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Physio-chemical and Microbial Characteristics of Treated Sewage Effluent Used for Crop Irrigation in the Kingdom of Bahrain

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ABSTRACT: Treated sewage effluent (TSE) is used in the Kingdom of Bahrain (KoB) heavily in irrigating lots of vegetables and fruits gardens due to the shortage in water resources. The aim of this study is to examine the waste water quality regarding pathogenic bacteria and parasites, trace metals (Fe, Cu, Zn, Pb, Ni, Cd and Ag) and physio-chemical properties (E-conductivity, pH, NH_3, NO_2, NO_3, BOD, COD and TSS). TSE is supplied from Tubli wastewater treatment plant (Tubli-WTP) in KoB as a tertiary stage effluent (TSE). The supplied TSE was free of pathogenic bacteria as no bacterium was grown on selective media (EMB, MC, DCA and XLD). TSE as well was free of pathogenic parasites when examined according to the American Standard for the Examination of Water and Waste Water (APHA). In addition, the chemical properties and heavy metals of TSE were within APHA standard. However, some pathogens were found in an irrigation storage reservoir used to collect TSE which was supplied from Tubli-WTP to a local farm (Farm-TSE). From this reservoir, eleven pathogens were identified (*Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Aeromonas hydrophyla*, *Pseudomonas Paucimobilis*, *Klebsiella Oxytoca*, *Salmonella sp.*, *Acinetobacter lwoffii*, *Serratia sp.* and *Pseudomonas Paucimobilis*) by using API 20E system. The possible contamination source of Farm-TSE was the storage condition of the reservoir and/or the supply process from Tubli-WTP to the local farm.

KEYWORDS: Irrigation, Pathogen, Trace metals, Treated sewage effluent.

1 INTRODUCTION

The Kingdom of Bahrain (KoB) is characterized by arid environment, low rainfall (annual rainfall is about 70 mm), high evaporation rate and poor groundwater recharge [1]. The renewable water reserves reached the limit of survival with no fresh water available for agriculture [2]. In the year 2025, the available amount of water from natural resources will be 10 to 20 m^3/person/year which is less than the actual domestic need (35 to 70 m^3/person/year) [2]. Due to the low availability of water; it is necessary to reuse sewage treated effluent (STE) to cope with water shortage in urban areas in the Kingdom of Bahrain.

Tubli sewage treatment plant is serving a population Equivalent of 800,000 (Curtis Calliva, Bluewater international, press release in filtration and separation, 2013). It performs three levels of treatments: Primary, secondary and tertiary treatments. Primary treatment involves removing of the suspended solid waste from the wastewater reaching the plant via scraping tanks. The remaining water contains dissolved organic compounds such as proteins, carbohydrates and fats in addition to microbes including bacteria, viruses and parasites [1]. In the secondary treatment phase; the water will be mixed with activated sludge in an aerated tank. Within a process between 12-14 hours, the bacteria will decompose the organic compounds into simpler materials in the aeration tank. Then the content is directed to the settle tanks, where materials are separated and the solids are pumped again to the aeration tank to ensure continuous biological processes. This process is followed by condensing the solids and drying them to form activated sludge. In the tertiary treatment, water in the settling tanks is passed through sandy filters to remove the purities. Disinfection process will be carried out to remove bacteria, parasites and viruses by the addition of chlorine and ozone. After the completion of this process, the treated water is pumped into a storage reservoir for irrigation purpose [1].

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Majority (550) of farmers at KoB are using TSE to irrigate their vegetable fields (Showqi Al-Manai, Ministry of Municipalities affairs & Urban Planning, KoB, Al-Ayam, issue 8234, 2011). The presence of microbial pathogens in polluted and Sewage treated effluent (STE) poses a considerable health risk to the public health beside the presence of trace metals. Sewage water is a major potential health risk, as fecal contamination is a major source of many microbial pathogens [3]. Moreover, the trace metals contamination and their health risk present emerging challenges affecting the world. Controlling the pathogens is particularly important regarding wastewater treatment and the reuse of wastewaters [4]. To avoid the spread of waterborne diseases and to protect public health; microbiological monitoring of water for pathogenic microbes and trace metal determination are needed. Four groups of microbial pathogens are commonly found in water and wastewaters including bacteria, viruses, pathogenic protozoa, and pathogenic helminthes [3].

This study will focus on the detection of the viable pathogenic bacteria -which can be cultured in the lab- from TSE samples which are used for irrigation. For an appropriate risk assessment to be made, the types of microbial pathogens which are present in TSE and their relative numbers will be determined. The detection, enumeration and identification of bacterial pathogens have focused on the use of selective culture and standard biochemical test. In addition, the quality of STE in its tertiary stage in terms of physio-chemical properties and trace metals concentration will be examined.

2 MATERIALS AND METHODS

2.1 MICROBIAL EXAMINATION OF STE

2.1.1 WATER USED FOR IRRIGATION (TSE FROM TUBLI SEWAGE TREATMENT STATION, TSE FROM LOCAL FARM RESERVOIR AND TAB WATER)

Sterile bottles (1L) were used to collect water samples from Tubli-WTP which is used for irrigation (Tubli-STE), local farm using STE (Farm-STE) and Tap water. Pour plate method was used to enumerate the total count of bacteria (CFU/ml) using a complete medium (nutrient agar, NA). the same method was used to determine the type of bacteria by using different selective agar media including Eosin Methylene Blue (EMB), MacConkey's (MC), Deoxycholate Citrate (DC) and Xylose Lysine Deoxycholate (XLD). EMB is both a selective and differential media used for the detection and isolation of Gram-negative intestinal pathogens. *E. coli* produce small, dark colonies with a green metallic sheen. MacConkey's agar is both a selective and differential media; it is selective for Gram negative bacteria and can differentiate those bacteria which have the ability to ferment lactose. DC is useful for the isolation of *salmonella* strains that cause food poisoning and *Salmonella paratyphi*. XLD is used for further selection of *Salmonella* and *Shigella* as well.

After obtaining a negative result for Tubli-STE water, another test was carried out to examine the presence of choliform in Tubli-STE samlpe. A standard filtration method was used in which 100 ml of Tubli- STE water sample was filtered under a sterile condition. The filter paper was placed on NA and EMB agar as a selective medium of *E. coli*. Then they were incubated at 37^0C for 24 h.

For enumeration of microbes, APHA Standard techniques were followed for microbial analysis of water samples [5]. The water samples were analysed for total parasite (T. parasite), total coliforms (TC) and *E. coli*.

2.1.2 IDENTIFICATION OF PATHOGENS BY GRAM STAIN, OXIDASE TEST AND API TEST

Enterobactericae are used as an indicator for the contaminated water, therefore, testing the presence of those types of bacteria in the water samples is necessary for determining the health risk. Enterobactericae are gram negative and oxidase negative. All of the grown bacteria were isolated from Farm-TSE on selective media (MC, EMB, DC and XLD). Lots of those bacteria (31 isolate) were examined by Gram stain. API 20E test was performed for the gram negative and oxidase negative bacteria.

2.2 PHYSIO-CHEMICAL ANALYSIS OF TUBLI-TSE

The physico-chemical parameters, such as pH and E-conductivity of the water samples (Tubli-TSE, farm-TSE and chlorinated Tap water) were determined. In addition total suspended solids (TSS), biological oxygen demand (BOD5, i.e. BOD5 is the amount of oxygen consumed biologically within 5 days), chemical oxygen demand (COD, i.e. the amount of oxygen required to completely oxidize the chemical species present, it includes both the biological and chemical reactions), total ammonia, total nitrate and total nitrite were analyzed following the standard methods for the examination of water and wastewater [6]. Three replicates were carried out for each analysis and the average value of the replicates was compared with the World Health Organization [7].

2.3 TRACE METAL ANALYSIS OF TUBLI-TSE

To determine the toxicity of Tubli-TSE, Chemical analysis of the trace metals concentration (Fe, Cu, Zn, Pb, Ni, Cd and Ag) was performed; following the American Standard for the Examination of Water and Waste Water [6].

3 RESULTS AND CONCLUSIONS

3.1 MICROBIAL EXAMINATION

To test the quality of TSE, three types of irrigation water samples (Tap water, Tubli-TSE and Farm-TSE collected from a reservoir at a local farm) were examined for total coliforms, total parasites and *E. coli* according to the standard methods for the examination of water and wastewater [6]. Total bacterial and parasite count (CFU/plate) were calculated for those types of irrigated water (Table 1). It was found that Farm-TSE was highly contaminated with total coliform including *E. coli* (Table 1). the presence of high dose of fecal coliform (*E. coli*) in Farm-TSE indicated a huge contamination of TSE which was supplied to the farm (Table 1). No parasite was detected in the three types of water (Table 1). The examination focused on growing the pathogens which were found in TSE on selective media. Samples were plated on complete medium (NA). Other water samples were tested for the presence of Enterobactericae on selective media such as *E. coli* (EMB, MC) and *Salmnella* (DC and XLD) (Fig. 1, Fig. 2). *E. coli* and/or *enterococcus* bacteria are pathogens indicators in water quality standards criteria by EPA [8]. It was found that no bacterium was detected in Tubli-TSE water (Table 1), whereas, there were high content of bacteria detected from Farm-TSE when grown on NA and the selective media including EMB, DC and MC. Those media were selective and differentiative media for Enterobactericae including *E. coli*, *Shigella* and *Salmonella*. In the tab water sample; insignificant number of bacterial colonies was detected on NA as compared to non bacterial growth on the desired selective media. The absence of colonies on the selective media in both Tubli-TSE and Tap water is an indicator for the high quality of water as no fecal coliform were detected in both irrigation water recourses.

Table 1. Determination of total number of parasite, coliform and E. coli by MPN in the three types of water (Tap water, Tubli-TSE and Farm-TSE collected from a storage reservoir at a local farm).

Microbe	Tab water	Tubli-TSE	Farm-TSE
Total parasite	0	0	0
Total coliform	<1	0	6131
E. coli	<1	0	345

Table 2. Total bacterial count (CFU/plate) which were found in the three types of water water (Tap water, Tubli-TSE and Farm-TSE collected from a storage reservoir at a local farm).

Media	Tubli–TSE (CFU/ml)	Farm-TSE (CFU/ml)	Tap water (CFU/ml)
NA	0	Uncountable	2
EMB	0	>400	0
DCA	0	196	0
MC	0	262	0

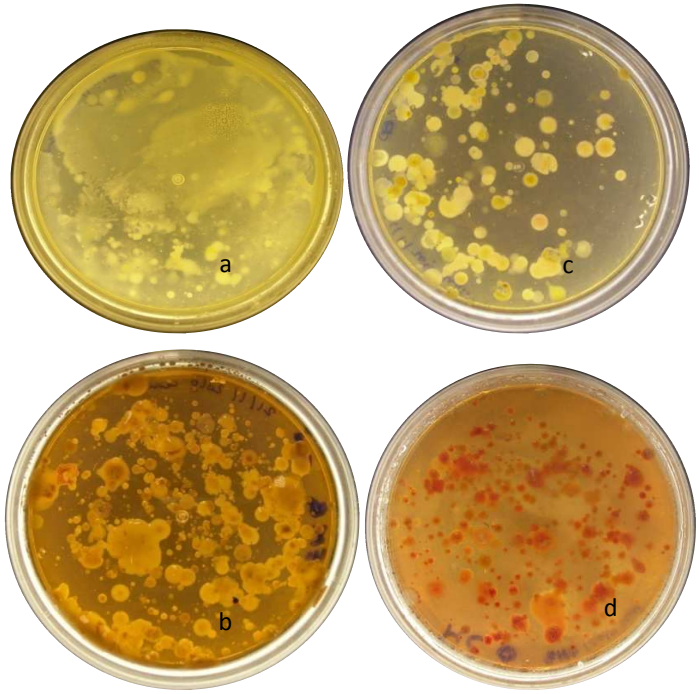

Fig. 1. Bacterial growth (24 hours) from Farm-TSE found in a reservoir at a local farm on different media (a: NA, b: EMB, c: DCA and d: Mc)

E. coli is one of the important microflora in the intestine of human and other mammals [9]. Most *E. coli* strains are harmless but several strains have virulence factors and can cause foodborne and waterborne illness e.g. diarrhea or hemorrhagic colitis [9] [10]. The presence of coliforms in water has been used as an indicator of fecal contamination, indicating the possible presence of fecal pathogens such as *Salmonella* and *Shigella* sp. [8]. Because of the difficulties in detecting many possible pathogens, further examinations for the occurrence of *E. coli* and *Salmonella* were performed. Several colonies which were grown on EMB, DCA and MC were streaked on EMB (i.e. for detecting *E. coli*) and XLD (i.e. for detecting *Salmonella*) (Fig. 2 and Fig. 3).

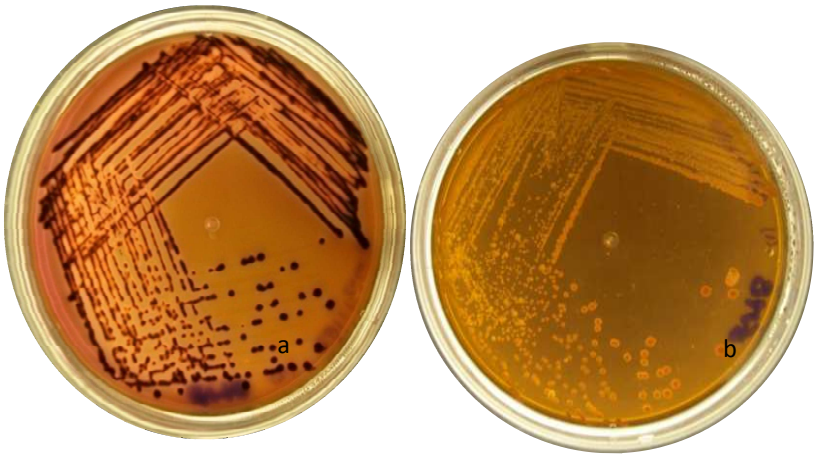

Fig. 2. Isolation of pure colonies from Fig.1 on EMB media which is selective for E. coli. a: the bacteria showed a metallic sheen appearance, which is a indication of pathogenic E. coli, b: Serratia sp.

According to literature [11] [12], *Salmonella* colonies appear red with black centre due to H_2S production, and *Shigella* colonies appear color less when grown on XLD. Based on XLD result, there was no evidence for the presence of neither *Salmonella* nor *Shigella* species (Fig. 3). Therefore, API test is necessary for further identification.

Fig. 3. Isolation of pure colonies from Fig. 1 on XLD medium.

3.2 IDENTIFICATION OF ISOLATED BACTERIA FROM FARM-TSE BY API

EMB examination confirmed the presence of coliforms in the Farm-TSE which are indicators for hygienic water quality and potential risk of infectious illness via water. Further identification of bacteria was done by API test which ensured the presence of particular pathogens (Table 3) in the reservoir of the local farm under investigation.

The presence of pathoges such as *E. coli*, *C. freundii*, *Ent. Cloacae*, *K. Pneumonia*, *A. hydrophyla*, *Ps. Paucimobilis*, *K. Oxytoca*, *Salmonella sp., A. lwoffii, Serratia sp and Ps. Paucimobilis.* were evidenced in a storage reservoir which is used for collecting the supplied TSE in a local farm. Generally, the presence of *E. coli* or coliforms in water is extensively used as an index of fecal contamination as well as to estimate the reservoir of exposure to other types of waterborne pathogens [13] [14]. Although the TSE is free of bacteria or parasite pathogens, it might be contaminated after being supplied by Tubli-WTP or during storage TSE in the local farm (Fig. 1-3, Table 1, 2). Therefore, great care must be taken when using TSE for vegetable or fruit irrigation, as it could be contaminated with pathogens. The open unclean reservoir and the distribution system could be the source of water borne pathogens.

Table 3. API identification of the pathogens which were isolated from the Farm-TSE (Tubli waste effluent collected from a reservoir at a local farm)

Pathogens	Potential health effect
E. coli	Enteric/diarrhoeal disease, urinary tract infections and sepsis/meningitis [9]
C. freundii	diarrhea, septicemia, meningitis, and respiratory and urinary tract infection [15]
Ent. Cloacae	Urinary tract and lower respiratory tract infections [16].
K. Pneumonia	Pneumonia, urinary catheters [17]
A. Hydrophyla	Respiratory tract, diarrhea alone or abdominal pain with mild fever [18].
S. paucimobilis	Septic arthritis and osteomyelitis [19].
K. Oxytoca	Colitis (inflammation of the colon) and mucocutaneous infections [20].
Salmonella sp.	Foodborne infections, enteric or typhoid fever, bacteremia, endovascular infections, focal infection and enterocolitis [21].
A. lwoffii	Bacteremia, pneumonia, meningitis, peritonitis, endocarditis, and infections of the urinary tract and skin [22].
Ps. Paucimobilis	nosocomial infections [19].
Serratia sp.	nosocomial infections, and has a mortality rate of ≤41% [23].

3.3 PHYSIO-CHEMICAL PROPERTIES OF TUBLI-STE, FARM-STE AND TAP WATER

Studying physico-chemical properties of water and comparing the results with standard values are very important to determine the quality of water [24]. Physio-chemical properties of Tubli-STE, Farm-STE and Tap water were examined. These properties include E-conductivity, pH, NH_3, NO_2, NO_3, BOD, COD and TSS according to APHA standard [6]. It was found that E-conductivity (3.9 $\mu s.cm^{-1}$) was close to the standard (3.5 $\mu s.cm^{-1}$) while pH value (pH 7.3-7.5) was below the standard (pH 8) (Fig. 4). There is an exception for E-conductivity of Farm-TSE which was below the standard, most probably due to the microbial activity present in the water sample. For NO_2, BOD, COD and TSS, they were below the acceptable standard. NH_3 in Tubli-TSE (16 $mg.l^{-1}$) and Farm-TSE (12.2 $mg.l^{-1}$) were higher than the acceptable standard (5 $mg.l^{-1}$) (Fig. 4). Total suspended solids (TSS- 2.3 $mg.l^{-1}$), BOD (8.68 $mg.l^{-1}$), COD (10.1 $mg.l^{-1}$), of the Farm-TSE water sample were found to be higher than

Tubli-TSE water sample. This high BOD value of Farm-TSE decreases the level of dissolved oxygen [24] and was in line with the presence of high bacterial content (Fig. 1, Table 1 and 2). This relationship between high BOD and high bacterial content indicates that the decrease in oxygen level was due to the action of biological process, which in turn increases COD.

Total nitrogen (16.5 mg.l^{-1}) of Tubli-TSE and Farm-TSE (16 mg.l^{-1}) are greater than the control tap water (4.6 mg.l^{-1}). Nitrate, ammonium and ammonia in addition to phosphate and other ions in TSE are considered to be essential nutrient elements for plants. These compounds enhance the agricultural productivity [25] and are of a particular advantage over the conventional irrigation water as they reduce the need for commercial fertilizers [26].

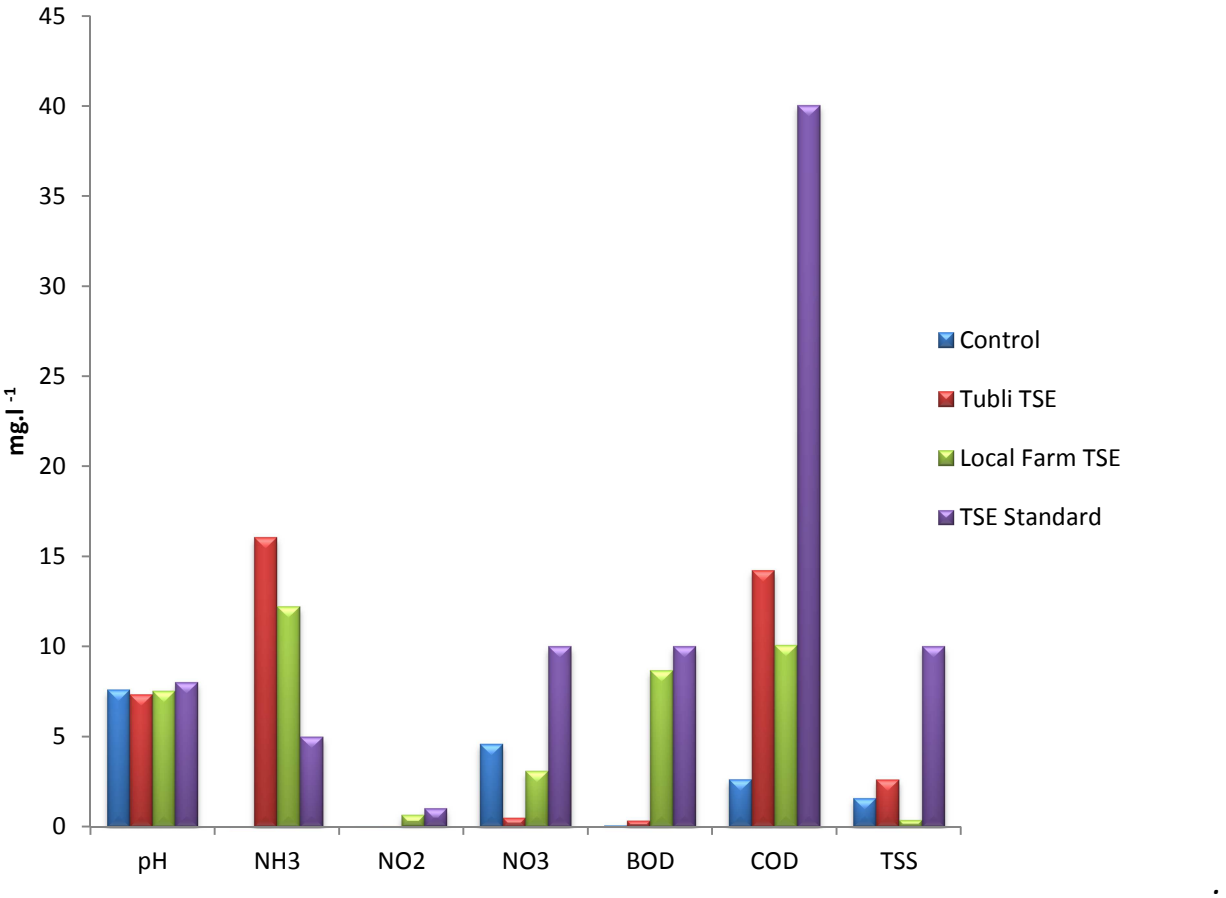

Fig. 4. Physio-chemical properties and bacterial characterization of Tubli-TSE, Farm-TSE and the control Tap water which were used for irrigation

3.4 EXAMINATION OF TRACE METALS IN TUBLI-STE IRRIGATION WATER

The presence of trace metals in the sewage treated effluent considered as a health risk; therefore, the content of trace metals were examined according to APHA standard. The level of trace metals in Tubli-STE is below APHA and WHO standard except for the cadmium which is almost near the standard (Fig. 4). Despite of the acceptable quality of Tubli-STE in terms of trace metal concentration, its health risk in irrigating crops is questionable. The effect of trace metal is accumulative. There is a great opportunity for the plant to absorb trace metals and concentrate them in the edible parts. So these edible parts of the plants which were irrigated by Tubli-STE must be analyzed for the accumulation of trace metals.

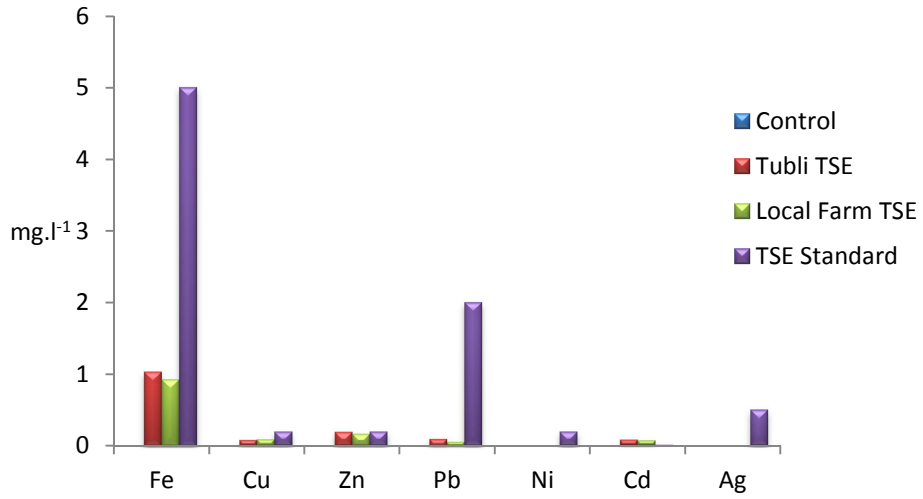

Fig. 5. Trace metals concentration (mg.l^{-1}) of Tubli-TSE, Farm-TSE and the control Tap water

Plants possess different mechanisms to maintain physiological concentrations of essential metals and to avoid non-essential trace metals [27]. They can automatically resist the occurrence of toxics including trace metals [28]. However, all plants differ in their resistance depending on the genetic makeup of the particular species [29]. At first, the plants tend to keep the toxic trace metals outside the cells. When metal ions do get into the cell, several detoxification mechanisms such as metal transport, chelation, trafficking, and impounding into the vacuole will be performed. Further lines of defense are followed by plant including the activation of stress defense mechanisms and the synthesis of signaling molecules, such as heat shock proteins, hormones and reactive oxygen molecules [27].

A study revealed that there is an accumulation of Cd, Zn, Cr, Pb, Cu, Ni, Mn and Fe in different parts of many species grown in contaminated areas. The accumulation of these trace metals in root is more than their accumulation in different parts of the plant [29]. Similar accumulation of trace metals was confirmed in another study, which showed the accumulation of Pb, Cu, Zn, Co, Ni and Cr in 16 plant species in contaminated soil in Pakistan [30]. A study done by Mustapha et al. (2014) [31] proved the presence of manganese and cadmium in the edible part of spinach which was irrigated by STE in Nigeria. This contamination with trace metals causes a serious threat to public health and food safety.

Despite of the sophisticated defense mechanism shown by different plant species to counteract the effect of trace metals on their physiology; there is a need to determine the metal accumulation in Tubli-TSE irrigated soils and crops. The authors recommend further examinations to assure the safety of using TSE for irrigation. It is necessary to monitor TSE irrigated soil and plants in terms of trace metals content in vegetable or fruits in addition to its viral contamination as viruses are a major cause for human waterborne illness. For the local farms, the farmers must be aware about keeping TSE water in a clean reservoir. Continuous monitoring and proper disinfection should be made for TSE effluent especially if it is used for irrigating fruits and vegetables eaten uncooked.

4 CONCLUSION

Tubli-TSE was found to be fit for human consumption in terms of bacterial and parasite content as it is pathogenic free. Conveying and storing the sewage treated effluent which is obtained from Tubli sewage wastewater treatment plant are very important issues. Tubli-TSE contains high content of macronutrients and micronutrients as compared to tap water. Therefore, it is more susceptible to microbial contamination. The bacterial contamination was evidenced in TSE which was supplied to a local farm (Farm-TSE). Farm-TSE was found to be unfit for human consumption due to the exceeding level of Coliforms. Having mainly excessive amounts of pathogens such as *E. coli*, *Salmonella*, *Serratia* and others are of special concern; because they cause diseases which are matters of deep public concern. The presence of certain pathogens in the sample which was collected from one farm indicates the risk of transporting and/or storing TSE water. Trace metals (Fe, Cu, Zn, Pb, Ni, Cd and Ag) and physio-chemical properties (E-conductivity, pH, NO_2, NO_3, BOD, COD and TSS) of TSE were within the American standard (APHA) except for NH_3 which exceeds APHA standard. It is difficult to admit that using Tubli-TSE is

completely safe for irrigation purpose, as further research must be carried out to monitor the accumulative effect of trace metals in plant tissue. In addition, viral examinations must be done on Tubli-TSE to assure its hygienic property.

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