Potential Risk Related To The Reuse of Faecal Sludge In Agriculture: Proposal For An Ecological Treatment Method Based On Active Charcoal

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Abstract

This study is aimed at highlighting the risks associated with the reuse of faecal sludge and proposed a sustainable treatment method. The sampling consisted of collecting samples of faecal sludge in 5L of sterilized containers and then transporting to the laboratory in a refrigerated chamber for the determination of helminth eggs using standard protocols. The experimental device consisted of two replicates, one test and one control. The test samples received active charcoal at different concentrations (C1, C2, C3, C4, C5, C6). The physico-chemical parameters were measured before and after treatment. The samples were then observed under the optical microscope at the 40X objective for morphological identifications. Molecular analysis was carried out using the Polymerase Chain Reaction technique. The viability of the eggs were determined using incubation and staining techniques. Analyses showed that the sludge used for irrigation contained eggs and larvae of 6 helminth species (*S. stercoralis, A. duodenale, N. americanus, T. trichiuria, H. nana* and *Ascaris* spp.) with viability percentages ranging from 57.72% to 74.46%. Treatment with active charcoal allowed significant adsorption of these parasites with yields ranging from 95 to 100%. In addition, the carbon used has favoured the alkaline stabilisation of the medium, which increases its absorption potential. It can therefore be used in the treatment of sludge because, unlike other chemical disinfectants, it does not present any toxic effects.

Introduction

Faecal sludge is increasingly used in agriculture because it offers multiple advantages (Montangero et al. 1999; Montangero and Strauss 2002). It partakes in the renewal of humus layer and has a positive effect on the soil structure, on its capacity to retain water and provides a variety of trace elements. In contrast to mineral fertilizers, which only have a single function of providing mineral elements, faecal sludge also has an organic function. The reuse of sludge is increasingly common (Luo et al. 2012; Zhou et al. 2013). It provides better yields from an economic and agronomic point of view. (keffala *et al*. 2012), it is also an important source of nutrients, allowing rapid plant growth (Aladawi et al. 2006; Jimenez 2007). Settling ponds, lagooning and desiccation are commonly used methods for the treatment of viruses, bacteria and parasites contained in faecal sludge prior to its use. These treatment methods have limitations because some eggs of low molecular weight sediment very slowly, while other parasites such as eggs of helminth can resist desiccation and remain viable when accumulated in sedimentation ponds. Helminths are distinctly different from bacteria and viruses because of their resistance in the environment and their low infective dose (Eisenberg et al. 2002). Their presence in sludge and sewage increases the risk of infestation directly to humans or indirectly through the reuse of sludge for agricultural purposes, especially if eggs are ingested at an infesting stage (WHO 1989). The WHO recommends the disposal of all helminth eggs in agricultural sludge (Blumenthal *et al*. 2000). To achieve these goals, several chemical treatment processes for sewage sludge have been developed (Mendez et al. 2002). These different chemical methods have many consequences as they can lead to the formation of disinfection by-products that are often highly toxic. Therefore, more effective sludge treatment methods must be developed to inactivate almost all parasites present in faecal sludge. Active charcoal is an

environmentally friendly alternative method for the optimal treatment of sewage sludge. It integrates adsorption and organic biodegradation of the sludge (Yu et al. 2014; Li et al. 2005). The use of active charcoal has shown very high yields on the abatement of certain physico-chemical parameters and on the bacterial load (De Jongue *et al*. 1991; Lim et al. 2002; Martin et al. 2004; Aziz et al. 2011; Ho et al. 2011; Faulconer et al. 2012; Qing *et al*. 2015), particularly on antibiotic resistant bacteria (Damiana *et al*. 2019). Very few studies have focused on the parasitic composition of faecal sludge (Madoux and Dupouy 2001) and adsorption from active charcoal. The objective of this study is therefore to assess the health risk associated with the reuse of faecal sludge in agriculture and to develop an ecological method for the treatment of helminths using active charcoal.

Materials And Methods

1.1 Study framework and sampling stations

This study took place from June 2018 to June 2019 and was carried out in two phases. The first phase, which lasted 3 months (June to August) consisted of a series of screening tests to determine the ranges of minimal observable effect concentrations on eggs. At the end of this screening, the active Carbon and 6 ranges of mass concentrations were obtained for the analysis, namely (0.1 g/L, 0.2 g/L, 0.3 g/L, 0.4 g/L, 0.5 g/L and 0.6 g/L).

1.2 Description of sampling station Discharge of Nomayos (No) With geographical coordinates 04°09'33.7" N and 11°22'08.9" E, and an altitude of 629 m, the Nomayos sewage sludge discharge station is located on the periphery of Yaounde. For the time being, it is the sole discharge point for sewage sludge removed from the septic tanks of households in this area (Fig. 1a). Trucks unload their contents on a surface assigned to them by the local authorities, these dumps are generally found in water course located at the downstream of the dump, the population living in the vicinity of this site practice food and market gardening and use the sludge from these excreta as fertilizer (Fig. 1b).

1.3. Preparation of disinfectant solution

We used charcoal particles of plant origin (Marcia *et al*. 2004; Zhang et al. 2005) which were ground into fine particles and then washed with distilled water, dried and sieved. Activation was carried out chemically using acid and base (Fig. 1c). First the coal crystals were soaked in 98% sulphuric acid (H_2SO_4) solution (desiccant, oxidant and mineral removal agent) for 24 h and then washed with distilled water until a pH of 6 was reached in the residual liquid. In a second step (25 g) of activated carbon was immersed in 100 ml KOH, the whole was brought under stirring to 85°C for 2 h. The resulting liquid was then filtered off and the fine active charcoal crystals obtained were dried at 120°C for 24 h (Mahmoud *et al*., 2018). The concentrations used for disinfection were weighed using a balance (0.1 g/L, 0.2 g/L, 0.3 g/L, 0.4 g/L, 0.5 g/L and 0.6 g/L).

1.4 Sampling

Sampling of wastewater and sewage sludge was carried out following the recommendations of Rodier *et al.* (2009) for physicochemical analysis and following the approach proposed by Keffala et al. (2012) for biological analysis. Samples were collected using sterile 5L bottles and returned to the laboratory. For wastewater, sampling was done directly on the effluent, for sewage sludge the sample was taken directly after discharge by trucks.

1.5. Physico-chemical analyses and determination of helminth eggs

1.5.1. Physico-chemical analyses

The physico-chemical parameters evaluated in this study were measured using conventional techniques described by Rodier *et al.* (2009) with appropriate reagents. The pH was measured using a multiparameter HANA HI 9829. Turbidity (FTU), suspended solids and colour were measured in the laboratory by colorimetry with a spectrophotometer HACH DR/3900. Measurements of ammoniacal nitrogen contents in mg/L of water were determined by colorimetry, using the spectrophotometer HACH DR/2900. The reagents used Nessler and Rochelle salt. The oxydability contents in mg/L of $KMnO_4$ were measured by volumetry.

1.5.2. Determination of helminth eggs

1.5.2.1 Sample pre-treatment

The sample brought back to the laboratory was sieved for the removal of large particles, then the viable eggs in the sample were previously quantified, non-viable eggs were removed by flotation with n-butanol. Approximately 1ml of n-butanol was added to 500ml of sample to allow the non-viable eggs which are less dense to float, and then these non-viable eggs were removed by suction of the supernatant.

1.5.2.2. Disinfection test

For the treatment and disinfection of the samples, we made a device comprising 1 series of 6 Erlenmeyer flasks, and then 500 mL of previously homogenized sample was introduced into each Erlenmeyer flask. Active carbon was introduced in Erlenmeyer flasks at the different concentrations (C1, C2, C3, C4, C5, C6). The samples were then homogenized using a magnetic device and a baro-magnet to ensure perfect contact between the Active charcoal and the sample. To allow the disinfectant to act, a contact time of 24 hours was observed for each sample (Akam et al., 2005). Then 5ml of pellet was taken from each sample and introduced into a test tube, this pellet was then washed with sterile water twice (Amoah *et al.*, 2017 a). To these 5mL of pellet were added 5mL of distilled water, shaken and centrifuged at 500 rpm for 7 min (Ibañez-Cervantes *et al.* 2013). The supernatant from this centrifugation was removed by aspiration. The resulting pellet was washed a second time with sodium thiosulphate solution to neutralize adsorption. The resulting pellet was subjected to viability testing by staining and incubation, after concentration of the eggs by sedimentation and McMaster technique. The test was repeated for all sampling campaigns conducted during this study, with observations repeated twice (Khallaayoune and Fatiha 1995).

1.5.3. Viability Analysis

1.5.3.1 Staining Viability Test

For the staining viability test we used the neutral red which is a vital dye (Merward et al. 2011; Karkashan et al. 2015). Neutral red is a dye that has the ability to bind to the structure of viable eggs and stain them red. After concentration of the parasitic elements 1 ml of neutral red was added to each sample and a contact time of 10 minutes was observed to allow the stain to penetrate the viable eggs. These samples were then placed on the McMaster slides for observation, the eggs stained red by the stain were considered viable, and the unstained eggs were considered potentially non-viable (Sarvel et al. 2006). The number of eggs per litre was calculated using the formula proposed by Sengupta et al. (2011).

$$N = AX/PV$$

Where N = number of eggs per litre of sample, X = volume of final product (ml), A = number of eggs counted on the McMaster slide or average of the numbers found in two or three slides, P = capacity of the McMaster slide (0,3 ml), V = volume of the initial sample (litres).

1.5.3.2 Incubation viability test

For this technique, 5ml of pellet were incubated on Petri dishes in an oven at 30°C for 30 days (Pecson et al. 2007), during these days the process of reduction of egg viability was demonstrated, starting from the destruction of the egg membrane to the inactivation of the egg. Then the eggs were examined under an optical microscope, so that non-viable eggs segmentation stopped while viable eggs continued their segmentation and development. Eggs containing at least eight blastomers and eggs containing mobile larvae were considered viable (Stien 1989; Keffala et al. 2012 Amoah *et al*. 2017 b). Identification was done through morphological analysis of egg size, shape and content (Řežábkováa *et al*. 2019). Egg content and wall changes were examined by light microscopy at 40X and 100X objectives. Measurements were made using an eyepiece micrometer and photos were taken using an Xploview model photographic device attached to one of the microscope's eyepieces. The number of eggs per litre was calculated using the formula proposed by Ajeagah et al. (2014) : $\mathbf{X}=\frac{y.Vx}{Vy}$

With Vx = volume of pellet in 1 L of sample, Vy = volume of pellet used for observation, y = number of eggs observed in Vy.

1.5.4. Molecular analyse by Polymerase Chain Reaction (PCR)

1.5.4.1. DNA isolation

Each wastewater sample (100 mL) was filtered through a sterile 0.2 µm Sterivex filter (Millipore, USA) and the genomic DNA (gDNA) was extracted from the filters using a PowerWater Sterivex DNA Isolation Kit (MOBIO Laboratories, California, USA). Extraction reagent blank controls (ExCs, *n* = 6) were included

alongside each batch of gDNA extractions. Purified DNA was stored at − 20°C prior to molecular analyses.

1.5.4.2. Next-generation sequencing library preparation

For NGS library preparation and sequencing, the 16S metagenomic sequencing library preparation protocol from Illumina (Part # 15044223 Rev. B; Illumina, USA) was followed, with only minor modifications to the first stage PCRs. The hypervariable 9 (V9) regions of the eukaryotic 18S were amplified, using 2 µl of template DNA (out of a total of 50 µl); these primers were modified to include Illumina MiSeq adapter sequences (Part # 15044223 Rev. B; Illumina, USA). Amplification of 18S V9 with the Euk1391F/EukBr primers was carried out using conventional PCR as per the 18S amplification protocol available from the Earth Microbiome Project. A mammalian blocking primer (Mammal_block_I-short_1391f) was used at a final concentration of 1.6 µM to reduce amplification of mammalian DNA. The libraries were sequenced on the Illumina Miseq platform (San Diego, CA, USA), with v2 sequencing chemistry for the eukaryotic 18S NGS.

1.6. Statistical analysis

The normality of the data was assessed using the Kolmogorov-Smirnov test, while data comparisons were made using the ANOVA test and Students T test. The correlations were made using the Pearson test. All these analyses were carried out using the SPSS version 17.0 software.

Results And Discussion

2.1.1. Morphological description of helminths

Figure 2 shows the images of the viable and non-viable helminths identified during the study. After incubation, the viable eggs of Hookworm (Figure 2a) and Necator (Figure 2b) had 4 and 8 blastomeres respectively, while the non-viable eggs of Hookworm (Figure 2a') and Necator (Figure 2b') had no blastomeres. Viable eggs of Ascaris (figure 2 c), Hymenolepis (figure 2 d) have a mobile larva, whereas in non-viable eggs of Ascaris, (figure 2 c') and Hymenolepis (figure d') there is no larva. Viable Trichuris eggs have a mobile hexacanth embryo (Figure 2 e) while non-viable Trichuris eggs (Figure 2 e') have an immobile hexacanth embryo. Viable Strongyloides larvae (Figure 2 f) are characterized by high mobility while non-viable Strongyloides larvae are immobile (Figure 2 f').

2.1.2. Variation in identified viable eggs

Figure 3 shows the variation in eggs identified during the study. *Ascaris* spp. had the highest number of eggs (9121 eggs/L) followed by *A. duodenale* species (7550 eggs/L), whereas *Hymenolepis nana* eggs had the lowest density (840 eggs/L) (Fig. 3). Viability tests carried out on these samples showed that larvae of *S. stercoralis* were the most viable (74.46%), followed by eggs of *A. duodenale* (73.37%), *N. americanus* (69.29%), *T. trichiuria* (67.14%), *H. nana* (63.69%), *Ascaris* spp. (57,72%).

2.1.3. Variation of egg density after treatment with active charcoal

The number of viable eggs of *Ascaris* and *N. americanus* obtained after application of active charcoal decreases considerably with increasing concentration. For *Ascaris* eggs the control values obtained are 7500 eggs/L (Test 1) and 4500 eggs/L (Test 2). After treatment, the lowest values of 327 eggs/L (Test 1) and 98 eggs/L (Test 2) were obtained at the C6 concentration (Fig. 4A). For *N. americanus* eggs the control values obtained are 4500 eggs/L (Test 1) and 1792 eggs/L (Test 2), after treatment the lowest values of 172 eggs/L (Test 1) and 102 eggs/L (Test 2) were obtained at C6 concentration (Fig. 4B).

2.1.4. Variation in the density of viable larvae of S. stercoralis and T. trichiuria before and after treatment

The number of viable larvae of *S. stercoralis* and eggs of *T. trichiuria* obtained after the application of activated charcoal drops considerably with increasing concentration. For *S. stercoralis* larvae the control values obtained before treatment are 2000 eggs/L (Test 1) and 1500 eggs/L (Test 2), after treatment the lowest values of 0 eggs/L (Test 1) and 38 eggs/L (Test 2) were obtained at the C6 concentration (Fig. 5A). For *T. trichiuria* eggs the control values obtained were 1982 eggs/L (Test 1) and 973 eggs/L (Test 2), after treatment, the lowest value namely 0 eggs/L (Test 1 and Test 2) were obtained at concentrations C5 and C6 (Fig. 5B).

2.1.5. Variation in the density of viable eggs of Hookworm and Hymenolepis nana counted before and after treatment

The number of viable eggs of *A. duodenale* and *H. nana* obtained after application of activated carbon decreases considerably with increasing concentration. For eggs of *A. duodenale* the control values obtained are 6350 eggs/L (Test 1) and 4730 eggs/L (Test 1), after treatment the lowest values of 0 eggs/L (Test 1 and Test 2) were obtained at concentrations C5 and C6 (Fig. 6A). For *H. nana* eggs, the control values obtained during Test 1 were 450 eggs/L (Test 1) and 620 eggs/L (Test 2), after treatment the lowest value of 0 eggs/L (Test 1 and Test 2) was obtained at concentrations C5 and C6 (Fig. 6B).

2.1.6. Hierarchical arrangement of the values obtained at the different concentrations

Figure 7 shows a hierarchical arrangement of the density of viable helminth eggs and larvae counted before and after treatment at different concentrations (Fig. 7). It can be seen that the control value differs significantly ($p < 5\%$), however after application of disinfectants the values obtained at concentrations C5, C6 differ significantly ($p < 5\%$) from the values obtained at the concentrations (Control, C1, C2, C3, C4).

2.1.7. Variation in physico-chemical parameters measured before and after application of disinfectants

The physico-chemical parameters measured vary considerably depending on the concentration of disinfectants (Table I). For ammonia nitrogen, nitrate and orthophosphate, the control values recorded before application of the disinfectants are 48 mg/L 163840mg/L, 16.7mg/L for ammonia nitrogen, nitrate and orthophosphate respectively. The lowest values obtained after disinfection are 1.6mg/L, 12mg/L, 1.7mg/L for ammonia nitrogen, nitrate and orthophosphate, respectively. All pH values obtained before and after application of disinfectants are slightly basic, the highest value (7.79) was obtained on the control sample, all values obtained after disinfection are below 7.51. The values of turbidity, color and TSS obtained on the control sample are 188416FTU, 870400Pt.Co and 8600 mg/L respectively for color,

turbidity and TSS, after treatment the lowest values obtained are 39.8 FTU, 11400Pt.Co and 1430 mg/L respectively for color, turbidity and TSS. The oxidability values obtained range from 450 mg/L (control sample) to 29.2 (C4).

Table I Variation in physico-chemical parameters measured before and after application of disinfectants

	Control	C1	C2	C3	C4	C5	C6
NH^{+}_{3}	48	31	22,4	9	11,1	3,3	1,6
pH	7,79	7,51	7,49	7,5	7,33	7,29	7,5
Turbidity	188416	75637,5	6789,3	2790,3	318,9	107,4	39,8
Oxydabillity	450	189	173	94	84,6	70	29,2
Color	870400	15789	14897	11786	12876	11267	11400
Orthophosphate	16,7	5,37	5,2	3,62	2,34	1,9	1,7
Nitrate	163840	1092	1072	962	273	87	12
Suspended solids	8600	3765	3176	2367	2467	1653	1430

2.1.7. Calculation of the charcoal efficiency yields on adsorption

Efficiency yields obtained with the different disinfectants R= [(initial value - residual value) / initial value] × 100

Table II presents the different efficiency yields of activated carbon on the adsorption of helminth eggs and physicochemical parameters. After treatment, it can be seen that the percentage of egg adsorption increases according to the concentration of active charcoal. Eggs of *T. trichiuria* and *A. duodenale* showed the highest percentage of adsorption (100%) at concentrations C5 and C6, followed by larvae of *S. stercoralis* with a percentage of adsorption of (98.914%), on the other hand the lowest efficiency yields were on eggs of A. lumbricoides (95.964) and on eggs of *N. americanus* (95.645%). With regard to physico-chemical parameters, nitrates and turbidity had the highest adsorption efficiencies (99.99%, 99.97% respectively for nitrates and turbidity), while suspended solids had the lowest adsorption efficiencies (83.372%).

Table II Efficiency performance of active charcoal on adsorption of physico-chemical and biological parameters

	C1	C2	C3	C4	C5	C6
A. lumbrioïdes	71,776	80,798	87,009	84,967	93,428	95,964
N. americanus	61,236	81,564	83,741	91,370	94,104	95,645
Strongyloides	80,371	84,914	87,457	93,314	95,543	98,914
Trichuris trichiuria	69,374	87,107	96,108	99,255	100,000	100,000
A. duodonale	16,886	56,922	90,135	97,978	100,000	100,000
H. nana	50,000	61,308	72,150	80,561	89,439	97,850
NH^+_4	35,417	53,333	81,250	76,875	93,125	96,667
Turbidity	59,856	96,397	98,519	99,831	99,943	99,979
Oxydability	58,000	61,556	79,111	81,200	84,444	93,511
Color	98,186	98,288	98,646	98,521	98,706	98,690
Orthophosphate	67,844	68,862	78,323	85,988	88,623	89,820
Nitrate	99,333	99,346	99,413	99,833	99,947	99,993
Suspended solids	56,221	63,070	72,477	71,314	80,779	83,372

Discussion

The morphological study allowed the identification of 6 species of helminths (*Ascaris* spp., *A. duodenale, T. trichiuria, S. stercoralis, Hymenolepis nana*) at variable densities, these identifications were confirmed by molecular analyses carried out by the Polymerase Chain Reaction technique. The high density of eggs of *Ascaris* spp. is thought to be due to the fact that Ascaris is a cosmopolitan species; in addition, *Ascaris* eggs have a triple membrane which enables them to withstand various environmental stresses for a long time. The viability study carried out on all identified eggs showed that although *Ascaris* is the most dominant species, it has the lowest viability rate (57.72%), compared to the other identified parasites. This low viability rate of *Ascaris* eggs compared to other species can be explained by the fact that in nature some infertile *Ascaris* eggs from unfertilized females are observed and therefore the ingestion of unfertilized eggs does not represent a real danger. This means that the risk of contamination by helminths in sewage sludge does not only depend on the presence of the eggs but also on the level of infestation of these eggs.

The species *S. stercoralis* has the highest percentage of viability (74.46%), this high percentage of viability can be explained by the fact that unlike other helminth species, *S. stercoralis* is present in nature as a larva, this larva can therefore use certain substrates in nature to feed and remain viable. However, in other species the embryogenesis process stops once the egg is in the wild.

The treatment of active charcoal-based sludges significantly reduced the concentration of viable helminth eggs and larvae present in these sludges, in fact the rate of viable helminths decreases with increasing concentration of active charcoal, similar effects were observed by Ariadna et al. (2016) on the treatment of Ascaris eggs. Ramakrishna et al. (1989); Marquez and Costa (1996) also obtained similar results on helminths isolated from wastewater. The highest efficiency rates were obtained at C6 concentration, on eggs of T trichiuria and A. duodenale (100%), S. stercoralis (98.91%) and H. nana 97.85%. This high efficiency would be due to the size of eggs of T trichiuria, A. duodenale and H. nana generally smaller than 60µm which can easily be adsorbed through the micro pores of active charcoal, unlike the eggs of Ascaris and N. americanus whose identified egg size was around 70µm and therefore require larger pores to facilitate their complete absorption. As for the larvae of S stercoralis, although they are large in size, they are very filiform in nature and can therefore easily be adsorbed through the micro pores of the activated carbon. This high efficiency of activated carbon is also due to its mode of action, in fact during the adsorption process there is oxidation of polypeptides, this mechanism is irreversible which leads to the inactivation of helminth eggs (Imlay 2008), in addition it does not lead to the formation of disinfection by-products often toxic to the organism (Li et al. 2017; Zheng et al. 2017).

In addition to the dose of product used, the mechanism for effective disinfection of faecal sludge relies on the rise in pH and ammonia nitrogen concentration during alkaline stabilization (Pescon and Nelson 2005). The nature of the pH and the adjuvants influence the effectiveness of the treatment (Qingyun 2018). During this study, the pH obtained before and after treatment remained basic (Table 2), which shows that active carbon did not significantly change the pH of the medium. This alkaline pH would have favoured the adsorption of microorganisms. According to Appels *et al.* (2008), the alkaline state of a solution better promotes the adsorption of microorganisms compared to the acid state.

In addition to pH, organic matter and insoluble particles can influence the active charcoal sludge treatment process. The values of Nitrate, Ammonia Nitrogen and Oxidability obtained on the control samples before disinfection are higher than the standards prescribed by Rodier *et al.* 2009. In fact, it is observed that after application of the disinfectants, there is a significant reduction in nitrogen, orthphosphate and insoluble particles. This adsorption of suspended particles and organic matter reduces the exchange and adsorption surface available for the adsorption of eggs, the more organic matter a water is loaded, the more difficult it is to treat. After application of disinfectants some of these values are reduced considerably; however, they remain present in all samples. The nitrate values obtained after treatment are all higher than 12 mg/l, the ammoniacal nitrogen values obtained after disinfection are also higher than 1.6 mg/l and the oxidability values remain higher than 29.2 mg/l. Indeed, ammonia is a parameter to be taken into account during water treatment. (Pecson and Nelson 2005; Fidjeland *et al*. 2015). The significant and positive correlations observed between ammoniacal nitrogen, nitrates and viable eggs counted confirm the role played by organic matter, during the adsorption process, organic matter can be absorbed by carbon and make the quantity of pores available to adsorb microorganisms unstable.

The values for suspended solids (1430 mg/l), turbidity (39.8 FTU) and color (11400 Pt.Co) recorded after application of the disinfectants remain high, these micro particles influence the treatment, as they are also adsorbed through the pores of the active charcoal. Significant and negative correlations were observed between suspended solids turbidity, color and number of viable eggs (r=-985). During disinfection, the particles present in the sludge constitute a potential barrier that can obstruct the pores of the active charcoal. Suspended particles can prevent permanent contact between the eggs and the disinfectant (Shimizu *et al*. 1997; Bougrier *et al*. 2005).

Conclusion

At the end of this work it appears that the sewage sludge dumped in the locality of Nomayos contains a high concentration of helminth resistance forms. This sludge is subjected to a summary treatment, before it is use, which does not allow for the elimination of helminth eggs before their use in agriculture. Viability testing of helminth eggs has shown a high concentration of viable eggs and larvae on sludge used to amend plantations, exposing populations at high risk of parasite contamination. To overcome this treatment deficit, an ecological helminth treatment technique based on active charcoal was developed, which eliminated 100% of the eggs of *T trichiuria* and *A. duodenale*, 98.91% of the larvae of *S. stercoralis* and 97.85% of the eggs of *Hymenolepis nana* 97.85% and 95% of the eggs of *Ascaris* spp. and *N. americanus*. This efficiency of active charcoal will make it possible to optimally treat the sewage sludge used in agriculture in order to limit the risks of contamination.

Declarations

Ethics approval and Not applicable

Consent to participate Not applicable.

Consent for publication Not applicable.

Authors' Contribution Gideon AJEAGAH is the person in charge of the parasitological analysis of water in the Hydrobiology and Environment Laboratory. He participated in the implementation of the protocol, the physico-chemical and biological analyses, the drafting and correction of the manuscript. Arnold Landry KOUAM FOTSEU participated in data collection, water disinfection, physicochemical and biological analyses, statistical tests and drafting of the manuscript. Isaac Dennis Amoah participated in the molecular analysis. Pierre TSOMENE NAMEKONG participated in the activation of the active charcoal. Thérèse Nadège OKOA AMOUGOU participated in the biological analyses and the correction of the manuscript.

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Competing interest The authors declare no competing interest.

Availability of data All data generated or analysed during this study are included in this published article.

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Figures

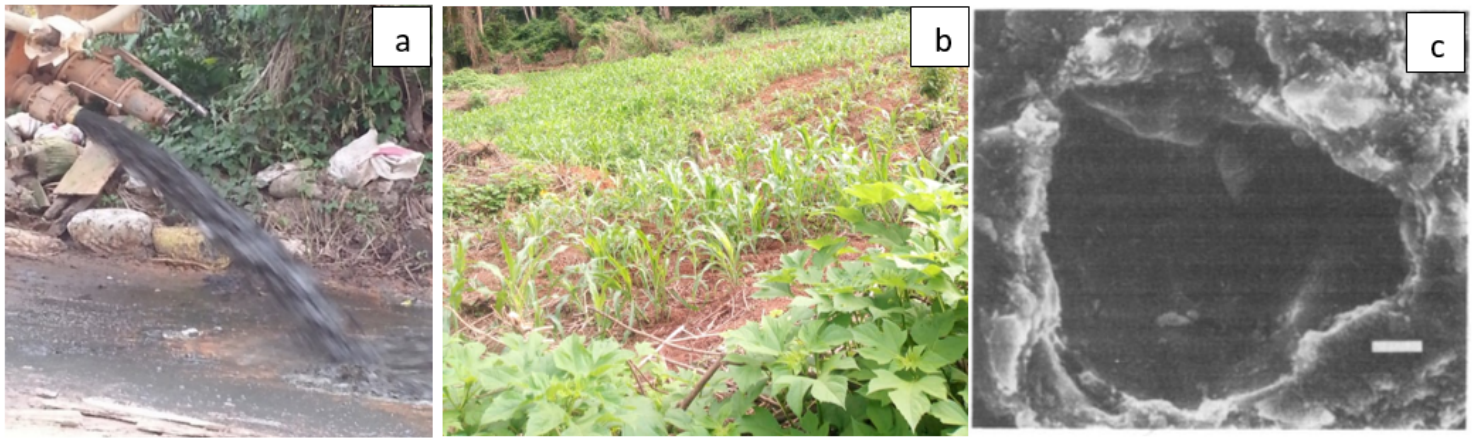

Figure 1

Discharge of faecal sludge (a), agricultural plantations using faecal sludge as fertilizer (b), macro pores of charcoal seen with an electron microscope (MEBX650, E: 650) (LAFRANCE et al. 1983).

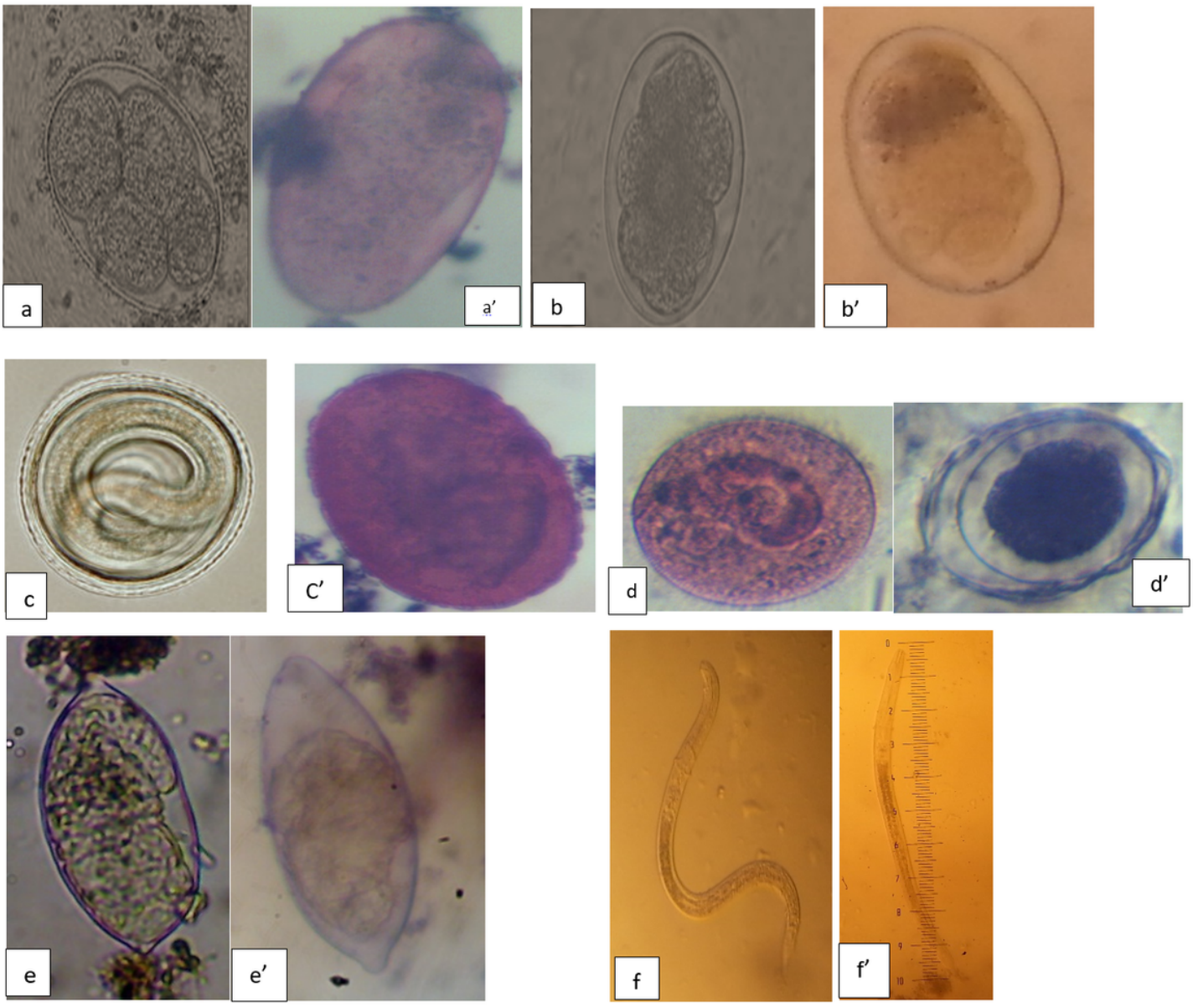

Figure 2

Images of viable helminths: Hookworms (a) Necator (b), Ascaris (c), Hymenolepis (d), Trichuris (e), Strongyloide (f); and image of non-viable helminths: Hookworms (a') Necator (b'), Ascaris (c'), Hymenolepis (d'), Trichuris (e'), Strongyloide (f').

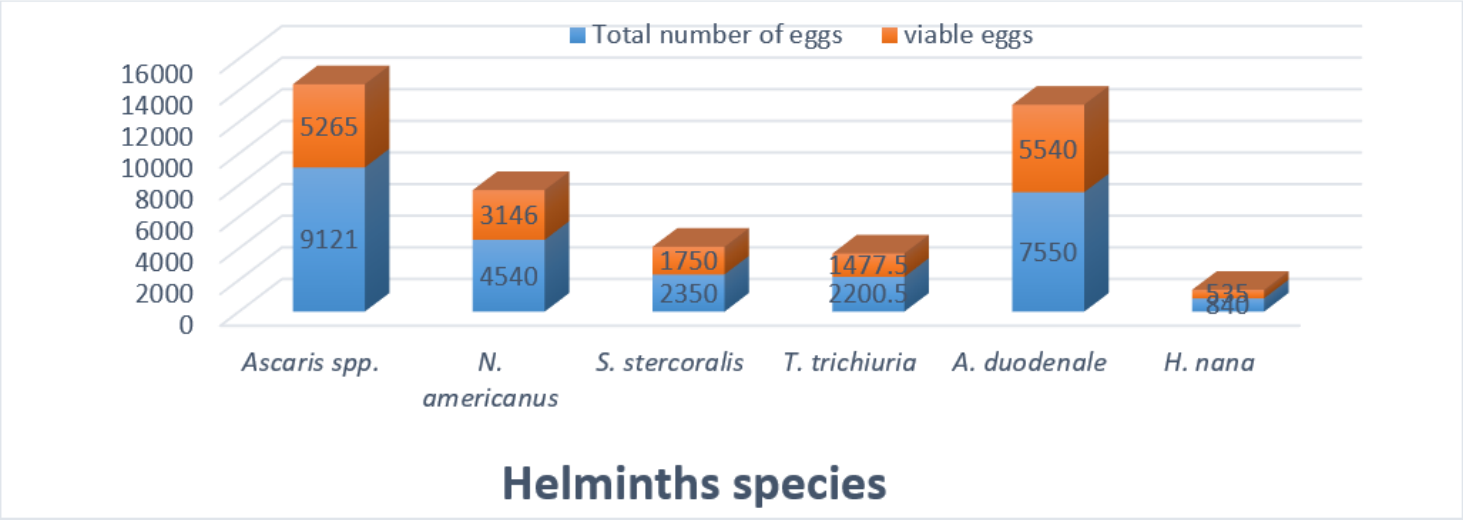

Figure 3

Variation in the number of viable and non-viable helminth eggs identified during the study

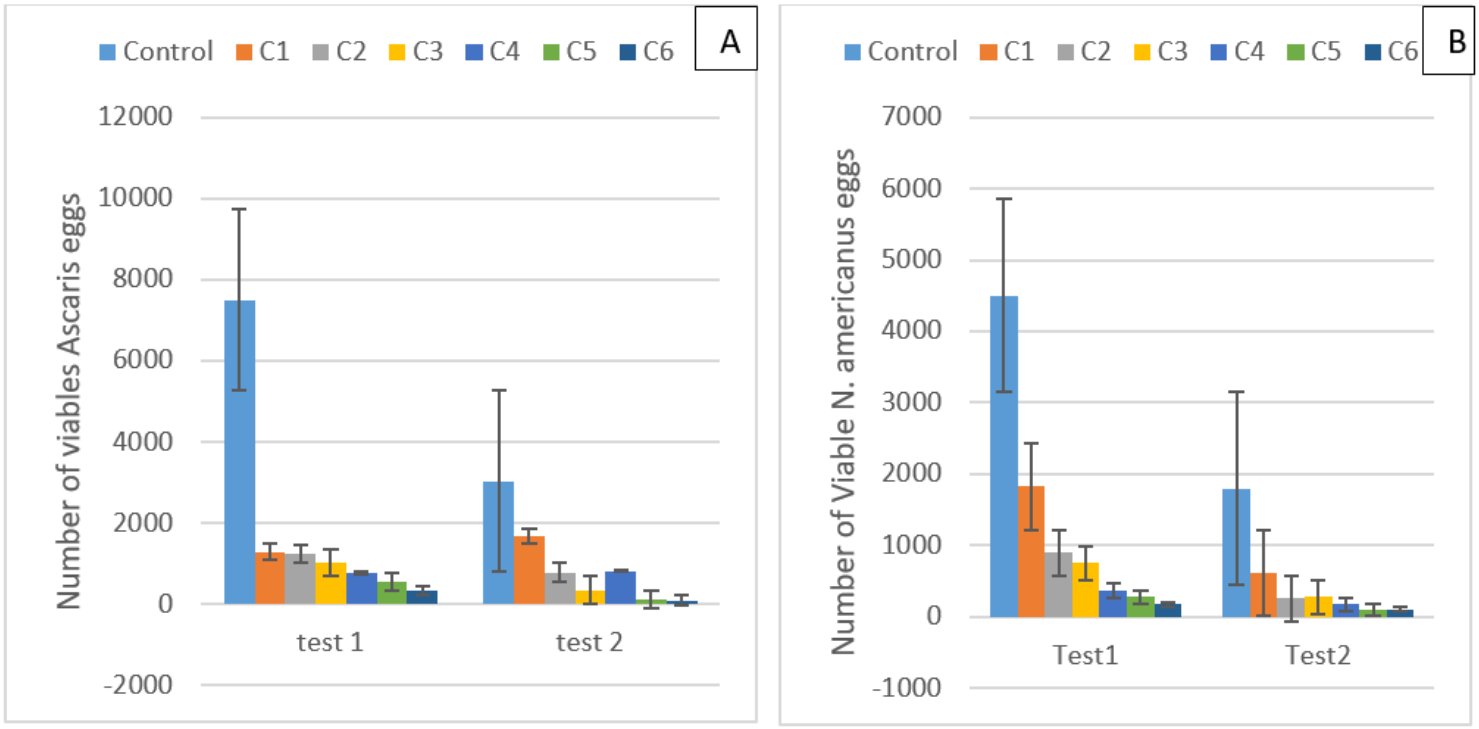

Figure 4

Variation in the number of Ascaris (A) and N. americanus (B) eggs obtained before and after treatment

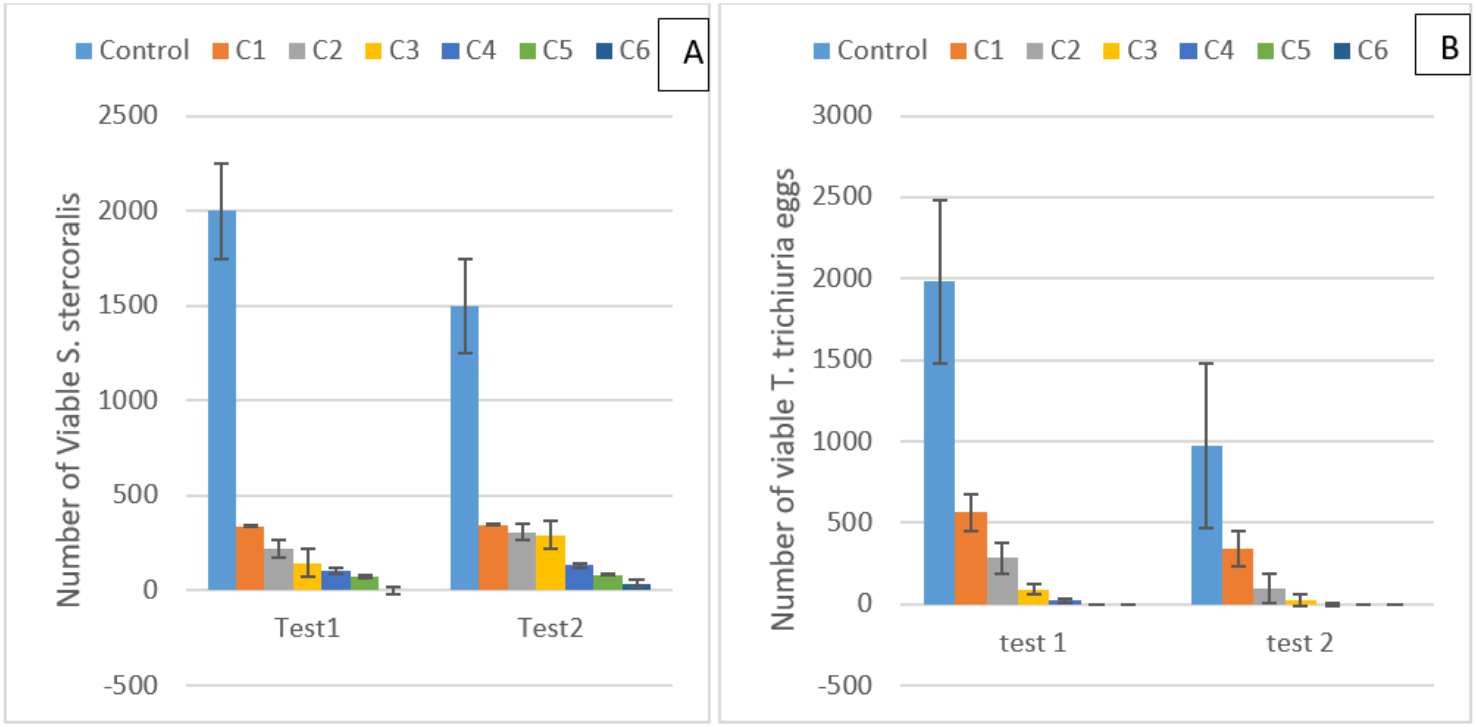

Figure 5

Variation in the number of viable larvae of S. stercoralis (A) and viable eggs of T trichiuria (B) obtained before and after charcoal treatment

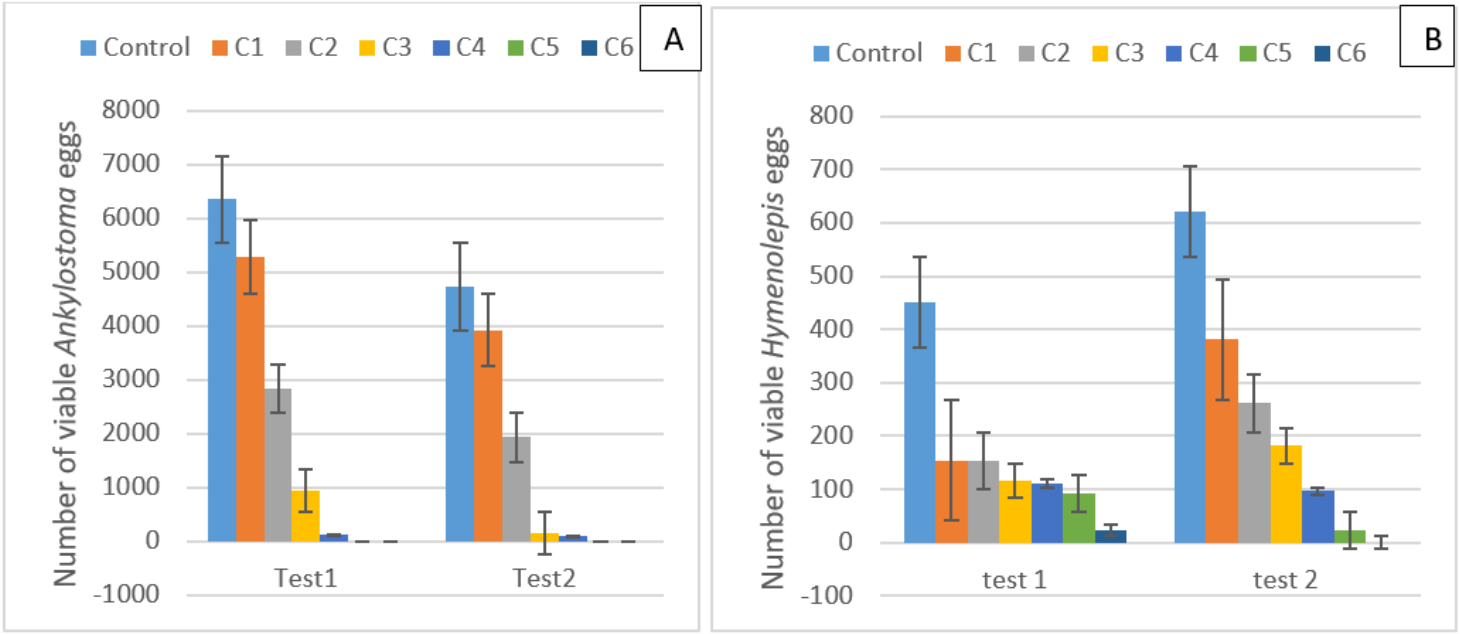

Figure 6

Variation in the number of Ankylostomes eggs (A) and H. nana (B) obtained before and after treatment

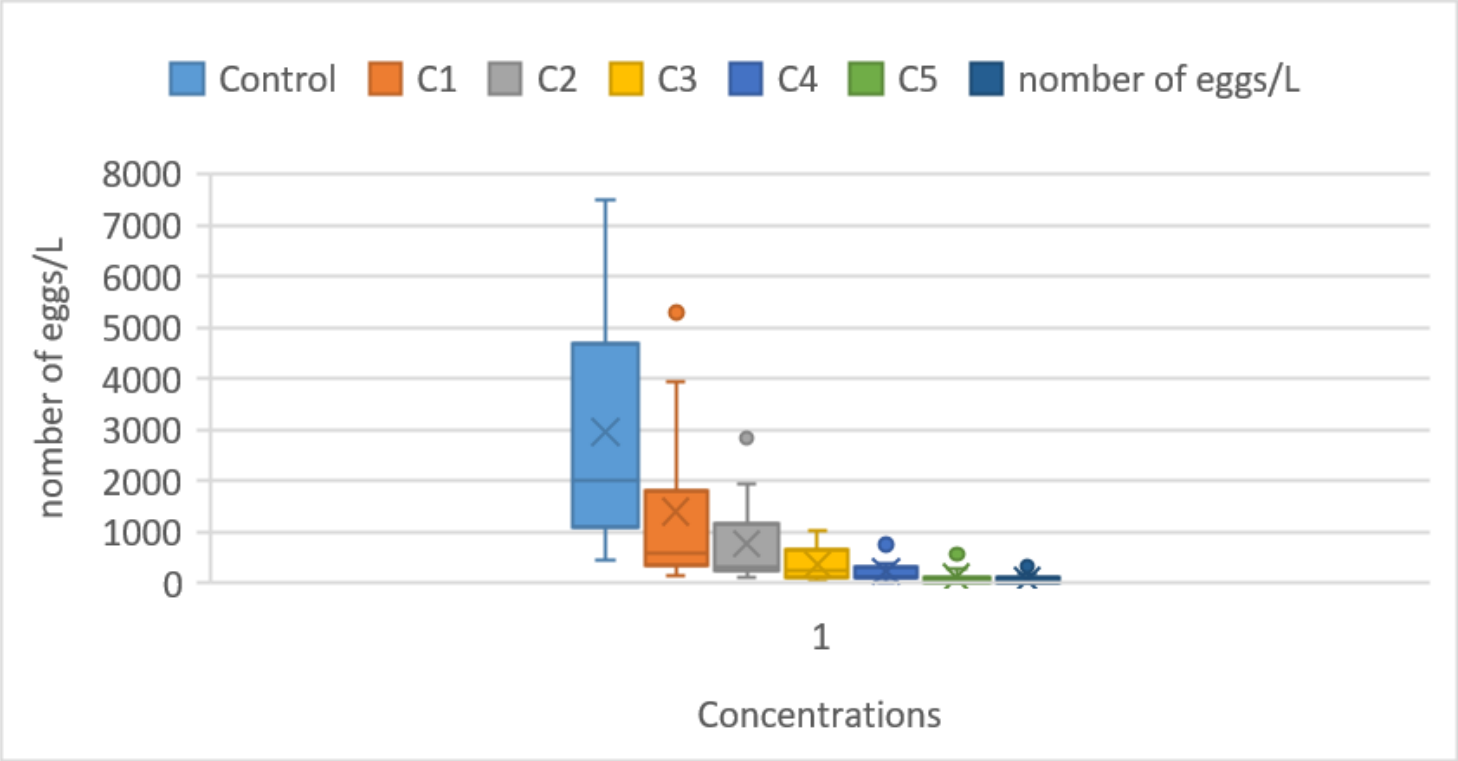

Figure 7

Hierarchical arrangement of the effect of different concentrations on helminth resistance patterns