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IJST Contact Information:
P.O. Box 2793 Amman 11953 Jordan
Tel. +962796543469
E-mails: info@ijst-jo.com / ijst.jordan@yahoo.com
URL: www.ijst-jo.com

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FORWARD

Dear Colleagues,

IJST was a fruitful effort issued by the International Centre for Advancement of Sciences and Technology – ICAST, which tries to take part in both globalization and revolution in information and communication technologies, because S&T development becoming not only the key elements of economic growth and industrial competitiveness, but also essential for improving the social development, the quality of life and global environment. ICAST took then a decision to establish a scientific alliance with TSTC (Tharwa for scientific Training & Consultations) and this alliance comes to support the efforts towards publishing IJST.

Today, we announce a new issue of our journal, that is the fourth issue from the fourteen volume of IJST, December, 2019.

Finally, I hope that all significant figures of sciences whom joined the editorial board, the researchers, and the readers of our journal will keep IJST between their eyes and contribute in continuing its journey, with their remarks, valuable recommendations and their researching outcomes.

Thanks a lot for all who support IJST.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

The Referees for this Issue

** The referees and advisory group below are listed according to alphabetical order, with deep appreciation for all.*

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A study of the bacteria forming the biological membranes in some liquefaction stations in the governorates of Nineveh and Salah Al-Din

Ranaa Wadullah Younus (1) Karkaz Mohammed Thalij (2) and Yasseen Hussain Owaied (3)

(1) Dept. of Biology/ Faculty of sciences/ University of Mosul (2) Dept. of Biotechnology/ College of Agriculture/ University of Tikrit (3) Dept. of Biology/ College of Education for Women/ University of Tikrit
Republic of Iraq

E- mail: wy555@yahoo.com

ABSTRACT

The study was conducted with the aim of isolating and diagnosing contaminated microorganisms from water samples taken from four areas extending from Northern of Mosul to Tikrit in the South represented by four liquefaction stations in Mosul, Qayyarah, Baiji and Tikrit in Iraq, and determining the component of these microorganisms' biofilms. Membrane filtration technique was used to isolate contaminated bacterial species with water samples.
The results showed that there are sixteen species that contain Germ-positive and negative bacteria, and it turns out that the dominant species is *Bacillus cereus*. *Salmonella paratyphi* and five dominant species were identified.
The most isolated microbial species consisting of biofilms are *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus, E. coli,* and *Citrobacter freundii*. The most important findings of the research are the possibility of the isolated types prevailing in the formation of biofilms on the walls of iron and plastic tubes in a laboratory, which can lead to corrosion of pipes and a change in the taste and smell of water.

Keywords: biological membranes. liquefaction stations.

INTRODUCTION

Biofilms formed due to their discovery in fossils more than three billion years ago (1). The Dutch scientist Anthone Van Levenhoke was credited with discovering it who discovered the simple microscope and observed the possibility of microorganisms sticking to the surfaces of the teeth (2). Attention has continued to know how these membranes form. In (1940) Heukelekian *et. al.* had observed that some types of bacteria can grow and multiply after adhering to the glass surfaces in the water, and that what enhances this is the binding and stability of cells with the surfaces in which they are attached (3). In 1943, Zobell also confirmed that the numbers of bacteria attached to surfaces were higher than in the surrounding medium. However, details of the nature of the biological membranes were not clarified until the discovery of the electron microscope, which distinguished the formation of the membrane more efficient than optical microscope (4).

Characklis (1973) showed that microbial cells forming membranes in industrial water systems are characterized by their ability to resist chlorine even at high concentrations (5).

These discoveries had aroused attention among researchers about the possibility of increasing the effect of microbial species that make up membranes in the health, industrial and environmental aspects, as Costerton *et. al.* (1978) had created four theories of biofilm formation by bacterial species that clarified the mechanisms in which these organisms adhere to living and non-living materials (6).

On the other hand, Studies have shown that the vast majority of bacterial species in the world can be present in the aquatic environment and biofilms are formed (7), as is the case when they are found in non-aqueous environments, as they were able to produce biofilms to be present on medical devices (8), likewise on dental plaques causing their necrosis (9), and also contributed to the formation of fibrous cysts for patients where the numbers of Pseudomonas aeruginosa disappear.

METERIALS AND METHODS

The study was conducted in the laboratories of the Department of Biological Sciences - College of Education for Women, University of Mosul during the period from July 1, 2011 until the end of June 2012, and was applied on four liquefaction stations, selected to follow water pollution in the riverbed through filtering stations to the liquefaction networks, in Mosul, Qayyarah, Baiji and Tikrit, to observe the microbial contamination of water and determine the types that make up the biological membrane from isolated microorganisms. The stations were as follows:

1. Zone 1 (Al-Arabi / New Extension Station).
2. Zone 2 (Qayyara Liquefaction Station).
3. Zone 3 (Baiji Liquefaction Station)
4. Zone 4 (Tikrit Liquefaction Station/ University).

Water sampling:

Samples were collected throughout the four seasons. As 300 samples were collected from the four stations (study areas), samples were taken from three locations in each station that included the riverbed with a depth of half a meter and from the water tanks in the filtering station. After purification, samples were taken from networks to liquefy through the water faucet immediately after a good cleaning of clay or calcined materials, then washed by tap water for about 3 minutes to get rid of stagnant water. The samples were taken and preserved in sterile 250 ml glass bottles with three replicates per zone. The samples were placed in a container of cork containing ice and sodium thiosulfate that was added to stop the effect of chlorine. It was brought to the laboratory for a transfusion procedure and record for each sample number, place of collection and date of collection (10).

RESULTS AND DISCUSSION

Isolation and diagnosis of microbial species

Membrane filtration technique was used for the purpose of isolation and after diagnosis it was found that sixteen bacterial types of microorganisms including Gram-positive and negative bacteria were found and the dominant type was *Bacillus cereus* and the least dominant was *Salmonella paratyphi* as shown in table (1). As the feed media has been used to detect aerobic microbial types from bacteria, as it is a general medium for bacterial growth, especially when the growth is at 37 o C and in air conditions that are suitable for most types of bacteria in their growth, therefore the types that have emerged are among the aerobic types of bacteria.

The tests described in table (2), are considered the most important tests through which the types of intestinal bacteria are diagnosed after their development on the MacConkey agar medium and observed on the culture medium in terms of shape and diameter, as well as their shape under the microscope and their ability to pigment in Gram stain. In addition to the IMViC tests and the test of catalyze and oxidase and their ability to move as well as their ability to produce urease enzyme and production of hydrogen sulfide in addition to its ability to ferment some types of sugars. The indicated tests are sufficient to reach the level of the type of intestinal bacteria and complete the diagnosis. After comparison through the tests indicated for the purpose of diagnosis, it was found that types of bacteria were *E. coli, S.paratyphi, Pro.Mirabilis., Ps.aeruginosa., Y. enterocolitica, Cit. freundii, Cit. diversus* and *Pro.alcalifaciens*.

Table (1): Numbers and percentages of bacteria species isolated from study sites

Isolated species		Study zones												Total number	%
		Mosul			Qayyara			Baiji			Tikrit				
		1	2	3	1	2	3	1	2	3	1	2	3		
Gram –ve	*Escherichia coli*	13	0	0	5	2	2	2	1	0	1	0	0	23	9.5
	Salmonella paratyphi	0	0	0	0	3	3	0	0	0	0	0	0	3	1.2
	Proteus mirabilis	1	2	0	1	1	1	1	0	0	2	1	1	10	4.1
	Yersinia pseudotuberculosis	0	0	0	3	1	1	0	0	0	1	0	0	5	2
	Yersinia enterocolitica	0	1	0	0	0	0	0	0	0	0	0	0	1	0.4
	Citrobacter freundii	0	0	0	3	3	3	1	0	0	0	4	0	15	6.2
	Citrobacter diversus	1	0	0	0	0	0	1	0	0	0	0	0	2	0.8
	Providencia rettegri	3	0	0	0	2	2	1	1	0	0	0	0	7	2.9
	Providencia alcalifaciens	0	1	1	0	0	0	0	0	0	1	0	0	3	1.2
Gram +ve	*Staphylococcus aureus*	4	0	1	5	1	0	4	0	0	1	1	0	17	7.0
	Staphylococcus epidermidis	0	2	0	0	0	2	0	0	0	0	0	0	4	1.6
	Staphylococcus Saprophyticus	0	0	0	0	0	0	1	0	0	0	0	0	1	0.7
	Bacillus cereus	23	9	5	19	14	3	18	11	8	17	11	7	145	60
	Streptococcus pyogenes	1	0	0	0	0	0	0	1	0	0	1	0	3	1.2
	Streptococcus mutans	0	0	0	0	1	0	1	0	0	0	0	0	2	0.8
	Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	1	0	1	0.4
														242	**100**

1 = river water sample 2 = liquefaction station water sample 3 = home water sample

Table (2): Results of biochemical tests for the diagnosis of isolates from different locations of the Tigris River

Bacterial species	IMVC	Catalase	Oxidase	Movement	Urease	TSI	H_2S	Sugar fermentation					
								Glucose	Lactose	Sucrose	Sorbitol	Arabinose	Xylose
E. coli	++ – –	+	–	+	–	A/A	–	+	+	V	+	+	+
S.paratyphi	– + – –	+	–	+	–	AIK/A	+	+	–	–	+	+	–
Pro. mirabilis	– + vv	+	–	+	+	AIK/A	+	+	–	V	–	–	–
Y. pseudotuberculosis	– + – –	+	–	+	+		–	–	–	–	–	+	+
Y. enterocolitica	v + – –	+	–	+	V		–	–	–	+	+	+	V
Cit. freundii	– + – +	+	–	+	V	A/A	+	+	V	V	+	+	+
Cit. diversus	++ – +	+	–	+	V	A/A	v	+	V	V	+	+	+
Pro. alcalifaciens	++ – +	+	–	+	–	AKV/A	–	v	–	+	–	–	–

For the diagnosis of microbial types of staphylococcus, its isolation was through its development on the Mannitol Salt Agar medium, as the susceptibility of isolates was tested on fermentation of mannitol sugar, and after making sure that it was positive for Gram stain, its ability to produce catalase and oxidase (table 3) were tested and its ability to Movement, urease production, hydrogen sulfide production and fermentability of some types of sugars. After comparing the development outcomes on the aforementioned media and tests, the diagnosed species included the following *Staph. aureus, Staph.epidermidis, Staph.saprophyticus, B.cereus, Str.pyogenes, Str.mutans* and *Pse.aeruginosa* The indicated species appear mostly in the composition of the biological membrane when they are grown on the medium suitable for growth. Figure (1) describes the numbers and percentages of bacterial species isolated from study zones, while figure (2) describes the most prevalent types of bacterial species isolated from the study zones.

Table (3): Results of biochemical tests for the diagnosis of staph, streptococcus and pseudomonas bacteria isolated from different locations of the Tigris River

Bacterial species	Catalase	Oxidase	Movement	Urease	Bacitracin	TSI	H_2S	Sugar decomposition					
								Glucose	Lactose	Sucrose	Sorbitol	Arabinose	Xylose
Staph.aureus	+	–	-	V	+	-	-	+	+	+	+	–	–
Staph.epidermidis	+	–	-	+	–	+	-	+	V	+		–	–
Staph.Saprophyticus	+	-	+	+	–	+	-	+	+	+	-	–	–
B. cereus	+	-	+	-	-		-	–	-	-	+		–
Str.Pyogenes					-.			+	+		–		
Str.Mutans			–		….			+	+	+	+		
Pse.aeruginosa	+	+	+	V	–	AIK/AK	–	+	–	–			

…… not tested , + positive, - negative results

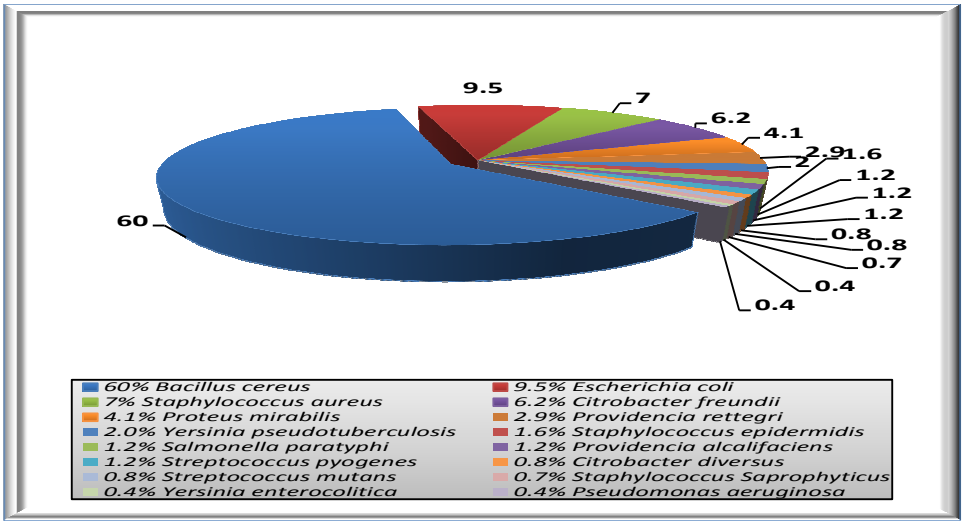

Figure (1): Numbers and percentages of bacteria isolated from study zones

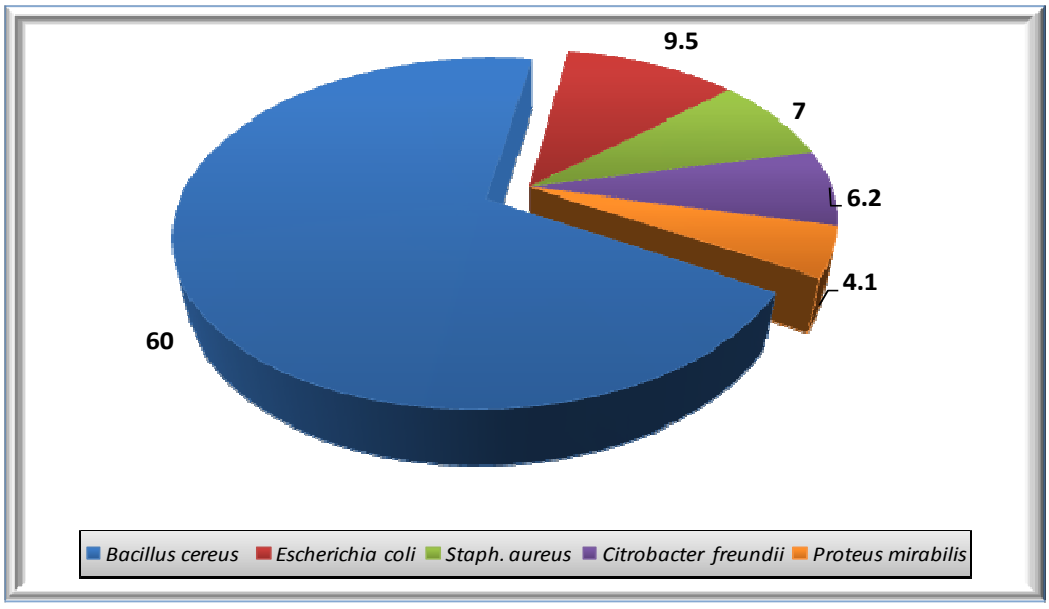

Figure (2): The most prevalent bacterial species

Biofilm production of bacterial species in test tubes

Biofilms have the potential to be formed on plastic and iron tubes in drinking water distribution systems when bacterial cells attach to the surface of tubes and are added to be a viscous film or layer on the surface of tubes (11), where (12) surveys of iron tubes removed from water distribution systems have been conducted and observed the scattered colonies on the surfaces of tubes associated with cracks in the mineral layer, the metabolism of bacteria in the biofilms leads to the production of hostile materials such as acids as well as natural patches of biofilms that generate a region of different oxygen content under the layer of the steel surface and lead to these properties (acid and Oxygen) to induce corrosion (13).

Characklis (14) had noticed that the biofilms were collected on the plastic tubes in the non-chlorinated water and find that the cells cover the area of $5x10^{10}$ M^2 / CELL.

The most prevalent bacterial isolates (*Proteu mirabilis, Staphylococcus aureus, Bacillus cereus, E. coli, Citrobacter freundii*) were activated on the Brain Heart Infusion Broth and 0.1 ml of bacterial suspension was added to each of them and added to containers filled with sterile drinking water and placed inside two tubes, one of them is of galvanized iron and the other is a 1/2 inch plastic and a length of 10 cm. The containers were incubated for 12 days using a water bath at a temperature of 35 ° C and used the method of publication by adding 0.5 microbial suspension after comparison with the Macferland tube 1.102 concentration and added to the implant media for a purpose. The colonies were counted and deployed with the L-Shape glass rod as indicated table (4).

The results showed the susceptibility of coliform semi-meters represented by *E. coli* and *Proteus mirabilis* on the formation of biofilms on iron tubes and this corresponds to a study (15) that showed the susceptibility of coliform semis isolated from the River Seoul in the formation of biofilms on galvanized iron tubes. According to (16) *E. coli* bacteria have the ability to form biofilms in the drinking water distribution network, whether it is iron or plastic tubes. Another study also found that plastic tubes are suitable for drinking water distribution tubes, and this is due to the lack of biofilms and the growing microbial diversity on them (17). The biofilms in Staphylococcus aureus found in drinking water distribution systems cause health problems and can pass on resistance to antiseptics, and they are known for their ability to stick to plastic surfaces (18). Chang *et. al.* explained that biofilms developing on tubes with rough surfaces such as galvanized iron and steel molds are more than developing on the smooth surfaces of plastic tubes (19) and this is what the results of the current study showed.

Table (4): The numbers of bacterial species forming biofilms in liquefaction tubes models

Serial of sample	Area	Bacterial species	Total numbers of bacteria species	
			In iron tubes	In plastic tubes
1	Mosul / after treatment	*Proteus mirabilis*	772	560
2	Tikrit / after treatment	*Proteus mirabilis*	789	750
3	Baiji/ before treatment	*Proteus mirabilis*	995	496
4	Qayyara / after treatment	*Proteus mirabilis*	1544	800
5	Mosul / after treatment	*Staphylococcus aureus*	2000	296
6	Qayyara / before treatment	*Staphylococcus aureus*	542	1360
7	Baiji/ before treatment	*Staphylococcus aureus*	864	511
8	Tikrit / after treatment	*Staphylococcus aureus*	457	541
9	Baiji/ before treatment	*Bacillus cereus*	850	493
10	Qayyara / before treatment	*Bacillus cereus*	384	118
11	Tikrit / before treatment	*Bacillus cereus*	1520	1360
12	Mosul / before treatment	*Bacillus cereus*	992	880
13	Qayyara / before treatment	*E. coli*	592	440
14	Baiji/ before treatment	*E. coli*	597	117
15	Tikrit / before treatment	*E. coli*	512	352
16	Mosul / before treatment	*E. coli*	1250	1048
17	Tikrit / after treatment	*Citrobacter freundii*	1000	240
18	Qayyara / before treatment	*Citrobacter freundii*	282	77
19	Qayyara / after treatment	*Citrobacter freundii*	400	1712
20	Baiji/ before treatment	*Citrobacter freundii*	260	985

The isolated species showed that the source of the river pollution has moved from it to the filtering stations and then to the distribution networks in the homes, due to the presence of the same isolated species in the river. The isolation of these species is evidence of the inefficiency of the filtering and sterilization process in the filtered and sterilized stations of the studied water and is of great importance as there is a link between isolation of these neighborhoods and diarrhea cases (20).

Sample culturing and enumeration

Bacillus cereus is the most sticky and fast type in adhesion, as the mechanism of adhesion is through its blackboards as a single layer on many types of surfaces (21). Stoodley *et. al.* found that *Bacillus cereus* and *E. coli* were good examples of biofilm formation, as changes in the genetic organization appear to cause the biofilms of these two species to appear outwardly and also different from the rest of the neighborhoods, such as phytoplankton (22). A study conducted by Harkes *et. al.* showed that the *E. Coli* cells adhere in an irreversible manner and have the ability to migrate on the hard surface (23). Among the most isolated species of different types of water, including drinking water distribution systems, is *Staph.aureus* and has the ability to form biofilms on different types of surfaces (24).

The most dominant bacterial isolates (*Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, E. coli, Citrobacter freundii*) were activated on the Brain- heart infusion broth and taken 0.1 ml of bacterial suspension for each of them and added to containers filled with sterile drinking water and placed inside the containers. Five glass slides were incubated in containers for 12 days using a 35 ° C water bath and used the diffusion method by adding 0.5 microbial suspension after its comparison with the Macferland Tube and it was found that its concentration $1x10^2$ was added to the culture media for the purpose of counting colonies and deployed with a glass rod (L-Shape)), And it turns out that these predominant species have the ability to form membranes Biofilms on glass slides (Table 5).

Table (5): Biofilms formed on glass slides

Serial of sample	Area	Bacterial species	Number of colonies after 12 days
1	Mosul / after treatment	*Proteus mirabilis*	300
2	Tikrit / after treatment	*Proteus mirabilis*	1200
3	Baiji/ before treatment	*Proteus mirabilis*	1000
4	Qayyara / after treatment	*Proteus mirabilis*	250
5	Mosul / after treatment	*Staphylococcus aureus*	1720
6	Qayyara / before treatment	*Staphylococcus aureus*	259
7	Baiji/ before treatment	*Staphylococcus aureus*	138
8	Tikrit / after treatment	*Staphylococcus aureus*	350
1	Baiji/ before treatment	*Bacillus cereus*	400
2	Qayyara / before treatment	*Bacillus cereus*	1096
3	Tikrit / before treatment	*Bacillus cereus*	656
4	Mosul / before treatment	*Bacillus cereus*	1040
1	Qayyara / before treatment	*E. coli*	592
2	Baiji/ before treatment	*E. coli*	101
3	Tikrit / before treatment	*E. coli*	445
4	Mosul / before treatment	*E. coli*	1080
5	Tikrit / after treatment	*Citrobacter freundii*	808
6	Qayyara / before treatment	*Citrobacter freundii*	744
7	Qayyara / after treatment	*Citrobacter freundii*	480
8	Baiji/ before treatment	*Citrobacter freundii*	1280

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Correlation between genital mycoplasma and infertility among Iraqi men and women: conventional and PCR methods

Eman A. K. Al-Fatlawy (1) and Abdul Jabbar N. Al-Shammari (2)

(1) Dept. of Central Veterinary Laboratory/ Veterinary Directorate/ Ministry of Agriculture/ Baghdad, Iraq, (2) Dept. of Medical laboratory Sciences/ Faculty of Science/ Al-Balqa' Applied University, Al-Salt, Jordan.

E –mail: shammari.icastjo@yahoo.com

ABSTRACT

The project for genital mycoplasma and its correlation with infertility among Iraqi males and females started in 1995. The project was continuing in intervals according to sample availability until 2016. The current study was focused on one of the more debatable issues about the correlation between the isolation of genital mycoplasma and infertility. To approach the goal of the study genital mycoplasma were isolated from infertile and fertile couples and related to infertility. A total of 1951 specimens were collected from 956 male s aged between 18-55 years and 995 females aged between 18-45 years, attended the infertility center in Alwya hospital, Central Diagnostic laboratory, Ministry of health and private sector clinical laboratories in Baghdad, Iraq. The conventional methods including; cultural, biochemical and serological methods. Six selective media for isolation of *Mycoplasma gentillium, Mycoplasma fermentans, Mycoplasma hominis and Ureaplasma urealyticum were used*. Growth inhibition test and direct immunofluorescences test using known specific antisera. Selected isolates identified by conventional methods was confirmed by PCR protocol with species-species primers for *M. gentilium, M. hominis and U. urealyticum*. Out of the 1951 patients studied, 951(48.7%) were positive for *U. urealyticum, M. hominis, Mycoplasma fermentans and M.gentillium*. The best media that support the growth of mycoplasma and ureaplasma from seminal fluid and endocervical swabs was Modified AE10 broth medium. The 951 isolates were grouped into six groups according to biochemical and serological methods. Of these isolates; 296 *M. hominis* (31.12 %), 255 *Ureaplasma urealyticum* (26.81%), 202 *Mycoplasma fermentans* (21.24 %), 76 *Acholeplasma laidlawii* (8.03%), 66 *M. ariginin* (6.94%) and 56 *M. gentillium* (5.85%). PCR assay was applied to confirm the identification of genital mycoplasmas identified by conventional methods. Six Mycoplasma, 3 Urea plasma and one mixed culture grown on modified AE 10 and were selected to amplification of DNA from 100 CFU of each. The results were reveals on ethidium bromide-stained agarose gels, shows identical species to that found on culture. The current studies confirm the isolation of genital mycoplasma from infertile patient and the significantly correlation between the frequencies of the isolation of mycoplasma and urea plasma and infertile couples.

Keywords: *U. urealyticum, M. hominis, Mycoplasma fermentans and M.gentillium*, infertility, PCR.

INTRODUCTION

Group of human genital mycoplasma was isolated from patients suffering from genital disorders, such as Bartholin's gland abscess (1), Vaginitis (2), non-specific gonococcal urethritis (3,4), urethra-prostatitis (5), Pregnancy-related conditions (6).
In Iraq, investigation of Mycoplasmas infection was started from the beginning of 1974 and concentrated on animals' mycoplasmas, later on at the mid ninety Al-Shammari team started to investigate the isolation of mycoplasma and ureaplasma from human (7-11). Researchers around the world investigated the correlation between the occurrence of genital mycoplasma in infertility male and female, because of difficulties in applied Koch' postulate; mostly the correlation depends upon finding these organisms in infertile vs fertile patients. This long debatable story started from the seventies when numerous reports reported the recovery of genital mycoplasma from infertile patients and response to antibiotics that eliminated mycoplasma (12-15). On the other hand, there were a few reports documented the lack of mycoplasma role in infertility (16,17). Even though the researcher around the world did not give up, but they continue digging to support their hypothesis and to find out the controversial tasks that support the role of mycoplasmas in involuntary infertility and pregnancy losses.
The current study collected a sufficient number of participants for 10 years and watched the outcome of this hypothesis depend on the outcome of the patient's status. new information and try to outline current concepts concerning the role of this unique group of Mollicutes microorganisms. To support the hypothesis, the experiments were designed to depend upon conventional and molecular methodology.

MATERIALS AND METHODS

Patients: A total of 1951 men and women with failure to conceive after one year of unprotected intercourse attending Alalwai Infertility Clinic of Gynecology outpatient department, Central Diagnostic laboratory, Ministry of health and private sector clinical laboratories in Baghdad, Iraq. Females aged between 18-45 years and males between 18-55 years. All couples were free from administered antibiotics for more than 2 months. Five hundred healthy fertile women and men who had pregnancy several times were used as a control in the experiments. Clinical history of the patients was taken including patient demographic social and if the patient suffers or exposed to venereal diseases, non-specific gonococcal urethritis (NSG), reproductive tract infections, spontaneous abortion, and hormonal disturbances.

Samples: Four hundred and three seminal fluids were collected from infertile males married for 1-8 years, and 548 endocervical swabs samples were collected from the infertile female for 1-8 years. All samples collected aseptically and transported to the laboratory in transport media (18).

Media, strain and antisera: Six media were used to cultivated genital mycoplasma; namely Arginine broth medium (19), modified Hayflick broth medium (11), Urease color test broth (U9c, U10c, UB) and modified AE 10 medium. All media were papered aseptically as mentioned by Al-Shammari and Al-Aubadi (19). Standard mycoplasmas species and their antisera were obtained from international laboratories have been sent to Al-Aubadi previous, and used as described in table (1).

Table (1): Standard mycoplasmas used for identification and characterization of isolates in this study

Genital mycoplasma	Strain	Source
Mycoplasma hominis	PG20	Al-Aubaidi (lemcke 1964)
Mycoplasma fermentans	PG18	Al-Aubaidi (Edward 1976)
Mycoplasma arginini	1L	Al-Aubaidi (Edward 1976)
Acholeplasma laidlawii	B6p	Al-Aubaidi 1972
Mycoplasma genitalium	PG6	Al-Aubaidi 1972
Ureaplasma urealyticum	Boston T-stain	Al-Aubaidi (Kundsin 1967)
Ureaplasma urealyticum	K12	Al-Aubaidi (Shepard 1974)
Ureaplasma urealyticum	P108 serotype	Al-Aubaidi (Romane 1980)

Cultivation:

Cultivation of semen samples: All six media were used to cultivate genital mycoplasma and Ureaplasma species as described previously (11,19), briefly by inoculate 0.1 and 0.2 ml from transport media containing seminal fluid on plates agar and 1.8 ml of broth media respectively. Broth were further diluted for three successive dilutions and incubated at 37C for 3-7 days. Color change unite (CCU) was used to evaluate the growth of ureaplasma. Loopful of tube gave highest dilution after CCU was inoculated on corresponding agar and incubated as mentioned above.

Cultivation of endocervical swabs: A 0.2 ml of transport media containing the endocervical swab was inoculated on 1.8 ml broth media mentioned above and incubated at 37C for 3-7 days.

Purification of mycoplasma and Ureaplasma colonies: Single colony was selected for further purification by transfer the agar block containing the colony and inoculated into cross ponding broth media (20).
Biochemical characterization: Biochemical tests including; sugar fermentation, Arginine deamination, tetrazolium reduction and film and spots as described previously (19,20).

Serological characterization:

Growth inhibition test: Test was performed as described by Clyde (21). Diluted mycoplasma and Ureaplasma broth were diluted to obtain that containing 106 CFU/ml**,** 0.1 ml was spread on agar. Sterile filter paper disk (6 mm in diameter) were impregnating with 0.025 ml of standard mycoplasma and Ureaplasma antisera (table 2). The zone of inhibition around known disk was measured in millimeters, positive results regards when the zone of inhibition more than 2 mm, less than 2 mm regards suspected and repeated again, negative result when there was no zone of inhibition.

Direct immunofluorescence: The test was performed as described by Al-Aubadi and Fabricant (20). Briefly; agar blocks containing isolated mycoplasma or Ureaplasma colonies treated with 2 drops of homologue's conjugated antibodies against *Mycoplasma hominis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum*. Heterologous antibodies were used as negative control.

Table (2): Frequency of Mycoplasma isolation from seminal fluid and endocervical specimens

Specimens origin	Total number	Positive / Negative		% of isolation
semen from infertile men	756	380	376	50.26
Semen from fertile men	200	23	177	11.50
Endocervical swab from infertile women	695	518	177	74.53
Endocervical swab from fertile women	300	30	277	10.00
Total number	1951	951	454	48.74

PCR assay:

DNA extraction from Mycoplasma and Ureplasma isolates: DNA was extracted from standard strains (*U. urealyticum, M. gentilium and M. hominis*) and identified mycoplasma and ureplasma by conventional methods in this study. Briefly, 1ml containing 100 CFU of each culture was centrifuged at 12000 ×g for 10 min. The pellet washed in PBS and resuspended in 50µl of distilled water. After boiling for 10 min, an aliquot of 7µl was used directly in PCR experiments (22, 23). PCR reactions was performed using the PCR master mix (Ampliqon Co, Skovlunde, Denmark) with an automated thermal cycler (Eppendorf, USA). The utilized primers which were capable of detecting *M. hominis, M. genitalium* and *U. urealyticum* simultaneously were as described by Stellrecht *et.al*. (23).

Statistical analysis:

Descriptive statistics method was used which included the percentage and mean, also the analysis of variance to shows comprehensive analysis and the significant value was designed to reveals the differences of isolation among mycoplasma species and their relation to cause of infertility between fertile and infertile patients (11).

RESULTS

Out of 756 seminal fluid taken from infertile men, 380 specimens were positive (50.26%), in contrast only 23 specimens were positive out of 200 seminal fluid taken from fertile men (11.50%). Out of 695 endocervical swabs from infertile women's, 518 were positive (74.53 %) while only 30 specimens were positive (10%) out of 300 fertile women. In

total number of 1951 specimens, 951 were positive (48.74 %) (Table 2). Table (3) compares different types of media used for isolation of mycoplasma and ureaplasma, modified AE 10 broth and agar media was found the favorable media for isolation of genital mycoplasma and ureaplasma.

Table (3): Comparison of different media used for isolation of genital mycoplasmas

Specimens origin	Total number	Arg*	Hay**	U9c***	U10c*****	UB*****	AE10******
semen	403	56	67	96	87	31	113
Endocervical swab	548	66	90	155	118	40	218

*Arg : Arginine broth medium, **Hay: Modified Hayflick broth medium, *** U9c: Urease color test broth medium, ****U10c: Urease color test broth medium, *****UB: Urease- bromothymol blue broth medium, ******AE10: Modified AE10 broth medium.

N.B. The same specimen may be growing in two or more media

According to biochemical characterization of the 951 isolates, isolated from men seminal fluids and women endocervical swabs taken from fertile and non-fertile patients. Six groups (A, B, C, D, E and F) were designed according to biochemical activities; glucose fermentation, arginine deamination, tetrazolium reduction (aerobic and anaerobic) and film and spots activity. Table (4) reveals the results of biochemical test.

Table (4): Biochemical characterization of men and women isolates

Group code	Number	Glucose	Arginine	Tetrazolium reduction. A/an	Urea hydrolysis	Film and spots
A	202	+	+	+/-	-	+
B	296	-	+	-/-	-	-
C	66	-	+	-/+	-	-
D	76	+	-	+/+	-	-
E	255	-	-	-/-	+	-
F	56	-	+	+/+ -	-	-
Total	951					

Table (5) shows the results of growth inhibition and immunofluorescent test used to identify the six groups mentioned in table (4). Group A was identified as *Mycoplasma fermentans*, while groups B, C, D, E and F were identified as *Mycoplasma hominis, Mycoplasma arginine, Acholeplasma laidlawii, Ureaplasma urealyticum* and *Mycoplasma genitalium* respectively.

The immunofluorescent test was accurate and showed the homogeneity and the morphology of immunofluorescent colonies of *Ureaplasma urealyticum* (group E) examined by fluorescent microscope (Figure 1).

Table (5): Identification of isolates by serological tests

Group code	Number	*M. fermentans*	*M.hominis*	*M. arginine*	*A. laidlawii*	*U. urealyticum*	*M. genitalium*
A	202	+	-	-	-	-	-
B	296	-	+				
C	66			+			
D	76				+		
E	255					+	
F	56						+
Total	951						

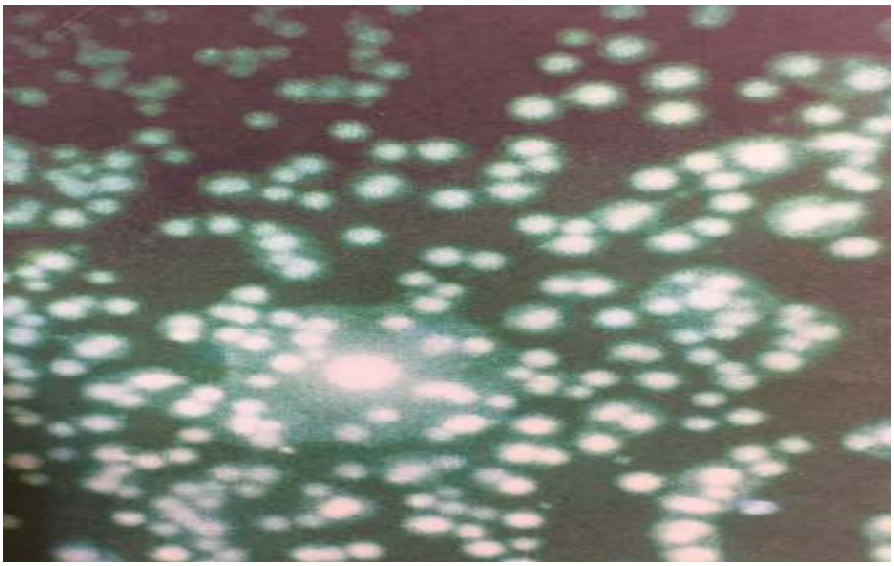

Figure (1): immunofluorescent colonies of Ureaplasma urealyticum (group E) examined by fluorescent microscope

The PCR assay's after the amplification of DNA taken from *M. genitalium* (group F), *M. hominis* (group B) and *U. urealyticum* (group E), shows distinct bands represented these mycoplasmas according to standard. Unfortunately, this assay did not used to discriminate the *M. fermentans* and *A. laidlawii* because used to confirm the diagnosis of genital mycoplasmas.

Out of these isolates; 296 *M. hominis* (31.12 %), 255 *Ureaplasma urealyticum* (26.81%), 202 *Mycoplasma fermentans* (21.24 %), 76 *Acholeplasma laidlawii* (8.03%), 66 *M. arginine* (6.94%) and 56 *M. genitalium* (5.85%) (Table 6).

Table (6): Percentage (%) of isolation according to species

Group code	Number	Mycoplasma species	Percentage %
A	202	*Mycoplasma fermentans*	21.24
B	296	*Mycoplasma hominis-*	31.12
C	66	*Mycoplasma arginin*	6.94
D	76	*Acholeplasma laidlawii*	8.03
E	255	*Ureaplasma urealiticum*	26.81
F	56	*Mycoplasma genitalium*	5.8 5
Total	951		

DISCUSSION

The correlation between the causative agents and pathological conditions in human being were investigated by the frequencies of isolation of causative agent's on diseased patients compared with non-diseased individuals. Numerous reports have appeared that document the recovery of mycoplasmas from infertile patients either by monitoring the cases after antibiotics treatment or by isolation. Although the debate over commensal versus pathogenic status of mycoplasma organisms continues. Indeed, among all prokaryotes'- mycoplasmas- has been so embroiled in controversy and in establishing a clear pathogenic niche. Their virulence determinants are undeniably complex, and their unique biological properties likely challenge the host differently from typical bacterial pathogens (24,25,26,27). For this reason, this study confirm that the large portion of the infertile individual's population carries *Ureaplasma urealyticum* and/or *Mycoplasma hominis, M. genitalium and M. fermentans* in their genital tracts has confounded those who ascribe an infertility inducing role to these organisms. Reports around the worlds try to found strong link between these organisms and reproductive failure diseases (3,4,6,28). Both *M.genitalium* and *U.urealyticum* have been detected to have clear role in reproductive failure (29,30) the isolation of *M. fermentans* more than *M. genitalium* and *M. Hominis* raised a lot of questions? Since *M.fermentans* was proved as the disease agent in chronic fatigue syndrome/fibromyalgia as well as in AIDS (30). Reports reveals that *M.fermentans* were first isolated from the lower genital tract early 1950s from adult men and women, but their role in human reproductive classical disorder were not established (31), but during 70's the picture of pathogenic *M.fermentans* was raised expectations for its pathogenic potential such as in rheumatoid arthritis patients and in bone marrow of children with leukemia (32). This study confirm the isolation of genital mycoplasma from patients attained the Gynecology clinic for infertility in Baghdad, Iraq; to treat their infertility problems, the percentage of occurrence of genital mycoplasma gave attentions, the highest frequencies were *M.hominis* (31%) followed by *Ureaplasma urealyticum* (27%), *Mycoplasma fermentans* (21.24 %) and *M. genitalium* (6%). A significantly higher rate of cervical Ureaplasma among women with unexplained infertility were reported by Khatamee and Decker (34) about (55%) more than among those with a known cause for their infertility (32%), but Rehewy *et.al* (35) mentioned a slightly higher rate of cervical ureaplasma isolation among population of infertile versus fertile women. Close isolation percentage (22%) was reported by Al-Kayat in Thi-Qar province in Iraq (36). On another hand, Nagata et al. failed to find a significant correlation between fertile and infertile women in Japan for both cervical *M.hominis* and *U.urealyticum* (37). The high isolation rate of *M.hominis* in this study seems very logic since the high concentration of this prokaryotes can lead to invasion of the endometrium and upper genital tract and can introduce a symbiotic relationship with protozoa like *Trichomonas vaginalis* and enhance the virulence of both organisms (24).

M.genitalium is a sexual transmitted organisms (38) it occurs in men more than women, results reported that the frequencies in current study about 6% and the world wide reports mentioned that the rate of *M.genitalium* infection ranges from 1-4% in men and from 1-6.4% in women, the prevalence rate in STI center ranges between 4-38% (39). Cazanave et al. (39) reported that the *M. genitalium* may cause female infertility, especially tubal infertility, but more studies are needed to confirm this finding. The presences of *U.urealyticum* and *M.hominis* in the women's genital tract may induce synergisms effect on reproductive system and cause reproductive failures, because treatment of infertile couples with tetracycline increase the change of fertility after elimination of both mycoplasmas (Abadi and Yawwar 2000, personal communication). We believed that the problem concerning the genital mycoplasma still debatable, whether they are responsible of infertility or just causes of several pathological conditions' in genital tracts. Isolation of mycoplasmas from lower genital

tract of women or respiratory tract of neonates means the ability to colonization of tissue and induces infertility. Another question is since the mycoplasma isolated from healthy individuals that mean it's commensals in genital tract, and also may be related to infection as in case of *M.genitalium* which sexually transmitted. Again, the isolation of mycoplasmas from genital tract of men and women support the hypothesis mentioned in introduction that mycoplasma causes several pathological conditions related to pregnancy.

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Evaluation of some heavy metals contamination in agricultural soil adjacent to the National Highway within Zakho District, Kurdistan of Iraq

Musher R. A. Al- Barwary

Dept. of Environmental Science/ Faculty of Science/ University of Zakho.
Republic of Iraq

E –mail: musheer.ahmed@uoz.edu.krd

ABSTRACT

Automobile exhausts can contribute to accumulation of Toxic metals in roadside soil. The soil pollution by toxic metals from transportation sources has become a serious environmental threat. The main objective in this research work was to assess the roadside soils contamination with Zn, Cd, Pb, Cu and Ni due to vehicular emissions. The samples were digested using wet method and heavy metals were analyzed using Atomic Absorption Spectrophotometry Technique. Monthly samples were collected from the agriculture soil around roadside during the period December to July 2018. A total of 96 soil samples were collected from different locations of villages around national roadside within Zakho District, Duhok governorate, Iraq. 48 samples were collected at 20 m from the roadway edge and 48 samples were collected at 40 m from the roadway edge. Physical and chemical factors believed to affect the mobility of toxic metals in the soil samples of the study area were tested such as; conductivity, TDS, pH and organic matter. The results showed that the soil pH was from 6.7 to 8.3and the organic matter percentages were from (0.8 to 2.6) EC, from (683 to 1475) µs/cm and TDS was from (437 to 944) mg/l. The soil samples of distance (20 and 40) m from roadside. The results of distance (20 and 40) m from roadside observed that concentration of Pb, Cu, Zn, Cd and Ni was found to vary between (1.1-7. 8) (7.7-16.4) (14.2-44.4) (0.10—0.37) (5.8-17.5) (1.0-4.5) (4.4-13.9) (10.1-38.5) (0.03-0.26) (4.7-13.7) mg /Kg respectively. This paper indicated that the heavy metals content decreased with increasing distances along the roadside. The concentrations (mg/kg) of the heavy metals recorded across the sampling sites follow the order: Zn > Ni > Cu > Pb> Cd, of 20 m distance and Zn > Cu> Ni> Pb> Cd, of 40 m distance.

Keywords: Agricultural highway, Heavy metals, road-side soil, Automobile exhausts.

INTRODUCTION

Zakho is a relatively small city having a population of 400 thousand individuals which is located 45km towards north of Duhok city Kurdistan of Iraq .This area enjoys a special status due to agricultural talent involved in various agricultural activities and multiple animal husbandries. At the same time, the main highways adjoining this area are the potential pollutant resources threatening the natural environment. Similarly, human induced pollutants from agriculture pesticides have considerable influence on the natural environment of this area (Figure 1). Vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals. Heavy metals have a potential to contaminate soil, which can be dispersed and accumulate in plants and animals, and taken in by humans through consumption. So heavy metal contamination has been a worldwide environmental concern with its potential ecological effect (1). Tire and break wear, oil spills, and erosion of road surfaces and building materials contribute to Zn, Cu, Cr, Cd, and Ni pollution. Vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals. Heavy metals have a potential to contaminate soil, which can be dispersed and accumulate in plants and animals, and taken in by humans through consumption. So heavy metal contamination has been a worldwide environmental concern with its potential ecological effect (2). Highways are known as the larger non-point source of creating pollution in urban environment. Emissions from highway operations cause many environmental and human health effects such as air ,water and soil pollution. Toxic metals are released during different operations of the road transport such as combustion, component wear, fluid leakage and corrosion of metals. Lead, cadmium, chromium and zinc are the major metal pollutants of the roadside environments and are released from fuel burning, wear out of tires, leakage of oils and corrosion of batteries and metallic parts such as radiators etc. Highway side soils and crops often contain high concentrations of metallic contamination. The bioavailability and environmental mobility of the metals are dependent upon the form in which the metal is associated with the soil. Analyses of highway side soil and crops revealed that they contain elevated levels of these heavy metals. The pollution of soil and vegetation by toxic metals from automobile exhaust is a serious environmental problem (3).

The objective of this paper is to understanding the contamination by toxic metals in agriculture soils due to vehicular emissions in Zakho District, Duhok governorate, Iraq.

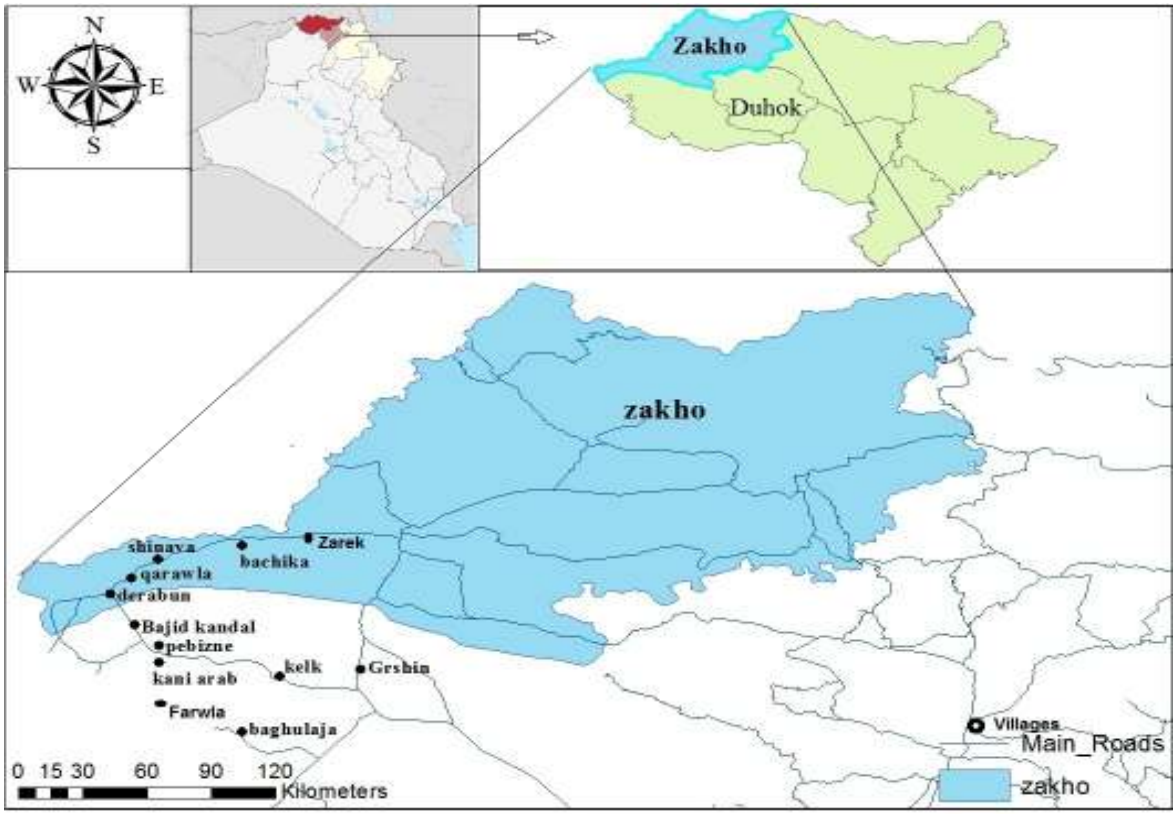

Figure (1): a map view of sampling locations of the study area

MATERIALS AND METHODS

Description of study area:

The study was conducted on major National high way. Zakho is a town in Iraq, at the center of the eponymous. Local Government Area of Zakho District of the Dohuk Governorate of Iraqi Kurdistan, located a few kilometers from the Iraqi–Turkish border. The city has a population of 400,000. The geographical coordinates of the study area lies between (Latitude: 37° 08' 55.36" N Longitude: 42° 41' 9.28" E). Zakho District is situated in a subtropical region; hot in the summer and cold in winter. Samples of roadside agricultural soils in this study were obtained in 2018, and the sampling sites are shown in Figure (1). The roadside locations are used for major economic crop production consisting of Wheat plants, maize, and rice.

Sample preparation and analysis:

A total of 96 soil samples were randomly collected from 12 rural agriculture areas adjacent the national roadside. The respective sample points are (Zarek, Pakloja, Kandaw, Frawla, Derabn, Kealk, Karshen, Pebazne , Shenava , Kanearab , Bagedkndil and Bageka. Soil samples were collected at a depth of 0-15 cm from the surface. 96 samples of agriculture soil were collected from 12 villages of two distance from roadside 20 m and 40 m adjacent the national roadside within Zakho District, Duhok governorate , Northern Iraq. Monthly samples were collected from the agriculture soil during the period December to July 2018. A method described by (4) was used to determine the heavy metals in the sample. All samples were air dried and passed through a 2-mm polyethylene sieve to remove rocks, leaves, and other debris, then ground with an agate mortar and sieved through a 0.15-mm polyethylene sieve. All handling procedures were carried out without contacting any metals to avoid potential cross-contamination of the samples. Approximately 0.5 g of each soil sample was digested primarily in a mixture solution of HNO3, HCl (1:3).Then added HClO4 for further digestion (5). The total concentrations of Zn, Cd, Pb, Cu and Ni, in the digested samples were determined using flame atomic absorption spectrophotometry.

Statistical Analysis:

Standard deviations and means were used to evaluate heavy metals concentration in soil samples. Linear regression analysis was conducted to estimation the relationships between effect of sampling distance and heavy metals concentration. In order to study the characteristics of roadside soils, the analyses were performed using SPSS to determine the value between different locations.

RESULTS AND DISCUSSION

Table (1) shows the mean concentrations of pb, Cu, Zn, Cd and Ni in the roadside soil along the Zakho District, that were (2.70 ± 623), (11.6 ±2.682), (16.5± 1.211), (0.05± 0.01808) and (11.0± 2.132) mg kg-1 of distance 40 m, respectively. While the highest concentration of pb, Cu, Zn, Cd and Ni was (4.0 ±0.974) , (14.4 ± 2.87), (19.6 ± 1.876) (0.23 ± 0.0803) and (13.6 ± 2.106) mg kg-1 respectively and were placed within the normal levels in agricultural soils. Zinc was reported to dominate in the agricultural soils. Metals concentrations in different distance showed significant variations. The high values of Pb observed, could be attributed to gasoline combustion which consequently settles on roadside soils. The concentrations of Cu, Zn and Ni were the highest along the study area. The sequence of the heavy metal values was generally as Zn > Cu > Ni > Pb > Cd. The concentrations of heavy metals observed in the study area were within the safe limit provided by (6). Similar results were reported by (7).

Table (1): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Zarek (mg /kg)

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	4.063	0.974	0.948
	40m	2.725	0.623	0.388
Cu in soil	20m	14.43	2.87	8.24
	40m	11.613	2.682	7.196
Zn in soil	20m	19.600	1.876	3.520
	40m	16.587	1.211	1.467
Cd in soil	20m	0.2338	0.0803	0.0065
	40m	0.05875	0.01808	0.00033
Ni in soil	20m	13.625	2.106	4.434
	40m	11.012	2.132	4.547

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates Means vertically have significant difference at $p \leq 0.05$

Table (2) shows the mean concentrations and standard deviation of Pb, Cu, Zn, Cd, and Ni was (4.100 ±1.365)(11.825 ± 2.293),(11.825 ± 2.293) (0.2612 ± 0.1059), and (14.55 ±2.91) mg/kg-1 at distance 20 m respectively. Metals values in distance 40 m showed mean concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soils was (2.525 ± 0.732) (9.513 ±1.968) (15.36 ± 2.91) (0.06875 ± 0.01808) and (11.925 ±1.603) mg/kg-1 respectively. The result indicates that the Automobile exhausts contribute to the pollution of these agricultural soils.

Metals in table (2) showed that the contents of heavy metals decreased with increasing the distance from the roadside. In this study are significantly lower than those reported by (8) .The permissible limit of pb recommended by (6) is 85 mg/kg. The permissible limit of Cu recommended by (6) is 36 mg/kg. Recommended limit for Ni by (6) is 35 mg/kg. Permissible level of Cadmium in soil by (6) is 0.8 mg/kg. The sequence of the heavy metal values was generally as Zn > Ni > Cu > pb> Cd.

Table (2): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Kealk mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	4.100	1.365	1.863
	40m	2.525	0.732	0.536
Cu in soil	20m	11.825	2.293	5.259
	40m	9.513	1.968	3.873
Zn in soil	20m	19.175	2.529	6.396
	40m	15.36	2.91	8.49
Cd in soil	20m	0.2612	0.1059	0.0112
	40m	0.06875	0.01808	0.00033
Ni in soil	20m	14.55	2.91	8.45
	40m	11.925	1.603	2.571

Heavy metal contents estimated according to distances from roadside were shown in table (3).
The mean concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soils along the roadside were (2.587± 0.923), (11.150 ± 2.268),(21.48 ± 5.19),(0.2213 ± 0.0636), (12.050 ± 1.679) mg/kg, -1 respectively with standard deviation for distance 20 m . While the distance 40 m recorded the following (1.925 ± 0.623), (9.450 ±1.869), (18.02 ± 4.53), (0.1088 ± 0.0327), (10.225 ±1.722) mg/kg -1 respectively, Pb, Cu, Zn, Cd, and Ni concentrations which were found in the agricultural soils are regarding the acceptable limits of heavy metals in soil and according to (9). The major sources of Cd pollution in most agricultural soils are atmospheric depositions during mineral fertilization. Copper was primarily the result of wear and tear of automobile tires which is mainly because Cu is hardness additive of automobile tires (10). The concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soil were similar to those found by (11).

Table (3): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Pebazne mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	2.587	0.923	0.853
	40m	1.925	0.623	0.388
Cu in soil	20m	11.150	2.268	5.143
	40m	9.450	1.869	3.494
Zn in soil	20m	21.48	5.19	26.95
	40m	18.02	4.53	20.51
Cd in soil	20m	0.2213	0.0636	0.0040
	40m	0.1088	0.0327	0.0011
Ni in soil	20m	12.050	1.679	2.820
	40m	10.225	1.722	2.965

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates Means vertically have significant difference at $p \leq 0.05$.

Table (4) the concentrations of Pb, Cu, Zn, Cd, and Ni of distance 20 m was (2.813± 1.124),(10.237± 2.254), (27.14 ± 4.42), (0.2188 ± 0.0804) , (12.80 ± 3.37) mg/kg-1 respectively. While from distance 40 m was (2.113 ± 0.666), (8.337± 2.124), (22.93 ± 3.63), (0.1113 ± 0.0872), (10.288 ± 2.684) mg/kg-1 respectively. Little variations were observed in the values of the heavy metals collected from the 12 sites from roadside soils (figure 1). The contents of heavy metals in soils sample was found in the following sequences: Zn > Ni > Cu > Pb > Cd . The Pb, Cu, Zn, Cd, and Ni concentrations which were found in agricultural soils were within the acceptable limits of heavy metals in soil according

to (9). This result indicated that Pb, Cu, Zn, Cd, and Ni contents decreased with increasing distances along the national roadside within Zakho District. Similar results were reported by (12).

Table (4): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Shenava mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	2.813	1.124	1.264
	40m	2.113	0.666	0.444
Cu in soil	20m	10.237	2.254	5.080
	40m	8.337	2.124	4.511
Zn in soil	20m	27.14	4.42	19.58
	40m	22.93	3.63	13.18
Cd in soil	20m	0.2188	0.0804	0.0065
	40m	0.1113	0.0872	0.0076
Ni in soil	20m	12.80	3.37	11.37
	40m	10.288	2.684	7.201

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates . Means vertically have significant difference at $p \leq 0.05$

Table (5) shows comparisons of the results of this study with results from other studies according to European Union standards, Table (5) compare the variations in the values of Pb, Cu, Zn, Cd, and Ni in the agricultural soils over 11 months. In this study the concentration of heavy metals Pb, Cu, Zn, Cd, and Ni in distance 20 m was (2.738 ± 0.996), (12.44 ±2.98), (37.70 ± 4.96), (0.3038± 0.0590), (8.700±1.755) mg/kg-1 respectively. Heavy metals in agricultural soils was significantly higher at 20 m distance than 40 m distance pass roadside, in this study are significantly lower than those reported by (15). Metals concentrations in two distances showed significant variations at $p \leq 0.05$. Cadmium levels in exhaust emissions have been related to the composition of gasoline, motor oil, wear-and-tear of tires and roadside deposition of the residues of those materials as well as traffic density (16). The results indicate that all soil samples lie within the permissible limits according to (European Union's Standards, 2006).

Table (5): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Pakhloja mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	2.738	0.996	0.991
	40m	2.200	0.906	0.820
Cu in soil	20m	12.44	2.98	8.90
	40m	10.27	2.92	8.54
Zn in soil	20m	37.70	4.96	24.61
	40m	30.36	4.67	21.81
Cd in soil	20m	0.3038	0.0590	0.0035
	40m	0.2012	0.0364	0.0013
Ni in soil	20m	8.700	1.755	3.080
	40m	6.512	1.910	3.650

The mean concentrations of Pb, Cu, Zn, Cd, and Ni in distance 20 m in roadside soils along the Zakho highway were (3.600 ±1.361), (11.775 ±1.628), (23.38 ±3.68),(0.15500 ± 0.02673), (11.638 ±1.641) mg/kg, respectively (table 6). Accumulation of heavy metals in agricultural soils is greatly influenced by vehicular emissions and all the metals exhibited a significant reduction in the agricultural soils with increasing distance from the road and the mean concentrations of Pb, Cu, Zn, Cd, and Ni in distance 40 m in roadside soils along the Zakho highway were (2.688± 0.908), (10.175± 2.157), (20.89 ± 3.33),(0.06375 ± 0.01685), (9.925 ±1.492) mg/kg-1 respectively. Heavy metal concentrations in the roadside soils of 20 distance followed order of Zn > Cu > Ni > Pb > Cd. While the distance 40 m followed order of Zn > Ni > Cu > Pb> Cd. the results showed that the studied agricultural soils contains higher levels of metals nearer the roadside. The vehicular emission played a significant role in the value of zinc on the roadside soil. Heavy metal concentrations in different distance showed significant variations, similar results reported by (14).

Table (6): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Kanearab

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	3.600	1.361	1.851
	40m	2.688	0.908	0.824
Cu in soil	20m	11.775	1.628	2.651
	40m	10.175	2.157	4.654
Zn in soil	20m	23.38	3.68	13.57
	40m	20.89	3.33	11.12
Cd in soil	20m	0.15500	0.02673	0.00071
	40m	0.06375	0.01685	0.00028
Ni in soil	20m	11.638	1.641	2.691
	40m	9.925	1.492	2.225

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates
Means vertically have significant difference at $p \leq 0.05$

The contents of heavy metals estimated according to distances from roadside were shown in table (7). The mean concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soils in the order of Zn > Ni> Cu > Pb> Cd for distance 20 m. While the mean concentrations of Pb, Cu, Cd, Ni, and Zn in roadside soils for distance 40 were (3.200 ± 0.463), (8.463 ± 2.675,(17.54 ±5.89), (0.0800± 0.0334),(9.862 ±1.846) mg/kg -1, respectively. These results indicate clearly that agricultural soils along the roadside are significantly polluted by vehicular exhust. Heavy metal concentrations in different distance showed significant variations, in this study are significantly higher than those reported by (12). The results indicate that the soil samples lie within the permissible limits according to (9).

Table (7): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Karshen mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	4.800	1.176	1.383
	40m	3.200	0.463	0.214
Cu in soil	20m	9.963	2.769	7.666
	40m	8.463	2.675	7.157
Zn in soil	20m	21.90	7.15	51.15
	40m	17.54	5.89	34.68
Cd in soil	20m	0.1875	0.0492	0.0024
	40m	0.0800	0.0334	0.0011
Ni in soil	20m	12.112	1.490	2.221
	40m	9.862	1.846	3.408

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates
Means vertically have significant difference at $p \leq 0.05$

Table (8) shows the mean concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soils in residential area that were in the order of Zn > Cu > Ni > Pb > Cd .This work indicated that the heavy metals content decreased with increasing distances along the national roadside within Zakho District. The results indicate that the soil samples lie within the permissible limits according to (11) Heavy metal concentrations in two distances showed significant variations *at P – value > 0.05***.** In this study are significantly higher than those reported by (12).

Table (8): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Baged kndil

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	4.350	1.350	1.823
	40m	2.875	0.723	0.522
Cu in soil	20m	12.338	1.455	2.117
	40m	10.675	1.743	3.039
Zn in soil	20m	36.23	5.70	32.55
	40m	31.51	4.74	22.51
Cd in soil	20m	0.1850	0.0481	0.0023
	40m	0.0788	0.0295	0.0009
Ni in soil	20m	11.263	1.556	2.420
	40m	8.200	1.514	2.291

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates
Means vertically have significant difference at $p \leq 0.05$

Table (9) shows the mean concentrations of Pb, Cu, Zn, Cd, and Ni in agricultural soils along the roadside that were (2.637±1.065),(8.825 ±1.816), (22.788 ±2.600), (0.2312± 0.0730), (0.2312± 0.0730) mg/kg of distance 20 m respectively. But the mean concentrations of same heavy metals from distance 40 m were (1.850 ± 0.641), (6.250 ±1.650), (19.463 ±2.176), (0.2312 ± 0.0730),(5.512 ±1.185) mg/kg-1 respectively. This work indicated that the heavy metals content decreased with increasing distances along the national roadside within Zakho District. Heavy metal concentrations in different distance showed significant variations at P – value > 0.05. The results indicate that the soil samples lie within the permissible limits according to (European Union's Standards, 2006) In this study, they were significantly lower than those reported by (13).

Table (9): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Derabon

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	2.637	1.065	1.134
	40m	1.850	0.641	0.411
Cu in soil	20m	8.825	1.816	3.296
	40m	6.250	1.650	2.723
Zn in soil	20m	22.788	2.600	6.761
	40m	19.463	2.176	4.737
Cd in soil	20m	0.2312	0.0730	0.0053
	40m	0.1612	0.0387	0.0015
Ni in soil	20m	8.138	0.721	0.520
	40m	5.512	1.185	1.404

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates
Means vertically have significant difference at $p \leq 0.05$

Table (10) shows the concentrations of heavy metals in the present study was in the order of Zn > Cu > Ni > Pb > Cd mg/kg-1 respectively. Heavy metal concentrations in different distance showed significant variations at P – value > 0.05**.** The high concentration of Pb, Cu, Zn, Cd, and Ni may be due to leaded gasoline emissions. While the concentration of Pb, Cu, Zn, Cd, and Ni of 40 m were (2.225 ± 0.824), (9.938 ± 2.015), (23.29 ±5.13), (0.1563± 0.0650),(8.675± 0.910) mg/kg-1 respectively. In this study the concentration of heavy metals generally decreases with increasing distance along the national roadside. The results indicate that the soil samples lie within the permissible limits according to (9) in this work are significantly lower than those reported by (14) and higher than those reported by (15).

Table (10): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Bageka mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	2.962	1.101	1.211
	40m	2.225	0.824	0.679
Cu in soil	20m	12.512	1.835	3.367
	40m	9.938	2.015	4.060
Zn in soil	20m	26.02	5.26	27.68
	40m	23.29	5.13	26.32
Cd in soil	20m	0.2188	0.0591	0.0035
	40m	0.1563	0.0650	0.0042
Ni in soil	20m	10.813	0.936	0.876
	40m	8.675	0.910	0.828

Means vertically have significant difference at $p \leq 0.05$

Table (11) shows heavy metals in soil shows that the mean concentrations of Pb, Cu, Zn, Cd, and Ni in roadside soils were (5.000 ± 2.295), (8.875 ± 1.795), (24.613 ± 2.635), (0.1513± 0.0368),(8.20 ± 0.971) mg/kg, respectively for distance 20 m. Contamination of agricultural soils within the study area may be due anthropogenic sources such as vehicle emissions beside of fertilizers. Heavy metal concentrations in two distances showed significant variations *at* P – value > 0.05. Automobile exhausts can contribute to accumulation of heavy metals in roadside soil. In this study the concentration of heavy metals generally decreases with increasing distance along the national roadside. The results indicate that the soil samples lie within the permissible limits according to (16) in this work are significantly higher than those reported by (17).

Table (11): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Kandaw mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	5.000	2.295	5.269
	40m	3.200	1.074	1.154
Cu in soil	20m	8.875	1.795	3.222
	40m	7.175	1.693	2.865
Zn in soil	20m	24.613	2.635	6.944
	40m	20.775	2.583	6.674
Cd in soil	20m	0.1513	0.0368	0.0014
	40m	0.0725	0.0369	0.0014
Ni in soil	20m	8.200	0.971	0.943
	40m	6.237	0.920	0.846

Mean value ± standard deviation .SD- standard deviation; VC- variation coefficient, Values from three replicates
Means vertically have significant difference at $p \leq 0.05$

Table (12) shows the mean concentrations of Pb, Cu, Cd, Ni, and Zn in roadside soils along the roadside soils were (3.000 ±1.213), (12.73 ± 2.94) (17. 950, ± 2.286) (0.25875 ± 0.02642), (12.125± 1.462) mg/kg, respectively for distance 20 m. The concentration of heavy metals in roadside soil was in the order of Zn > Cu > Ni > Pb > Cd . Vehicular exhausts can contribute to accumulation of heavy metals in roadside soil. In this study the concentration of heavy metals generally decreases with increasing distance along the national roadside. Heavy metal concentrations in two distances showed significant variations *at* P – value > 0.05. in this study are significantly higher than those reported by (18).

Table (12): Statistical description of pb, Cu, Zn, Cd and Ni in soil samples of two distances in site Kandaw mg/kg

Heavy metals	Distance	Mean	S.D.	variation coefficient
Pb in soil	20m	3.000	1.213	1.471
	40m	2.188	0.849	0.721
Cu in soil	20m	12.73	2.94	8.64
	40m	10.725	2.490	6.199
Zn in soil	20m	17.950	2.286	5.226
	40m	14.800	2.303	5.306
Cd in soil	20m	0.25875	0.02642	0.00070
	40m	0.1775	0.0396	0.0016
Ni in soil	20m	12.125	1.462	2.136
	40m	10.262	1.409	1.986

Average values of Pb, Cu, Zn, Cd, and Ni in roadside soils along the study area were (2.5 to 5.0) (4.2 to 12.7) (17.8 to37.7) (0.15 to 0.30) (7.6 to 14.1) mg/kg, respectively, for distance 20 m, while in distance 40 m the Average values were (1.9 to 3.2) (6.5 to11.6) (0.05 to 0.18) (5.4 to11.3) mg/kg, respectively. In this study the concentration of heavy metals generally decreases with increasing distance along the national roadside (table 13).

Table (13): Average concentrations of heavy metals (mg/kg) in 12 cities along the highway of 2 distances

Parameter / Sites	Pb 20M	Pb 40M	Cu 20M	Cu 40M	Cd 20M	Cd40M	Ni20M	Ni40M	Zn 20M	Zn 40M
1-Zarek	4.0	2.7	14.4	11.6	0.23	0.06	13.6	11.0	18.4	16.5
2-Kealk	4.1	2.4	11.8	9.5	0.26	0.07	14.1	11.3	19.1	16.3
3-Pebazne	2.5	1.9	4.2	8.8	0.22	0.11	10.6	9.7	17.8	18.0
4-Shenva	2.8	2.1	9.7	8.3	0.21	0.11	12.4	10.3	28.8	20.2
5-Pakloja	2.7	2.0	12.4	10.2	0.30	0.20	7.6	6.5	37.7	35.3
6-Kanearab	3.6	2.6	11.7	10.1	0.15	0.06	11.6	9.3	23.3	20.8
7-Karshen	4.8	3.1	9.9	8.4	0.18	0.08	12.1	34.8	21.9	17.5
8Bagedkndil	4.3	2.8	12.3	9.9	0.18	0.05	8.8	7.8	36.2	31.5
9-Derabon	2.6	1.8	7.7	6.5	0.23	0.16	8.1	5.4	22.7	14.4
10-Bageka	2.9	2.2	12.5	8.4	0.21	0.15	10.8	8.6	24.9	23.3
11-Kandaw	5.0	3.2	8.8	6.8	0.15	0.06	8.2	6.6	24.6	20.6
12-Farwla	3.0	2.2	12.7	14.2	0.25	0.18	12.1	10.2	17.9	14.8

CONCLUSION

Roadside soils from different sites in Zakho District Northern Duhok Government . The study area was examined for Pb, Cu, Zn, Cd, and Ni. Results from the study area showed that Zn, Pb, Cu, were the most abundant metals especially in agriculture soil adjacent to the national high way. The concentration of these toxic metals in roadside soil, increases with increasing traffic volumes. The pattern of total metal concentrations in the roadside soils followed Zn > Cu > Ni > Pb > Cd.at site Zarek,(Zn > Ni > Cu > pb> Cd) at site Keklak, Zn > Ni > Cu > Pb > Cd .at site Shenafa, Zn > Ni > Cu > Pb> Cd.at Knearab. In this study, the concentration of Zn was found to be higher than all toxic metals in this study of aroadside soils. The high Zn and slightly high Ni show that heavy metal contamination at the agricultural soil of study area. In this study the concentration of heavy metals generally decreases with increasing distance along the national roadside. The results of all locations indicate that the soil samples lie within the permissible limits according to (European Union's Standards, 2006).

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New record of *Trypanosoma* sp. from *Bubalus bubalis* in Basrah province, Iraq

Noor Ghanim Hameed and Muna Mohammed Jori

Dept. of Microbiology and Veterinary Parasitology / College of Veterinary Medicine / University of Basrah.
Republic of Iraq

E-mail: ijst.jordan@yahoo.com

ABSTRACT

Microscopic examination of blood smears for 175 bovine (60 buffalo and 115 cattle) was done from 6 regions of Basrah province (AL-Qurna, AL-Hartha, Qarmat- Ali) with cases in the college of Veterinary Medicine and from Agriculture college farm and AL-Faw. Two specimens of *Trypanasoma* sp. were detected through February and March 2019 from blood smear of alive water buffalo *Bubalus bubalis* infected naturally in north of Basrah province in February and March with *P*. value (0.4) between bovine and prevalence (3.3), and *P*. value (0.9) between the sexes of buffaloes with prevalence (3.4) in female and (3.2) in male at $p \leq 0.05$. Identification of *Trypanosoma* sp. was based on morphological characteristic and biometrical analysis of Giemsa stain with trypomastigote stage.
The detection of *Trypanosoma* sp. from Buffalos in the present study regarded Buffalo as a new host of trypanosomiasis in Iraq, and Basrah province as a new region for appearance of trypanosomes in bovine.

Keywords: *Trypanosoma* sp., *Bubalus bubalis* , Basrah

INTRODUCTION

The economic losses by parasitic diseases are attributed mainly to reduced weight gain, lower milk production, high mortality, and high costs of veterinary care (1,2). Trypanosomosis in bovine caused by 3 main species; *Trypanosoma brucei*, *T. congolense* and *T. vivax*, naturally transmitted wherever tsetse-flies (*Glossina* spp.) are prevalent, but may also be transmitted mechanically by other hematophagous flies and the disease occurs (3).

In the last three decades, numerous reports from Asia have shown that surra is still, and maybe "again," an important disease in cattle and buffaloes, especially in Indonesia, the Philippines, Thailand, and Vietnam (4). Also (5) and (6) reported fever, abortion, and decreased milk production are frequently in dairy cattle.

It was stated that among parasitic diseases of cattle haemoparasitism constitute a disease entity of great economic importance (7). The impact of haemoparasites on cattle productivity is also difficult to quantify (8). But (9) mentioned that losses in traction power, milk and meat production and costs of control programs have been ascribed to haemoparasites.

In Iraq, Mosul city (10) recorded fever, pale mucous membranes, anorexia, dullness, emaciation, short and moist cough, moist rales, mucopurulent ocular discharges and, uncoordinated movements in all infected calves with *T.brucei*.

For the little data available about parasites of bovine in south region of Iraq the study design for investigate the bovine haemoparasites.

MATERIALS AND METHODS

A total of 115 cattle and 60 buffalos of both sexes and ages (< 1 year to >2 years) were randomly collected from different region of Basrah province(AL-Qurna, AL-Hartha, Qarmat- Ali with cases in the college of Veterinary Medicine and from Agriculture college farm and AL-Faw). The study was carried out between September 2018 and May 2019. The bloods were collected from the jugular vein, and put into a labelled Ethylene diamine tetra acetic acid (EDTA) tubes placed in ice piece. The analyses of samples were done in the Parasitological Laboratory of the Veterinary Medicine College/ University of Basrah. Thin and thick blood smears were dried on air, Fixed in absolute methanol, Stained with Giemsa stain and examined under 400x and 1000x objective magnification microscopically according to (11).

Statistical analysis:

The data were subjected to statistical analysis with $p \leq 0.05$ considered significant in comparison with reference ranges of bovine (12).

RESULTS

Two samples from water buffalo (*Bubalus bubalis*) detected *Trypanasoma* sp. through February and March 2019, and identified according to (13-15).

Description and measurements:

Table (1) showed the measurements by microns for the detected specimens. the parasite appear as monomorphic organism in the blood smears of the buffalos, pointed, slender, elongated fusiform in shape with short free flagellum, tapering beyond the nucleus in the ventral side of the last half of the body and the undulating membrane was inconspicuous (figure 1). The kinetoplast was small and subterminal at the posterior extremity.

Nucleus located in the end of third anterior part of the body look like Horseshoe with nucleolus. Parasite contains small molecules (volutin granules) distributed along the body, and the anterior part staining as dark pigmentation, with some vacuoles (figure 2).

Table (2) shows the differences between the currently registered type and *T. theileri* in terms of total length, PN/KN to PN/NA, strains and host.

While table (3) shows the comparison among the current recorded type of *trypanosoma* with other species in terms of description features and measurements.

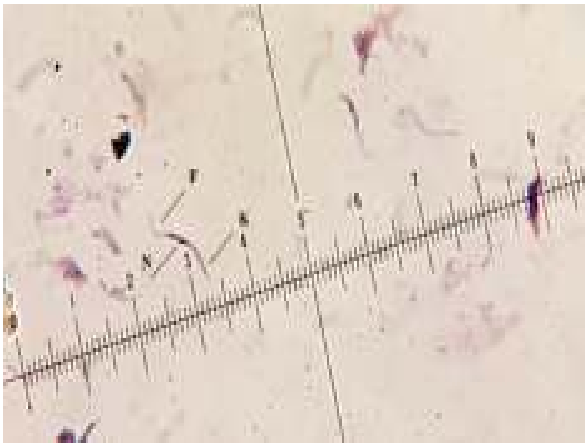

Figure (1): Blood smear of *Trypanasoma* sp. with (F) free flagellum, (N) nucleus, and (K) kinetoplast. Giemsa stain 400x with ocular standard lens (compound light microscope)

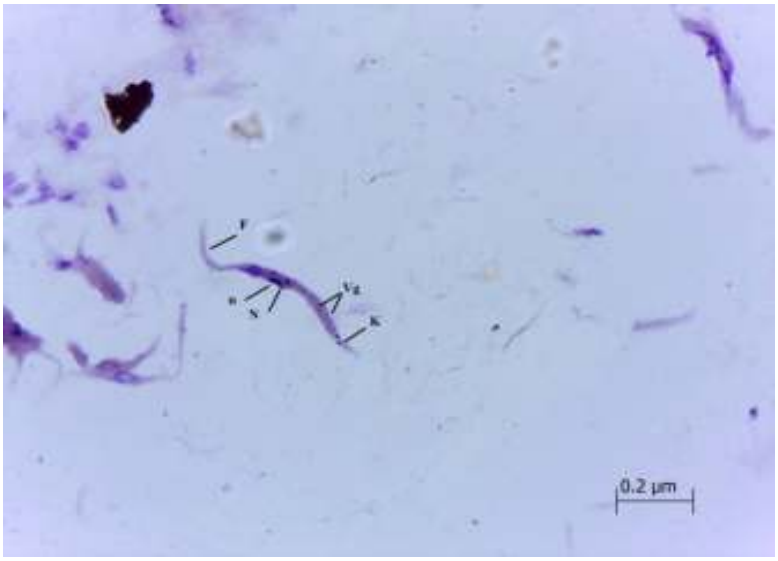

Figure (2): blood smear of water buffalo with Trypanasoma sp. (F) Free flagellum, (N)Nucleus, (n) nucleolus, (K) kinetoplast, (Vg) volutin granules. Giemsa stain. Oil immersion (1000x) (Leica imaging microscope

Table (1): The Measurements of *Trypanasoma* sp., of water buffalo (mean (µm))

Biometrical data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
Mean	3.125	15.625	18.75	3.75	10	51.25	1.2	5

PK= Distance from the posterior end to kinetoplast. KN= Distance from Kinetoplast to middle of nucleus; PN= Distance from the posterior end to middle of nucleus. NA= Distance from nucleus to anterior extremity. F= Free flagellum length. L= Total length, including free flagellum

Table (2): Comparison between the currently recorded species and *T. theileri* (16)

Description	*T.(Megatrypanum) theileri* LAVERAN, 1902	*Trypanasoma* sp.
Total length	69-109 µm but sometimes comparable (25 µm)	51.25µm
PN/KN To PN/NA	2.5-5.8 µm	1.2-5 µm
Strains	All continents (Asia and South America)	North of Basrah
Host	Cattle and buffaloes	Water Buffaloes (*Bubalus bubalis)*

Table (3): Comparison between the currently record species of *Trypanasoma* and some species recorded in previous studies (17)

Description	*T. bruci*	*T. congolense*	*T. vivax*	*Trypanasoma* sp.
Length (L)	20-30 µm	9-22 µm	18-26 µm	51.25µm
Nucleus (N)	central	central	central	Before the central
Undulating membrane	conspicuous	modest	modest	inconspicuous
Flagellum (F)	free	no	free	free
Kinetoplast	small	sub terminal	terminal large	sub terminal
Polymorphic /monomorphic	Long slender& stumpy	monomorphic	monomorphic	monomorphic
Posterior end	pointed	rounded	rounded	pointed

Biometrical data of tables (4), (5) and (6) refer to the comparisons of the previous species measurements detected in cattle from Mosul city with the new recorded species from water buffaloes in Iraq.

Table (4): Mean of *T. brucei, T. congolense* of cattle in Mosul city (10) with *Trypanosoma* sp. in Basrah province of Iraq

Biometrical data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
T. brucei	4	2.3	4.5	5.2	8	23.9	1.9	0.85
T. congolense	1.9	4.2	5.8	3.4	1.7	17	1.8	0.75
***Trypanosoma* sp.**	3.125	15.625	18.75	3.75	10	51.25	1.2	5

Table (5): Mean of *Trypanosoma vivax* of cattle in Mosul city (18) with *Trypanosoma* sp. in Basrah province of Iraq

Biometrical data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
T. vivax	1.24	3.84	3.84	6.33	4.32	18.33	0.93	0.56
***Trypanosoma* sp.**	3.125	15.625	18.75	3.75	10	51.25	1.2	5

Table (6): Comparison between the currently registered species and some species recorded in previous studies of cattle in Mosul city (19)

Biometrica data	PK	KN	PN	NA	F	L	PN/KN	PN/NA
T. theileri	32.02	5.53	17.16	-	11.26	77.34	-	-
T. uniform	0.86	3.16	3.02	-	4.75	17.42	-	-
T. evansi	4.35	3.91	7.12	-	4.01	25.89	-	-
***Trypanosoma* sp.**	3.125	15.625	18.75	3.75	10	51.25	1.2	5

Clinical examination:

The infected buffalos with *Trypanosoma* sp. showed clinical signs of trypanosomosis, which noted combined with fever more than 41Cº, pale mucous membranes, anorexia, dry hair coat, lethargy, emaciation, and depression with severe anaemia.

Prevalence study:

Tables (7) and (8) explain the results of present identified species of *Trypanosoma* indicated there were no significant difference associated with the parasite infection between bovine with *P*. value (0.4) and prevalence (3.3). However there are significant differences between the sexes of buffalo at *P*. value (0.9) and prevalence (3.4) in female and (3.2) in male with no significant difference($p \leq 0.05$).

Table (7): Prevalence of *Trypanosoma* sp. of cattle and buffalo in Basrah province ($p \leq 0.05$)

Type of infection	Categorized animal species		
	115 Cattle (%)	60 Buffalo (%)	*P*. value
***Trypanosoma* sp.**	0 (0.0)	2 (3.3%)	0.4

Table (8): Distribution of *Trypanosoma* sp. in cattle and buffaloes in Basrah Province ($p \leq 0.05$)

Type of infection	Cattle			Buffalo		
	Female (%)	Male (%)	*P*. value	Female (%)	Male (%)	*P*. value
Infected Uninfected	-	-	-	1 (3.4) 28 (96.5)	1 (3.2) 30 (96.7)	0.9

From the comparisons with other species of Trypanasomes, the results of present study was the first reported of *Trypanasoma* sp. in blood smears from naturally infected water buffalo. Buffalo considered as a new host of trypanosomiasis in Iraq, and a new host of bovine in Basrah province.

DISCUSSION

There is a large group of mammalian infective with trypanosomatid species that are extracellular during proliferation in the mammalian host, infection of cattle is most likely mediated by ingestion of infected flies and also through vertical transmission (20). In 1841, (21) found flagellates that today are included in *Trypanoplasma* in the blood of trout (22). Genus (*T. sanguinis*) was named by (23) after parasites in the blood of frogs. David Bruce in 1903 identified the protozoan parasite and the tsetse fly vector of African trypanosomiasis (24). By Griffith Evans, in the blood of Indian equines and dromedaries in 1880, *Trypanosoma evansi* (Steel 1885) Balbiani, 1888, is the first pathogenic mammalian trypanosome to be described in the world (25).

In Asia, for the last 3 decades, numerous reports have shown that surra is still, and may be "again," an important disease in cattle and buffalo, especially in Indonesia, Philippines, Thailand, and Vietnam (4).While (26) mentioned that *T. evansi* in very low numbers (although able to induce immunosuppressive effects), or when it is absent from the host blood stream (although present in the nervous system), identification of the etiological agent and evaluation of its pathogenic effects and the impact are especially difficult. The information available to trypanosomoses determined the comparative economic impact between themselves and in relation to other haemoparasites, concomitant anaplasmosis and babesiosis can hinder the clinical diagnosis of livestock trypanosomosis (27-29).

The technical considerations that measures genetic divergences neglect the most important concern must have for trypanosomes parasites: their host range, pathogenicity, consequent geographical distribution and vectors, for these reasons it seems to be reasonable and less confusing to keep the taxonomy as it is by consider the particular parasitic niche of *Trypanasoma* spp.(30).

The recorded specimens in the present study is similar to *T. theileri* according to the measurements shown in the table (1), but it differs from *T. theileri* in the long of free flagellum of *T. theileri*, since the last species longer than the present recorded one, and *T. theileri* has obvious undulating membrane unlike the present specimens, location of nucleus in *T. theileri* in the middle of the body but in the our species located before middle of the body (in the end of first third of anterior end), in *T. theileri* the posterior part more pointed than present one, and in presence of granules that distributed through the inner edges of the posterior end of the *Trypanasoma* sp.(table 2).

The comparison among the current recorded type of *Trypanosoma* sp. (Table 3) with other species (***T. brucei, T. congolense*** and ***T. vivax***) is shows *Trypanosoma* sp**.** was the longest between them, similar to *T. brucei* and *T. vivax* in the presence of a free flagellum**,** at the kinetoplast site and in form of posterior end with *T. brucei*. The differences are in his lack of undulating membrane, site of nucleus and general shape of the body.

Volutin granules were showed in figure (2) agrees with (10) species in description of the *T. congolense* in cows.

In another studies from Mosul city in Iraq for the same species in cattle (Table 4 and 5) there are two different studies (18 and 10) appeared comparison among *T. congolense, T. vivax* and *T. brucei* with *Trypanasoma* sp., which was not identical with biometrical data and general features with current recorded one. (18) mentioned to the differences observed may related to biometrical data that could be related to the phase of the disease (acute or chronic).

The study of (19) in Mosul city compared the differences in biometric data among three species (*T. theileri, T. uniform* and *T. evansi*), the current species of *Trypanosama* defers in measurements comparisons with them data (Table 6).

There is a suggestion that, the disease might have occurred due to introduction of a susceptible herd which was imported from Brazil, India, Turkey, Iran and other regions associated with trypanosomiasis as (10) mentioned about *T. brucei* and *T. congolense* or might have occurred due to introduction of a suspected herd which came from Brazil. Since parasite numbers in infected cattle can rapidly increase in immunocompromised, ill, or stressed animals, the parasitaemia in healthy animals is probably limited by the host immune system (31) and symptoms of disease are infrequent (32 and 33). Surveys in United States and Europe, and most recently United Kingdom, indicate that ***T. theileri*** is present in>80% cattle but at very low parasitaemias within the blood and tissues of infected animals (34-37).

With rise in body temperature above 41Cº, emaciation, pale mucous membranes, depression and anorexia observed by (10) resembles in symptoms with recorded ones in the present study and complies with (38) in experimentally infected sheep with *T. evansi*, *T. vivax*, and *T. equiperdum*. The finding data in respect with anemia agreement with (39-41) results. While the fever, depression and lethargy can cause by anemia and this finding similar to (42) observations.

The resulted prevalence of *Trypanasoma* sp. was 3.3% in buffalo; 3.4% in female and 3.2% in male through February and March, this study is inconsistent with (19) whose recorded 23.07%, 15.38 and 23.07% respectively to *T. theileri*, *T. uniform* and *T. evansi* in cattle, it also disagreement with study in Thailand for (43) whose recorded the

overall prevalence of *T. evansi* in dairy cows 8.1%. Although there are other types of insects that act as the mediator of infection, *T. (Megatrypanum) theileri* (transmitted by tabanid flies, where it undergoes a developmental cycle (44).

This finding of prevalence was lower than the other reported recorded parasites. The appearance of trypanosome during February and March following the months with high rain due the emergency of biting flies to high rate. (45) also refer to the importance of rainy months in encouragement activity of flies.

In conclusion, the Trypanosomiasis remains a parasite for all types of organisms and is not limited to specific species. The centuries-old studies are still important for researchers in blood infections of organisms. The information about the occurrence of these pathogens may provide basis for further research about water buffalo blood parasites of Basrah province in Iraq.

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