



Studies on Some Heavy Metals and Bacterial Pollutants in Tilapia Fish of El Salam Canal, Northern Sinai, Egypt.

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ABSTRACT

The present study was conducted to investigate the distribution of bacteria and selected heavy metals (Al, Fe, Zn, Mn, Cu, Cd, Pb, Co, Ni and Cr) in water samples and in the gills and muscles of the Nile tilapia (*Oreochromis niloticus*) grown along El- Salam Canal zone. Samples were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES). The concentrations of heavy metals in water samples showed the high levels of heavy metals in the studying area. The levels of heavy metals in the fish samples can be considered as a serious matter of concern as it may be consumed and it signifies the degradation of canal quality. Regarding to microbiological analysis, the results showed an increase in some bacterial pollutants which linked with human disease. The counts of pathogenic bacteria *Pseudomonas aeruginosa* ranged from 80 to 200 MPN/100 ml, while that of Salmonella- Shigella varied from 0 to 30 CFU/ml and *E.coli* colony number range from 0 to 10. In addition, the fish caught from the study area revealed the presence of different bacterial species. A total of eight bacterial species were isolated from fish and identified biochemically as *Salmonella* spp., *Enterobacter cloacae*, *Rhizobium radiobacte.*, *Fluorescens* spp., *Listeria monocytogenes*, *Streptococcus agalactiae*, *Streptococcus iniae* and *Aeromonas hydrophila*. The bacterial count from each sample revealed 44.5×10^7 cfug⁻¹ from the gills, 20×10^8 cf ug⁻¹ from the intestine and 38.75×10^8 cfug⁻¹ from the skin. The isolates were found to be of medical importance, so restricted measurements should be taken to inhibit the spreading of pollutant sources in El-Salam Canal.

INTRODUCTION

Fish is an important source of food for people and provide us for about 60% of the world's supply of protein, 60% of the developing countries received 30% of their annual protein from fish (Abisoye *et al.*, 2011). It is one of the cheapest sources of protein in Africa (Clauca *et al.*, 1996). The advantage of fish as its easy digestibility, and it has high nutritional value. Nile tilapia (*Oreochromis niloticus*) is the most important aquatic species produced and consumed in Egypt. It is a teleost fish with a worldwide distribution; therefore it is a good model for assessing the impacts of different environmental pollutants on aquatic ecosystems (Akan *et al.*, 2009).

Heavy metal residues and its hazard effects on the health of people are a matter of great concern to food hygienists.

They accumulated in fish not only have a bad influence on fish but they also affect the health of human beings through the food chain (Desta *et al.*, 2012). Bioaccumulation of trace elements in living organisms and bio-magnification in them describes the processes and pathways of these possible pollutants from one trophic level to another, exhibiting the higher bioaccumulation ability in the organisms concerned. Increasing concentration through the food chain caused higher retention time of toxic substances than that of the other normal food components. Therefore, various fish species are widely used as bio-indicators of metal contamination (Svobodova *et al.*, 2004). Under acidic conditions the free divalent ions of many metals may be absorbed by fish gills directly from the water. Hence, concentrations of heavy metals in organs of fish determined primarily the level of pollution in the water and food (Farkas *at al.*, 2000). The concentration of pollutants in water samples only indicate the situation at the time of sampling, while concentrations in the organism are the result of past as well as current pollution levels in the environment in which the organism lives (Ravera *et al.*, 2003). Water that resides in rivers, streams and lakes are major sources of drinking water. Thus, the pollution of these natural waters is one of the most critical environmental issues today. Inorganic compounds from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose a serious threat because of their toxicity, long time persistence, bioaccumulation, and bio magnifications in the food chain. Many groups of organisms can be used as a bioindicators of environmental and ecological change, but numerous publications attest that fish (in situ) are a good indicator of aquatic environmental change and ecosystem health, especially in the case of toxic water pollution (El-Shafei, 2015)). In aquatic ecosystems, heavy metals have received considerable attention due to their toxicity, accumulation in biota and biomagnification in the food chain (Emara *et al.*, 2015). For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediment. These essential metals as Cu, Fe, Mn, Zn can also produce toxic effects when the metal intake is excessively elevated (Canli and Atli, 2003). The concentration of heavy metals in fish as a good indicator of aquatic environmental has been recommended by several researchers (Abdel-Mohsein and Mahmoud, 2015, Bayomy *et al.*, 2015 and El-Shafei., 2015). Because health safety is very an important aspect of food quality, so the present study was carried out to investigate the rate of infection of tilapia with bacteria, as well as the extent of its presence in the water of the Nile. Also to estimate bioaccumulation of heavy metals and to produce baseline data on the distribution of heavy metals (AL, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Cr, Co) in water and edible parts of commonly consumed fish species (*Oreochromis niloticus*) obtained from El-Salam Canal, Egypt. Analyses of these heavy metals in water and fish samples were used to evaluate the magnitude, impacts and possible sources of heavy metals contamination on the canal. Consequently tilapia is susceptible to a wide variety of bacterial pollutant, most of them causing disease and is considered by some researches to be saprophytic in nature (Lipp and Rose, 1997). The pathogenic organisms that cause mortality in tilapia are bacteria such as *Aeromonas* spp., *Flavobacterium columnare*., *Francisella* spp., *Streptococcus agalactiae*., *Escherichia coli*, *Clostridium botulinum*, *Shigelladynteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella*. Some of these pathogens are distributed in warm water, such as River Nile, The disease is related to the interaction between fish, pathogens and their waterways (Snieszko, 1975 ; Lipp and Rose, 1997). When a fish is exposed to a bacterial

pathogen in an unfavorable environment (as poor water quality or excess of organic matter) disease percentage is higher because the balance between the pathogen, host and aquatic environment destabilizes. However, fish contain a high bacterial diversity which results from a symbiotic effect among bacteria and tolerance by fish that protect them when adapting to nutritional changes and present of food in the digestive tract (Huong *et al.*, 2014). Regarding to the microbiological diversity of the fresh fish muscle depends on the fishing grounds and environmental factors around it. The bacteria in fish only become pathogens when fish are physiologically unbalanced nutritionally deficient, or there are other stresses (Cahill, 1990). Therefore, it is important to identify the extent of heavy metal and bacterial content in fish samples by monitoring them to explain potential impacts on the food chain and human health risks.

MATERIALS AND METHODS

Study Area: the total length of El-Salam Canal is 242 km, updated performed parts include 87 km West Suez Canal and 35 km East Suez Canal. The East Canal is known as El-Sheikh Gaber Canal on which different sampling locations were selected to cover the implement study area.

Laboratory Analysis: water and Tilapia fish (*Oreochromis niloticus*) samples were collected during one year [2015-2016]. The selection of sites was chosen to represent El-Salam Canal area as shown in Figure (1).

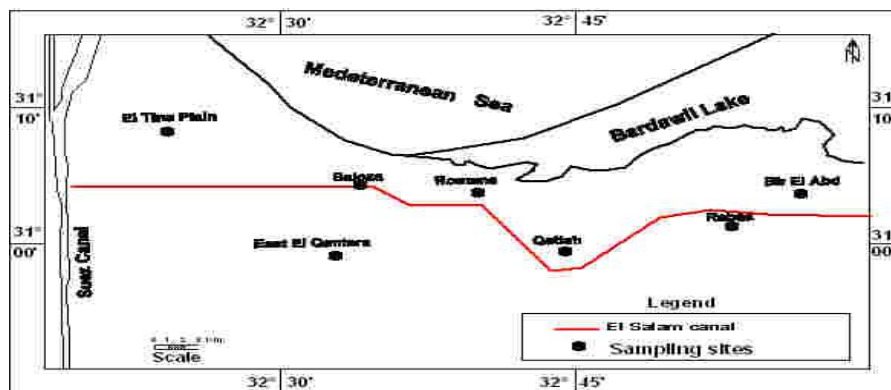


Fig. 1: Schematic Diagram for study area.

Water samples

For heavy metals analysis: about 100 ml of each water sample were filtered through nitrocellulose filter membrane of 0.45 μ m pore size prior dried in 105⁰C for 2 hours. The filtrate and unfiltered water samples were preserved in 2 ml concentrated nitric acid (HNO₃) to prevent precipitation of metals and growth of algae. Samples were collected according to international standard methods for examination of water and wastewater (APHA, 2005) to evaluate the concentrations of selected heavy metals. Samples were collected in stopper polyethylene plastic bottles, stored in an iced cooler box and delivered immediately to the laboratory for analyses. The plastic bottles were cleaned by soaking in 10% HNO₃ and the procedural blanks of standard solutions were prepared under clean laboratory environment.

For bacteriological analysis: about ten ml of each water sample was subjected to serially dilutions (10⁻¹ to 10⁻⁵) with sterile physiological saline (0.85% wt/vol. NaCl) in deionized water. The pour plate technique on nutrient agar medium was used for

the enumeration of total bacterial counts at both 22°C and 37°C incubation temperatures. For total spore-forming bacteria, water samples and its serial dilutions were pasteurized for 15 min at 80°C, prior to plating in nutrient medium and incubating at 30°C. MPN technique was used for enumerated total and faecal coliforms (using MacConky broth medium) and faecal streptococci (using Azidedextrose broth medium), then incubated at 37°C for 24-48 h. Eosin Methylene Blue (EMB) was used for enumerated *Escherichiacoli* and Violet Red Bile agar (VRB) for enumerated *Enterobacterease*. Asparagine broth medium was used for enumerated *Pseudomonas aeruginosa* as well as Salmonella Shigella agar (SS agar) was used for enumerated *Salmonella* spp. and *Shigella* spp. (APHA, 2005).

Fish Samples

For heavy metals analysis: fish samples were collected from different sites along El-Salam Canal area, they were washed with de-ionized distilled water to remove slime and/or ice, drained and water removed with tissue paper. Pieces from gills and muscles were taken and kept frozen at -20 °C then transported to laboratory for digestion and heavy metals analysis.

Samples Digestion: samples were prepared by mixing 0.5 g of wet weight fish tissues (gills and muscles) for 24 hours at 70 °C with 6ml concentrated 65% HNO₃ and 1ml 30% hydrogen peroxide (H₂O₂) for digestion, using microwave digestion system (model Milestone, MLS-1200 mega, Germany) then ventilated for 5 min and filtrated to 100 ml by using deionized water.

Analytical Procedures: laboratory analysis, all reagents were used for analytical grade and deionized water was used for all the prepared reagent solutions. Heavy metals (Al, Fe, Zn, Mn, Cu, Cd, Pb, Co, Ni and Cr), the samples were filtered by filtration system through membrane filter of pore size 0.45 µ and were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) and their recovery studies for the trace elements ranged between 99 and 102%.

For bacteriological analysis: one hundred and twenty tilapia fish were freshly captured (b.w. 350 gm), collected and examined. They were collected aseptically then immediately transported in a cleaning bag to the laboratory and processed within 3hrs. Clinical and post-mortem examination were done by using the methods described by (Buller, 2004).

Samples for total viable aerobic bacterial counts:

Skin Surfaces: sample from different locations of the skin of all fish was taken by rubbing a sterilized cotton swab over the skin and inoculated into 9 ml of Nutrient broth. 10 serial dilution of the suspended bacterial already inoculated in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated using standard plate count agar after incubated at 37°C for 48 hrs as described by (Slaby *et al.*, 1981).

Intestines and Gills: samples from the intestines and gills were taken out, blended and mixed completely in a mortar. It was aseptically transferred to a sample bottle containing 9 ml of 0.1ml sterile peptone water. The bottle was closed and shaken strongly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold of serial dilution was carried out in duplicates and viable aerobic bacterial counts were counted in standard plate count agar after incubation at 37°C for 48 hrs. as recommended by (Slaby *et al.*, 1981).

Samples for bacterial isolation and identification:

Samples were collected from gills, intestine and skin from fishes and cultured on general and specific media; brain heart agar, tryptic soy agar and tryptic soy broth (Difco) added to it 1.5% (w/v) NaCl, aeromonas agar base medium supplemented

with ampicillin, pseudomonas agar base medium supplemented 2% NaCl and azide blood agar supplemented with 2% NaCl and S.S agar medium and all media were incubated at 22°C for 48 hours. Identification of *Listeria monocytogenes* was carried out according the outlines recommended by Mc Clain *et al.* (1998). *Enterococcus* spp. were isolated by plating on KF Agar and incubated at 37°C for 48 hours. Enterobacteriaceae species were isolated by plating on Hecktoen Agar (Merck, Darmstadt, Germany) and incubated at 37°C for 48 hours. Colonies were subjected to Gram Stain and biochemical tests (API 20E, BioMérieux, France), *Salmonella* spp. was investigated by pre-enrichment in Buffered Peptone Water (Biolife, Milano, Italy), incubated at 37°C for 18 hours, and enrichment in Selenite Cystin Broth and Rappaport and Vassiliadis Broth (Merck, Darmstadt, Germany), both incubated at 37°C for 24 hours. Selenite Cystine and Rappaport & Vassiliadis cultures were streaked on to Rambach agar (Merck, Darmstadt, Germany) and Hecktoen agar (Merck, Darmstadt, Germany) and incubated at 37°C for 48 hours. Colonies suspected of being *Salmonella* spp. were transferred to Lysine Iron Agar and Triple Sugar Iron Agar (Merck, Darmstadt, Germany) and incubated at 37°C for 48 hours (FDA, 2001; HOLT, 1994 and Macfiddan, 2000). *Staphylococcus* spp. were isolated by plating on Baird-Parker Agar (Biolife, Milano, Italy) and incubated at 37 °C for 48 hours. The colonies were subjected to Gram stain and biochemical tests (API Staphy, BioMérieux, France) (FDA, 2001; HOLT, 1994 and MAC FADDIN, 1980).

Identification of the isolates: pure cultures of the isolates were identified biochemically following the criteria proposed by those described by Garrity, (2001). Confirmation of each strain was reached by using the analytical profile index of the API20-E system (Buller, 2004). Complete identification was done by using VITEK 2 instrument at Biological and chemical war management, Ministry of Military, Egypt.

Statistical analysis: data are presented as means ± standard deviations, according to Statistical Package for Social Science (SPSS) Ver. 14.

Bioaccumulation Factor (BAF): bioaccumulation factor; a major concern for environmental contamination is the extent to which pollutants concentrate from water into aquatic organisms such as fish. The extent of such concentration, termed the bioaccumulation factor (BAF), is given by the ratio of the pollutant concentration in fish to that in water (Chiou, 2002). The BAF for muscle was calculated as follows:

$$BAF = \frac{\text{Mean concentration in wet fish muscle mg/kg}}{\text{Mean concentration of water mg/l}}$$

Ecological characteristics of fish samples:

Common name	English name	Scientific name	Habitat*	Feeding* mode	Food* source
Bolti	Tilapia fish	<i>Oreochromis niloticus</i>	Benthopelagic	Herbivorous	Mainly algae

* According to Dsikowitzky *et al.* (2013).

RESULTS AND DISCUSSION

Results from clinical and post-mortem examination revealed that; symptoms detected in the naturally diseased fish included an increase in mucus secretion, exophthalmia and some fish showed hemorrhages in the eye, and large irregular hemorrhagic areas in many parts of the body, at the base of fins, on the gill cover, at

the anal region, anal fin. There were abdominal distensions observed. The quantity of mineral nutrients in diet should be balanced to promote optimum response in human metabolism. Heavy metals are persistent contaminants in the environment causing serious illness in fish, animals and human. Regionally, industrial and agricultural runoffs are considered the primary source of metal poisoning for fish and other aquatic animals in Egypt (Badr *et al.*, 2014). The results of the investigation have been summarized in Tables (1-9) and Figures (2-6). The mean values of heavy metals in water and fish tissues are presented in Tables 1, 2, 3 and 4 respectively. While the calculated bio-accumulation factors (BAF) for the various heavy metals are also inserted in Table (5).

Environmental pollution is a worldwide problem; heavy metals belonging to the most important pollutants, the progress of industries has led to increased emission of pollutants into ecosystems. The mean concentrations and associated standard deviations of HMs in water were presented in Tables 1&2, according to the analysis results, the following findings were obtained for the concentration of the metals: 0.63 ± 0.002 for Al, 0.16 ± 0.008 for Cu, 0.60 ± 0.002 for Fe, 0.38 ± 0.001 for Mn and 0.47 ± 0.007 for Zn mg/l respectively, meanwhile 0.001 ± 0.00 for Cr, 0.003 ± 0.0004 for Co, 0.0024 ± 0.0001 for Ni, 0.012 ± 0.0002 for Pb and 0.01 ± 0.0005 for Cd were found. Heavy metal concentrations in El-Salam Canal water were decreased in the sequence of Al>Fe>Zn>Mn>Cu> Cd>Pb>Co>Ni>Cr. Results for levels in water were compared with international water quality guidelines. The levels of Al, Cu, Fe, Mn, Zn, Co, Cr and Ni recorded in El-Salam Canal area in this investigation were higher than maximum acceptable limits recommended by Canadian water quality guidelines for the protection of aquatic life (CCME, 2001) but lower than the maximum acceptable limits in livestock drinking water recommended by WHO, (2008) and US EPA, (2011) for irrigation (Table1) respectively. However, the levels of Pb (0.012) and Cd (0.01) exceeded these limits. Certainly, extending El-Salam Canal through the semi-arid desert of north Sinai is an attraction for human & animal activities and fisheries uses.

Table 1: Mean concentration (mg/l) of heavy metals in water samples from the study area.

Element	Al	Cu	Fe	Mn	Zn
Samples					
water	0.63 ± 0.002	0.16 ± 0.008	0.60 ± 0.002	0.38 ± 0.001	0.47 ± 0.007
WHO (2008)	-	2	0.3	0.2	5
US EPA (2011)	0.087	1.3	0.3	0.05	5
CCME(2001)	0.005	0.01	0.3	-	0.05

Table 2: Mean concentration (mg/l) of heavy metals in water samples from the study area.

Element	Cr	Co	Ni	Pb	Cd
Samples					
water	0.001 ± 0.00	0.003 ± 0.0004	0.0024 ± 0.0001	$.012 \pm 0.0002$	$.01 \pm 0.0005$
WHO (2008)	0.05	0.005	0.3	0.003	0.003
US EPA (2011)	0.1	0.1	0.3	0.055	0.005
CCME (2001)	0.05	-	0.02	0.03	-

World Health Organization (WHO, 2008), US Environmental Protection Agency (USEPA, 2011) and Canadian water quality guidelines for the protection of aquatic life (CCME, 2001).

Therefore, its water quality for different purposes is of much concern, and justifies including bacteriological analyses in the present study. Our results agree

with Agrama and Amer, (2012) who concluded that chemical analysis for heavy metals and the water quality index of El-Salam Canal water indicated that concentrations are within the permissible levels for irrigation and livestock but is not suitable for fisheries water. While Hafez *et al.* (2008) remarkable that heavy metal concentrations limit in their studying area are not acceptable for animal drinking or irrigation purposes. The quality of El-Salam Canal water should be addressed to help monitoring and mitigating the negative impacts of the reused drainage water of the canal on the surrounding environment of north Sinai (Othman *et al.*, 2012). The later author found that Cu, Zn concentrations are within the permissible levels for irrigation and drinking water, while Cd and Fe concentrations exceeded the permissible levels for both irrigation and drinking livestock at El-Salam canal water.

Moreover, the concentrations of Al, Fe, Mn, Ni and Pb were above the permissible limits recommended by EOSQS (1993), and WHO (2008) referring to bad condition of the aquatic ecosystems. In many developed countries, the limits of heavy metals concentrations in fish have been set to safeguard public health. Egypt, for example, has set maximum limits of contamination for heavy metals based on permissible limits recommended by EOSQS, (1993). So this study is focused on determination of selected heavy metals concentrations in the selected tissues of tilapia fish (gills and muscles). In this study, the heavy metal permissible levels in fish tissues were also compared with others such as FAO (1983), EOSQS (1993) and FDA, (2001) (Tables 3&4). In aquatic ecosystem, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Several studies indicated that metal bioaccumulation in fish tissues depend on a number of factors such as food habits and foraging behavior of the fish; trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the environment; food availability; bio-magnification and/or bio-diminishing of a particular metal; metal detoxifying proteins in the body of the fish; temperature, transport of the metals across the membrane and the metabolic rate of the animal; species, age, size of fish and exposure time (Ogbeibu and Ezeunara, 2002 and Desta *et al.*, 2012).

Table 3: Mean concentration (mg/kg wet weight ± SD) of heavy metals in different tissues of Nile tilapia (*Oreochromis niloticus*).

Element Samples	Al	Cu	Fe	Mn	Zn
Fish Gill	193±72.01	5.45±3.581	406.3±66.68	9.429±3.415	22.4±10.11
Fish muscle	93.22±47.41	3.32±3.68	189±9.875	11.79±17.03	18.28±4.59
FAO(1983)	30	3	100	2.0-9.0	10-75
EOSQS (1993)	50	20	30	10	40
FDA (2001)	---	0.1	100	100	75

FAO (Food and Agricultural Organization), (1983) -FDA (Food and Drug Agency), (1993) & EOSQS (Egyptian Organization for Standaridization Quality and Control) (2001).

Table 4: Mean concentration (mg/kg wet weight ± SD) of heavy metals in different tissues of Nile tilapia (*Oreochromis niloticus*).

Element Samples	Cr	Co	Ni	Pb	Cd
Fish Gill	4.22±0.39	0.19±0.32	2.501±1.093	3.618±1.09	0.56±0.22
Fish muscle	1.47±1.014	0.183±0.3	1.588±0.285	1.138±0.56	0.39±0.20
FAO (1983)	0.15	0.1	0.6	0.2	2.0
EOSQS (1993)	20	10	10	2	0.5
FDA (2001)	0.15	---	0.5	0.2	2.0

FAO (Food and Agricultural organization), (1983)-FDA (Food and Drug Agency), (2001) & EOSQS (Egyptian Organization for Standardization Quality and Control)

The same heavy metals (Al, Cu, Fe, Mn, Zn, Cr, Co, Ni, Pb and Cd) were analyzed and the levels of their concentration were measured in fish samples (gills and muscle) from El-Salam Canal area using ICP-MS. The results of the analysis are presented in Tables 3 & 4 and further illustrated in Figure 2. In our study we found that accumulation of each heavy metal varied between the fish muscle and gills. Data of HMs concentration showed that HMs were accumulated in the gills of *Oreochromis niloticus* in the order of Fe (406.3) > Al (193) > Zn (22.4) > Mn (9.43) > Cu (5.45) > Cr (4.22) > Pb (3.618) > Ni (2.50) > Cd (0.56) > Co(0.19) ppm respectively, meanwhile these concentration were accumulated in the muscle samples in the order of Fe (189) > Al (93.22) > Zn (18.28) > Mn (11.79) > Cu (3.32) > Ni (1.58) > Cr (1.47) > Pb (1.038) > Cd (0.39) > Co (0.183) ppm respectively. The present results are good agree with the finding of El-Deek *et al.* (1994) and Authman *et al.* (2013) who mentioned that fish muscle tissue is generally low in trace metal content.

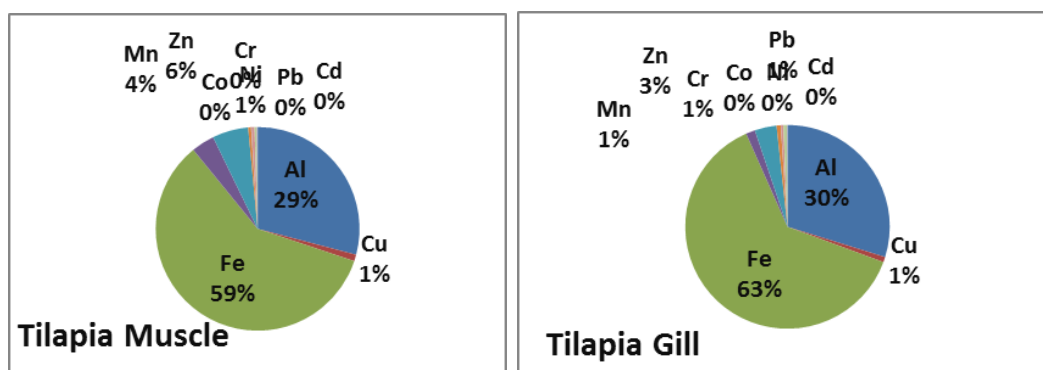


Fig. 2: Content (%) of the studied HMs in muscle and gill of *Oreochromis niloticus*

Trace elements in fish tissues were always higher than water that confirmed with Abumourad *et al.* (2014) and Riani, (2015) and that may be due to the variety of occurrences on fish body and other aquatic biota, including the regular diffusion, bio-magnifications, and bioconcentration. The highest concentrations of aluminum, iron, copper and zinc were detected in gill samples, while the minimum values of the same elements were recorded in muscle samples (Table 3). Aluminum has been shown to have deleterious effects on the central nervous, skeletal, and hematopoietic systems of humans. It is worth mentioned that its accumulation in brain altered amino acid neurotransmitters, so it has been suggested to be an associated phenomenon in various neurological disorders as dementia, senile dementia, and Alzheimer disease (Donia, 2015). Comparing our results with the previously reported metals data in fish tissues, it can be noticed that Al levels were higher than those reported by Authman *et al.* (2013) who recorded that aluminum concentrations were found to be in the ranges of 4.046 and 9.886, 4.042–9.854 and 8.069–14.204 mg/kg dry wt. for skin, muscles and kidney, respectively. Comparing our results with the permissible limits (Table 3) recommended by FAO (1983), EOSQS (1993) and FDA, (2001), levels of Al in muscles and gills samples are higher than the permissible limits (Table 3). Fe is an abundant and important element, unsurpassed by any other heavy metals in the earth's crust (Badr *et al.*, 2014). It is worth mentioned that Fe levels are higher than the permissible limits recommended by FAO (1983), EOSQS (1993) and FDA, (2001) (Table 3). The increase of Fe accumulation in fish in all examined tissues was higher than the other metals possibly due to the increase of total dissolved Fe in the

canal and consequently increases the free metal Fe concentration and there by lead to an increase in metal uptake by different organs. Regarding to copper, the highest concentration (5.45mg/kg) was recorded in gill samples. Copper is an essential metal which is important for the normal metabolism of fish, and non-essential metals may accumulate in their organs. Moreover, highest concentration of manganese was detected in muscle samples, while the minimum value was recorded in gill samples. Our results showed that the Mn is highly accumulated in muscles than gills, regarding to manganese concentration in fish samples was 9.429 ± 3.415 mg/kg in gill samples and 11.79 ± 17.03 mg/kg in muscle samples. Mn concentration was high in muscles samples than gill samples than, these results are disagree with Badr *et al.* (2014), Osman and Kloas, (2010) who demonstrated that Mn was highly concentrated in the gills of fish from early six sites along the whole River Nile. Manganese is a very common compound that can be found everywhere on earth. It is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, but it is also toxic when too high concentrations are present in a human body (Donia, 2015). It is obvious that Mn levels are lower than that recommended by FAO (1983), EOSQS (1993) and FDA, (2001) except fish muscles samples were higher than the permissible limits recommended by EOSQS (1993). Concerning zinc, it is constituent of all cells and is an essential trace element for fishes, it acts as a co-factor for number of enzymes and it is involved in well over one hundred different reactions in the body. It is known that toxicity of zinc is close to toxicity of lead (Kenzhin, 2014). The concentration of zinc in fish organs was 22.4 mg/kg in gill samples and 18.28mg/kg in the muscle samples. Zn concentrations were within permissible limits recommended by FAO (1983), EOSQS (1993) and FDA, (2001). In this study, it is obvious that concentrations were 0.001for Cr, 0.003 for Co, 0.0024 for Ni, 0.012 for Pb and 0.01for Cd. For the gills samples, it may be due to the fact that freshwater fish's gills might be expected to be the primary route for the uptake of water borne pollutants. Essential metals include Fe, Cu, Zn and manganese (Mn), whereas non-essential metals are Hg, Pb, nickel (Ni) and Cd (Hashim *et al.*, 2014) Chromium (Cr) is a naturally occurring element found in rocks, animals, plants, and soil, predominantly in its insoluble trivalent form [Cr(III)]. Unfortunately, excessive industrialization and other anthropogenic activities have led to the global occurrence of soluble Cr (VI) in concentrations above permissible levels.

Regarding to the Bioaccumulation Factor, Table (5) shows it (BAF) for different heavy metals (HMs) from water to Tilapia fish were as follows: 20.75 for Cu, 31.02 for Mn, 38.89 for Zn, 39 for Cd, 61 for Co, 94.83 for Pb, 147.96 for Al, 315 for Fe, 661.66 for Ni and 1470 for Cr were found. The result indicating Cr with a maximum BAF followed by Ni, Fe, Al, Pb, Co, Cd, Zn, Mn and Cu. BAF for HMs from water to Tilapia fish decreased in the sequence of $Cr > Ni > Fe > Al > Pb > Co > Cd > Zn > Mn > Cu$. This results were emphasizes on a large amounts of domestic and agriculture waste water disposal directly or indirectly in the Canal without any treatments.

Table 5: Bioaccumulation Factor (BAF) for different heavy metals from water to fish.

Al	Cu	Fe	Mn	Zn
147.96	20.75	315	31.02	38.89
Cr	Co	Ni	Pb	Cd
1470	61	661.66	94.83	39

Concerning the bacteriological analysis for water, bacterial counts are shown in Table (6) & Figure (3). The number of the distribution and seasonal variations of the total aerobic bacteria at 22°C ranged between 15×10^6 - 940×10^6 CFU ml⁻¹, while the bacteria developing at 37°C varied from 19×10^6 - 870×10^6 CFU ml⁻¹. The maximum bacterial counts were detected during spring 2016 reflecting the effect of high content of organic matter due to flourishing of phytoplankton which increased active multiplication of the bacteria. This agrees with explanation for increase bacterial counts in spring after year (Sabae *et al.*, 2008) but the numbers that we obtained at the same site of El Salam Canal are more than those written in previous research, and this may be due to increased pollution and climate change.

Table 6: Total viable bacterial counts $\times 10^6$ CFU ml⁻¹, water at 22°C and 37°C

Spring		Summer		Autumn		Winter		Spring	
22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C
39	45	32	36	15	19	350	250	940	870

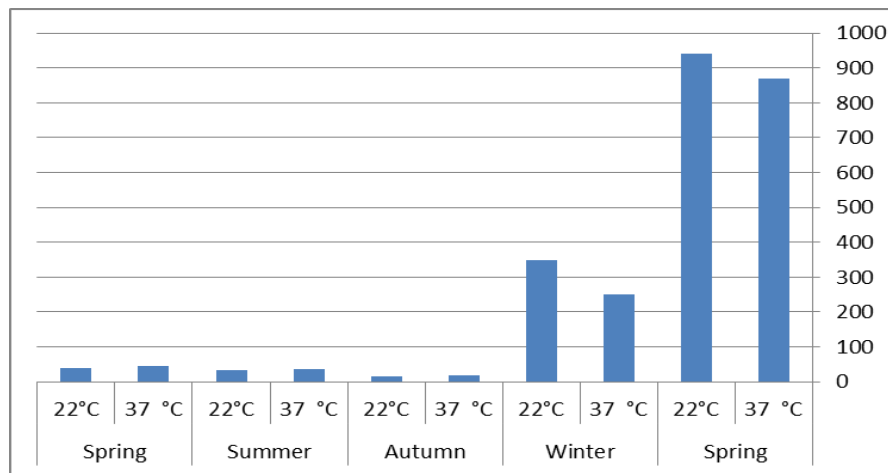


Fig. 3: Total viable bacterial counts $\times 10^6$ CFU ml⁻¹ water at 22°C and 37°C

The most probable numbers (MPN) of indicator bacteria are recorded in Table 7 and Figure (4). Their numbers were in the ranges of 11×10^2 - 90×10^3 , 5×10^2 - 32×10^3 and 3×10^2 - 15×10^3 MPN/ 100ml water for total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS), respectively. Generally the result of the this study showed increased in the indicator bacteria as compared with the previous results reported by Othman *et al.* (2008) and yehia and Sabae, (2011) we also realized that the environmental conditions as solar radiation, turbidity, temperature and salinity, they are affect the presence of indicator bacteria in El-Salam Canal. The minimum counts of the indicator bacteria were recorded in the warmer seasons which due to increasing of solar radiation and high temperature (Kocacy, 1989 and El-Shenawy, 2005). Some officials present FC and FS data as a ratio in an attempt to indicate the origin of bacterial pollution. FC/FS ratio of 4 or greater has been said to indicate a human source. The corresponding ratio for domestic animals is on average of 0.1- 0.6 and for wild animals less the ratio than 0.1. As this ratio has been applied to surface and ground water samples, these numbers hold true only for recent fecal contamination (US EPA, 2001) In the present study FC/FS ratios were in the ranges of 1.4, 3.6, 0.38, 2 and 2.1 for the studied seasons, respectively, which indicated mixed origin of fecal pollution and are agree with those previously reported by Sabae and Abdel- Rahman, (2008).

Table 7: The most probable number of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) × 10 MPN /100ml water

Spring			Summer			Autumn			Winter			Spring		
TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS
11	13	9	15	11	3	11	5	13	90	24	11	48	32	15

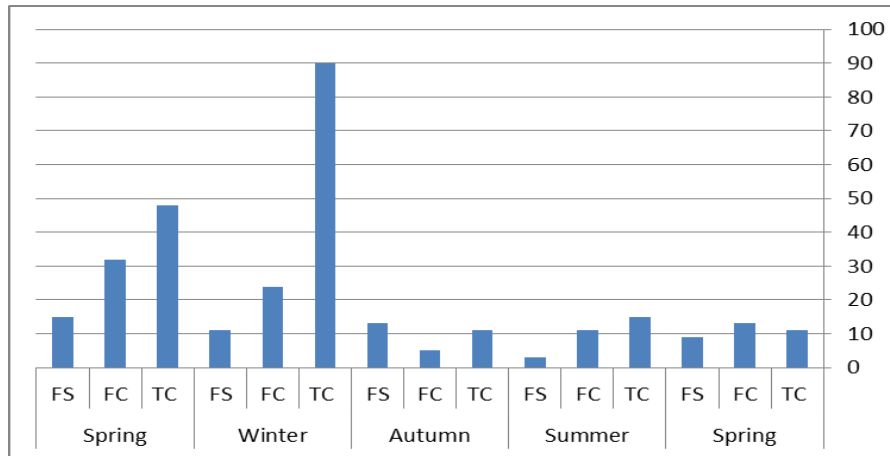


Fig. 4: The most probable number of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) × 10 MPN /100ml water

During the present study, the counts of pathogenic bacteria *Pseudomonas aeruginosa* (Table 8 and Figure 5) ranged in El-Salam Canal waters from 80 to 200 MPN/100 ml, while the numbers of *Salmonella- Shigella* varied from 0 to 30 CFU ml and E.coli colony number range from 0 to 10 this result come is agree with yehia and Sabae, (2011). This might be due to the effects of different environmental factors like amount of suspended matter in addition to the amount of wastewater discharge by the drains into the canal.

Table 8: The numbers of pathogenic bacteria (*Pseudomonas aeruginosa* MPN/100ml and *Salmonella- Shigella* CFU ml water and *E. coli* colony)

Spring			Summer			Autumn			Winter			Spring		
Ps.	s. s	E. coli	Ps.	s.s	E. coli	Ps.	s.s	E.coli	Ps.	s.s	E. coli	Ps.	s.s	E-coli
110	0	0	90	10	3	100	20	5	200	30	10	80	0	0

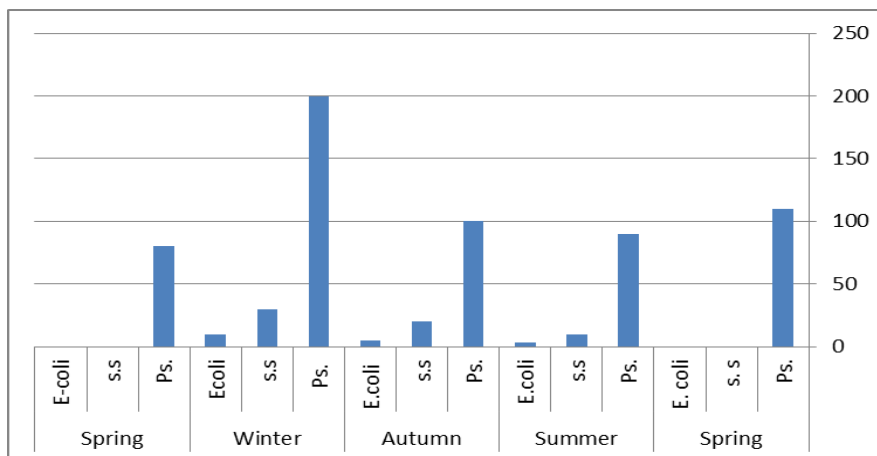


Fig. 5: The numbers of pathogenic bacteria (*Pseudomonas aeruginosa* MPN/100 ml and *Salmonella- Shigella* CFU ml water and *E.coli* colony).

Concerning the results and discussion of bacteriological analysis, for fish a range of total viable count of the three sites analyzed revealed 38.75×10^8 cfug⁻¹ from the skin, 20×10^8 cfug⁻¹ from the intestine 44.5×10^7 cfug⁻¹ and from the gills, this come agree with (Shinkafi and Ukwaja, 2010) they confirm that skin is one of the most common segment that contains bacteria. The frequency of occurrence of bacterial isolates is shown in Table (9) and Figure (6):

Table 9: Frequency of occurrence of bacterial isolates

Bacterial isolates	Number of occurrences	% occurrence
<i>Salmonella</i> spp	8	5.6%
<i>Enterobacter cloacae</i>	9	6 %
<i>Rhizobium radiobacter</i>	12	8.4%
<i>Ps. fluorescens</i>	14	9.7%
<i>Listeria monocytogenes</i>	21	14.7%
<i>Streptococcus agalactiae</i>	22	15.4%
<i>Streptococcus iniae</i>	25	17.5%
<i>Aeromonas hydrophila</i>	32	22.4%
total	143	

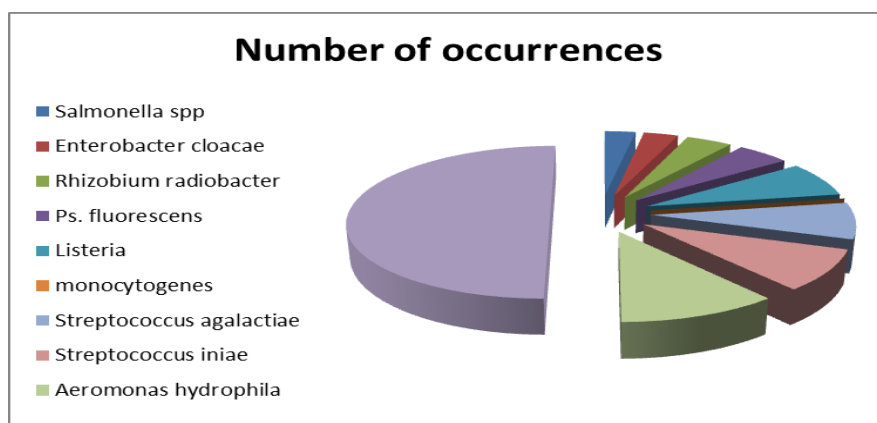


Fig. 6: Frequency of occurrence of bacterial isolates

The results from this research indicate that the bacterial load variations in the three segments of the fishes analyzed the skin, intestine and gills. The bacterial load in all samples was high and may be explained to the high ambient temperature in the river where it was close to optimum for many bacteria. Bacterial load in fish might increase with the increase of water temperature (Fernandes *et al.*, 1997). Choudhury *et al.*, (1989) reported an intestinal bacterial load of Tilapia fish as 5.5×10^9 cfu/g, this count is less than our results (20×10^8) in this research at similar temperature this may due to increase in pollution during the year. The high bacterial count on the skin may be due to increase contamination by genuinely aquatic species as well as those that contaminate the commodity during handling. The gills had the lowest bacterial population, compared to the intestine and skin. According to Trust, (1975) and Shinkafi & Ukwaja, (2010) the number of bacteria associated with the gills are actively maintained at a low level, thereby implying that fish probably has a mechanism which enables it to keep the bacteria number low and therefore afford it some degree of protection against bacterial invasion by the gill microflora (Ezeriet *al.*, 2001) Shinkafi and Ukwaja. (2010). Based on the percentage frequency of occurrence of *Salmonella* spp showed the least frequency of 5.6% and *Enterobacter cloacae* 6%. Presence of *Salmonella* spp. and *Enterobacter cloacae* in the fish tissue and in the water suggest pollution this result come with (Burras, 1993) who suggest

possibly from contamination with animal waste from chicken as observed by Abed El-Aziz and Ehab, (2003). *Salmonella* spp. has been reported to cause enteritis and systematic disease, *Rhizobium radiobacter* bacteria isolated by 8.4% percent, it may be due to legumes are in ingredients for fish feed which may contaminated by *Rhizobium radiobacter* or the water is contaminated with these bacteria and thus contaminated the fish skin. *Ps. Fluorescens* is widely distributed in natural sources of water and considered as one of the primary cause of septicemia in both freshwater and marine fish throughout the world and causes severe economic losses and decreases fish farm efficiencies (Olsson *et al.*, 1998) and it isolated by 9.7%. These results came in accordance with those obtained by Atwa, (2007) and Saad El-Deen, (2014). The percentage frequency of occurrence of *Listeria monocytogenes* 14.7% these findings is in agreement with other previous studies of Shinkafi and Ukwaja (2010), this may due to two sources of fish contamination which includes; the attack of *Listeria* from intestinal contents to other fish tissues like muscles, especially when the time from fish death till removing the viscera is more than a few hours (Ertas *et al.*, 2005), cross contamination (fish manipulation, using contaminated equipments and inappropriate transport) (Gudbjornsdottir *et al.*, 2004), it's importance come due to it produces a series of toxins hemolytic, lipolytic, hemorrhagic and pyrogenic which are involved in the disease process, the bacterium can also invade the eye and skin cause conjunctivitis and skin lesions (Bahk, and Marth, 1990). Regarding to *Streptococcus agalactiae*, a gram-positive bacterium which isolated by 15.4%, that can infect a number of fish species, including freshwater and seawater species, the percent was in accordance with earlier reports of (Baeck *et al.* 2006; Garcia *et al.* 2008; Ye *et al.* 2011; Chen *et al.* 2012). Tilapia is extremely susceptible to infection by *St. agalactiae* and is able to cause disease and death in Nile tilapia (Ye *et al.* 2011; Chen *et al.* 2012), *Streptococcus iniae* 17.5% an important pathogen in aquaculture, which may cause considerable economic losses and infect different species of marine and freshwater fish. Furthermore, it is an emerging zoonotic pathogen as previously reported by Agnew and Barnes (2007) & Baiano and Barnes (2009). In contrast, *Aeromonas hydrophila* record high percentage this may due to is related to sudden changes in temperature, dissolved oxygen and inadequate nutrition as pointed out by Conroy, (2014) who indicate that the constant variation of physicochemical parameters is a stress factor that benefits the outbreak of disease caused by opportunistic bacteria. According to Tryfinopoulo *et al.* (2002) both *Pseudomonas* spp. and *Aeromonas* spp. show high capacity to produce exoenzymes as lipases and proteases whose actions are associated to fish product deterioration.

CONCLUSIONS

According to the uses of El-Salam Canal in fisheries the results of the present study clearly demonstrate that it is contaminated with Al, Cu, Fe, Mn and Zn due to the continuous discharge of different pollutants as a result of mixing drainage water with the Nile water. Metal contamination in water followed the order of Al>Fe>Zn>Mn>Cu> Cd>Pb>Co>Ni>Cr. While these concentrations in fish organs followed the order of Fe> Al> Zn>Mn> Cu> Cr>Pb> Ni> Cd> Co. Metal accumulation in *Oreochromis niloticus* reflects the degree of water pollution. Also, this research sheds considerable light on the bacterial species associated with fresh Tilapia fish and water which they are potentially pathogenic to humans. Hence, adequate measures should be taken in processing the fish before consumption, great efforts and cooperation between different authorities are needed to protect the Canal from pollution and reduce environmental risk. This can be achieved by treatment of the agricultural, industrial, and sewage discharge. Regular evaluation of pollutants in the Canal is

also very important. Finally, the high levels of bioaccumulation factor of Cr, Ni, Fe, Al and Mn shows that they were good bioindicator to monitor pollution in this study. The quantities of HMs in fish muscles and gills measured in this study provide baseline information on concentrations and distribution of heavy metals and light on the bacterial species in *Oreochromis niloticus* from El-Salam Canal, Northern Sinai Egypt. So, our results clearly indicate the urgent need for effective strategies for the treatment of the drainage water resources before mixing with the Nile water, and the water in the area of study used with some restriction for the parameters which not comply with different guidelines. Therefore, further studies are strongly recommended for monitoring environmental pollution in this area.

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ARABIC SUMMARY

دراسات على بعض المعادن الثقيلة والملوثات البكتيرية في أسماك البلطي بترعة السلام - شمال سيناء، مصر.

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أجريت الدراسة الحالية لدراسة توزيع البكتيريا وبعض المعادن الثقيلة (الالومنيوم، الحديد، الزنك، المنجنيز، النحاس، الكاديوم، الرصاص، الكوبلت، النيكل والكريوم) في عينات المياه وخياشيم وعضلات البلطي النيلي (*Oreochromis niloticus*) التي تنمو على امتداد ترعة السلام. تم تحليل العينات باستخدام جهاز الحث المزدوج للبلازما (ICP-OES). تركيزات العناصر الثقيلة في عينات المياه كانت عالية. ويمكن اعتبار مستويات المعادن الثقيلة في عينات الأسماك مصدر قلق لأنها قد تستهلك عن طريق الإنسان، وهو ما يدل على تدهور جودة نوعية مياه القناة. وفيما يتعلق بالملوثات البكتيرية فقد أظهرت التحاليل الميكروبيولوجية عن وجود عدد من العزلات البكتيرية الممرضة للإنسان. وقد تراوحت نسبة السالمونيلا- شيغلا بين 0 إلى 30 وحدة مستعمرة بكتيرية في الملي الواحد (cfu/ml) و الميكروب القولوني كانت عدد مستعمراته من 0 إلى 10 وحدة مستعمرة بكتيرية في الملي الواحد (cfu/ml) وايضا أعداد بكتريا *Pseudomonas aeruginosa* كانت من 80 إلى 200 من 100 مليلتر بطريقة العدد الاكثر احتمالاً (MPN). بالإضافة إلى ان أسماك البلطي التي تم صيدها من مواقع الدراسة كشفت على نسبة عالية من المحتوى البكتيري حيث كشف العد البكتيري الكلي على $44,5 \times 10^7$ cfug⁻¹ من الخياشيم، و 20×10^8 cfug⁻¹ من الأمعاء و $10^8 \times 10^8$ cfug⁻¹ من الجلد. أما أنواع البكتيريا المعزولة من الأسماك والمصنفة بيوكيميائياً فهي:

Salmonella spp., *Enterobacter cloacae*, *Rhizobium radiobacte.*, *Fluorescens* spp., *Listeria monocytogenes*, *Streptococcus agalactiae*, *Streptococcus iniae* and *Aeromonas hydrophila*.

لذلك فإن مزيد من الاجراءات يجب ان تتخذ للحد من مصادر تلوث ترعة السلام.