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Microbiological Assessment of Sludge Treatment at the 6th October Wastewater Treatment Plant, Giza, Egypt

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THIS STUDY was carried out to assess the sludge treatment processes applied at the 6th October wastewater treatment plant (WWTP), Giza, Egypt. The applied processes included aerobic digestion followed by gravity thickening, filter-pressing and drying beds (in emergency cases). Bacteriological, virological and parasitological evaluation was carried out. Results showed that aerobic digestion removed two log units of total and faecal coliforms and one log unit of *Escherichia coli* (*E. coli*) and faecal streptococci. The removal percentages of coliphage and enteroviruses after aerobic digestion were 97.0 and 58.3 %, respectively. The salmonellae disappeared from sludge after aerobic digestion. Qualitative and quantitative decrease in helminth ova (*Ascaris*, *Trichuris*, *Trichostrongylus*, *Taenia* and *Hymenolepis*) was observed after aerobic digestion and thickening stages. An average decrease of 4 log units for total and faecal coliforms, 3 log units for each of faecal streptococci and coliphage and 2 log units for *E. coli* through sludge treatment process was observed. Enteroviruses, helminth ova and enteric protozoa were completely eliminated from sludge after 6 months in drying beds. The loads of faecal coliform, *E. coli*, faecal streptococci and coliphage in the dried sludge were 6.5×10^2, 1.6×10^2, 4.5×10^2 cfu and 8.5×10^1 pfu/g, respectively. Microbiological quality of the final treated sludge complied with the regulations of United States Environmental Protection Agency.

Keywords: Sludge Treatment, Bacterial indicators, Coliphage, Enteroviruses, Protozoa and Helminth ova .

Wastewater treatment results in the formation of slurries, high in suspended solids, commonly called sludge. The application of sewage sludge in agriculture is beneficial, being a source of nutrients and improving soil properties (Gantzer *et al.*, 2001). Sludge use in agriculture is acceptable only if its sanitary quality is sufficiently guaranteed. This is achieved by treatment of sludge, aiming at the reduction of both water content and pathogenic organisms that may cause public health problems (Nowak *et al* , 2003).

Faecal microorganisms, whether pathogenic or nonpathogenic, are present in sludge in concentrations higher than that of wastewater. Regarding pathogens the regulations for use of sludge in agricultural purposes are based on three principles: a) the requirement for treatment including thermal treatment to reduce the amount of pathogens, b) validation of the treatment and c) assurance of the microbiological quality of sludge (U.S. EPA, 1993; Moce-Llivina *et al*, 2003 and Nelson *et al.* 2004). With the implementation of European directives concerning municipal wastewater purification, the quantity of sludge produced will be doubled over the next 10 years (Gantzer *et al.*, 2001).

This study aimed to assess the application of a treatment system (aerobic digestion followed by gravity thickeners, filter-pressing and drying beds for emergency cases) to the sludge produced from the 6^{th} October wastewater treatment plant (WWTP). The study focused on monitoring the sludge quality after the different treatment steps and evaluating the efficiency of the successive operating units for the removal of biological contaminants.

Material and Methods

Description of the treatment plant

The city of 6^{th} October is located in the northern west of Giza Governorate, Egypt. It is considered one of the largest new residential and industrial communities in the country. Treatment of the wastewater and the corresponding produced sludge of the city are conducted in a newly constructed wastewater treatment plant (6^{th} October WWTP). The starting overall capacity of the plant is 90000 m^3/day of municipal wastewater that can be increased later to 180000 m^3/day. The plant was designed to contain several units for sludge treatment as follows:

* One square aerobic sludge digester of 45 m in length and equipped with 9 turbine aerators each of 90 K.W. power.
* Circular gravity thickeners (18 m in diameter with average depth of 3 m).
* Mechanical dewatering (filter-pressing) plant with a capacity of 30 m^3/h.
* Forty eight drying bed units (each of 10 m x 20 m equipped with 40 cm gravel filter layer and a maximum sludge layer of 35 cm).

The drying beds were designed to act as a complementary step in emergency cases when the mechanical dewatering plant is out of order or as a further stabilization step of pressed sludge to reach safe reuse of sludge as agricultural fertilizer.

Figure 1 representes general schematic diagram of the applied sewage sludge treatment steps as well as the corresponding sampling sites in the 6^{th} October WWTP.

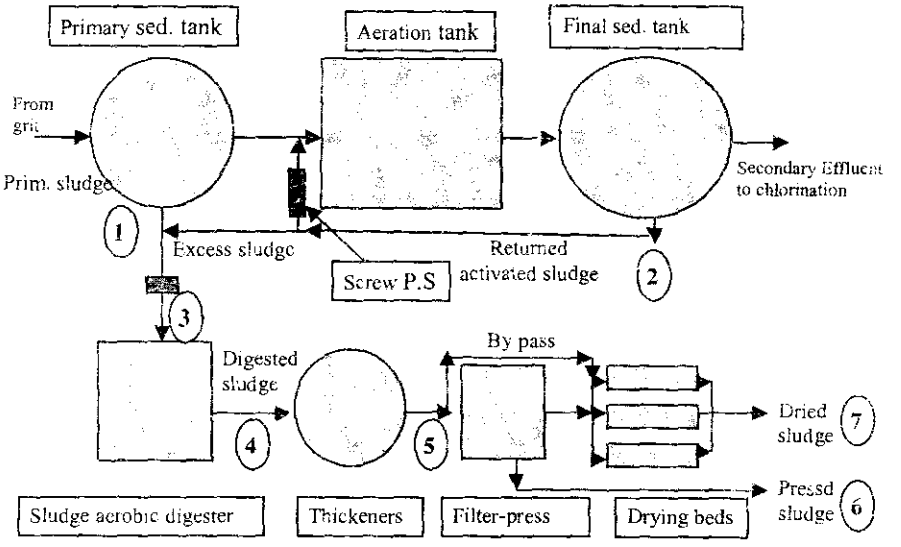

Fig.1. Schematic diagram and sampling locations of sludge treatment process in the 6th October WWTP, Giza, Egypt.

Samples and sampling sites

Seven locations, in the 6th October WWTP, were selected and sampled to evaluate the sludge treatment process as indicated in Table 1. Briefly locations no. 1 and 2 denoted sludge obtained from primary and secondary sedimentation tanks, respectively. Location no. 3 represented mixture of primary and secondary sludge (1+2), while location no. 4 (aerobic digestion) was considered the prime stage of sludge treatment that was followed by thickening (location no. 5), filter pressing (location no. 6) and drying beds (location no. 7). Each location was examined monthly along the period from June to September 2004.

TABLE 1. Sampling locations at the 6th October WWT .

Location No.	Sampling location
1	Primary sedimented sludge
2	Secondary sedimented sludge
3	Mixture of primary and secondary sedimented sludge (1+2)
4	Aerobically digested sludge
5	Thickened sludge
6	Filter-pressed sludge (Mechanically dewatered sludge)
7	Sludge after 6 months drying in drying beds

Determination of dry weight

Dry weight contents of the collected sludge samples were determined and calculated as the method of APHA (1998).

Bacteriological analysis

Total coliform (TC) bacteria were determined by the most probable number (MPN) technique as recommended by APHA (1998) using MacConkey broth (Merck, Germany) as a presumptive test and EMB agar for confirmation (El-Abagy *et al.*, 1994).

Faecal coliform (FC) bacteria were detected by re-inoculation of gas-positive tubes in MacConkey broth followed by incubation at 44.5^{0}C for another 24 hr. *Escherichia coli* (*E. coli*) bacteria were detected according to Lee *et al.* (1997). Fecal streptococcus (FS) bacteria were determined by MPN technique. Salmonellae were detected by the qualitative method according to APHA (1998). Coliphage was determined by using *E. coli* ATCC 13706 as a host according to APHA (1998).

Virological analysis

a) Virus concentration was determined according to Schwartzbrod and Mathieu (1986). Briefly, liquid sludge samples were first centrifuged (3000 rpm for 15 min) to obtain a pellet which was then eluted as follows:

Three hundred and sixty milliliters of 10 % beef extract, pH 9 (Oxoid, London, England) were added to 40 g of solid sludge (the pellet) followed by agitation for 3 min, sonication on ice for 5 min and centrifugation at 3000 rpm for 15 min. Then, the organic flocculation technique according to Katzenelson *et al.* (1976) was carried out. The supernatant was adjusted to pH 3.5 followed by magnetic stirring for 30 min and centrifugation at 3500 rpm for 20 min. The final pellet was dissolved in 10 ml 0.15 M Na_2HPO_4 buffer (BDH Limited Poole, England) (pH 7.2) to give the virus concentrate. Decontamination and detoxification of the samples were done using chloroform (BDH Chemicals Ltd Poole England) as follows:

One hundred µl of chloroform was added to 300 µl of concentrated samples, mixed by vortexing and let in contact for 10 min, followed by centrifugation at 16000 xg for 20 min. The upper layer was transferred to a new eppendorf tube. Aeration was done gently to get rid of the residues of chloroform (El-Senousy *et al.*, 2004).

b) Viral assays: Plaque assay was used with a minor modification of the technique originally described by Hausing and Melnick (1957). Titres were measured using BGM continuous cell line cultivated in Eagle's minimum essential medium (MEM, Gibco, England) supplemented with 10 % fetal calf serum (Sigma, Germany). Volumes of 200 µl were inoculated onto BGM monolayer grown in 12 multi well plates. After 1 hr incubation at 37^{0}C, the inoculum was removed and the cells were overlaid with agarose (BDH Limited Poole, England) and 2X MEM (1:1). Plaques were counted after 5 days

incubation at 37 ^{0}C. Viral concentrations were expressed as pfu/g of dry weight sludge.

Parasitological analysis

The U.S. EPA method (U.S. EPA, 1999) was used to concentrate, detect and enumerate helminth ova. Samples were mixed with buffered water containing a surfactant (Triton X-100, Aldrich Chemical Co. Ltd., England) and large particles were removed. The solids were allowed to precipitate and the supernatant was decanted. The sediment was subjected to a density gradient centrifugation using magnesium sulphate (BDH Limited Poole England) (specific gravity 1.2). Small particles were removed by a second screening on a small mesh size screen and proteinous material by an acid alcohol/ethyl ether extraction. The concentrate was subsequently examined microscopically for the presence of helminth eggs by using Sedgwick-Rafter counting chamber (Hausser Scientific, Horsham Pa, 19044 USA). Identification of the detected helminth ova was performed on the bases of morphological and dimensional criteria by the aid of Garcia and Bruckner (1997). Stained smears from formalin-fixed pellets of concentrate were prepared and examined microscopically for detection of *Giardia* cysts (by chlorazol black E), *Cryptosporidium* oocysts and spores of microsporidia (by modified Kinyoun acid-fast) according to Alles *et al.* (1995).

Statistical analysis

The arithmetic averages of percent removal and descriptive statistics were applied to the collected data using Microsoft Excel version 5.

Results

Dry weight of sludge samples, indicating sludge water content and settlement efficiency, are shown in Table 2. The dry weight of primary sludge was always higher than the dry weight of the secondary sludge. Gradual increase in sludge dry weight was observed after aerobic digestion, thickening and filter-pressing stages. Sludge dry weight of traditional drying beds was much higher than that produced from the filter-press.

TABLE 2. Total solids (g/l) of sludge from different treatment stages.

Location No.	Total solids g/l (dry weight basis)				
	1st run	2nd run	3rd run	4th run	Mean
1	36.1	56.6	45.2	38.7	44.2
2	14.3	16.5	3.4	21.3	13.9
3	62.2	63.5	56.0	64.4	61.5
4	43.3	95.0	82.2	91.8	78.1
5	73.6	155.0	113.8	94.5	109.2
6	174.9	122.0	108.9	134.3	135.0
7*	423.7	727.0	388.4	579.7	529.7

* Drying time : six months .

The mean values of classical bioindicators of faecal pollution (Table 3) as well as alternative enteric microorganisms (Table 4) were determined at the different treatment stages of sludge. The stepwise removal percentages of the detected microorganisms were summarized in Table 5.

TABLE 3. Classical bacteriological and helminthological examination of sludge during different treatment stages.

Location No.	Total coliform	Faecal coliform	*E. coli*	Faecal streptococci	S. (+/-)*	Helminthes ova (Mean count/ 5g**)
	MPN index (mean cfu/g**)					
1	$4.4x10^9 \pm 2.4x10^9$	$4.7x107 \pm 1.6x107$	$3.2x106 \pm 8.2x105$	$8.8x10^6 \pm 4.2x10^6$	+	*Ascaris* 24.5 ± 6.1 *Trichuris* 4 ± 2.5 *Trichostrongylus* 1.3 ± 1.3 *Taenia* 1.5 ± 0.9 *Hymenoleps* 2 ± 1.2
2	$2.8x10^8 \pm 2.1x10^8$	$2.7x10^6 \pm 1.8x10^6$	$7x10^4 \pm 2.5x10^4$	$6.9x10^5 \pm 2.5x10^5$	+	*Ascaris* 6.8 ± 1.9 *Trichuris* 0.8 ± 0.5 *Taenia* 0.3 ± 0.3
3	$2.5x10^8 \pm 2x10^8$	$2.4x10^6 \pm 2x10^6$	$4.1x10^4 \pm 1.3x10^4$	$2.4x10^5 \pm 1.6x10^5$	+	*Ascaris* 17 ± 4.3 *Trichuris* 2.8 ± 1.6 *Trichstrongylus* 1 ± 0.7 *Taenia* 0.5 ± 0.5 *Hymenolepis* 1 ± 0.6
4	$3.2x10^6 \pm 1.7x10^6$	$5.5x10^4 \pm 1.5x10^4$	$5.1x10^3 \pm 8.9x10^2$	$3.6x10^4 \pm 1.9x10^4$	-	*Ascaris* 4 ± 2.3 *Trichuris* 0.3 ± 0.3 *Hymenolepis* 0.3 ± 0.3
5	$3.2x10^6 \pm 1.7x10^6$	$4.2x10^4 \pm 2.1x10^4$	$4.7x10^3 \pm 1.2x10^3$	$2.3x10^4 \pm 1.8x10^4$	-	*Ascaris* 0.8 ± 0.5
6	$3.2x10^6 \pm 2x10^6$	$9.8x10^3 \pm 3.8x10^3$	$9.5x10^2 \pm 2.6x10$	$3.3x10^3 \pm 1.5x10^3$	-	0
7	$1.2x10^5 \pm 8.2x10^4$	$6.5x10^2 \pm 3x10^2$	$1.6x10^2 \pm 1x10^2$	$4.5x10^2 \pm 1.5x10^2$	-	0

* Salmonellae (presence /absence)
** Total solids (dry weight basis)

TABLE 4. Alternative viral and protozoal examination of sludge during different treatment stages.

Location No.	Coliphage (pfu/g*)	Enteroviruses (pfu/g*)	Pathogenic protozoa (count/5g*)
1	$2.3x10^6 \pm 7.0x10^5$	28.8 ± 16.0	*Cryptosporidium 24.0 ± 3.4* *Giardia* 1.8 ± *0.9* Microsporidia *2.5 ± 1.0* *Isospora 0.3 ± 0.3* *Entamoeba 0.3 ± 0.3*
2	$3.3x10^5 \pm 1.8x10^5$	10.3 ± 5.5	*Cryptosporidium 9.5 ± 1.0* *Giardia 0.3 ± 0.3* Microsporidia *0.8 ± 0.5* *Entamoeba 0.3 ± 0.3*
3	$1.2x10^5 \pm 9.6x10^4$	10.5 ± 5.1	*Cryptosporidium 13.5 ± 1.7* *Giardia 1.0 ± 0.7* Microsporidia *2.0 ± 0.8*
4	$3.6x10^3 \pm 1.5x10^3$	4.3 ± 3.6	*Cryptosporidium 6.8 ± 1.2* *Giardia 0.3 ± 0.3* Microsporidia *0.3 ± 0.3*
5	$1.1x10^3 \pm 3.2x10^2$	0.9 ± 0.6	*Cryptosporidium 3.3 ± 1.1*
6	$6.7x10^2 \pm 1.7x10^2$	0.1 ± 0.1	*Cryptosporidium 1.0 ± 0.4*
7	$8.5x10^1 \pm 3.9x10^0$	0	0

*Total solids (dry weight basis).

TABLE 5. Stepwise removal percentages of detected microorganisms through sludge treatment stages.

Organisms		Removal (%)			
		Aerobic digestion	Thickening	Filter-pressing	Drying beds
Bacterial indicators	Total coliform	98.7	0	0	96.3
	Faecal coliform	97.7	23.6	76.7	98.5
	Escherichia coli	87.6	7.8	79.8	96.6
	Faecal streptococci	85.0	36.1	85.7	98.0
Viruses	Coliphages	97.0	69.4	39.1	92.3
	Enteroviruses	58.3	79.9	85.3	100
Helminth ova	*Ascaris*	76.5	81.3	100	100
	Trichuris	89.8	100	100	100
	Trichostrongylus	100	100	100	100
	Taenia	100	100	100	100
	Hymenolepis	66.7	100	100	100
Protozoa	*Cryptosporidium*	50.0	51.9	69.3	100
	Giardia	75.0	100	100	100
	Microsporidia	87.5	100	100	100

The mean values of TC, FC, *E.coli*, FS, coliphage and enteroviruses (counting around 10^8, 10^6, 10^4, 10^5, 10^5 and 10^1 pfu/g, respectively) were similar in both secondary and mixed sludge. Also pathogenic protozoa in mixed sludge were qualitatively related to that of secondary sludge. On the contrary, helminth ova in mixed sludge were qualitatively related to that of primary sludge. Salmonellae and ova of both *Trichuris* and *Taenia* were detected only in raw sludge in samples taken from locations 1, 2 and 3.

Through acrobic digestion the average densities of classical bacterial indicators and coliphage decreased by two logs for TC (98.7 %), FC (97.7 %) and coliphage (97.0 %) and one log unit for *E.coli* (87.1 %) and FS (85.0 %) (Tables 3 and 5). The removal percentages of *Trichuris*, *Ascaris* and *Hymenolepis* ova reached 89.8, 76.4 and 66.7 % while microsporidial spores, *Giardia* cysts and *Cryptosporidium* oocysts were 87.5, 75.0 and 5.0 %, respectively. The removal percentage of enteroviruses was 58.3 % (Table 5).

The thickening stage resulted in the removal of all helminth ova and intestinal protozoa, except 18.7 % of *Ascaris* ova and 48.1 % of *Cryptosporidium* oocysts. The removal percentages of enteroviruses, coliphage, FS, FC and *E. coli* through thickening process reached 79.9, 69.4, 36.1, 23.6 and 7.8%, respectively.

The filter-pressing stage removed *Ascaris* ova completely, while *Cryptosporidium* oocysts were still present. The classical bacterial indicators as well as coliphage were reduced by one log unit in the filter-pressing stage. The stepwise removal percentage of enteroviruses reached 85.2% by filter-pressing stage. While aerobic digestion removed 98.7 % of total coliforms, the residual coliforms were not removed by sludge thickening and/or pressing.

After 6 months drying in drying beds, complete removal of enteroviruses and *Cryptosporidium* oocysts was achieved, while TC, FC, FS, *E. coli* and coliphage were reduced by 96.3, 91.9, 86.4, 83.2 and 87.3 %, respectively.

Discussion

The process of sludge treatment is mainly based on the reduction of both water content and pathogenic microorganisms that may cause public health problems (Vesilind *et al.*, 1986).

Aerobic digestion of sludge for 1 day detention time resulted in the removal of classical bacterial indicators of pollution by ≥ 97 % for total and faecal coliforms and ≥ 85 % for both *E. coli* and faecal streptococci with complete removal of Salmonellae. This process had also a considerable effect on the removal of coliphage (97.0 %) and enteroviruses (58.3 %) with a qualitative and quantitative decrease in helminth ova and intestinal protozoa. Thermophilic sludge treatment via aerobic digestion reduced the number of somatic coliphages by 0.5 and one log unit which may guarantee reduction in the number of infectious human viruses and cysts or oocysts of protozoa to levels below the limits recommended by some guidelines (U.S. EPA, 1992 and 1993) which were not reached in our results. Bitton (1994) reported that aerobic digestion of sludge for 1 day detention time generated a temperature around 55–60^{0}C due to released free energy during oxidation and degradation bio-reactions. Other workers found that *Ascaris* ova were completely inactivated through aerobic digestion at ≥ 55^{0}C for 2 days (Plachy *et al.*, 1995; Plym-Forshell, 1995 and Reimers *et al.*, 1998). In general the reduction in densities of enteric bacteria, viruses and parasites during aerobic digestion of sludge depends on both detention time and elevated temperatures of the medium (Scheuerman *et al.*, 1991).

Our results showed that the thickening stage could eliminate all helminth ova (except *Ascaris*) and intestinal protozoa (except *Cryptosporidium*). *Ascaris* ova predominated in sludge and persisted through treatment processes more than other helminth ova, and this finding was supported by other workers (Gaspard *et al.*, 1997; Shamma & Al-Adawi, 2002 and Capizzi-Banas *et al.*, 2004). The egg shell of all helminth ova consists of three basic layers: a lipoidal inner layer, a chitinous middle layer and a proteinous outer layer. Variations in consistency and resistance between different helminth ova depend mainly on the number of amino acids incorporated into shell layers (Rojas-Valencia *et al.*, 2004). The

thickening stage also decreased the counts of FC, *E. coli*, FS, enteroviruses and coliphage. This reduction may be due to the dewatering process (Ahmed and Sorensen, 1995 and Monpoeho *et al.*, 2004). The density of total coliform was unaffected either through this stage or through the pressing process. This may be due to better survival of microorganisms associated with solids than when they are suspended in water (Scheuerman *et al.*, 1991 and Moce-Llivina *et al.*, 2003).

On the other hand, *Ascaris* ova disappeared completely during the filter-pressing process while *Cryptosporidium* oocysts were still present. The pressing stage had a great effect on the removal of FS, FC, *E.coli* and enteroviruses. This has been attributed to decrease of free water content (Straub *et al.*, 1992 and Monpoeho *et al.*, 2004).

The final stage of sludge treatment (drying beds) had a high removal of classical bacterial indicators and coliphage with complete removal of enteroviruses and *Cryptosporidium* oocysts. Sun light appears to be the most important mechanism in the inactivation of sewage microorganisms due to the damaging effect of UV and photo-oxidation. Enteroviruses, coliphage and pathogenic organisms were found to be highly susceptible to wave lengths below 342 mm UV and susceptible to photo-oxidation by a wide range of inactivating wave lengths (Sinton *et al.*, 2002).

In conclusion, from the microbiological point of view, our result showed that the treated sludge obtained only from drying beds was acceptable for reuse in agriculture according to the U.S. EPA guidelines. We should recommend that coliphage, enteroviruses and enteric protozoa are parameters that should not be neglected in the assessment of sludge treatment processes.

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التقييم الميكروبيولوجي لمعالجة الحمأة في محطة السادس من أكتوبر لمعالجة مياه الصرف الصحي ، جيزه ، مصر

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تناول البحث دراسة تقييم المراحل المختلفة لعمليات معالجة الحمأة الناتجة من محطة معالجة مياه الصرف الصحي والحمأة بمدينة ٦ أكتوبر من الناحية الميكروبيولوجية (بكتريولوجية -- باراسيتولوجية – فيرولوجية) وذلك من خلال عدة مراحل مختلفة وهي: الهضم الهوائي (aerobic digestion) – التغليظ باستخدام الجاذبية (gravity thickening) – الضغط بالترشيح (filter-pressing) – وأحواض التجفيف (drying beds).

وأظهرت النتائج ان مرحلة الهضم الهوائي ذات فاعلية كبيرة في الإزالة الميكروبية حيث وصلت نسبة الإزالة لبكتيريا القولون الكلية والبرازية (total and faecal coliforms) بواقع اثنين وحدة لوغاريتمية لكل مجموعة، كما كانت نسبة الإزالة لبكتيريا ايشريشيا كولاي (*E. coli*) والبكتيريا السبحية البرازية (faecal streptococci) بواقع وحدة لوغاريتمية واحدة لكل منهما. وفي هذه المرحلة اختفت مجموعة السالمونيلا (salmonellae) اما بخصوص الفيروسات المعوية والكوليفاج فكانت النسبة المئوية للازالة في هذه المرحلة ٥٨,٣ و٩٧% علي التوالي. وقد لوحظ في هذه المرحلة ايضا (الهضم الهوائي) الانخفاض الكمي والنوعي لبويضات الديدان المعوية (*Ascaris, Trichuris, Trichostrongylus, Taenia,* and *Hymenolepis*). كما اظهرت مرحلة التغليظ نفس الكفاءة في ازالة هذه الديدان المعوية.

وبصورة عامة في ضوء النتائج المتحصل عليها أوضحت المراحل الأخرى لمعالجة الحمأة الدور الفعلي في إزالة بكتريا القولون الكلية والبرازية بواقع أربع وحدات لوغاريتمية لكل منهما ، أما إزالة البكتريا السبحية البرارية والكوليفاج فكانت بواقع ثلاث وحدات لوغاريتمية لكل مجموعة منهما واثنين وحدة لوغاريتمية بالنسبة لبكتريا إيشريشيا كولاي. وكان لهذه المراحل من المعالجة الدور الرائد في الإزالة الكاملة للفيروسات المعوية وبويضات الديدان والبروتوزوا المعوية حيث اختفت هذه الكائنات تماما بعد عمليات التجفيف لمدة ستة أشهر في أحواض التجفيف.

وقد وصل الحمل الميكروبي للحمأة بعد تمام عملية التجفيف (في أحواض التجفيف) ٦,٥ x ١٠^{٢} لبكتريا القولون البرازية ، ٤,٥ x ١٠^{٢} لمجموعة البكتريا السبحية البرازية ، أما بكتريا إيشريشيا كولاي فكانت ١,٦ x ١٠^{٢} والكوليفاج ٨٥ وحدة (pfu) .

وبهذا تكون نوعية المنتج النهائي للحمأة المعالجة مطابقة ومتوافقة للمواصفات المصرية والعالمية من الناحية الميكروبيولوجية.