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Research Article

Evaluation of Groundwater Quality against Effluent of a Slaughterhouse

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ABSTRACT

Water intended for domestic use must be free from chemical substances and micro-organisms in amounts which would provide a hazard to health is universally accepted. Livestock wastes result from slaughtering and processing procedures introduced zoonotic enteric pathogens and excess chemicals into groundwater. Physicochemical and bacteriological characters of 400 shallow well groundwater samples (zones A; B; C and D) were evaluated against the effluent of Ismailia's slaughterhouse. Physicochemical; Electrolyte; Heavy metals and bacteriological examinations of groundwater samples revealed that; groundwater sources at zone A contained a higher levels of pollutants with a gradual highly significant (P<0.01) improvement at zone C and D although they contained fecal contamination indictors. Bacteriological assessment revealed that E. coli; Enterococcus fecalis; Salmonella and Klebsiella predominated in zones A; B; C; and D (100%; 100%; 71%; 46%, P<0.01, 100%; 100%; 77%; 49%, P<0.01, 69%; 40%; 12%; 7%, P<0.01 and 69%; 46%; 7%; 5%, P<0.01; respectively). Shigella and Campylobacter predominated in zones A; B; and C (40%; 20%; 8%, P<0.01 and 28%; 19%; 5%, P<0.01; respectively). A highly significant strong positive correlations ($r \ge 0.7$, P<0.01) were revealed in pH; electrical conductivity; total dissolved solids; alkalinity; total hardness; phosphate; sulfate and nitrate. On the other hand; dissolved oxygen revealed a highly significant strong negative correlations (r \geq -0.9, P<0.01). Heavy metals and electrolyte analysis revealed a highly significant strong positive correlations ($r \ge 0.9$, P<0.01). In conclusion; high pollutants concentrations near discharge point confirmed a pointed pollution from slaughterhouse effluent, the decline pattern at zones C and D suggest self-purification abilities of groundwater sources.

Key words: Water, Bacterial count, Organoleptic, Slaughterhouse

INTRODUCTION

About 13-30% of the total freshwater volume on earth is groundwater (Dragoni and Sukhija, 2008); which considered one of the major sources of drinking water for over 50% of the world's population. Self-purification process of groundwater is the main function of soil depth with the concentration of pollutants in the percolating water (Makwe and Chup, 2013). The water used for cleaning procedures must meet drinking water standards (Fonseca *et al.*, 2000), meanwhile potable water is the one doesn't contain chemical substances or microorganisms in amounts that could cause hazards to health.

The continuous elevated levels of urbanization; as well as increased population contributed higher demands for surface and ground water, and even greater amount of organic and inorganic wastes were speared back into water sources, so less potable water becomes available (Adeyemo *et al.*, 2002). Livestock wastes result from slaughtering and processing procedures introduced zoonotic enteric pathogens and excess chemicals into the surface water and sometimes can contaminate ground water (Meadows, 1995).

Heavy metal contamination of groundwater is one of the most urgent issues in the present time (Schwarzenbach *et al.*, 2006), mainly resulting from anthropogenic activities (Bakis and Tuncan, 2011; Ağca *et al.*, 2014; Wongsasuluk *et al.*, 2014). Thus, the increased heavy metal levels and microbial contaminants in groundwater would pose potential threats to human health and survival (Hofman *et al.*, 2015; Zhang *et al.*, 2015). Also, groundwater is being influenced by climatic changes (Klove *et al.*, 2014).

The physicochemical as well as microbiological analysis of surface and groundwater are important tools for assessment of domestic and industrial activities impact

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on water sources (Amund and Odubella, 1991). The aim of this study was to evaluate the physiochemical and microbiological characteristics of groundwater against the effluent of a slaughterhouse (Abo-Khalifa) in Ismailia governorate; which can influence these water bodies, as well as detection of some indicator for water contamination as *Enterococcus fecalis*.

MATERIALS AND METHODS

Sampling

A total of 400 shallow well groundwater samples of two litter each were collected during the study period. The sampling area was divided in accordance to its distance from Ismailia's slaughterhouse into: zone A (at discharge point); zone B (at 500 m from slaughterhouse); zone C (at 3 Km from slaughterhouse) and zone D (at 5 Km from slaughterhouse) in Ismailia governorate. Samples collection was carried-out using plastic bottles; they were thoroughly washed, rinsed with deionized water and soaked for 48 h in 50% HNO₃, then rinsed thoroughly with deionized water and air-dried. Temperature, Electrical Conductivity (EC) and PH were determined at point of samples collection in the field.

Sample preparation

Each sample was divided as following: one L was assigned for physical and chemical analysis; 500 mL was preserved by adding 0.2 mL nitric acid, and kept for heavy metal analysis, and the last 500 mL was assigned for microbiological examination. Samples assigned for bacteriological examination were prepared by transferring one mL from the original dilution of these samples aseptically to a test tube containing 9 mL sterile 0.1% buffered peptone water (w/v) to prepare a dilution of 10^{-2} , then from which tenfold decimal serial dilution up to 10^{-6} were prepared to cover the expected range of samples contamination which could be easily counted.

Water physical and chemical examination

Organoleptic properties such as color, odor and taste were assessed. PH was determined using a portable PH meter. Conductivity (EC μ S / cm) was measured with a conductivity meter calibrated with potassium chloride solution before the water samples were preserved. Total Dissolved Solids (TDS mg / L) were calculated from the Electrical conductivity (EC) using correlation factor 0.67 (0.55-0.8). Dissolved oxygen (DO mg / L) was determined by Winkler's titration method using standard sodium thio-sulfate solution; manganous sulphate solution and alkali-iodide-azide reagents were added to preserve the dissolved oxygen (DO) of the samples. Alkalinity were determined by potentiometric titration using sulfuric 0.1N and mixed indicators. Total Hardness (mg / L CaCO₃) was determined by titration using EDTA solution 0.01 mol / L. Phosphate (PO_4^{2-} mg / L) was determined calorimetrically by ascorbic acid- molybdenum blue method. Sulfate $(SO_4^{2-} mg / L)$ was determined by a turbidimetric method. Nitrate (NO₃²⁻ mg / L) was determined using phenol disulphanic acid method; APHA, (2012). Electrolytes (Sodium; Potassium; Calcium; Magnesium and Chloride mg / L) were determined by Electrolyte Analyzer (Roche 9180). Heavy metals (Iron;

Copper; Zinc and Lead mg/L) were determined by atomic absorption spectrophotometer.

Bacteriological Examination and Viable Counts

Bacterial counts (TBC; TEC and TSfC) in water samples were applied using drop Plate Method (Zelver et al., 1999; Herigstad et al., 2001); plates showed 30-300 CFU were counted (Cruickshank et al., 1975-1980). Total Bacterial Count (TBC) was carried-out using standard plate count agar (SPCA); inoculated as well as uninoculated control plates were incubated at 37 °C for 24-48 h. Total Enterobacterieacae Count (TEC) was conducted using Eosine Methylene Blue Agar (EMB); plates were incubated at 37°C for 24-48 h. Metallic green colonies were counted and five typical colonies were selected and cultured onto MacConkey agar plates, incubated at 37 °C for 24 h; pure colonies on MacConkey agar plates were inoculated onto nutrient slant and incubated at 37°C for 24 h and kept for further identification. Total Enterococcus fecalis Count (TEfC) enumeration and isolation was carried out using Azide Blood Agar; inoculated and control plates were incubated at 37°C for 24-48 h. Counting the colonies surrounded with greenish discoloration of alpha (α) hemolysis (Bohm, 1971; Ruoff and Beighton, 1999). Enterococcus selective agar (Slanetz and Bartley, 1957) supplemented with 10 µg /mL tetracycline was also used in enumeration of Enterococcus fecalis and to isolate tetracycline resistant enterococci (Knudtson and Hartman, 1992-1993).

Microbial isolation

Samples were cultured on blood agar plates by striking; incubated at 37 °C for 24-48 h. The growing colonies were picked and kept for biochemical confirmation using traditional biochemical set including indole test; Methyl Red; Voges Proskauer; Cimmon Citrate (IMVIC) and TSI / LIA reactions.

Statistical analysis

The data obtained were assessed using SPSS 10.01 (version 20). Differences between water samples from different zones were determined with the one-way analysis of variance (ANOVA) test, while the nonparametric Qui-Square was used to detect the frequencies of all micro-organisms (*E. coli; Salmonella; Shigella; Klebsiella; Campylobacter* and *Enterococcus fecalis*). The data were expressed as mean±standard error of the mean (SEM). Differences were considered significant at $P \leq 0.05$ and P < 0.01 (Levesque, 2007). Bacterial count logarithmic transformation were done before analysis. The correlation co-efficient was calculated to compare the influence of each measured parameter mean values on each other (Fulekar, 2009).

RESULTS

Physicochemical examination of groundwater samples that were collected at discharge point (zone A) of Ismailia slaughterhouse (Tables 1, 2, 3) revealed the highest levels of chemical pollution; these levels are much higher than the standard levels set by WHO (Maximum permissible limits- MPL). Thus; water in these areas must not be used for human and animal consumption.

Table	1: Physicochemical	analysis of	groundwater sam	ples collected from a	different zones around	slaughterhouse
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Parameters /	Sampling area	Zone A	Zone B	Zone C	Zone D	MPL
Temp.	Range	24-26	24-26	24-26	24-26	
(°C)	Mean±SE	24.82±0.05ª	24.68±0.06 ^{ab}	24.61±0.05 ^b	24.70±0.05 ^{ab}	25
pH	Range	7.5-9.4	7.3-7.9	7.1-7.5	7.1-7.4	
(mg/L)	Mean±SE	8.71±0.04 ^a	7.51±0.01 ^b	7.32±0.01°	7.31±0.00°	8.5
EC	Range	845-1210	512-681	300-502	98-289	
(µS / cm)	Mean±SE	1028.6±8.70 ^a	580.5±4.69 ^b	389.0±5.18°	172.9±3.87 ^d	200
TDS	Range	566.1-810.7	343.0-456.2	201-336.3	65.6-193.6	
(mg/L)	Mean±SE	689.1±5.83 ^a	388.9±3.14 ^b	260.6±3.47°	115.8±2.59 ^d	500
DO	Range	0.0-0.3	2.5-3.7	4.1-5.2	6.5-7.6	
(mg/L)	Mean±SE	0.052 ± 0.00^{d}	3.20±0.02°	4.75±0.02 ^b	7.23±0.02 a	10
Alkalinity	Range	6.1-7.6	4.7-5.5	3.4-4.2	2.4-3.1	
(mval/L)	Mean±SE	7.00±0.03 ^a	5.03±0.01 ^b	3.78±0.02°	2.64±0.01 ^d	-
T. Hardness	Range	2421-2865	1625-2235	710-1352	150-632	
(mg/L)	Mean±SE	2623.7±11.4 ^a	1957.0±14.6 ^b	1061.8±12.5°	396.0±14.8 ^d	500
PO_4	Range	0.7-0.9	0.3-0.6	0.1-0.3	0.0	
(mg/L)	Mean±SE	0.85 ± 0.00^{a}	0.45±0.00 ^b	$0.14\pm0.00^{\circ}$	0.00 ± 0.00^{d}	0.1
SO_4	Range	321-721	245-398	103-278	15-110	
(mg/L)	Mean±SE	492.5±4.99 ^a	305.6±4.21 b	169.1±2.65°	55.31±1.92 ^d	250
NO3	Range	12.0-25.0	8.2-15.7	5.0-6.7	0.4-5.1	
(mg/L)	Mean±SE	19.6±0.26 ^a	10.6±0.12 ^b	5.54±0.04°	2.22 ± 0.10^d	45

Zone A (at discharge point); Zone B (500 m); Zone C (3 Km); Zone D (5 Km); MPL maximum permissible limits (WHO, 2004); Means carrying different superscripts in the same row are significantly different at (P<0.05) or highly significantly different at P<0.01); Means carrying the same superscripts in the same row are non-significantly different at (P>0.05).

Table 2: Electrolyte analysis of groundwater samples collected from different zones around slaughterhouse

Parameter	s / Sampling area	Zone A	Zone B	Zone C	Zone D	MPL
Na	Range	29.0-40.0	19.1-29.8	10.0-19.8	2.3-9.1	
(mg/L)	Mean±SE	35.85±0.23ª	26.61±0.26 ^b	15.15±0.26°	6.42 ± 0.18^{d}	200
K	Range	4.6-5.8	3.1-4.8	2.1-3.2	1.2-1.9	
(mg/L)	Mean±SE	5.31±0.02 ^a	3.98±0.03 ^b	2.57±0.02°	1.53 ± 0.01^{d}	10
Ca	Range	325-406	200-298	115-196	48-98	
(mg/L)	Mean±SE	373.4±2.13 ^a	263.0±2.23b	164.9±2.06°	66.4±1.25 ^d	75
Mg	Range	65.3-85.4	54.4-65.4	32.1-42.1	16.5-31.1	
(mg/L)	Mean±SE	75.30±0.36 ^a	59.97±0.24 ^b	$36.87 \pm 0.24^{\circ}$	23.06±0.38 ^d	50
Cl	Range	2136-5896	1700-2105	845-1250	123-395	
(mg / L)	Mean±SE	2841.2±34.47 ^a	1941.0±10.23b	1034.6±8.71°	289.5 ± 6.70^{d}	200

Zone A (at discharge point); Zone B (500 m); Zone C (3 Km); Zone D (5 Km); MPL maximum permissible limits (WHO, 2004); Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at P<0.01). Means carrying the same superscripts in the same row are non-significantly different at (P>0.05).

Table 3: Heavy metals concentrations of groundwater samples collected from different zones around slaughterhouse

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Parameters	/ Sampling area	Zone A	Zone B	Zone C	Zone D	MPL
Fe	Range	1.71-1.78	1.41-1.61	1.15-1.27	1.01-1.11	
(mg / L)	Mean±SE	1.74±0.001 ^a	1.51±0.004 ^b	1.23±0.001°	1.02 ± 0.001^{d}	0.1
Си	Range	0.17-0.19	0.13-0.15	0.11-0.12	0.08-0.10	
(mg/L)	Mean±SE	0.179±0.000 ^a	0.135±0.000b	0.113±0.000°	0.088 ± 0.000^{d}	1.0
Zn	Range	5.6-6.5	3.8-4.5	1.5-2.2	1.1-1.3	
(mg / L)	Mean±SE	6.00±0.023 ^a	4.16±0.022b	1.83±0.023°	1.18 ± 0.008^{d}	5.0
Pb	Range	0.19-0.22	0.15-0.18	0.12-0.13	0.01-0.11	
(mg/L)	Mean±SE	0.211±0.001 ^a	0.163±0.000 ^b	0.123±0.000°	0.09 ± 0.001^{d}	0.05

Zone A (at discharge point): Zone B (500 m); Zone C (3 Km); Zone D (5 Km); MPL maximum permissible limits (WHO, 2004); Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at P<0.01). Means carrying the same superscripts in the same row are non-significantly different at (P>0.05).

Analysis of water samples collected from zone A (at discharge point) revealed that; pH (Table 1) was shifted to alkaline side (P<0.01) creating suitable condition for microbial flourishing; as well as TDS (Table 1) showed a highly significant increase (P<0.01) compared to the maximum permissible limit (MPL) 500 mg / L and to the proceeding zones ensuring the low quality of water. Dissolved oxygen (DO) as shown in Table 1 and as a result of the heavy chemical and microbial existence was brought to a minimum (0.052 mg / L, P<0.01). Nitrates (NO₃) in zone A revealed a highly significant increase (19.6 mg / L, P<0.01) compared to zones B; C and D; although the levels are lower than MPL. Total Hardness; Phosphate (PO₄) and Sulfate (SO₄) recorded a highly significant increase (2623.7 mg / L, P<0.01; 0.85 mg / L, P<0.01; 492.5 mg / L, P<0.01; respectively) in water samples collected from zone A compared to MPL (500 mg / L CaCO₃; 0.1 mg / L; 250 mg / L; respectively) as well as to the other zones (Table 1).

Physicochemical characters of water samples collected from zone B (Tables 1) revealed that electrical conductivity; total hardness; phosphate and sulfate showed a highly significant increase (580.5 μ S / cm, P<0.01; 1957.0 mg / L CaCO3; 0.45 mg / L, P<0.01;

305.6 mg / L, P<0.01; respectively) compared to MPL and to the proceeding zones. Temperature; pH; TDS and nitrates were normal compared to MPL although the parameters revealed a highly significant increase (P<0.01) compared to proceeding zones.

Zone C water samples analysis (Table 1) revealed that only both of EC and total hardness showed a highly significant increase (389.0 μ S / cm, P<0.01; 1061.8 mg / L CaCO₃; respectively) compared to MPL and zone D. On the other hand; temperature; pH; TDS; phosphate and sulfate were normal compared to MPL although; these parameters revealed a highly significant increase (P<0.01) compared to zone D.

Physicochemical analysis of zone D water samples (Table 1) reveled a highly significant normal values in temp (24.7 °C, P<0.01); pH (7.31 mg / L, P<0.01); EC (172.9 μ S / cm, P<0.01);TDS (115.8 mg /L, P<0.01); total hardness (396.0 mg / L, P<0.01); phosphate (zero mg / L, P<0.01); sulfate (55.3 mg / L, P<0.01) and nitrate (2.22 mg / L, P<0.01) when compared to MPL.

Electrolyte analysis in Table 2 revealed a highly significant sodium (Na) and potassium (K) levels (35.8; 26.6; 15.1; 6.4 mg / L, P<0.01 and 5.3; 3.9; 2.5; 1.5 mg / L, P<0.01) in zones A; B; C and D; respectively. Although; these levels are normal when compared to MPL. Calcium (Ca) levels (373.4; 263 and 164.9 mg / L in zones A; B; and C; respectively); Magnesium (Mg) levels (75.3; 59.9 mg / L in zones A and B; respectively) and Chloride levels (Cl) (2841.2; 1941.0; 1034.6; and 289.5 mg / L in zones A; B; C and D; respectively) revealed a highly significant increase (P<0.01) compared to MPL.

Heavy metal concentrations (Table 3) revealed that iron (Fe) and lead (Pb) showed a highly significant increase (1.74; 1.51; 1.23 and 1.0 mg / L, P<0.01 and 0.21; 0.16; 0.12 and 0.09 mg / L, P<0.01) in zones A; B; C and D; respectively. Zinc (Zn) revealed a highly significant increase (6.0 mg / L, P<0.01) in zone A only compared to MPL. Meanwhile, Copper (Cu) maintained normal levels in all zone with a highly significant decline (0.17; 0.13; 0.11 and 0.08 mg / L, P<0.01) compared to MPL.

The physicochemical analysis; Electrolyte and Heavy metals examinations of zones A; B; C and D (Table 1, 2, 3) revealed a good improvement of groundwater quality and lower levels of all pollutants and contaminants as distance proceed from the discharge point, but the accepted levels were achieved with a highly significant decrease (P<0.01) starting from zone C (at 3 Km distance from slaughterhouse) and zone D (at 5 Km distance from slaughterhouse) when their values compared to MPL.

Physicochemical parameters correlation (Table 4) revealed weak correlations in temperature with pH (r = 0.141, P<0.01); EC (r = 0.105, P \leq 0.05); TDS (r = 0.105, P \leq 0.05); dissolved oxygen (r = -0.084, P>0.05); Alkalinity (r = 0.098, P>0.05); T. Hardness (r = 0.091, P>0.05); PO₄ (r = 0.107, P \leq 0.05); SO₄ (r = 0.106, P \leq 0.05), and NO₃ (r = 0.101, P \leq 0.05). A strong highly significant positive correlations (r \geq 0.7, P<0.01) were revealed in pH; electrical conductivity; total dissolved solids; alkalinity; hardness; phosphate; sulfate and nitrate. On the other hand; dissolved oxygen revealed a strong highly significant negative correlations (r \geq -0.9, P<0.01). Heavy metals and electrolyte analysis revealed a highly significant strong positive correlations (r \geq 0.9, P<0.01). Bacteriological examination of water samples (Table 5) revealed a highly significant (P<0.01) bacterial count at zone A (log TBC: 6.78 CFU / ml; log TEC: 4.14 CFU / mL, and log TEfC: 4.69 CFU / mL); these bacterial counts subjected to a highly significant decline (P<0.01) until reached the minimal counts detected at zone D (log TBC: 3.53 CFU / mL; log TEC: 0.78 CFU / mL, and log TEfC: 0.81 CFU / mL). Compared to WHO standards; neither of the four zones met the required levels.

Microbial isolation in Table 6 revealed that E. coli and Enterococcus fecalis were predominating with a high significant decline as zones proceed in zone A; B; C; and D (100%; 100%; 71%; 46%, P<0.01; 100%; 100%; 77%; 49%, P<0.01; respectively). Salmonella and Klebsiella predominated with a highly significant decline as zones proceed in zones A; B compared to zone C and D (69%; 40%; 12%; 7%, P<0.01 and 69%; 46%; 7%; 5%, P<0.01; Shigella and respectively). Campylobacter Spp predominated with a highly significant decline as zones proceed in zone A; B; and C (40%; 20%; 8%, P<0.01 and 28%; 19%; 5%, P<0.01; respectively); meanwhile both Shigella and Campylobacter were completely absent in zone D; confirming the highly significant improvement in water quality as zones proceed and become far away from the source of pollution.

DISCUSSION

The various watershed perturbations including input of city effluents have the potential impact of water quality degradation which can adversely affect wellbeing. Point and non-point city effluents have been found to result in deterioration in groundwater quality near to the discharge points; as well as physicochemical characteristics of groundwater varied in response to anthropogenic influences. The deterioration in groundwater quality was found to be more pronounced in regions which received urban runoff in addition to agricultural land drainage and runoff from refuse dump sites and slaughter house (Akpan, 2004). In our results; water quality at zone A (at discharge point of slaughterhouse effluent) revealed the highest levels of chemical and bacterial pollution when compared to the standards set by WHO, (2004), the condition that was clearly reflected on organoleptic characters of water.

Slaughterhouse wastes are potential sources of environmental contamination due to their high organic matter (Marchaim and Klinger, 1987). Such wastes were found to contain animal manure (dung), blood, intestinal contents and other remains which decayed and considerably increased the BOD and COD of the water. The wastes are released without treatment into the water bodies and are likely to contain high load of pathogenic micro-organisms (including Escherichia coli) which are harmful to human. Much of the effluents are discharged in intermittent pulses which can be described as episodic and the rate of flow is controlled by precipitation. The present study revealed that all the pollutants (chemical and bacterial) were exciting in higher levels at water sources that were near to the discharge point. These higher levels of pollutants were subjected to significant decline as distance proceed a way from discharge point.

 Table 4; Physicochemical parameters correlation (Below Diagonal) and Heavy metals-Electrolyte concentrations correlation (Above Diagonal) of groundwater samples

r	Fe	Cu	Zn	Pb	Na	K	Ca	Mg	Cl	
Temp.	1	0.964**	0.976**	0.967**	0.975**	0.983**	0.981**	0.982**	0.977**	
pH	0.141**	1	0.957**	0.960**	0.946**	0.956**	0.961**	0.946**	0.956**	
EC	0.105*	0.850**	1	0.960**	0.960**	0.969**	0.962**	0.974**	0.964**	
TDS	0.105*	0.850**	1.000**	1	0.949**	0.958**	0.962**	0.957**	0.959**	
DO	-0.084	-0.811**	-0.974**	-0.974**	1	0.965**	0.959**	0.964**	0.958**	
Alkalinity	0.098	0.826**	0.972**	0.972**	-0.981**	1	0.971**	0.969**	0.965**	
Hardness	0.091	0.746**	0.943**	0.943**	-0.969**	0.964**	1	0.971**	0.965**	
PO_4	0.107*	0.841**	0.966**	0.966**	-0.970**	0.973**	0.958**	1	0.967**	
SO_4	0.106*	0.809**	0.951**	0.951**	-0.965**	0.968**	0.953**	0.961**	1	
NO3	0.101*	0.846**	0.949**	0.949**	-0.956**	0.963**	0.933**	0.958**	0.946**	1
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**. Correlation is highly significant (P<0.01); *. Correlation is significant (P \leq 0.05). ^{NS}. Correlation is non-significant (P>0.05). r = 0.1-0.39 represent weak correlation; r = 0.40-0.69 represent intermediate correlation; r = 0.70-0.99 represent strong correlation

Table 5; Bacteriological counts (Mean±*SE* log TBC; log TEC; log TEfC) of groundwater samples collected from different zones around slaughterhouse

Bacterial count (CFU / mL) / Sampling area	Zone A	Zone B	Zone C	Zone D	MPL
Log TBC	6.786±0.006 ^a	5.769±0.007 ^b	4.738±0.008°	3.532±0.014 ^d	0
Log TEC	4.145±0.010 ^a	3.992±0.014 ^a	1.932±0.126 ^b	$0.785 \pm 0.088^{\circ}$	0
Log TEfC	4.695±0.005 ^a	4.466 ± 0.010^{b}	1.786±0.098°	0.817 ± 0.086^{d}	0

Zone A (at discharge point); Zone B (500 m); Zone C (3 Km); Zone D (5 Km); MPL maximum permissible limits (WHO, 2004); Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at P < 0.01). Means carrying the same superscripts in the same row are non-significantly different at ($P \ge 0.05$).

 Table 6; Intensity and frequencies of micro-organisms in groundwater samples collected from different zones around slaughterhouse

Sampling Area	Zoi	ne A	Zone B		Zone C		Zone D	
M.Os	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
E1	100	0	100	0	71	29	46	54
E. coll			P=0.0	01	$\chi^2 = 1$	23.7**		
V 1 - 1: -11 -	69	31	46	54	7	93	5	95
Klebslella			P=0.0	01	$\chi^2 = 134.7^{**}$			
Calmon alla	69	31	40	60	12	88	7	93
Saimoneila			P=0.0	01	$\chi^2 = 1$	12.9**		
Chinalla	40	60	20	80	8	92	0	100
Snigella			P=0.0	001	$\chi^2 =$	64.3**		
Campulahaatan	28	72	19	81	5	95	0	100
Campyiobacier			P=0.0	001	$\chi^2 = -$	43.6**		
Entre foodlig	100	0	100	0	77	23	49	51
Entro. Jecaits			P=0.0	01	$\chi^2 = 1$	16.7**		

Zone A (at discharge point); Zone B (500 m); Zone C (3 Km); Zone D (5 Km); Total chi-square = 96.95**; Significant qui square statistics imply a highly significant association between micro-organisms and zones from which samples were collected.

Drinking water is monitored for microbial quality as coliforms, including total and fecal coliforms (Escherichia coli) are the primary method of assessing contamination. In the European Union (EU), enterococci are used as indicators of drinking water contamination (The Council of the European Union, 1998). In the EU, enterococci are not permitted in a 100 mL sample of tested drinking water that flows from a tap, and they are not permitted in a 250 mL sample of bottled water. Enterococci are also used as indicators of fecal contamination of drinking and recreational waters throughout the world; (Stevenson, 1953). Climate is changing and will continue to change in the future affecting the level of downward movement; recharge and well discharge (Dragoni and Sukhija, 2008). Anthropogenic activities such as the release of CO₂ are accelerating global warming and affecting the hydrological cycle (Maxwell et al., 2009). The current results showed how the isolated micro-organisms as E. coli and Enterococcus fecalis were predominated in all zones from which the water samples were collected with a highly significant decline as zones proceed; the decline in the levels of the isolated micro-organisms (E. coli; Enterococcus fecalis; Salmonella; Shegella; Campylo*bacter* and *Klebsiella*) suggest the water ability for selfpurification at the depth of the soil.

Conclusion and recommendation

The elevated levels of pollutants were a live evidence for the pointed source of pollution arises from the effluent of slaughterhouses; and unfortunately the adverse effect that can be noticed on the wellbeing of animal and human if this water source were used for consumption. Advanced control measures have to be taken against slaughterhouse effluent and livestock waste to prevent the pointed pollution of groundwater sources. These control measure includes; the efficient treatment of slaughterhouse effluent using physical means as precipitation and filtration.

The present study revealed that despite of elevated degrees of chemical and bacteriological pollution in groundwater sources at different points from slaughterhouse starting from discharge points; there were a decline of these levels as the distance proceed away from the slaughterhouse. The declared pattern in decline of these pollutants (chemical and bacterial) prove the water self-purification abilities that were revealed in the promising improvement of groundwater quality until achieving the accepted levels of most bacterial and chemical pollutants at zone C (at 3 Km distance from slaughterhouse) and zone D (at 5 Km distance from slaughterhouse).

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