International Journal of Environment 3(4): 212-220, 2014
ISSN: 2077-4508

Survival of Enterotoxigenic *E. coli* O157: H7 Strains in Different Water Sources

[1]M. Azab El-Leithy, [2]Einas H. El- Shatoury, [2]Mohamed A. Abou-Zeid, [1]Hemdan B. A., [1]Samhan F. A., [1]Gamila E. El-Taweel.

[1]Water Pollution Research Department, Environmental Research Division, National Research Center, Dokki, Cairo. 12311, Egypt.
[2]Microbiology Department, Faculty of Science, Ain Shams University. El- Khalifa El-Mamon St., Abbassia, Cairo. 11566, Egypt.

ABSTRACT

The aims of this work are to evaluate the survival of *E. coli* O157:H7 in ground, River Nile and drainage water, to compare with the same water after sterilization and also, to determine tolerability of different *E. coli* O157:H7 strains in tested water. It was achieved by inoculation 10^6 CFU from three *E. coli* O157:H7 strains; ATCC 35150 (Strain 1), (Strain 2) and (Strain 3) isolated from River Nile and El-Rahawy Drain, respectively into two portion; sterile and non-sterile portion of ground, River Nile and El- Rahawy Drain water. All strains possesses five virulence genes (*stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *eae* (intimin), *rfbE* (O157 antigen), and *fliC* (flagellar antigen)). It was found that, the prolonged survival *E. coli* O157:H7 was observed up till 98 days in sterilized wastewater followed by survival for 84 days in both sterilized ground and River Nile water. *E. coli* O157:H7 survived in all sterilized tested water types longer than non sterilized water. It was found that the *E. coli* O157:H7 isolated from wastewater sample had the longest survival time (98 days) in sterilized wastewater compared to the other strain (reference strain and isolate of River Nile).

***Key words*:** Survival, *E. coli* O157:H7, River Nile water, El-Rahawy Drain water

Introduction

E. coli O157:H7 causes different types of diseases for human, including non-bloody and bloody diarrhea, hemorrhagic colitis (HC), in some cases kidney failure; hemolytic uremic syndrome (HUS) and death at times in sever cases. *E. coli* O157:H7 is a normal inhabitant in the intestinal tract of cattle and transmission by consumption of raw or undercooked contaminated vegetable and bovine food products (Kassenborg *et al.,* 2004). In addition to these, outbreaks related to consumption of contaminated drinking water or to the use of surface water for recreational and irrigation purposes have been reported as well (Bruneau *et al.,* 2004; Bidet *et al.,* 2005).

Water characteristics can effect on bacterial survival; for example, availability of nutrients and concentrations has been shown to affect survival of non-pathogenic *E. coli* in water (Lessard and Sieburth, 1983). Moreover, there are many factors which effected on microbial survival in the aquatic environments includes temperature, light, pH and the presence of predators (Flint, 1987; Davies and Evison, 1991; Medema *et al.,* 1997). In case of soil and water environments, survival is influenced by moisture content of soil type, nutrients and competing microbes (Zhai *et al.,* 1955; Reddy *et al.,* 1981).

When following bacteria strain had been isolated from environmental samples and cultured in the laboratory as example for growth model, it was found that, they grow will be as same as a growth model. According to this model, bacteria isolate introduced to a brief lag phase by an abundance of nutrients, then followed by replication at an exponential phase until the nutrient supply is decreased, a period which, such as *E. coli*, occurs in <24 h. When follows the exponential phase, the cells then enter the stationary phase which can last for many days, after which the death phase ended and 99% of the cells die from starvation. Those cells entered to the long term stationary phase, where they can remain viable and capable of renewed growth when reintroduced to nutrient medium may be reached more than >5 years. These cells are recognized to possess the growth advantage in stationary phase phenotype, which confers the ability to manage extended periods of nutrient deprivation via a complex interaction of metabolic and genetic adaptations (Finkel, 2006). It should be noted that it is difficult to decide with assurance which of the above growth stages is being entertained by a given bacteria present in an aquatic environments, such as fresh water (Higgins and Hohn, 2008).

Daubner in (1975) designed survival experiments in three different water types by using *E. coli* strains isolated from stool of healthy people and inoculated in sterile demineralized, Danube, and highly mineralized water. He observed that, there were many changes carried out in the biochemical activity of the cells and also observed cell shrinking and the reduction of cytoplasmatic content of *E. coli* had been recovered from both distilled and Danube water and damage to the integrity of the cell in mineralized water. Kerr *et al.* in (1999) also observed widespread damage to the majority of cells of *E. coli* O157:H7, there were large spaces between cell wall and cell membrane.

Corresponding author M. Azab El-Leithy, Water Pollution Research Department, Environmental Research Division, National Research Center, Dokki, Cairo. 12311, Egypt.
E-Mail: mohamedazabr@yahoo.com

The survival of *E. coli* in river sediments was studied by Davies *et al.* (1991), they found that after a 60 days of incubation period at 20°C. *E. coli* either died completely or their number declined to the level close to 7 logs CFU/ml down to around 3 logs CFU/ml, depending on sediment type. Wang and Doyle in (1998) noted that, *E. coli* O157:H7 survived for 21 days in a lake water source and 77 days in reservoir water, both held at 15°C. Grad *et al.* in (2000) found that, *E. coli* O157:H7 survived in natural mineral water for 70 incubation days. A few studies have addressed the viability or survival of *E. coli* O157:H7 in surface water including lakes, rivers, marine water, animal trough water and farm water (Wang and Doyle, 1998; Rice and Johnson, 2000; McGee *et al.,* 2002; Williams *et al.,* 2007). Czajkowska *et al.* in (2005) found that, COD level as well as pH of water and muddy water did not play any important role in the survival of *E. coli* O157:H7. Czajkowska *et al.* in (2005) studied that, survival of *E. coli* serotype O157:H7 in bottom-shore sediments in relation to incubation temperature of sample. It was evident that, at 6°C undetectable level (< 1 CFU/g) of pathogenic bacteria after agar plating method were noticed after 73-100 incubation days, whereas, 30-60 incubation days at 24°C after gave similar results.

Materials and Methods

Isolation and identification of E. coli O157:H7 strains

Three selected strains of *E. coli* O157:H7 were used in this experiment. *E. coli* O157:H7 strains ATCC 35150 (Strain 1) was obtained from (*VACSERA*, Co., Egypt), Strain (2) was isolated from River Nile Rossita Brunch at the mixing point with El-Rahawy Drain and Strain 3 was isolated from El-Rahawy Drain water. Identification of *E. coli* O157:H7 was carried out according to ISO (1991) and APHA (2005), presumptively positive isolate from HiCrome EC O157:H7 selective agar was tested for sorbitol fermentation, indole and oxidase tests. Indole positive, non sorbitol fermenting isolates and oxidase negative were considered suspect *E. coli* O157:H7. Furthermore, *E. coli* O157:H7 isolates were confirmed by somatic agglutination serological test EC O157:H7. Also, *E. coli* O157:H7 isolates were confirmed using BIOLOG GN III according to manufacturers' instructions. All *E. coli* O157:H7 strains were confirmed by multiplex PCR according to Bai *et al.* (2010) to determine six virulence genes (*stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *eae* (intimin), *rfbE* (O157 antigen), and *fliC* (flagellar antigen) and *hlyA* (hemolysin)).

Preparation of E. coli O157:H7 inoculums

Loopful of each strain was subcultured into 100 ml TSB and incubated at 37°C for 24 h then centrifuged at 3000 rpm for 20 min. The pellets were washed three times using sterilized distilled water. The pellet was suspended in 10 ml sterililized distilled water. This suspension was used as inoculum to avoid introducing additional nutrients or minerals to the water. The inoculum density (initial count) of strains was determined by ten folds serial dilutions and counted using both HiCrome MacConky sorbitol agar as a selective media and plate count agar as an enrichment media.

Water tested

Three types of water samples used in the survival experiment were collected according to APHA (2005) in duplicate a one liter wide mouth, sterile glass sampling bottles. Groundwater was collected from Tokh drinking water treatment plant, Qalyubia Governorate. River Nile water were collected from the main stream of Rossita Branch, Giza Governorate before the mixing point with El-Rahawy Drain. Some physicochemical parameter were determined such as (pH = 8, EC= 394 µs, TDS= 224 mg/l, NO_2= 0.01 mg/l, NO_3 = 0.18 mg/l, Turbidity = 3.13 NTU, and COD= 19 mg/l). Drainage water was collected from El- Rahawy Drain, Giza governorate. Some physicochemical parameter were determined such as (pH =7.3, EC =1378 µs, TDS =740 mg/l, NO_2 =0.00 mg/l, NO_3= 0.2 mg/l, Turbidity= 10 NTU, and COD = 71.5 mg/l). Each water type was divided into 4 flasks (each flask contains 200 ml). Two flasks from 4 flasks were autoclaved at 121°C for 15 min.

Inoculation of different water types by tested strains

The survival experiment was carried out for each tested strains by adding one ml of inoculum strain into sterilized and non sterilized 200 ml of groundwater (SGW, NSGW), River Nile water (SRN, NSRN), wastewater flasks (SWW, NSWW), respectively. Two flasks sterilized and non sterilized containing 200ml from groundwater, River Nile water, wastewater flasks, respectively were used as control (uninoculated) for the three strains. The inoculated flasks and control flasks were incubated at 20±2°C lab. temperature (the mean annual temperature of the Egyptian water), as the same as this experiment was repeated in that with strains (2) and (3).

Determination of tested strains counts

Determination of tested strains counts was carried out in the first and second days after inoculation, then once a week interval by ten folds serial dilution from each flask. Total viable bacterial count (TVBC) was determined by transferring one ml from appropriate flask into a sterile Petri dish using plate count agar medium. *E. coli* O157:H7 was determined by spreading appropriate dilution onto the surface of HiCrome MacConky

sorbitol agar. The plates were incubated at 37°C for 24 h. The relationship between the survival of tested *E. coli* O157:H7 strains in different water types (groundwater, River Nile and treated wastewater) was carried out using linear correlation (Person correlation) ($P \leq 0.005$ and $P \leq 0.05$).

Results

Survival experiment of *E. coli* O157:H7 was carried out using three different strains. *E. coli* O157:H7 ATCC 35150 (strain 1), strain 2 River Nile isolated strain and strain 3 El-Rahawy Drain isolated strain were inoculated in sterile and non-sterile ground, River Nile and drainage water. The survived *E. coli* O157:H7 were counted in the first and second days then counted at weekly interval (7, 14, 21, and 28 for 105 days) using HiCrome MacConky Sorbitol agar and plate count agar.

Control experiments for all strains at different water types

Total viable bacterial counts (TVBC) (CFU/ml) were not detected in sterile un-inoculated groundwater, River Nile water and treated wastewater along the period of study (105 days) which was used as a control for contamination. In addition to this, the TVBC (CFU/ml) were not detected in nonsterilized un-inoculated groundwater, River Nile water and treated wastewater after 35, 63 and 77 days, respectively. *E. coli* O157:H7 was not detected in both groundwater and River Nile water during the period of the study. Their numbers declined in wastewater to be absent after 56 days (data not shown).

Survival of strain (1) in different water types

Strain (1) survived in sterile wastewater (SWW) up to 91 days longer than (non-sterile) wastewater (NSWW), groundwater (NSGW) and River Nile water (NSRN) (Figures 1 to 4). The survival time of strain (1) in both SGW and SRN was longer than its survival in NSGW and NSRN (Figures 1 and 2). Strain (1) survived in SGW and SRN up to 84 days. It survived longer in NSGW and NSRN. The shortest survival for strain (1) was shown in NSRN (Figures 1 and 4).

From statistical analyses linear correlation between the survival of tested strain (1) in different water types and survival time (days) using plate count agar medium. There are positive correlations with high significance between different water types ($p \leq 0.005$). SRN showed positive correlation with significance with NSGW, NSRN, and NSWW ($p \leq 0.05$). *E. coli* O157:H7 (strain 1) counts was inversely proportioned with survival time (days), which represented in negative correlations with high significance as follow; (r= -.650, -.786, -.647, -.642, -.622 and -.660 for NSGW, SGW, NSRN, SRN, NSWW and SWW, respectively). In addition to this, linear correlation between survival of tested strain (1) in different water types and survival time (days) using HiCrome MacConky sorbitol medium. There are positive correlations with high significance between different water types ($p \leq 0.005$). *E. coli* O157:H7 (strain 1) counts was inversely proportioned with survival time (days), which represented in negative correlations with high significance as follow; (r= -.640, -.772, -.636, -.746, -.628 and -.745 for NSGW, SGW, NSRN, SRN, NSWW and SWW, respectively).

Survival of strain (2) in different water types

Survival of *E. coli* O157:H7 River Nile isolate (strain 2) in SWW was 91 days, followed by SGW (84 days), then in SRN (70 days) using plate count agar. While using HiCrome MacConky Sorbitol agar survival was recorded up to 91, 84 and 63 days in SWW, SGW and SRN, respectively. Regarding, non- sterile water samples, using plate count agar, strain (2) survived in NSWW (77 days) followed by NSRN (63 days) and in NSGW (56 days), respectively. In case of using HiCrome media it survived up to 70 days and 56 days in NSWW, NSGW and NSRN, respectively (Figures 5 to 8). There are linear correlation between the survival of tested strain (2) in different water types and time (days) using plate count agar medium. There are positive correlations with high significance between different water types ($p \leq 0.005$). Strain (2) counts were inversely proportioned with survival time (days), which represented in negative correlations with high significance with survival time (days) (r= -.642, -.704, -.664, and -.748 for NSGW, SGW, NSRN, and SRN, respectively). Moreover, in case of NSWW, SWW, strain (2) counts were inversely proportioned, which represented in negative correlations with significance with survival time (days) (r=-.594 and -.604). Also, linear correlation between survival of tested strain (2) in different water types and survival time (days) using HiCrome MacConky sorbitol agar. There are positive correlations with high significance between different water types ($p \leq 0.005$). Strain (2) counts were inversely proportioned with survival time (days), which represented in negative correlations with high significance between different water types and survival time (days) (r= -.645, -.704, -.709, and -.738 for NSGW, SGW, NSRN, and SRN, respectively). But, in case of NSWW and SWW, strain (2) counts were inversely proportioned, which represented in significance negative correlations with survival time (days) (r= -.565 and -.590).

Int. j. Environ. 3(4): 212-220, 2014

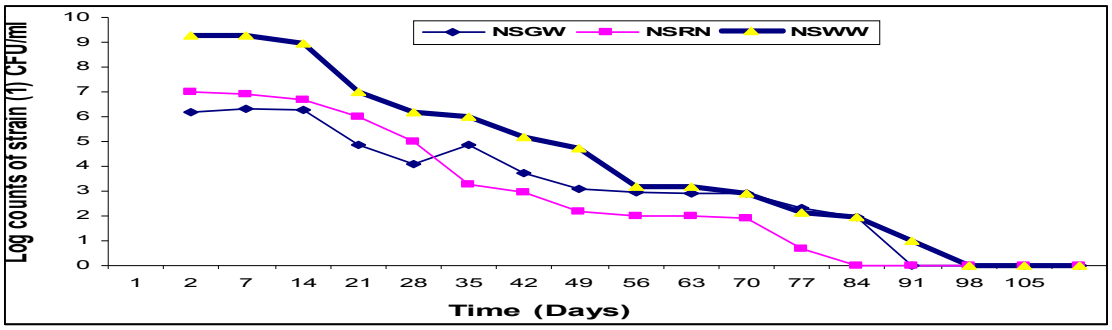

Fig. 1. Log counts of strain (1) in non-sterile different water types using plate count agar.

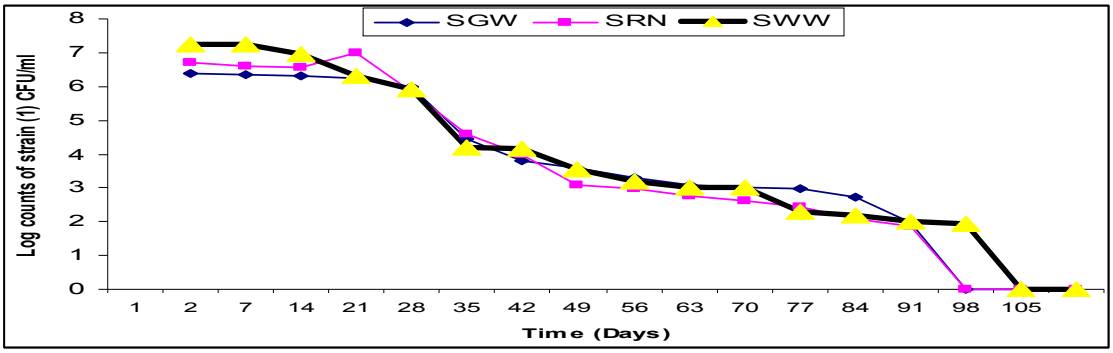

Fig. 2. Log counts of strain (1) sterile different water types using plate count agar.

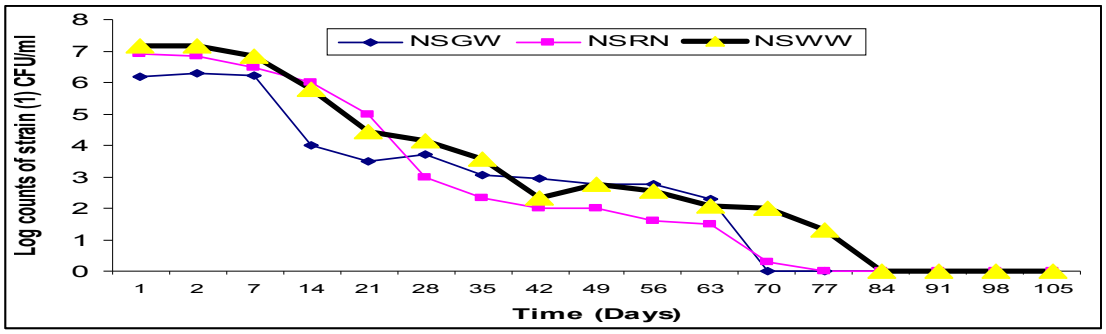

Fig.3. Log counts of strain (1) in non -sterile different water types using HiCrome MacConky Sorbitol agar

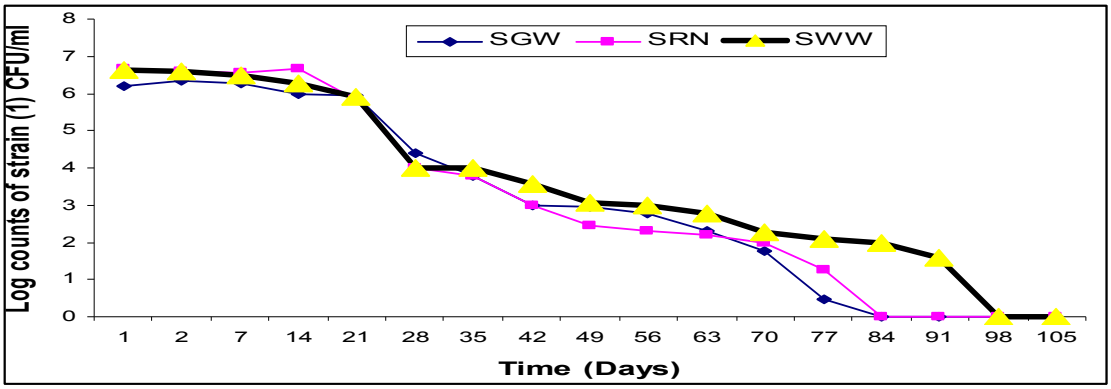

Fig. 4. Log counts of strain (1) in sterile different water types using HiCrome MacConky Sorbitol agar

SGW: Sterilized groundwater, NSGW: Nonsterilized groundwater. SRN: Sterilized River Nile, NSRN: Nonsterilized River Nile, SWW: Sterilized wastewater, NSWW: Nonsterilized wastewater.

Int. j. Environ. 3(4): 212-220, 2014

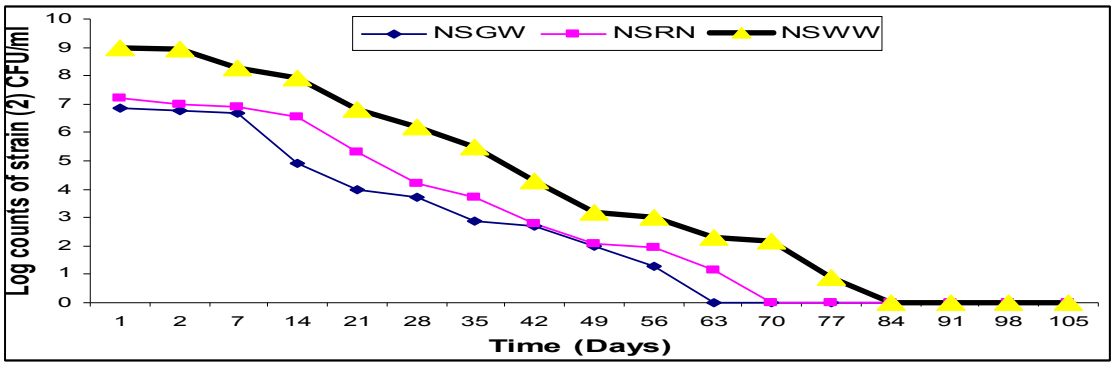

Fig. 5. Log counts of strain (2) in non- sterile different water types using plate count agar

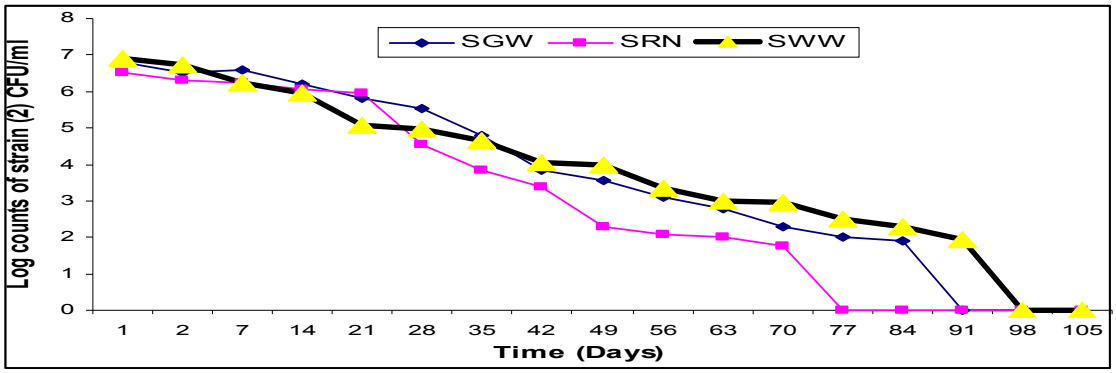

Fig. 6. Log counts of strain (2) sterile different water types using plate count agar

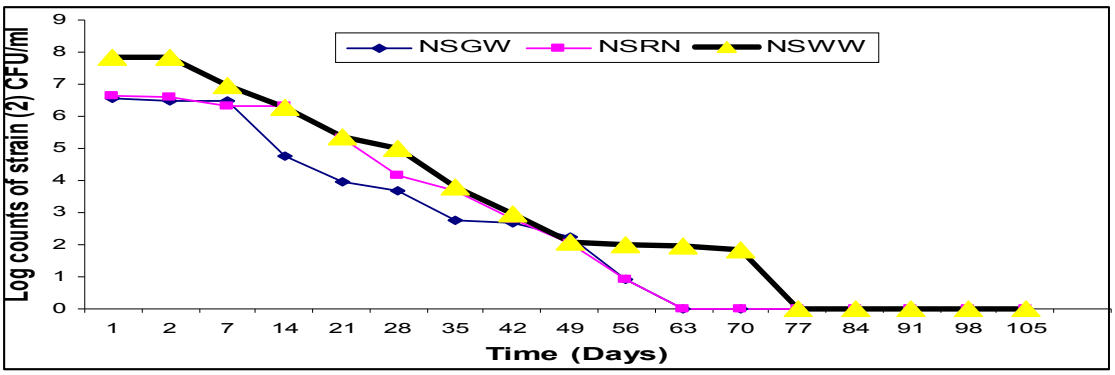

Fig. 7. Log counts of strain (2) in non -sterile different water types using HiCrome MacConky Sorbitol agar

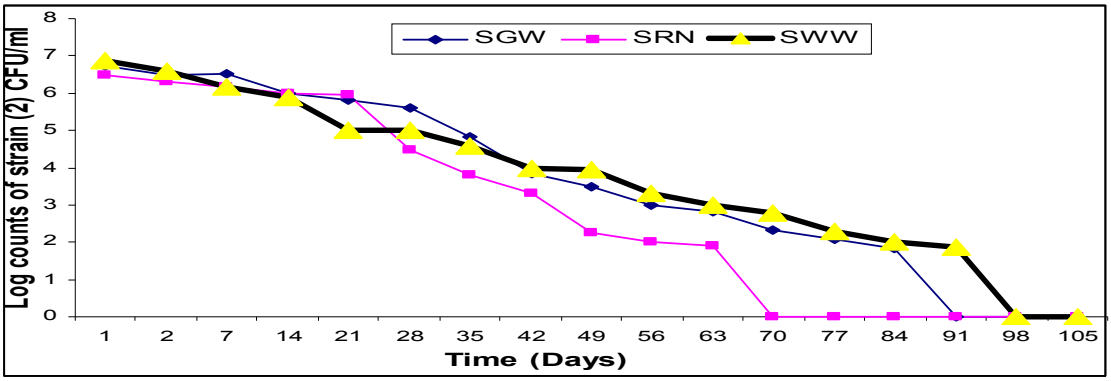

Fig. 8. Log counts of strain (2) in sterile different water types using HiCrome MacConky Sorbitol agar

Int. j. Environ. 3(4): 212-220, 2014

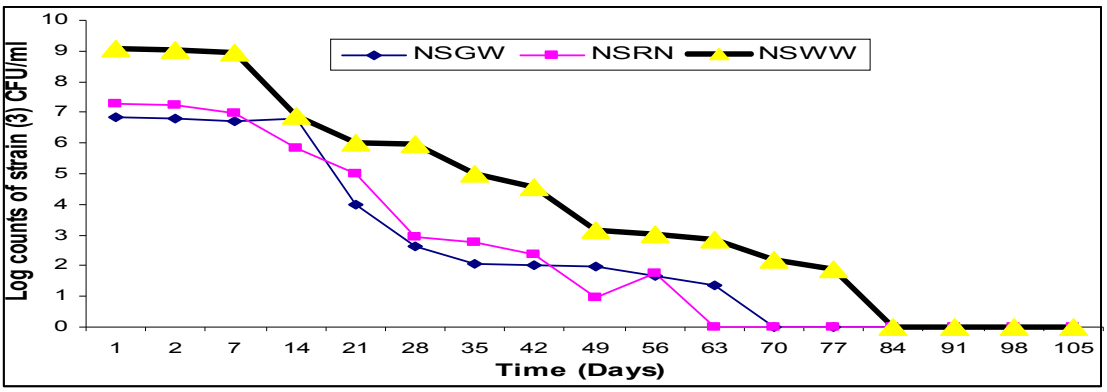

Fig. 9. Log counts of strain (3) in non- sterile different water types using plate count agar

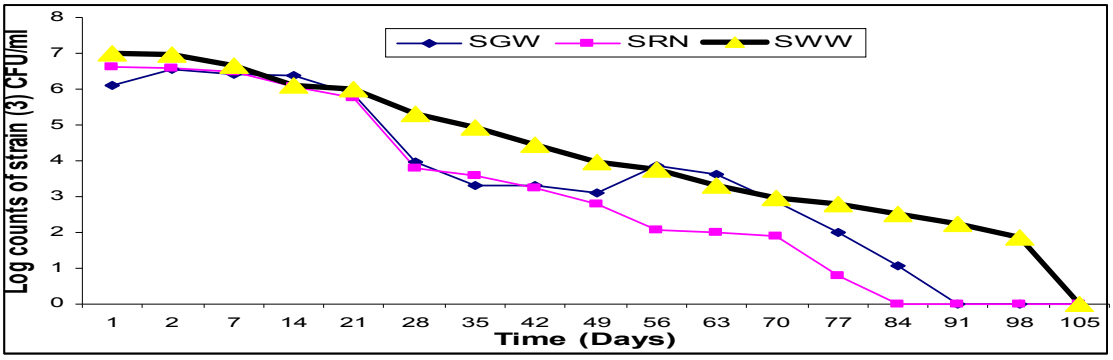

Fig. 10. Log counts of strain (3) sterile different water types using plate count agar

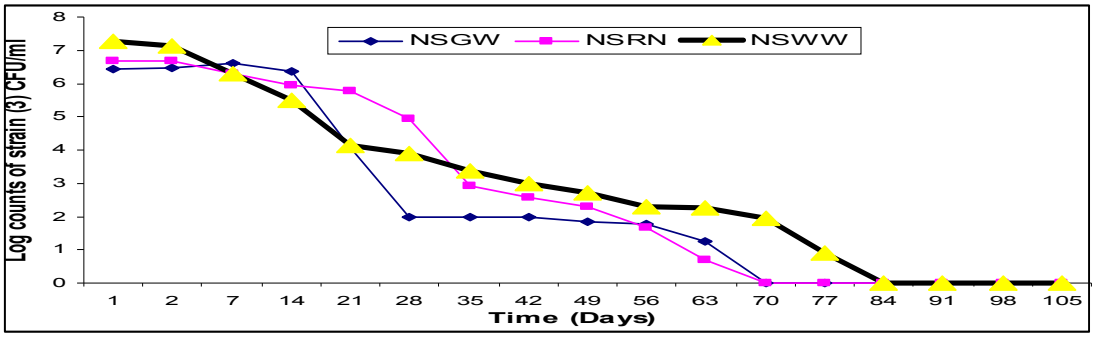

Fig. 11. Log counts of strain (3) in non-sterile different water types using HiCrome MacConky Sorbitol agar

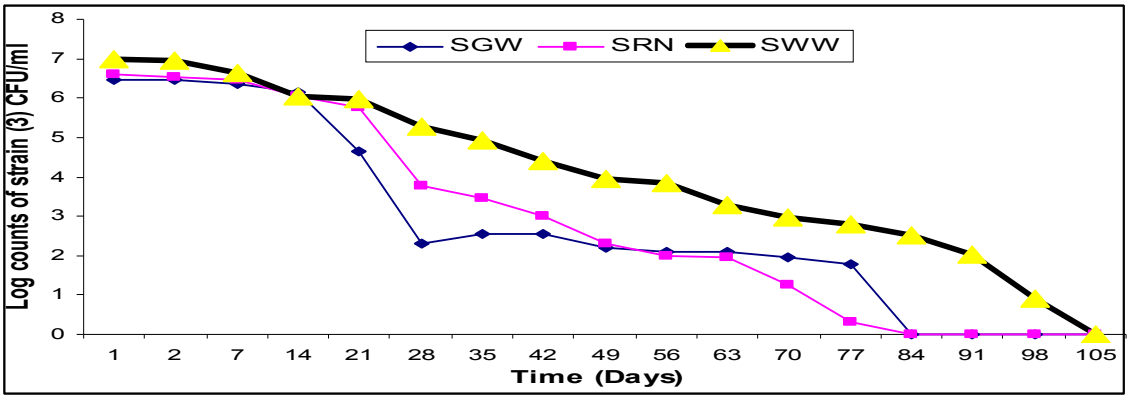

Fig. 12. Log counts of strain (3) in sterile different water types using HiCrome MacConky Sorbitol agar

Survival of strain (3) in different water types

El-Rahawy Drain *E. coli* O157:H7 isolate strain (strain 3) survived longer than other two strain. It survived the longest survived in SWW (98 days), followed in SGW, using plate count agar. In non-sterile water it is shortest survival time (days) was in NSRN (56 days) (Figures 9 to 12).

There are linear correlation between the survival of tested strain (3) in different water types and (survival time) days using plate count agar medium. There are positive correlations with high significance between different water types ($p \leq 0.005$). Also, there are negative correlations with high significance between different water types and survival time (days) (r= -.721, -.715, -.628, -.721, -.640, and -.672 for NSGW, SGW, NSRN, SRN, NSWW and SWW, respectively). Regarding to the linear correlation between the survival of tested strain (3) in different water types and (survival time) days using HiCrome MacConky sorbitol agar, it was found that, there are positive correlations with high significance between different water types ($p \leq 0.005$). Strain (3) counts were inversely proportioned with survival time (days), which is represented in the negative correlations with high significance as follow; (r= -.711, -.717, -.675, -.721, and -.669 for NSGW, SGW, NSRN, SRN, and SWW, respectively). Also, with significance in NSWW and survival time (days) (r= -.556).

Generally, it is clear that, the three *E. coli* O157:H7 strains survived longer in the selected three sterile water types than non-sterile water. Whereas, in non-sterile water, it rapidly declined in River Nile (between 56-70 days) followed in groundwater (65-77 days) and treated wastewater (between 70-77 days). *E. coli* O157:H7 which isolated from El-Rahawy Drain (strain 3) had the longest survival in wastewater (98 days) than other two strains (reference strain (strain 1) and the strain (2) isolated from River Nile water both survived for 91 days.

Discussion

Waterborne pathogens remains a critical risk factor in water overall the world, municipal sewage become the main source for the passage of pathogens into surface water (Irvine *et al.,* 1995). *E. coli* O157:H7 is waterborne pathogen that has emerged as a major cause of HC and is transmitted to humans either by food or water, also, it can cause HUS mainly by secretion of shiga toxins encoded by the virulence genes *stx1* and/or *stx2* and others variants (Bidet *et al.,* 2005). The human infectious dose of *E. coli* O157:H7 is very low, and ingestion of amounts as few as 10 bacterial cells is sufficient to cause infection and illness (Chart, 2000). Considering the *E. coli* O157:H7 survival in water, usually, when *E. coli* O157:H7 bacteria are introduced into water environments, they gradually die and this process is accompanied by changes in their characteristics. Daubner in (1975) carried out survival experiments using *E. coli* strains freshly isolated from the excreta of healthy people in different water types. He noticed changes in the physiological and morphological properties of *E. coli* during die-off, such as shrinking of the cell and damage to the integrity of the cell or the reduction of cytoplasmatic content. Moreover, he observed changes in the biochemical activity of the cells, the most important ones being an immediate decrease in respiration and dehydrogenase activity of the cells when introduced into the water environments. Also, Kerr *et al.* in (1999) observed widespread damage to the majority of *E. coli* O157:H7 cells, with large spaces between cell wall and cell membrane. Thus in this study, the survival of three *E. coli* O157:H7 strains in three different water types was carried out to evaluate the longest survival time of *E. coli* O157:H7 in groundwater, River Nile water and wastewater (sterile and non- sterile) and to determine the viability of these strains in the tested water.

In this study, *E. coli* O157:H7 survived in all sterile water types longer than non sterile water this may be due to the fact that, all natural flora in water types were killed by sterilization and the dead cells are considered as nutrients for the added strains. Moreover, killing natural flora prevents the competition on nutrients. Flint (1987) noticed that, the autochthonous flora of the sterile distilled water (SDW) was eliminated by sterilization which allowed the proliferation of entered organism. Also, Korhonen and Martikainen in (1991) documented that the autochthonous microbes of the water has a very high effect on the survival of *E. coli* through competition for nutrients or predation.

Enteric pathogens are commonly assumed to die after they are shed from the human or animal hosts into the natural environment. However, a few reports point to that some of them, such as pathogenic *Escherichia coli*, are able to survive for a long time or even grow under certain conditions in water and soil (Ishii *et al.,* 2007; Vital *et al.,* 2008). There are numerous a biotic and biotic factors which control in the ability of enteric pathogens to grow or survive, but it well known that substantially is limited for most of those factors (Winfield and Groisman, 2003). In the present study, the longest survival time of *E. coli* O157:H7 was observed in sterile wastewater were survived for 98 days and non-sterile wastewater were survived for 77 days followed by sterile and non-sterile groundwater for 84 days then sterile River Nile water for 84 days and non-sterile River Nile water 70 days. *E. coli* O157:H7 which was isolated from wastewater showed the longest survival time in wastewater (98 days) than the other two strains (reference strain and the strain which was isolated from River Nile), these results are in agreement with Avery *et al.* in (2008) who found that, *E. coli* O157:H7 survived better in low nutrient lake water and high-nutrient fecally contaminated puddle water than in livestock-drinking troughs and river water. Wang and Doyle in (1998) noted that, *E. coli* O157:H7 survived for 21 days in a lake

water source as opposed to 77 days in reservoir water, both held at 15ºC. Flint in (1987**)** showed that, the natural microbial flora from river water increased the survival of *E. coli* till 250 days.

Relatively few studies are available which describes the survival of pathogenic bacteria in environment, especially in water that may potentially be contaminated. Much of the emphasis has been placed on cattle manures, manure-amended soils, and surface water, with less emphasis on groundwater. In general, pathogens tend to survive longer in cold rather than warm temperatures and in water rather than in manures or soils. This may be problematic as manures and soils are stationary whereas water is a significant transport medium for pathogens (Kudva *et al.,* 1998).

In this study, non-selective media (plate count agar) recovers more *E. coli* O157:H7 than selective media (MacConky Sorbitol agar) in survival experiment, this may be due the fact that plate count agar is considered high enrichment media moreover, selective media was supplemented with antibiotic which might have inhibit the injured *E. coli* O157:H7 cells, however, statistical analysis showed no significance differences in *E. coli* O157:H7 recovery between the used media. Degirmenci *et al.* in (2012) reported that, sub-lethally injured cells can fail during growth on selective media, thus; it is recommended to use non-selective media was for their recovery. Although, decreasing numbers were recorded when the selective media compared with the non selective media; no significant difference was found between these recovered populations (P>0.05) (Rocelle *et al.,* 1996; Silk and Donnelly, 1997).

References

APHA (American Public Health Association), 2005. Standard methods for the examination of water and wastewater, 21st ed**.** Washington, D.C.

Avery, L.M., A.P. Williams, K. Killham and D.L. Jones, 2008. Survival of *Escherichia coli* O157:H7 in waters from lakes, rivers, puddles and animal drinking troughs*. Sci. Total Environ.,* 389: 378-385.

Bai, J., X.Shi, T.G. Nagaraja, 2010. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. *Microbiol. Meth.,* 82: 85-89.

Bidet, P., P. Mariani-Kurkdjian, F. Grimont, C. Brahimi, P. Grimont and E. Bingen, 2005. Characterization of *E. coli* O157:H7 isolates causing haemolytic uraemic syndrome in France. *Medical Microbiol.,* 54:71-75.

Bruneau, A., H. Rodrigue, J. Ismael, R. Dion and R. Allard, 2004. Outbreak of *E. coli* O157:H7 associated with bathing at a public beach in the Montreal-Centre region. *Can. Commun. Dis. Rep.,* 30: 133-136.

Chart H. 2000. VTEC enteropathogeicity. *Appl. Microbiol*., 88: 12-23.

Czajkowska, D., A.Witkowska-Gwiazdowskka, I. Sikopska, H. Boszczyk-Maleszak, and M.Horoch, 2005. Survival of *Escherichia coli* serotype O157:H7 in water and in bottom-shore sediments . *Pol. J. Environ. Stu.,* 14: 423-430.

Daubner, I., 1975. Changes in the Properties of *Escherichia coli* under the influence of water environments. *Intern. Verein. Limnologie Verhandlungen.,* 19; 2650-2657.

Davies, C., L. Evison, 1991. Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.,*70: 265-274.

Degirmenci, H., M. Karapinar and S. Karabiyikli, 2012. The survival of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in black carrot (Daucus carota) juice. *Inter. J. Food Microbiol.,* 153: 212-215.

Finkel, S.E., 2006. Long-term survival during stationary phase: evolution and the GASP phenotype. *Nat. Rev. Microbiol.,* 4: 113-120.

Flint, K.P., 1987. The long-term survival of *Escherichia coli* in river water. *J. Appl. Bacteriol.,* 63: 261-270.

Grad, M.K., M. Fitzerald, J.J. Sheridan, 2000. The survival of added *Escherichia coli* O157:H7 in natural mineral water and its products and the development of rapid method for enumeration of the heterotrophic bacteria in natural mineral water. *Bacteriol.,* 30: 291-305.

Higgins, J. and C. Hohn, 2008. Effects of prevalent freshwater chemical contaminants on in vitro growth of *Escherichia coli* and *Klebsiella pneumonia. Environ. Poll.,* 152: 259-266.

Irvine, K.N., G.W. Pettibone, I.G. Droppo, 1995. Indicator bacteria sediment relationships: implications for water quality modeling and monitoring, p. 205–230. In James, W. (ed.), Modern methods for modeling the management of stormwater impacts. Computational Hydraulics International, Guelph, Ontario, Canada.

Ishii, S., D.L. Hansen, R.E. Hicks and M.J. Sadowsky, 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. *Environ. Sci. Technol.,* 41: 2203-2209.

ISO (International Organization for Standardization), 1991. Guidance on sampling of drinking water and water used for food and beverage processing (ISO 5667-5), Geneva.

Kassenborg, H. D., C. W. Hedberg, M. Hoekstra, M. C. Evans, A. E. Chin, R. Marcus, D. J. Vugia, K. Smith, S. D. Ahuja, L. Slutsker, and P. M. Griffin, 2004. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 Food Net sites. *Clin. Infect. Dis.,* 38: S271–S278.

Kerr, M., M. Fitzgerald, J.J. Sheridan, D.A. McDowell, and I.S. Blair, 1999. Survival of *Escherichia coli* O157:H7 in bottled natural mineral water. *J. Appl. Microbiol.,* 87: 833- 841.

Korhonen, L.K. and P.J. Martikaine, 1991. Survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water. *J. Appl. Bacteriol.,* 71: 379-382.

Kudva, I.T., K. Blanch, C.J. Hovde, 1998. Analysis of *Escherichia coli* O157:H7 in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.,* 64: 3166-3174.

Lessard, E.J., J.M. Sieburth, 1983. Survival of natural sewage populations of enteric bacteria in diffusion and batch chambers in the marine-environment. *Appl. Environ. Microbiol.,* 45: 950-959.

McGee, P., D.J. Bolton, J.J. Sheridan, B. Earley, G. Kelly, N. Leonard, 2002. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. *J Appl. Microbiol.,* 93: 706-713.

Medema, G., M. Bahar, F. Schets, 1997. Survival of *Cryptosporidium parvum*, *Escherichia coli*, *Faecal Enterococci*, and *Clostridium perfringens* in river water: Influence of temperature and autochthonous microorganisms. *Water Sci. Technol.,* 35: 249-252.

Reddy, K.R., R. Khaleel, and M.R. Overcash, 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *J. Environ. Qual.,* 10: 255-266.

Rice, E.W., C.H. Johnson, 2000. Survival of *Escherichia coli* O157:H7 in dairy cattle drinking water. *J. Dairy Sci.,* 83: 2021-2033.

Rocelle, M., S. Clavero and L.R. Beuchat, 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity and temperature and suitability of media for its recovery. *Appl. Environ. Microbiol.,* 62: 2735-2740.

Silk, T.M., C.W. Donnelly, 1997. Increased detection of acid-injured *Escherichia coli* O157:H7 in autoclaved apple cider by using nonselective repair on trypticase soy agar. *J. Food Protec.,* 60: 1483-1486.

Vital, M., F. Hammes, and T. Egli, 2008. *Escherichia coli* O157 can grow in natural fresh water at low carbon concentrations. *Environ. Microbiol.,* 10: 2387-2396.

Wang, G., M.P. Doyle, 1998. Survival of enterohemorrhargic *Escherichia coli* O157:H7 in water. *J. Food Prot.,* 61: 662-667.

Williams, A.P., L.M. Avery, K. Killham, and D.L. Jones, 2007. Persistence, dissipation, and activity of *Escherichia coli* O157:H7 within sand and seawater environments. *FEMS Microbiol. Ecol.,* 60: 24-32.

Winfield, M.D. and E.A. Groisman, 2003. Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl. Environ. Microbiol.,* 69: 3687-3694.

Zhai, Q., M.S. Coyne, and R.J. Barnhisel, 1955. Mortality rates of fecal bacteria in subsoil amended with poultry manure. *Bioresource. Technol.,* 54:165-169.