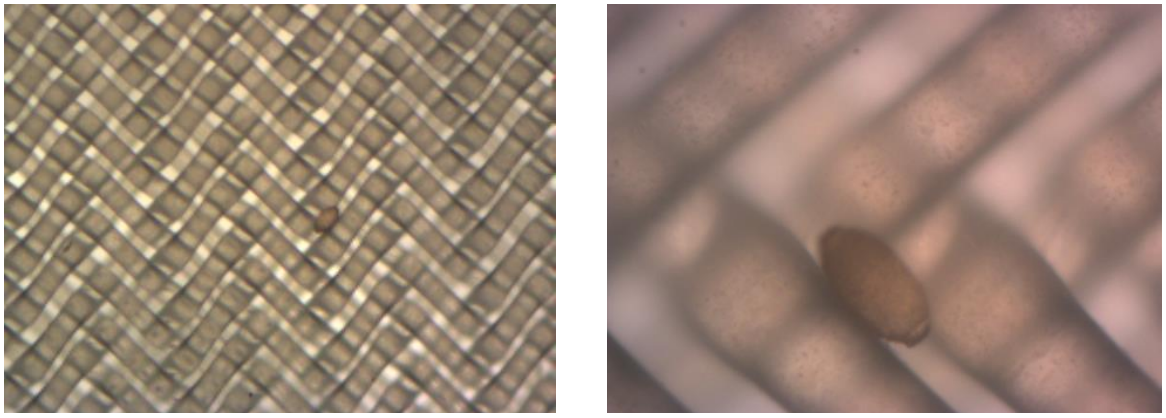

Figure 1 Egg of *Trichuris trichiura* retained by 17 μm PET filter cloth. Magnification: 100x (left image) and 630x (right image).

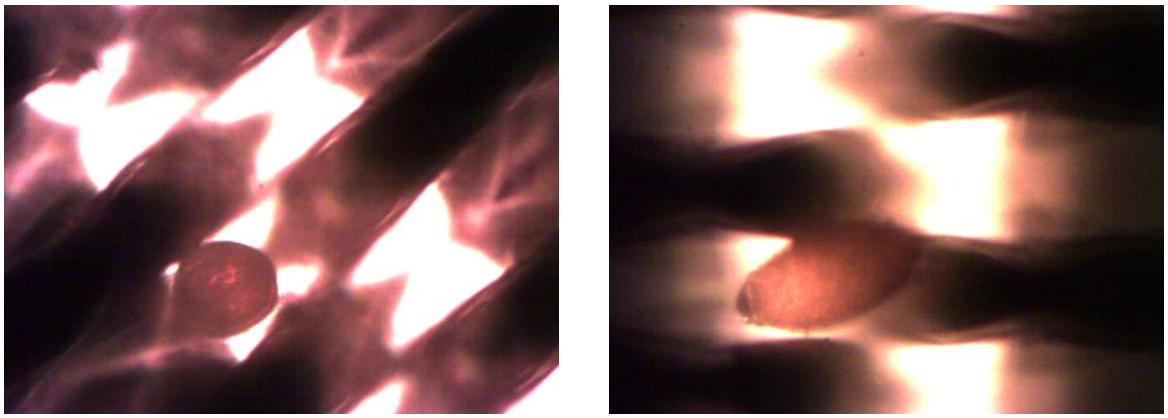

Figure 2 Eggs of *Taenia solium* (left image) and *Trichuris trichiura* (right image) retained by 18 μm stainless steel filter cloth. Magnification: 630x (left image) and 630x (right image).

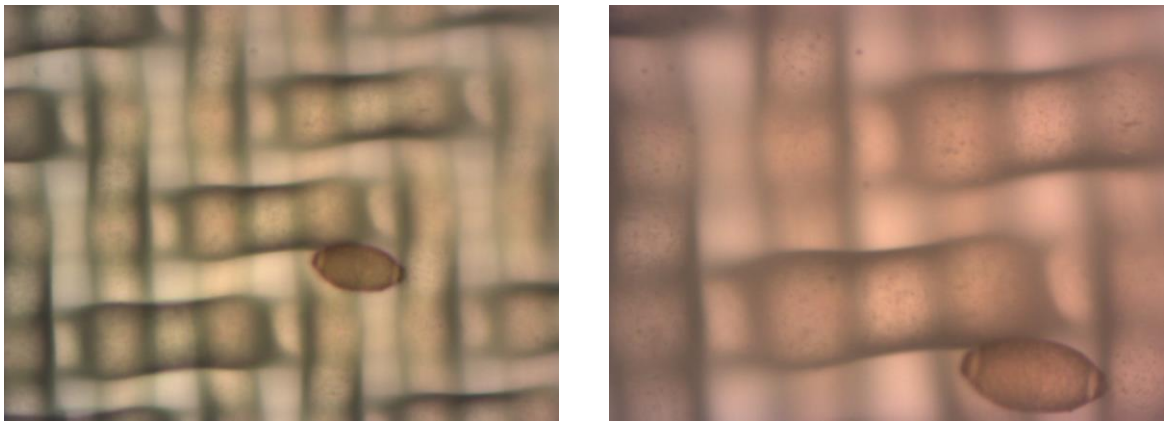

Figure 3 Egg of *Trichuris trichiura* retained by a 20 μm PET filter cloth. Magnification: 400x (left image) and 630x (right image).

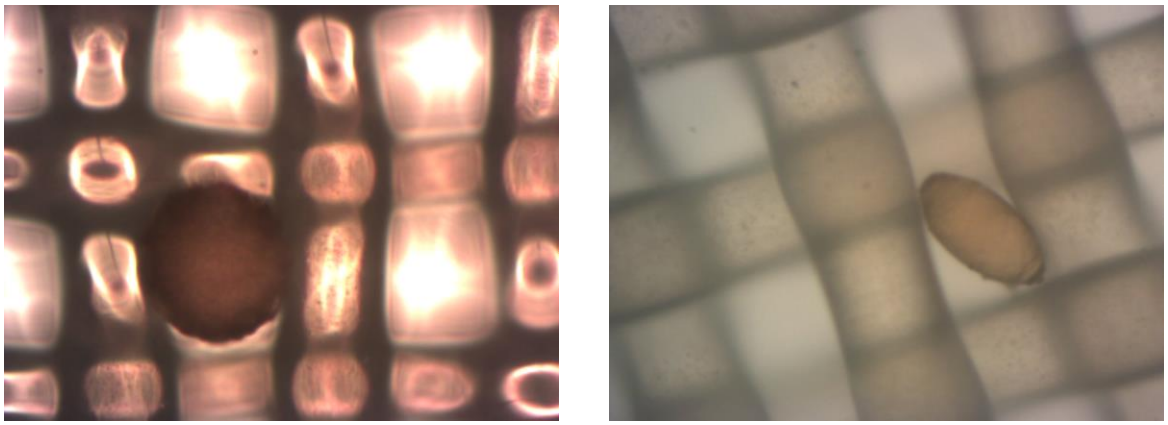

Figure 4 Eggs of *Ascaris lumbricoides* (left image) and *Trichuris trichiura* (right image) retained by 33 µm PET filter cloth. Magnification: 630x (left image) and 630x (right image).

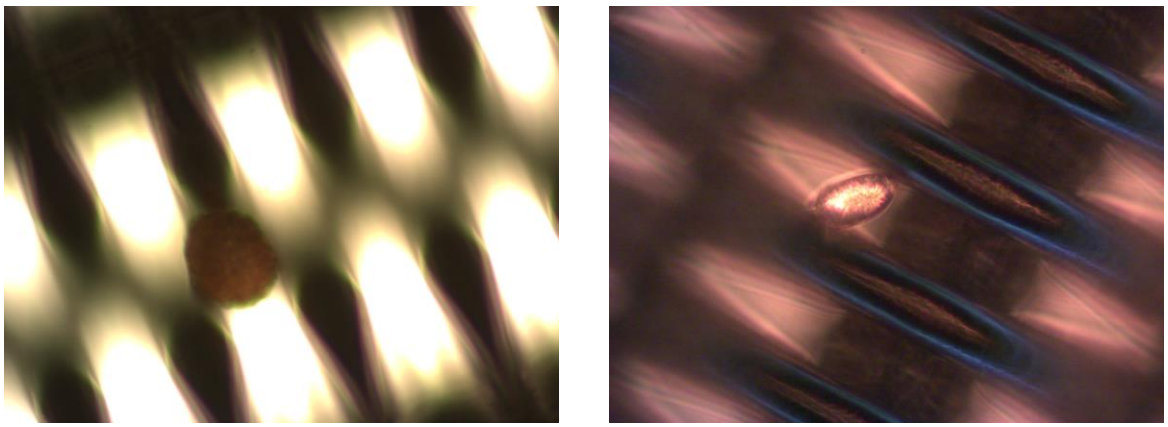

Figure 5 Eggs of *Ascaris lumbricoides* (left image) and *Trichuris trichiura* (right image) retained by 37 µm stainless filter cloth. Magnification: 400x (left image) and 400x (right image).

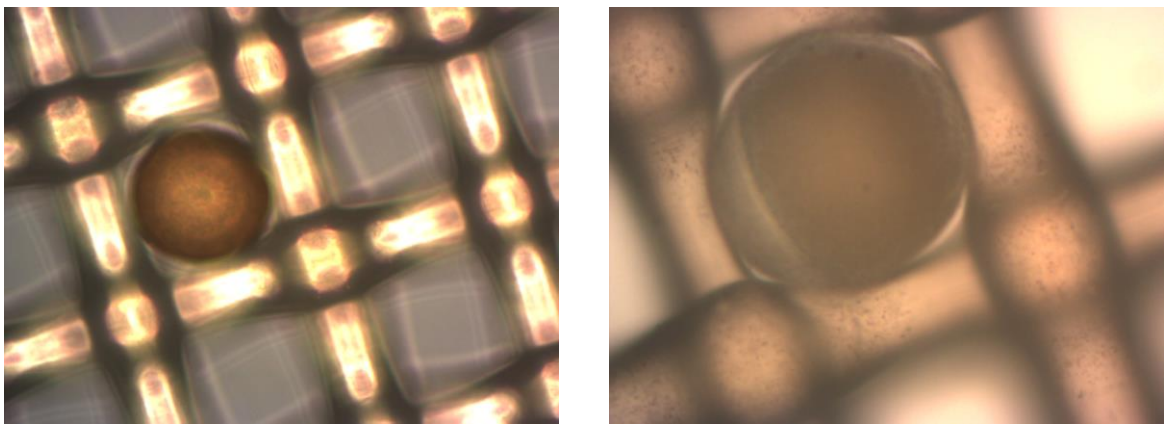

Figure 6 Eggs of *Hymenolepis diminuta* (left image) and *Toxocara canis* (right image) retained by a 54 µm PET filter cloth. Magnification: 400x (left image) and 630x (right image).

Furthermore, filter tests with the above mentioned wastewater using 20, 33, 37, and 54 µm filter cloths were carried out. As shown in Table 3, regarding the filter tests with 20, 37, and 54 µm filter cloths a lower quantity of helminth eggs was detected in the filtrate using domestic wastewater as test solution. However, in case of the filter tests using 33 µm filter cloths a lower quantity of helminth eggs was detected using tap water as test solution. For the 54 µm PET filter cloth particularly, an increased removal of *Ascaris lumbricoides, Trichuris*

trichiura and *Hymenolepis nana* was observed. For instance, 28 *Trichuris trichiura* eggs were detected in the filtrate of the wastewater sample instead of 123 in the tap water filtrate.

Table 3 Results of the filter tests using domestic wastewater (TSS = 11.2 mg/L). Concentrations are stated in helminth eggs/1.65 L. The results of the filter tests using tap water are stated in brackets.

Species		*Ascaris lumbricoides*	*Trichuris trichiura*	*Hymenolepis nana*	*Hymenolepis diminuta*	*Taenia solium*	*Toxocara canis*
Initial concentration		**76.6**	**120.3**	**124.6**	**16**	**6**	**5**
Filtrate concentration	20 µm (PET)	N.D. (N.D.)	N.D. (1)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
	33 µm (PET)	N.D. (N.D.)	53 (30)	13 (4)	N.D. (N.D.)	1 (1)	N.D. (N.D.)
	37 µm (Steel)	N.D. (N.D.)	12 (35)	3 (13)	N.D. (N.D.)	0 (4)	N.D. (N.D.)
	54 µm (PET)	44 (69)	28 (123)	14 (77)	N.D. (N.D.)	4 (5)	N.D. (N.D.)

N.D. = not detected

Due to the fact that it was not possible to find a *Trichuris trichiura* or a *Hymenolepis nana* egg by microscopic analysis of the used filter cloths, the separation mechanisms cannot be explained adequately. Figure 7 on the left shows an *Ascaris lumbricoides* egg removed by a 54 µm filter cloth in the presence of suspended solids. It can be seen that the removal of this egg might be induced by the attachment to particulate matter. Furthermore, clogging of filter pores resp. the formation of a *filter cake* could be observed on the used filter cloths. Both phenomena might have led to the observed higher removal performance.

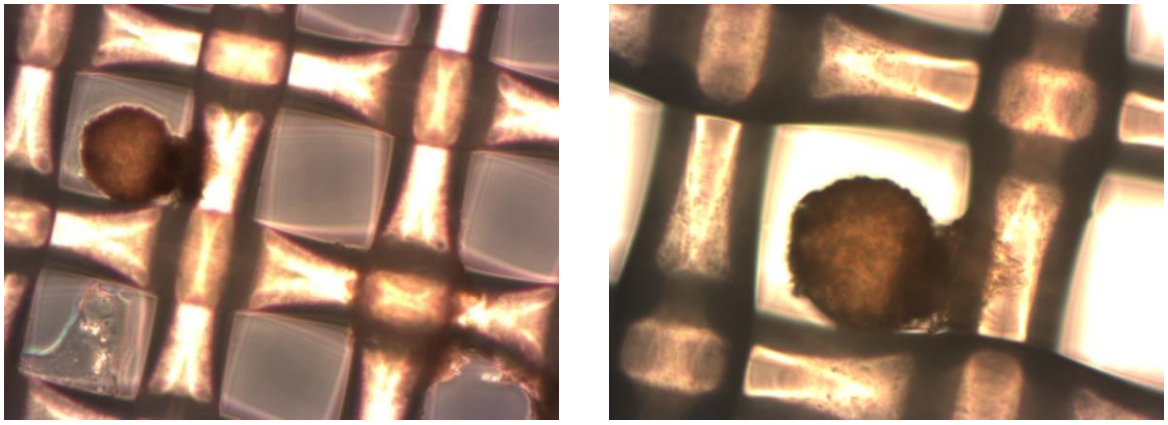

Figure 7 Egg of *Ascaris lumbricoides* attached to particulate matter retained by a 54 µm PET filter cloth. Magnification: 400x (left image) and 630x (right image).

Conclusions:

This study focuses on the removal of helminth eggs by micro screening. Woven filter cloths of PET and stainless steel with pore sizes of 17, 18, 20, 33, 37, and 54 µm were tested in laboratory scale. The following main conclusions can be made:

- 20 µm filter cloths do not ensure the removal of *Trichuris trichiura* eggs. In case of strict threshold values the use of filter cloths with smaller pores is recommended.
- Eggs of *Ascaris lumbricoides*, the most common parasitic species, were not detected in the filtrate of filter cloths with pore sizes smaller or equal than 37 µm.
- A higher removal performance is expected in the presence of particulate matter. It is supposed that helminth eggs tend to attach to particulate matter and form larger stable

aggregates that are easier to remove than single helminth eggs. Furthermore, the formation of a *filter cake* might lead to higher removal performance.

- Further long-term experiments are necessary to predict the removal of helminth eggs in pilot- and large-scale disc filters.

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