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# EFFECT OF CO-COMPOSTING ON HELMINTH EGGS REMOVAL

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## Abstract

This study concerns the effectiveness of helminth eggs elimination during co-composting of activated sludge from a wastewater treatment plant mixed with ligno-cellulosic waste in a ratio of either 1/3-2/3 or 1/2-1/2 for 180 days. The Analysis of the initial sludge showed a load of 4-27 nematode eggs /g fresh matter identified as *Ascaris* sp., *Capillaria* sp. and *Trichuris* sp. During the co-composting stabilization phase, the reduction of the number of eggs varied between 50 and 90%. At the end of the process, reduction reached 100% for all the helminth ova. These results confirm the effectiveness of co-composting on their removal. Thereby, the final compost complied with the WHO guidelines for the safe reuse of fecal sludge.

*Key words:* co-composting, helminth eggs, palm tree waste, sewage sludge

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## 1. Introduction

According to the World Health Organization (WHO, 2006) fecal matter contains mainly enteric pathogenic agents such as bacteria, viruses and parasites (protozoa and helminthes); which are responsible for many diseases, particularly in children. The most common parasitic infections are intestinal nematodes, which can persist in natural environment (Gunawardena et al., 2004). The fecal oral route is considered to be the main path of parasitic infection in humans, through soil and/or water (Brooker et al., 2010). In addition, the growing usage of treated wastewaters for crop irrigation has led to the contamination of salads and other vegetables with helminth eggs (Ezatpour et al., 2013; Mercanoglu and Halkman, 2011). According to Amoah et al. (2006) vegetables irrigated with polluted urban wastewater contain higher concentrations of *Ascaris* (55-60%) than *Trichuris* or *Schistosoma eggs* (2-3%). Gupta et al. (2009) identified the presence of *Ascaris lumbricoides* (36%), *Trichuris trichiura (1.7%)* and hookworms *(6.4%)* on Lettuce, Parsley, Spinach, Mint, Celery and Coriander irrigated by wastewaters. With low water-quality and consumption of contaminated irrigated vegetables, these authors report intestinal worm infections with high persistence and resistance especially of *Ascaris* eggs.

Many studies showed that raw wastewater presents evident contents of parasites in several taxa (Amahmid et al., 2002; Bouhoum et al., 2002; Scott-Emuakpor et al., 2016; Sylla and Belghyti, 2008). Among the identified parasites, we found *Ascaris* in abundance. According to Cisse et al. (2011), this abundance is linked to both their resistance as well as to their transmission mode (direct cycle). Feachem et al. (1983) explained the predominance of *Ascaris* in the environment by their extremely abundant egg production and their ability to survive. Indeed, the female *Ascaris* produce 200,000 eggs per day, compared to the 2000–10,000 eggs produced by *Trichuris trichiura*. Several authors showed 100%

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elimination of *Ascaris eggs* during wastewater treatments (Konaté et al., 2013; Lakshminarayana and Abdulappa, 1969; Maya et al., 2012). Amahmid et al. (2002) attributed this reduction of parasite eggs during the treatment of wastewaters to their accumulation in the solid phase (sludge). The sedimentation speed is estimated by Shuval et al. (1986) at 65cm/h for *Ascaris* eggs. Dehydration and liming of sludge are often used as solutions to reduce and/or to eliminate their load of parasites (Cappizzi-banas et al., 2004; Gaspard and Schwartzbrod, 2003).

During the agricultural usage of the activated sludge, many studies have focused on the verification of their harmlessness in terms of organic pollutants (Phathalates, HAPs, PCB, etc.) and metallic pollutants (Amir et al., 2005). Nevertheless data relating to the fate of parasites during the composting of sludge are rare or non-existent. In this perspective, the objective of the present study was to assess the quantities of helminth eggs in dehydratated sludge from the activated sludge plant of Marrakesh city and to follow their removal during composting of the sludge with date palm waste. We also determined the parameters involved in the effectiveness of helminth eggs deactivation by the co-composting procedure.

## 2. Material and methods

### *2.1. Co-composting trials*

Two trials of composting were run with activated sewage sludge from industrial, urban and slaughterhouses (with urban and slaughterhouses predominance) wastewater treatment plant (WWTP) and date palm waste from Marrakesh city green spaces mixed (v/v) in different proportions according to El Fels et al. (2014):

-Mixture A: 1/3 activated sludge + 2/3 date palm tree waste, total volume: 4 $m^3$

-Mixture B: 1/2 activated sludge + 1/2 date palm tree waste, total volume: 4 $m^3$.

Windrows were turned over manually each week to aerate the mixture. 1Kg of homogeneous samples was taken by quartering method at $T_0$ (first day of composting) and after each aerating of the mixture. The samples were kept at -20°C until analysis.

### *2.2. Physico-chemical analysis*

The main physico-chemical parameters were measured such as temperature, pH, moisture, total organic carbon and nitrogen according to El Fels et al. (2014). The temperature was measured every day with sensors with data memory (PH0700115 model 1.20, Ector-Traçability software, ECTOR France). Moisture contents were determined by drying 100 g of co-compost at 105 °C for 48 h. The pH was measured in an aqueous extract of the compost (1g / 10 mL of distilled water). Total organic carbon and ash content were determined after 6h of calcination in a muffle furnace at 600 °C. Total Kjeldahl Nitrogen (TKN) was assayed by using classical Kjeldahl procedure. Likewise, ammonium ion content was assayed by alkaline distillation and nitrates after reduction by Dewarda alloying (Barje et al., 2012; El Fels et al., 2014).

### *2.3. Method of extraction and identification of helminth eggs*

Owing to their relatively low number density for direct microscopic examination (Thevenot et al., 1985), the parasite eggs were concentrated by various means. 5 g of fresh compost were used for each count. To concentrate the eggs, we used the flotation method for the analysis of biowaste (Bowman et al., 2003; US EPA Protocol, 1999) modified by Koné et al. (2007) and Schwartzbrod (2003). After disruption of the particles by ammonium bicarbonate (11.9 %) for a few minutes, and because of the low density of *Ascaris* and *Trichuris* eggs, 1.10 and 1.15 respectively (David and Lindquist, 1982), and after centrifugation at 1389 g/ 3 min, the pellet was re-suspended in 40 ml of $ZnSO_4$ (56.81%). After equilibration for 10 min, the mixture was centrifuged again at 617 g/ 3min, and the supernatant recovered centrifuge tubes then washed with distilled water and centrifuged at 964 g/ 3 min. These tubes were combined by rinsing into a test tube to recover all the eggs. Then, a final centrifugation at 964 g for 3 minutes yielded a pellet suitable for analysis. The helminth eggs were identified at 400× magnification. The eggs were then counted using a two-chambered MacMaster slide with a capacity of 0.3 ml (0.15 ml under each grid). Photomicrographs were made using a binocular microscope (camera: Moticam 1000, 1.3 M Pixel USB 2.0, lens 16 MM, ø28). The total number of helminth eggs was expressed per gram of fresh sample.

## 3. Results and discussions

### *3.1. Quantitative and qualitative analysis of the parasitic load of raw sludge*

The results of helminth eggs microscopic identification (Table 1) show that the sludge contained 4 to 27 eggs/g of fresh sludge. These findings are in agreement to that observed by several authors. Ayres et al. (1993) reported egg concentrations from 9 to 41.5 eggs /g, and Koné et al. (2007) isolated up to 22-38 helminth eggs (*Ascaris* and *Trichuris*) /g of sludge, of which 25 to 50% were still viable after the dehydration of the sludge. These levels are above those authorized for the usage of non-treated sludge according to WHO guidelines 2006 for the safe reuse of fecal sludge.

The parasites identified were essentially the nematodes *Ascaris* sp., *Capillaria* sp. and *Trichuris* sp. The predominance of nematode eggs compared to those of other groups has been mentioned in other works (Schwartzbrod and Banas, 2003). These authors attributed this result to the greater resistance of intestinal nematode eggs (in comparison to the eggs of

cestodes) in wastewater, leading to higher concentrations.

The difference in the concentrations of the identified nematodes can be explained by the initial load of these parasites in wastewater and consequently in the sludge, that is in relationship with the effluent nature especially those from urban and slaughterhouses. In the present case, *Capillaria* sp. eggs were the most numerous, followed by *Trichuris* sp. and finally *Ascaris* sp. the latter appearing at higher densities in many studies (Maurer et al., 2009; Tonner-Klank et al., 2007). The high *Capillaria* sp. prevalence could be due to a multitude of factors, such as seasonal variation, and their load variation, which could be directly explained by changes in food availability. On the other hand, besides being of human origin, *Capillaria* are the most common parasites in animals such as badgers (Delahay et al., 2006; McDonald et al., 2008; Wobeser, 2002). In this study the high prevalence of *Capillaria* sp. is likely due to wildlife, especially sewer animals. Horak (1992) identified *Hymenolepis* sp. in sludge, and attributed it to rats living in the sewer systems.

According to Vosta (1958), *Hymenolepis* sp. is known to be quickly destroyed and cannot survive in wastewater or sludge. This probably accounts for the absence of this parasite in our samples. Furthermore, physical phenomena (decantation in the pools or formation of organic matter-parasite aggregates), the nature of activities carried out in the area feeding the sewer systems, demographic evolution and the state of health of the population, systematically influences the quality and quantitative composition of sludge. In our case, the Marrakesh activated sludge plant receives the wastewaters of the city especially urban, and of the slaughterhouse, this accounts for the high load of helminth eggs in the sludge samples.

The sludge had previously undergone treatment by dehydration, but this did not appear to be effective for the complete elimination of helminth eggs. Koné et al. (2007) showed that the process of dehydration of fecal sludge on drying beds is not effective for the inactivation of all the helminth eggs. They also showed that nematode ova of *Ascaris and Trichuris* are 25 - 50% viable, after the dehydration of sludge. The WHO directives for irrigation with wastewater require a norm of less than 1 egg of *Ascaris, Trichuris* or *Hookworm* per liter. The persistence of parasite eggs in sufficient quantities after dehydration and the sanitary risks that they represent, suggest the utility of additional treatment to ensure the harmlessness of the sludge.

Tables 2 and 3 presented the mean physic-chemical characteristics of co-composting samples, according to El Fels et al. (2014). The results show that the composting is a good way to stabilize the organic matter (Perteghella and Vaccari, 2017).

During the co-composting, the identified helminth eggs were the same as those found initially in dehydrated sludge namely: *Ascaris* sp., *Capillaria* sp., and *Trichuris* sp. The presence and the abundance of the eggs of *Capillaria* sp. followed by *Trichuris* sp. and *Ascaris*sp. are well marked in the initial co-composting mixtures with a lower concentration of the total helminths than in the sludge only (Fig. 1). The variability in the numbers of the helminth eggs observed at $T_0$ in the two mixtures A and B (Table 1, Fig. 1, Fig. 2), is due to the different proportions of mixed sludge with the palm tree waste.

The random distribution of these two substrates (sludge and green waste) for mixtures A and B and the structure of the ligno-cellulosic matrix onto which the helminth eggs can be adsorbed, partially explains the differences noted in helminth eggs numbers. Similarly, Koné et al. (2007) showed that when the materials are digested and the particles are finer, this variability of egg numbers diminishes because of the greater consistency of the sample.

**Table 1.** Numbers of helminth eggs versus co-composting time in mixtures A and B

| ***Helminth eggs*** | ***Sludge /g (FM)*** | ***Mixture A*** | | | | ***Mixture B*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***T0*** | ***T1*** | ***T2*** | ***T3*** | ***T0*** | ***T1*** | ***T2*** | ***T3*** |
| | | ***MS*** | ***TH*** | ***MT*** | | ***MS*** | ***TH*** | ***MT*** | |
| Total nematods eggs | 4-27 | 8 | 1 | 0.3 | - | 12 | 2.3 | 0.3 | - |
| *Ascaris* sp | 0-3 | 1 | 0.3 | - | - | 1 | 0.5 | - | - |
| *Capillaria* sp | 3.5-16.3 | 5 | 0.3 | 0.3 | - | 7 | 0.6 | 0.3 | - |
| *Trichuris* sp | 0.5-7.6 | 2 | 0.3 | - | - | 4 | 0.3 | - | - |

*FWt: Fresh matter, T0:initial stage, T1: One month, T2: Two months, T3: Three months.MS: Mesophilic phase, TH, Thermophilic phase, MT, Maturation phase*

**Table 2.** Main features of the substrates co-composted

| ***Parameters*** | ***Palm waste*** | ***Sludge*** |
|---|---|---|
| pH | 6.31 ±0.08 | 6.45 ±0.09 |
| Moisture/(% FWt) | 25.1 ±0.21 | 46.46±0.26 |
| Ash content (% DWt) | 9 ±0.1 | 43.80±0.02 |
| TOC (% DWt) | 50.5 ±0.61 | 31.20±0.11 |
| TKN (% DWt) | 1.36 ±0.24 | 1.50 ±0.2 |
| C/N | 37.13 | 20.8 |

*FWt: Fresh weight; DWt: dry weight; TOC: total organic carbon; KTN:Kjeldahl total nitrogen*

**Table 3.** Physico-chemical parameters during the co-composting of mixtures A and B

| *Mixtures* | *Time of co-composting (months)* | *%TKN** | *Moisture* | *$NO_3^-$* | *$NH_4^+$* | *C/N* | *DEC (%)** | *pH* |
|---|---|---|---|---|---|---|---|---|
| **A** | 0 | 1.31±1.2 | 58.83 | 0.016±0 | 0.22±0.03 | 26.2 | - | 6.34±0.03 |
| | 1 | 2.29±1.32 | 69.5 | 0.046±0.01 | 0.12±0.04 | 12.8 | 20.03±0.9 | 6.33±0.3 |
| | 2 | 2.79±1.5 | 66.8 | 0.075±0.02 | 0.05±0.02 | 9.92 | 28±1.2 | 6.51±0.2 |
| | 3 | 2.2±1.42 | 66.94 | 0.065±0.01 | 0.02±0 | 11.2 | 33.4±1.17 | 6.59±0.37 |
| | 6 | 2.18±1.1 | 66 | 0.075±0.01 | 0.009±0 | 10.09 | 40.07±1.1 | 6.79±0.06 |
| **B** | 0 | 1.28±1.09 | 60.97 | 0.028±0.03 | 0.21±0.01 | 27.4 | - | 6.04±0.28 |
| | 1 | 2.16±1.22 | 68.8 | 0.06±0.02 | 0.15±0.01 | 14.39 | 18.22±0.2 | 6.4±0.56 |
| | 2 | 2.72±1.51 | 66.2 | 0.1±0.01 | 0.05±0.01 | 10.8 | 23.4±0.12 | 6.65±0.27 |
| | 3 | 2.21±1.08 | 66.56 | 0.6±0. 1 | 0.02±0 | 11.5 | 33.39±1.75 | 6.99±0.28 |
| | 6 | 2.28±1.21 | 66 | 0.07±0.02 | 0.009±0 | 10.08 | 40±1.49 | 7.03±0.08 |

*(*): Results expressed per unit weight dry matter.TKN = Total Kjeldahl Nitrogen; DEC = Decomposition rate*

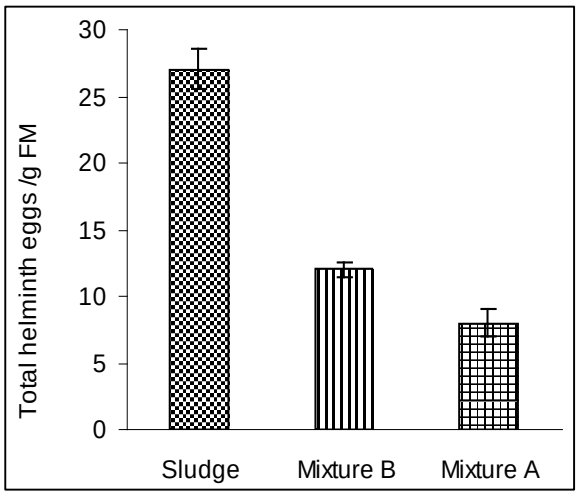
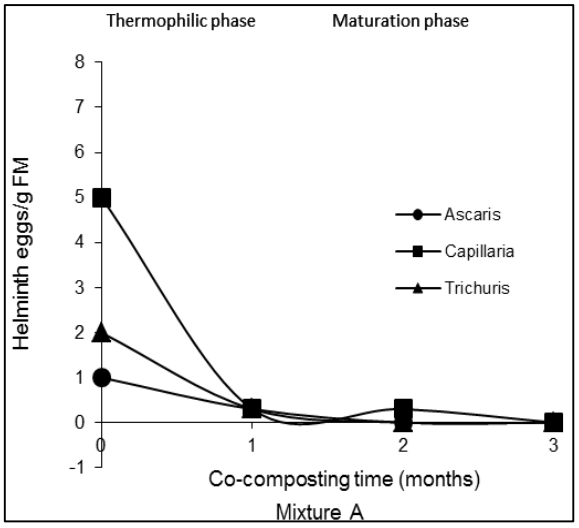



**Fig. 1.** Total helminth eggs per g of fresh sludge material and of mixture A and B at the first stage ($T_0$)

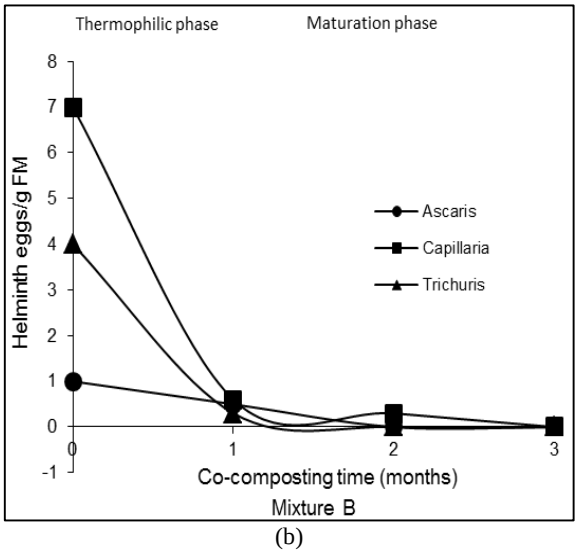
During co-composting, we observed a decrease in the concentration of total helminth eggs (Table 1). This corresponds to a reduction of 88 and 81% during the thermophilic phase for mixtures A and B respectively. At this co-composting phase, the reduction in the numbers of *Ascaris* sp. eggs was 70 and 50%, *Trichuris* sp. was 85 and 92% and *Capillaria* sp. was 94 and 91%, for mixtures A and B respectively. At the maturation phase of co-composting, 100% for all helminth eggs was obtained (Fig. 2).

The strong reduction in the number of helminth eggs during the stabilization phase is explained by the increase in temperature of the windrows (over 65° C) (Fig. 3).

Many physical-chemical parameters could inactive the helminth eggs, such as temperature and ammonia content. High temperatures are damaging the cells of the helminths and their capacity to survive. In our case, the helminth eggs were exposed to such temperatures for several days, which effectively inactivated them. Other authors (Cappizzi-Banas et al., 2004; Gaspard and Schwartzbrod, 2003) showed that high temperatures accelerate the rate of desiccation of *Ascaris* cells. Koné et al. (2007) showed that a temperature of over 45° C for more than 30 days destroyed the cells and inactivated the *Ascaris* eggs. This composting phase is called the stabilization and hygienization phase (El Fels et al., 2014; Zeng et al., 2011). The range of thermal inactivation of the pathogens depends on the lethal temperatures, the length of exposure, and the proportions of biosolids in the mixture (Hay, 1996).

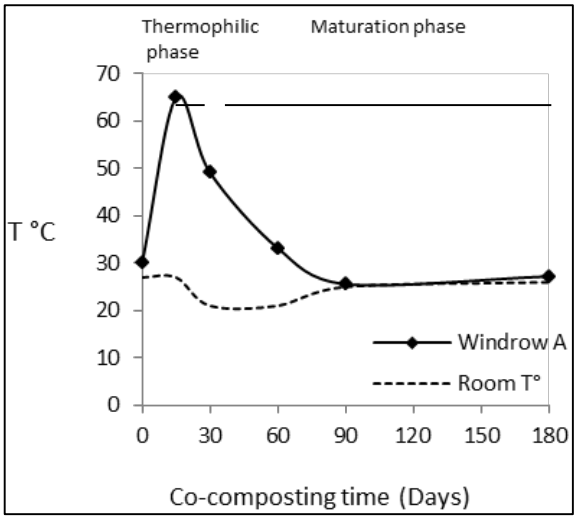



(a)

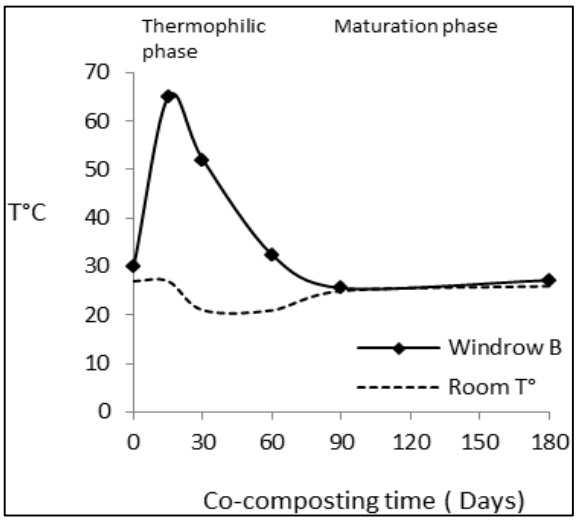



(b)

**Fig. 2.** Numbers helminth eggs versus co-composting time for mixtures A(a) and B (b)




(a)




(b)

**Fig. 3.** Temperature versus co-composting time for mixtures A (a) and B (b)(El Fels et al., 2014)

The time necessary to attain high levels of helminth inactivation (> 95%) is very variable in the literature, reports varying from 2 hours to 180 days, according to the temperature level (Brewster et al., 2003; Gantzer et al., 2001). Mesophilic temperatures (<40° C), either with aerobic or anaerobic digestion, will have a partial effect on the inactivation of *Ascaris* eggs. Reimers et al. (1998) determined the time required to completely inactivate the *Ascaris* eggs by aerobic digestion. They showed that at 25° C, complete inactivation requires aerobic digestion of 130 days while at 57° C, it only requires only 2 days.

In order to confirm the relationship between the temperatures reached during composting and the length of exposure of the parasites, Feachem et al. (1983) described a theory linking time and temperature and the inactivation of the pathogens. Further, Vinnerås et al. (2003) reported an equation (Eq. 1) for *Ascaris* sp. to determine the time (in hours) necessary, at any temperature, to obtain a total inactivation of these pathogens (Table 4). This equation is shown below:

$$t = 177 * 10^{-0.1922\,(T-45)} \quad (1)$$

where: $t$ is temperature; $T$ is time.

**Table 4.** Equations derived from (Feachem et al., 1983; Vinnerås et al., 2003) for the time in hours (t) required to attain absence of viable organisms (equal to 12 $\log_{10}$ inactivation) of different helminths at different temperatures (T°C) above 45°C

| ***Helminths*** | ***Equation*** |
|---|---|
| ***Ancylostoma*** | $t = 9.31*10^{-0.1340(T-45)}$ |
| ***Ascaris*** | $t = 177*10^{-0.1944(T-45)}$ |
| ***Schistosoma*** | $t = 10*10^{-0.1844(T-45)}$ |
| ***Taenia*** | $t = 6.6*10^{-0.1306(T-45)}$ |

Based on the inactivation figures of Feachem et al. (1983) and Vinnerås et al. (2003) we can say that theoretically, the inactivation of all *Ascaris* eggs will take place if the temperature in the composting windrows remains above 45° C for at least 5 days. The same result can obtained with 8 days at 44° C, 12 days at 43° C, 19 days at 42° C, a month at 41°C or 1.5 months at 40° C. While referring to this theoretical curve, we can conclude that the conditions of temperature attained in our case are in favor of the inactivation of the *Ascaris* sp. eggs; as well as *Capillaria* sp. *and Trichuris* sp. that are less resistant.

Other compounds can play a role in the inactivation of pathogens, including organic acids, aldehydes and alcohols (Reimers et al., 2001). Pecson and Nelson (2005) showed that the presence of ammonia at concentrations usually found in sludge led to 99% of eggs inactivation. The increase in ammonium content during stabilization phase of co-composting (Table 3) contributed to the inactivation of the parasite eggs. Ammonia is present naturally in wastewater after the hydrolysis of urea and the degradation of proteins and other nitrogen-containing compounds. Other authors (Ghiglietti et al., 1997; Mendez et al., 2002) also studied the effect of ammonia $NH_3$ on the inactivation of *Ascaris* eggs. Indeed, the permeability of the *Ascaris* shell is principally controlled by membrane lipids, the permeability of which increases with an increase in the temperature of composting (Barrett, 1976; Fairbairn and Passey, 1955). This could make the eggs very sensitive to the $NH_3$ effects, which leads to an increase in the intracellular pH, and consequently induces cell inactivation (Jenkins et al., 1998).

On the other hand, the decrease in the level of moisture during the second phase of co-composting and the increase in pH that became stabilized at around neutrality, can contribute to the inactivation of the remaining helminth eggs, to obtain a reduction of 100%. Sanguinetti et al. (2005) showed that the decrease in water of the environment favors the inactivation of the parasites. The reduction of helminth eggs (99-100%) (Fig. 2) is obtained at the end of two months of co-composting, for both mixtures A and B. It is linked to the different parameters of co-composting (temperature, pH,

ammonium, etc.) and produces helminth eggs-free composts which is in accordance with the recommendations of the WHO (2006) for the reuse of sludge set at 1 egg /g or less.

## 4. Conclusions

The activated sludge was initially loaded with *Ascaris* sp. (0-3 ova/g), *Capillaria* sp. (3.5 – 16.3 ova/g) and *Trichuris* sp. eggs (0.5 – 7.6 ova/g). The treatment of the sludge by co-composting with the ligno-cellulosic waste totally eliminated the helminth eggs after 6 months of co-composting process.

The inactivation of the helminth eggs is linked to the different parameters of co-composting, in particular the temperature, ammonia content, pH and moisture.

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