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Bacteriological and Physicochemical Studies of Ground-water in Beith Lahia-Gaza Strip– Palestine

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TWENTY wells of groundwater located in the vicinity of wastewater ponds were investigated to indicate the levels of water pollution during the four seasons of year (2000- 2001). Water samples were tested for the indicators of faecal pollution, heterotrophic plate count (HPC) at 25 and 37°C, total coliform, faecal coliform, faecal streptococci, *Clostridium perfringens, Pseudomonas aeruginosa, Campylobacter* and *Salmonella*. Bacteriological analysis revealed that HPC at 25°C exceeds that was counted at 37°C in the examined water samples. A marked seasonal variation on the bacterial counts was observed where the high counts of most organisms were obtained in winter months. It recorded 130,120 CFU/ml for TC and FC, respectively in the most contaminant well No. (13), corresponding to 110, 90 CFU/ ml in autumn season. Also, the secondary indicator microorganisms appeared relatively low counts while *Salmonella* sp. was not detected.

High significant differences were observed between the tested beacterial counts, wells depth, distance from the wastewater ponds.

Physicochemical analysis of water samples showed that some parameters such as turbidity, NH_3^+, No_2^-, No_3^-, K^+, BOD_5, COD and hardness exceeded the permissible level in many of tested wells. A relationship between the bacterial counts and physicochemical parameters was observed.

Prepared questionnaire indicate that most the participants used to wells water without treatments suffering from various illnesses such as diarrhea, vomiting and other gastrointestinal problems specially in winter season.

Keywords: Groundwater wells, Wastewater ponds, Indicator microorganisms, Physicochemical parameters and seasonal variations.

Groundwater in Gaza Strip is the only source for drinking water and agriculture. It is recharged from several sources include rain water, sewage irrigation, sewage infiltration and sea water intrusion. Gaza Strip suffers from pollution due to human activities on the ground surface such as intensive agriculturing (MOA, 1997 and PWA, 1999b). The major source of groundwater pollution is the infiltration of sewage, solid waste leachates and agricultural chemicals (Enhassi, 2000). Faecal contamination can reach groundwater from many routes failing septic system, leaking sewer lines and cesspools (Macler and Merkle, 2000).

Organisms commonly used as bacterial indicators for faecal pollution are the coliform group and faecal coliforms especially *Escherichia coli* (Kabler & Clark, 1960 and Hectar *et al.,* 1998). Total coliforms are good indicators for water contamination because most enteric pathogenic bacteria die off very rapidly outside the human gut, whereas indicator bacteria such as *E. coli* will persist for a certain period of time (Dutka, 1973 and Charles, 1981).

A large number of microbial pathogens are known to contaminate groundwater (Herwaldt *et al.*, 1992; Moore *et al.*, 1993 and Kramer *et al.*, 1996). These pathogens are believed to be a result of discharge of faecal material from humans and some animals into the subsurface.

Faecal streptococci and *Pseudomonus aeruginosa* are considered to be pathogens and supplementary indicators of faecal pollution in addition to *Clostridium perfringens* (Geldreich & Kenner, 1969 and Kenner, 1978). *Clostridium* spores serve as a useful long-lived indicator which are more resistant to various environmental effects in all depths (Bezirtzoglou *et al.,* 1997). Also, occurrence of *Campylobacter* in natural water is extremely variable and it can persist in cold water for up to several weeks (Blaser *et al.*, 1980).

The depth of groundwater from the discharge point of the sewage system was the main factors in influencing the pattern and severity of groundwater contamination (Chen, 1988 and Appleyared, 1996). Evidence exists that bacteria and viruses are similarly filtered out or adsorbed by some soil types under certain conditions (Bales *et al.*, 1989 and Gerba *et al.*, 1991).

The aim of the study is to evaluate the extent of bacteriological and chemical contamination of groundwater of twenty wells located in the vicinity of wastewater ponds in Beith Lahia and to study the impacts of groundwater pollution on the health of users.

Material and Methods

Sample collection

Groundwater samples were obtained from existing drilled wells. The samples were collected by electrical motor pumps already installed on the wells. Three replicate samples of the twenty wells were collected in sterile, screw-cap bottles to prevent contamination. All sample bottles were transported in an ice box at 4 $\pm$ 1°C to the analytical lab for direct bacteriological and chemical examination.

The samples were collected during the four seasons of the year starting at April 2000.

Membrane filter (MF)

This was used for the isolation of bacteria from different water samples according to APHA (1995) and Jamie & Richard (1996).

Media and growth condition

The bacteriological media used throughout this study were prepared according to Difco manual (1985). The procedures used for the detection of different bacterial counts followed the Standard Methods for Examination of Water and Wastewater (APHA, 1995).

a- Nutrient agar (Oxoid) was used and incubated at 25 and 37°C during 24 hr.
b- M-Endo agar (Difco) was used and incubated at 37 ± 0.2°C during 24 hr.
c- M-FC medium agar (Biolife) was used and incubated at 44.5 ± 0.2°C during 24 hr.
d- M-Enterococcus agar (Difco) was used and incubated at 37 ± 0.2°C during 24hr.
e- Violet Red bile agar and MacConkey agar (Difco) were used for recovery and detection of *Pseudomonas aeruginosa* at 37 + 0.02°C for 24hr. Oxidase test and Analytical Profile Index (API 20E) strips (BioMerieux) were used to confirm the isolates.
f- Selenite F-broth and S.S agar (Difco) were used for recovery and detection of *Salmonella* at 37°C for 24hr. Serological Kits and (API 20E) were used to confirm the isolates.
g- Campy-thio medium and Camplybacter Agar Base (Oxide) were used for recovery and detection of *Campylobacter* at 42 ± 0.2°C for 48hr in a microaerophlic environment (5% O_2 and 10% Co_2). Serological kits were used to confirm the test.

Physicochemical analysis

All water samples were analyzed physically and chemically according to Chapman & Pratt (1978) and APHA (1995).

A close-ended questionnaire for health impact of water pollution on the people who live and use the tested water was prepared.

Statistical analysis was done by simple correlation and regression coefficient according to Sndecor and Cochran (1981).

Results

Effect of seasonal variations, well depth, location and distance from the ponds on the level of water contamination were observed.

Effect of seasonal variation

Bacterial counts of the tested water samples obtained from twenty wells were assessed during the four seasons.

Heterotrophic plate count (HPC) incubated at 25 and / or 37 °C given in Fig. 1 showed high viable counts were observed at 25 °C throughout the four seasons especially in well No. 12. The degree of contamination was higher in winter season than the others, it recorded (750 CFU/ml) at 25 °C.

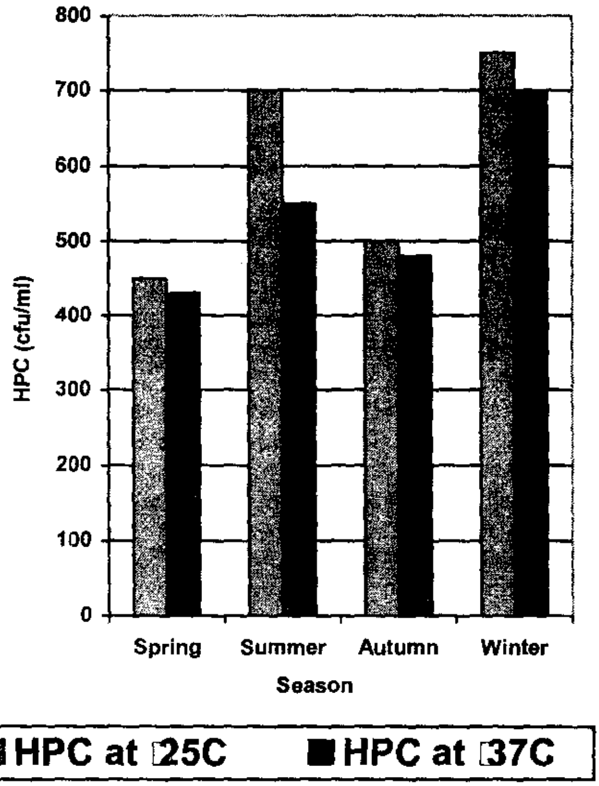

Fig. 1. Heterotrophic plate count (HPC) at 25 °C & 37 °C in well No.12 over the four seasons.

Total coliform (TC), faecal coliform (FC) and faecal streptococci (FS) counts in all tested wells over the four seasons were recorded in Fig. 2. In winter, summer and winter seasons 90, 65 and 35% of the wells contain high counts of TC, FC and FS respectively than the allowed standard.

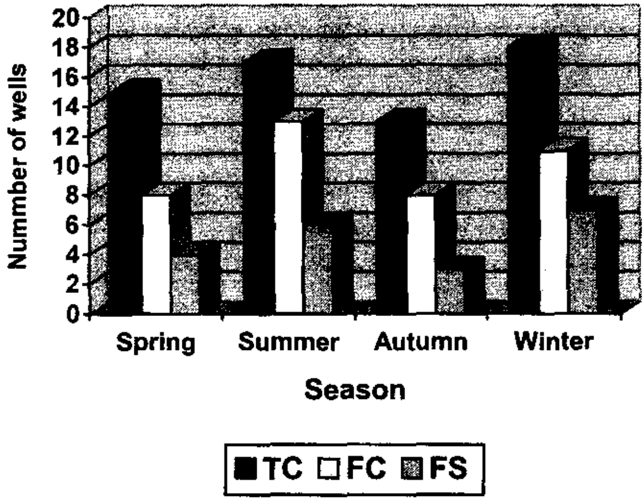

Fig. 2. Number of wells with contaminated water samples over the four seasons.

Low counts of *Clostridium perfringens* were observed in some wells (Fig. 3). The highest count (12 CFU/100ml) was obtained in well (13) during winter season. *Campylobacter* sp. was detected only in wells (12,13) while *Pseudomonas aeruginosa* and *Salmonella* were absent throughout the study.

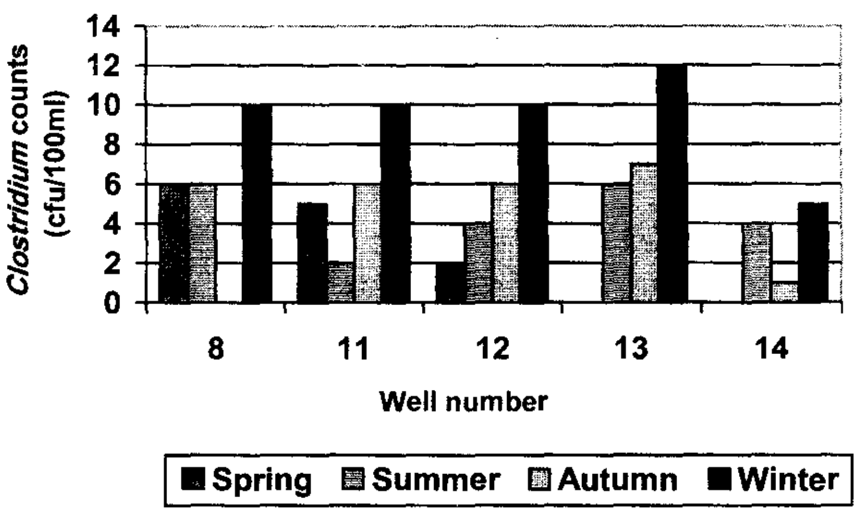

Fig. 3. *Clostridium* counts in positive wells over the four seasons.

Statistical analysis of the relationship between (TC) and (FS) in the twenty water samples indicated a strong correlation between the two organisms (Fig. 4). The faecal streptococci increase as the total coliform incrêae over the four seasons.

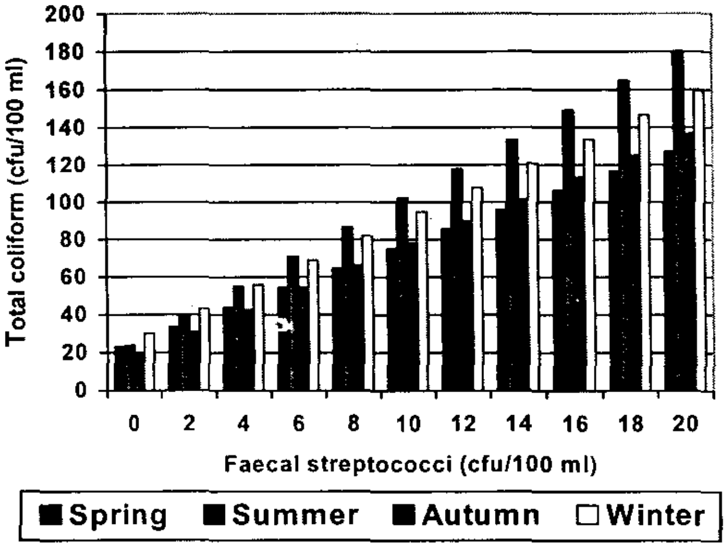

Fig. 4. Correlation between total coliform & faecal streptococci over the four seasons.

The obtained results showed increased bacterial counts in winter season than the other seasons, therefore the winter season was taken as period for further investigation.

Effect depth and distance

For winter season, the correlation between bacterial counts (HPC at 25, 37°C, TC, FC and FS), well depth and distance between the wells and the wastewater ponds were studied.

Increased level of the bacterial counts were observed as the wells depth and distance decrease, respectively. Statistical analysis at (5, 1%) levels indicate high significant differences between the bacterial counts and each of wells depth and distance (Table 1 & Fig. 5, 6, 7 and 8) .

TABLE 1. Correlation between wells depth & distance from wastewater ponds and certain microbiological parameters in winter season.

Parameters	HPC at 25 °C	HPC at 37 °C	TC	FC	FS
Depth	-0.781**	-0.505*	-0.514*	-0.433	-0.37
HPC at 25 °C		0.991**	0.932**	0.870**	0.676**
HPC at 37 °C			0.914**	0.845**	0.670**
TC				0.879**	0.746**
FC					0.837**
Distance	-0.713**	-0.725**	-0.742**	-0.691**	-0.645**
HPC at 25 °C		0.991**	0.932**	0.870**	0.676**
HPC at 37 °C			0.914**	0.845**	0.670**
TC				0.879**	0.746**
FC					0.837**

r (20-2) 5% = 0.4438 * = Significant
r (20-2) 1% = 0.5614 ** = Highly significant

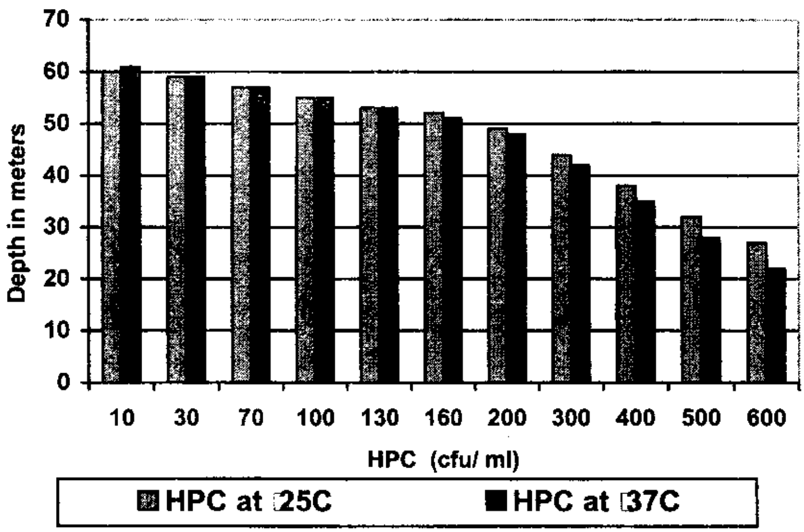

Fig. 5. Correlation between depth of the wells and heterotrophic plate counts at 25°C and 37°C in winter season.

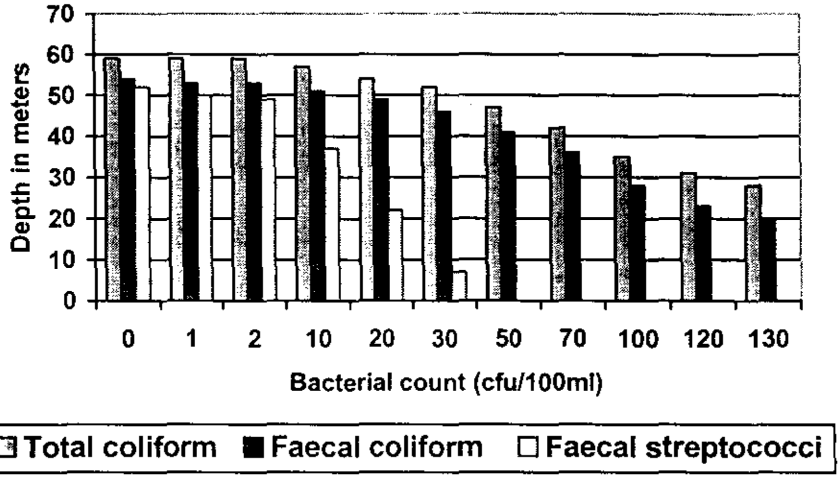

Fig. 6. Correlation between depth of the wells and each of total coliform, faecal coliform and faecal streptococci counts in winter season.

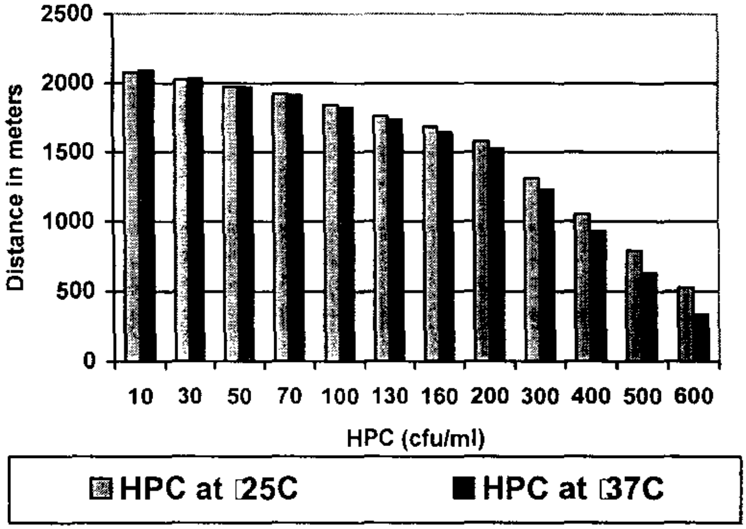

Fig. 7.Correlation between distance of the wells from wastewater ponds and heterotrophic plate count at 25 °C and 37 °C in winter season.

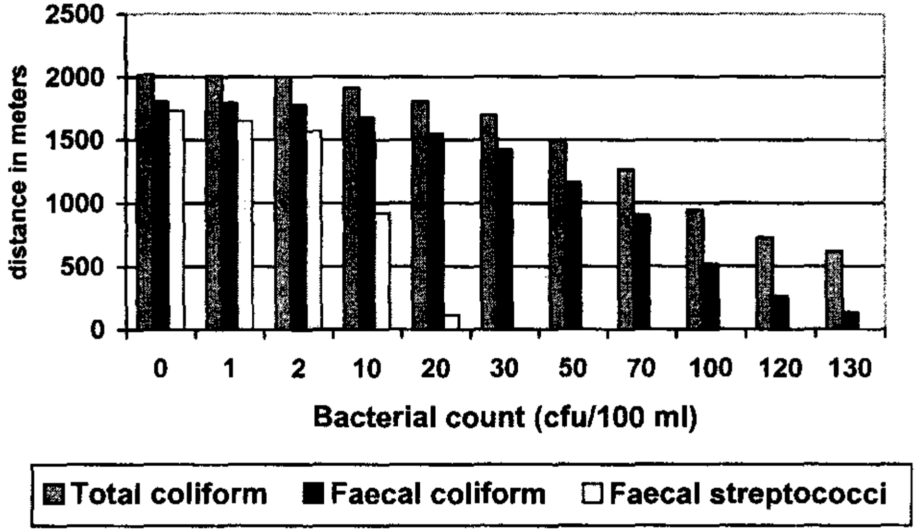

Fig. 8. Correlation between distance of the wells from wastewater ponds and total coliform, faecal coliform and faecal streptococci counts in wells water in the winter season.

Physicochemical characteristics

Physical and chemical analysis of the water samples were performed to make sure that the levels are within the limits of the World Health Organization (WHO) and Palestinian Standard (2000) of drinking water. In winter season, Table 2 indicated that, in some wells the levels of NH_3^+, NO_2^-, NO_3^-, K^+, BOD_5, COD, hardness as well as turbidity in wells (11, 12) exceeded the permissible limits.

Highly significant values at 5 & 1% levels were obtained between microbiological and physicochemical parameters (Table 3).

Discussion

Indicator microorganisms were detected in most of the sampled wells. Total coliforms were present in 90% of water wells in winter season. At the same time, HPC recorded 750 CFU/ml at 25°C and indicated variable counts in the examined wells, due to wells location, distance, depth and nutritional status.

Similar finding had been reported by Brooks & Ceck (1979); Robertson & Edberg (1997); Martino *et al.* (1998) and Cho & Kim (2000). Matthess and Pekdeger (1981) stated that many microorganisms can be transported from pollution point to a long distances (30 to 1000 m).

TABLE 2. Physicochemical analysis of twenty wells water in winter season.

Well No.	Distance	Depth	Physicochemical Analysis	Temp	pH	Turb	E.C	T.D.S	NH_4^+	NO_2	NO_3	Cl	SO_4^{2-}	Ca	[illegible]	K^+	Na^+	Alka	Hard	F	BOD_5	COD
								(mg/l)														
1	1300	127		18.8	7.06	0.8	610	406.6	0.1	0.02	22.1	55.1	40.2	48.8	30.9	1.3	16.6	173.2	233.3	0.5	0.5	3.0
2	1100	88.18		19.1	7.42	0.8	1435	956.6	0.1	0.04	41.5	198	121	129.1	54.2	1.8	78.9	306.2	568.2	0.2	0.5	3.0
3	1400	71		19.1	7.21	1	581	387.1	0.18	0.08	18.82	71.3	23.9	61.3	19.8	1.5	18.4	120.1	235.0	0.8	0.5	2.0
4	1700	60.87		18.8	7.02	0.35	512	341.3	0.06	0.02	54.32	112.2	23.7	54.4	23.8	1.8	13.8	180.2	290.9	0.6	0.0	0.0
5	2200	42.3		18.7	7.31	0.8	748	798.7	0.04	0.02	9.11	116.5	22.2	43.1	32.8	3.5	70.5	255.0	248.5	1.1	0.0	0.0
6	1750	47.72		19.2	7.31	1.08	1050	700	0.08	0.02	22.68	177	7.3	115.5	40.5	4.5	51.1	316.6	450.0	0.4	0.0	0.0
7	1900	53		19.6	7.33	1.02	382	254.6	0.1	0.04	5.31	37.9	10.8	35.8	20.3	0.8	10.6	133.6	172.1	0.5	0.0	2.0
8	450	33.55		18.9	7.06	3.41	1195	796.6	0.46	0.3	46.66	165.5	53.1	107.1	52.0	1.5	48.2	225.1	479.0	0.6	3.0	12.0
9	750	29.32		19.8	7.11	3.1	1220	813	0.46	1.2	32.5	202.2	21.1	124.1	50.7	1.5	56.9	288.1	514.6	0.4	3.5	20.0
10	1100	39.5		19.3	7.3	1.31	1080	720	0.16	0.08	25.8	1712	27.5	98.2	58.5	1.5	36.6	261.1	477.7	0.3	1.5	6.0
11	700	32.7		18.9	7.4	4.5	672	448.1	0.52	1.4	35.61	72.8	32.9	63.1	27.2	20.5	21.8	150.8	269.9	0.7	4.5	22.0
12	240	28.8		19.2	6.71	5.1	708	472	0.66	2	72.72	164	32.1	72.1	30.3	2.5	15.6	214.1	393.1	0.5	7.0	24.0
13	560	29.5		18.8	7.03	3.8	991	660.4	0.5	1	109.3	117.8	70.7	110.6	33.8	1.5	41.2	198.2	410.0	0.3	5.0	17.0
14	900	32.88		18.8	7.05	2.5	1334	889.3	0.3	0.2	20.32	228	31.4	112.4	62.1	2.3	62.7	310.0	530.1	0.5	3.5	14.0
15	1200	31.15		19.1	7.01	3.1	642	428	0.32	0.22	23.68	135.1	10.6	75.6	24.9	0.8	17.8	128.2	287.0	0.5	5.0	20.0
16	1800	32.2		18.8	6.98	0.95	381	253.6	0.22	0.12	20.61	38.2	10.2	48.1	18.3	0.8	12.1	146.3	190.1	0.6	1.5	5.0
17	2200	42.2		19.7	7.38	0.91	361	240.2	0.2	0.08	8.99	32.46	7.6	37.8	19.6	0.8	12.1	145.6	162.3	0.5	1.0	4.0
18	2500	30.9		18.9	7.23	0.81	1046	697.3	0.1	0.02	13.92	167.5	29.1	88.2	37.2	1.6	67.0	263.3	372.2	0.3	0.5	2.0
19	2600	39.23		19.2	7.2	0.92	1455	970	0.1	0.06	10.69	190	41.2	50.2	31.6	2.5	103	392.8	492.0	0.4	0.5	4.0
20	3000	47		19.3	7.02	0.66	472	314.6	0	0	21.27	44.4	21.4	59.1	24.1	1.3	12.8	154.4	248.0	0.2	0.0	0.0

Temp = Temprature (°C)
Turb = Turbidity (NTU)
E.C = Electrical Conductivity (micro sm/cm)
T.D.S = Total Disolve salts
Alk = Alkalinity as CO_3+HCO_3^-
Hard = Hardness as $CaCO_3$
BOD_5 = Biological Oxygen Demand
COD = Chemical Oxygen Demand

Distance = Distance of the well from Wastewater ponds in meter
Depth = Depth of the well from the ground level in meters
Micro sm/cm = Micromhos/cm
[shaded box] = Higher than W.H.O and Palestinian standard

TABLE 3. Correlation between certain microbiological and physicochemical parameters in winter season.

Parameters	HPC at 37 °C	TC	FC	FS	Temperature	pH	Turbidity	E.C	TDS	Ammonia	Nitrate	$B.O.D_5$	C.O.D
HPC at 25 °C	0.991**	0.932**	0.870**	0.676**	0.009	-0.517	0.933**	0.082	0.082	0.926**	0.598**	0.974**	0.956**
HPC at 37 °C		0.914**	0.845**	0.670**	0.031	-0.512	0.930**	0.094	0.094	0.919**	0.567**	0.978**	0.958**
TC			0.879**	0.746**	-0.660	-0.488	0.923**	0.131	0.131	0.961**	0.596**	0.928**	0.936**
FC				0.837**	0.053	-0.561	0.855**	0.158	0.158	0.864**	0.751**	0.868**	0.837**
FS					0.255	-0.465	0.723**	0.263	0.263	0.772**	0.494*	0.663**	0.705**
Temperature						0.229	-0.031	-0.062	-0.062	0.010	-0.265	-0.05	0.049
pH							-0.399	0.138	0.318	-0.380	-0.474	-0.528	-0.414
Turbidity								0.151	0.151	0.954**	0.600**	0.952**	0.954**
E.C									1	0.147	0.147	0.116	0.167
T.D.S										0.147	0.147	0.116	0.167
Ammonia											0.609**	0.923**	0.935**
Nitrate												0.611**	0.512*
$B.O.D_5$													0.969**
C.O.D													1

r (20 -2) 5% = 0.4438 * = Significant

r (20 -2) 1% = 0.5614 ** = Highly significant

Soil type and its structural matrix also influence the movement of microorganisms (Hagedoron, 1981 and Bales *et al.*, 1989). Cracks and fissures in clay soil facilitate the microorganisms movement (PEPA, 1994).

A marked seasonal variation on the bacterial density was observed. The highest level of contamination was detected in winter season. The rainfall could be one explanation for elevated contamination in winter months. Heavy rainfall has been shown to allow significant vertical migration of the microorganisms as well as increasing overflow wastewater from the ponds (Zyman and Sorber, 1988).

Heavily contamination in wells (12, 13) due to their flow direction with respect to the wastewater ponds (PWA, 1999a). Faecal coliform are detected in most wells with high count, and always associated with HPC densities. This is in agreement with the finding of Kabler & Clark (1960) and Blannon & Peterson (1974).

Houston (1900) reported that FS were consistently present in the faeces of all warm-blood animals. Cooper and Ramadan (1955) and Bartly & Slanetz (1960) demonstrated that FS were present in a greater numbers than coliform bacteria in faecal discharges of animals, in contrary with domestic wastewater (Hammit and Cole, 1998).

In winter season, the levels of *Clostridium perfringens* were relatively high in the positive wells. The natural resistance of its endospores may implicate old pollution of these wells, this makes a *Clostridium* a good indicator for faecal pollution (Bezirtzoglou *et al.*, 1997).

Campylobacter was detected in some of the tested wells. Taylor *et al.* (1983) confirmed the findings, *Campylobacter* sp. can be isolated from 23% of individuals with diarrheal disease acquired as water born.

Salmonella was not dtected because of the fastidious nature which exclude its persistance in groundwater. Nagaraju and Sastri (1999) stated that the prevalent environmental conditions have a strong effect on the recovery of *Salmonella*.

Physicochemical parameters showed significant correlation with the indicator microorganisms. The high level of NH_3 in groundwater was associated with the bacterial activity (Appleyared, 1996).

Nitrate is an indication of pollution from either sewage or fertilizers (Geoffery *et al.*, 1999). It is a threat to human health when present in excess in drinking water and leads to stomach cancer and blue-baby disease (Pandey and Mukherjee, 1994a,b).

Prepared questionnaire about the health impacts of contaminated wells water on the users indicated that 94.3% of the participants used to drink water without any prior treatment. Therefore most of them are suffering from various illnesses such as diarrhea, vomiting and other gastrointestinal problems. These results are in agreement with Gerba (1981); Bitton & Gerba (1982); Andersson *et al.*,(1997) and Macler & Merkle (2000).

Generally, it could be concluded that many types of bacterial contamination as well as some of metals pollution are present in most tested wells water samples. Therefore, wastewater treatment ponds are needed for maintenance and proper operations are made in order to prevent seepage and overflow of wastewater.

Also, wells located in the vicinity of the ponds which are vulnerable to contamination should be subjected to strict periodical monitoring by authorized peoples. Finally, awareness programs for consumers are necessary, and a suitable treatment such as chlorination should be recommended to eliminate pollutants.

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دراسات بكتريولوجية وفيزيقوكيميائية للمياه الجوفية في منطقة بيت لاهيا- قطاع غزة- فلسطين

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قسم النبات- كلية البنات- جامعة عين شمس- القاهرة - مصر و * قسم التحاليل الطبية- الجامعة الإسلامية- غزة - فلسطين .

يعتبر تلوث المياه الجوفية من أهم المشاكل البيئية في قطاع غزة حيث أنها منطقة شبة جافة ولذا تعد هذه المياه مصدر أساسي للشرب والزراعة. ونتيجة لقرب هذه الأبار الجوفية من أحواض الصرف الصحي ونظرا لطبيعة الأراضي الرملية في هذه المنطقة والتي تسمح بتسرب المياه بدرجة كبيرة وسريعة لذلك فقد أجريت هذه الدراسة لتقدير مدي التلوث البكتيري لمياه تلك الآبار القريبة من الأحواض وذلك لمعرفة مدي صلاحيتها للاستهلاك الآدمي من خلال إجراء بعض الفحوص البكتريولوجية والفيزيقوكيميائية.

تم في هذا البحث فحص ٢٠ بئر للمياه الجوفية والتي تقع قريبة من أحواض الصرف الصحي للكشف عن مدي تلوث المياه بها علي مدار الفصول الأربعة وذلك خلال سنة (٢٠٠٠- ٢٠٠١). تم فحص عينات المياه للكشف عن البكتريا التي تعتبر دليل علي وجود تلوث بفضلات الإنسان والحيوان وتشتمل علي العدد الكلي للبكتريا. مجموعة القولون- القولون البرازية- ستربتوكوكس البرازية- كلوستريديوم بيرفرينجس- سيدوموناس إريجونوزا- كمبيلوبكتر والسالمونيللا. أشار التحليل البكتريولوجي أن العدد الكلي للبكتريا النامية عند درجة حرارة ٢٥ منوية أعلي من تلك التي نمت عند ٣٧ درجة منوية.

لوحظ تأثير إختلاف الفصول علي الأعداد البكتيرية حيث تم الحصول علي أعداد كبيرة لمعظم الكائنات في أشهر الشتاء وقد سجلت ١٣٠، ١٢٠ مستعمرة / مللي لكل من مجموعة القولون والقولون البرازية علي التوالي في البئر رقم ١٣ علي اعتباره أكثرهم تلوثا مقابل ١١٠، ٩٠ مستعمرة/ مللي في فصل الخريف. أيضا الكائنات الثانوية الكاشفة للتلوث ظهرت بأعداد قليلة نسبيا بينما لم تكتشف السالمونيللا.

أوضح التحليل الإحصائي وجود علاقة معنوية قوية بين الأعداد البكتيرية المختبرة وكل من عمق الآبار والمسافة التي تبعدها عن أحواض الصرف الصحي وكذلك موقعها.

تم إجراء التحليل الفيزيقي والكيميائي لعينات المياه وأوضحت النتائج أن بعض القياسات مثل العكارة، الأمونيا، النيتريت، النترات، البوتاسيوم، احتياج الأكسجين الحيوي، إحتياج الأكسجين الكيميائي والعسر قد تعدت النسبة المسموحة وذلك في عدد من الآبار التي تم اختبارها. لوحظ وجود علاقة طردية بين كل من الأعداد البكتيرية والقياسات الفيزيقوكيميائية.

أوضح الاستبيان الذي تم إعداده علي المستهلكين لمياه تلك الآبار بدون معالجة أنهم يعانون من أمراض مرضية عديدة منها الإسهال والقيء ومشاكل معدية معوية أخري خاصة في فصل الشتاء.