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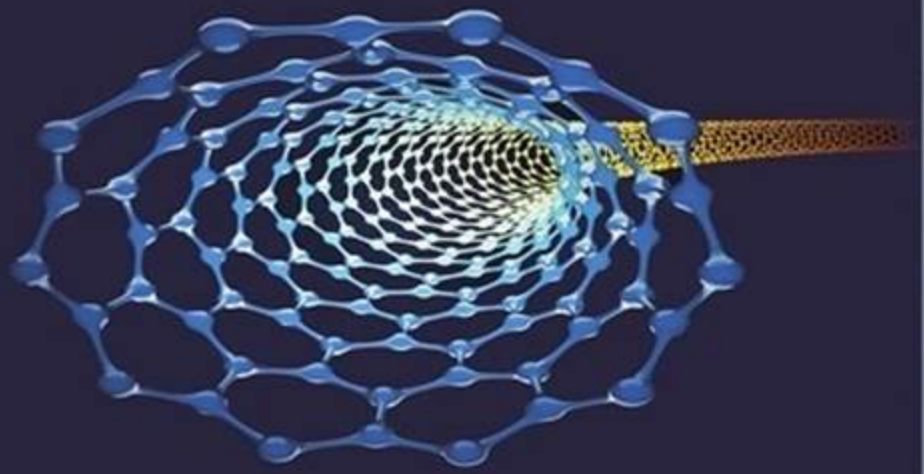

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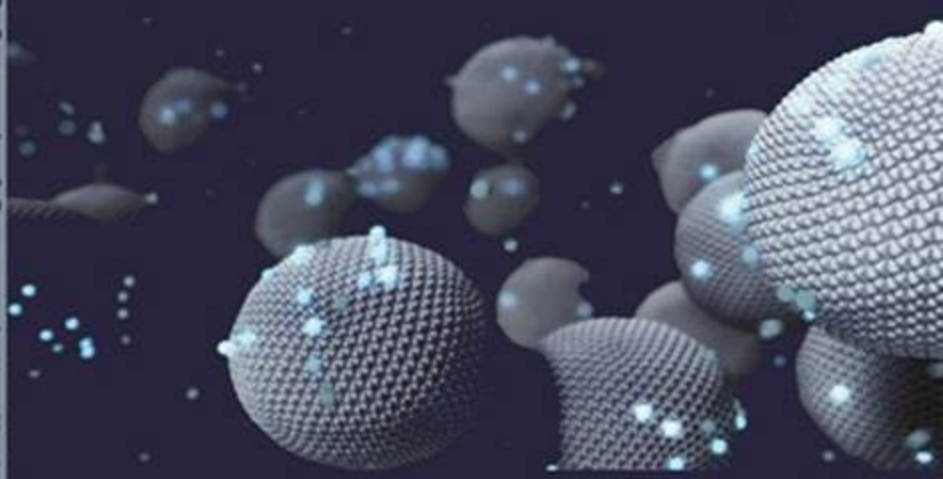

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Molecular Detection of pulmonary Tuberculosis using the Gene X pert MTB/RIF assay in Kurdistan of Iraq

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ABSTRACT

Tuberculosis has been potentially diagnosed by an innovative Gene Xpert Mycobacterium tuberculosis refampcin tool substantially impacted the quick detection of RIF resistance in clinical samples, Multi drug-resistant tuberculosis signify the remarkable resistance to the conventional (isoniazid, rifampicin against tuberculosis.

The gene x pert MTB/RIF has been clinically and microscopically utilized for the detection of the multivariate pulmonary specimens of early pulmonary tuberculosis cases. Robust screening study had been implemented at Erbil community in the chest and respiratory diseases centre in the period June 2017- June 2018.

Two hundred fifty Sputum samples were obtained from TB suspects. All samples were tested on Gene X pert for MTB/RIF detection after acid fast bacilli microscopy.75(30%) sputum samples were AFB smear positive and 175 (70%) were negative. In MTB/RIF assay 85 (34%) were MTB positive and 165 (66%) were negative. The MTB/RIF assay also detected 7 RIF-resistant specimen and 78 RIF-susceptible specimens, and the results were confirmed by drug susceptibility testing. Sensitivity and specificity of Gene x pert were 100% and 94.29% respectively, compared with the conventional method.

Hence from the overall conclusion, we can acknowledge that the MTB/RIF assay has simplified and solve the dramatic challenge of multiple TB cases and cut short with positive impact the dilemma of empirical TB treatment drugs.

1.INTRODUCTION

WHO has launched a new Cepheid Gene X pert diagnostic modality for *Mycobacterium tuberculosis* and refampcin resistant (Daum *et al.*, 2016).

This expedited identification of MTB/RIF resistant has a great impact on disease management overall owing to the easily and accessible disease transmission among the population.(Arzu *et al.*, 2011).

WHO,(2014) recorded about 9000000 people infected with TB in (2013), also the main threat of MDR/TB was roughly approximated to be 480000 patient posing 210000 cases-fatalities. (Keira *et al.*, 2016).

In order to supplant the conventional low diagnostic yields and modest sensitivity, the

newer modalities of nucleic acid tests have been evolved, the latter method has gained a high sensitivity for AFB positive specimen versus lower acid-fast bacilli negative specimen. (WHO 2010). (Panayiotis *et al.*, 2011).

Great diagnostic burden still sways on the issues related to the poor gain of smear microscopy and time-consuming culture and also to the labile sensitivities of molecular types. (Giulia et al., 2017; Lewinsohn et al., 2017).

The poor prognosis and down-running outcome are directly proportional to the ill-diagnosed lab. Moreover, microbiological settings, leading to the delayed clinician response in the wake of disease management, hence posing ill-treated patients with the emergence of widespread resistance.

The key core organ that plays a pivotal disease contraction for T.B. infection is the lung whether a new or relapsed cases are ranging between 54 to 97 % of the overall reported cases. (WHO 2017).

Delayed or faulty T.B. diagnosis has promoted disease transmission among the population, the main and critical strategy is to reduce or prevent the eruption of epidemic cases and to enhance rapid and precise diagnosis also identify and notify for premature T.B. cases through broadened and proper screening programs.

The conventional smear microscopy and culture methods have gained poor or modest sensitivities in addition to other limitations concerning the delayed timeframe and ill-equipped skeleton of the lab. Facilities in some countries leading to attenuated access for proper diagnosis and treatment. (Detjen *et al.*, 2015 ,Eutkemeyer *et al.*, 2016)

2. MATERIALS AND METHODS

At the Chest and Respiratory Diseases Center in Erbil city a statistical study employed between the time span June 2017_June 2018. Those patients who suffer of clinical and radiological (x-ray) were included in the study by obtaining sputum samples and sending for AFB staining and x pert MTB/RIF test.

2.1 Smear microscopy

All specimens were stained for acid-fast microscopic examination using Ziehl-Neelsen before sample concentration. The grade of acid-fast bacilli positivity was assigned to one of the four categories (1+, 2+, 3+, 4+) as per Clinical and Laboratory Standards Institute (CLSI) guidelines CLSI Publishes New Microbiology Guideline, M48—Laboratory Detection and Identification of Mycobacteria in (2018)

2.2 X pert MTB/RIF assay

Early morning coughing in the appropriate sterile container is paramount for our laboratory Test and study.

The assay utilises single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and hemi nested rt-PCR. (Zhang et al., 2016 ,Gayen et al., 2016).

The MTB/RIF assay was performed as described previously. (WHO 2017 ,Reje et al., 2015).

Sputum samples are treated with sample reagent (SR) containing NaOH and isopropanol. The SR is added using a 2 to 1 ratio of the sputum sample, homogenized and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the Gene X pert instrument, and an automatic process completes the remaining assay steps.

The system automatically interpreted all results from the measured fluorescent signal into the following categories: invalid, if PCR inhibitors were detected with amplification failure, negative or positive. Positive results were scaled into 4 categories (very low, low, medium, high) depending on bacterial load and defined susceptible or resistant to rifampicin depending on detection of mutations in RNA polymerase gene.

3. RESULTS AND DISCUSSION

Mycobacterium tuberculosis has been considered as the leading cause of huge threat upon millions population died annually, the critical significant of Gene x pert has been greatly substantiated worldwide owing to the critical key role used in 23 000000 tests in 130 countries via WHO. (Dereje et al., 2015).

The evolving mission of WHO with reach 2035 is to reduce 90% in incidence and 95% in mortality Among TB patient. (Wu et al., 2017.

ZN staining was done for 250 samples of the patients who were having a history suggestive of pulmonary tuberculosis. Out of these 75 (30%) sputum samples were AFB smear positive, and 175 (70%) were negative. Then all positive samples were performed on Gene X pert ® MTB/RIF assay. Out of the 250 sputum samples 85 (34%) were MTB (Mycobacterium tuberculosis) positive and 165 (66%) were negative. The results of Gene X pert and ZN staining are compared in our study. It is evident from the (table 1) that Gene X pert MTB/RIF is more useful than ZN staining. As compared to ZN staining it can detect MTB even in 1ml of sputum. The second important advantage of Gene X pert is that it also detects (RIF) rifampicin resistance and helps us to diagnose multidrug resistance tuberculosis (MDR TB).(Meyer et al., 2017) .

Table1: Comparison of sputum between the detection of tuberculosis by smear microscopy and X pert® MTB/RIF

	AFB +ve	AFB -ve	Total	
Gene x pert +ve	75	10	85	Ppv; 88.24%
Gene x pert –ve	0	165	165	NPV;100%
Total	75	175	250	
	Sensitivity=100%	Specificity= 94.29 %		

For the years 2017 to 2018 more negative results were found by Gene x pert because during this period even the negative microscopically cases had been tested as a routine without a clinical basis ,Although X pert showed high overall sensitivity and specificity with pulmonary samples, its sensitivity has been lower with smear-negative pulmonary samples. (Ngayen *et al.*, 2016) ,Eutkemeyer *et al.*, 2016).

The Gene X pert MTB/RIF test had a fair sensitivity and specificity for diagnosing smear-negative pulmonary TB. It may be useful for diagnosing pulmonary TB in patients with a negative sputum AFB smear. The assay is faster than culture and can detect rifampicin resistant strains of MTB. (Chakravorty *et al.*, 2017).

Combing smear microscopy and X pert MTB/RIF appears to be an accurate and cost-eff ective tool for the early diagnosis of pulmonary TB. (Xiaofu *et al.*, 2018).

Gene x pert plays a pivotal role in identifying multi drug resistance tuberculosis and rifampicin resistance. In this study, we find seven drug-resistant patients as illustrated in

(table 2) out of 250 (2.8%) cases being confirmed as drug-susceptible TB cases.

Table 2: RIF resistant and RIF not resistant Xert® MTB/RIF

	MTB +ve	MTB -ve	Total
RIF not resistant	78	165	243
RIF resistant	7	0	7
Total	85	165	250

Drug-resistant TB poses a huge threat and issue-related fatalities. (Singh *et al.*, 2016, Walters *et al.*, 2017).

The main obstacles for the occurrence and sustained acquisition of multi-drug resistant cases due to a shortage of diagnostic equipment's, they are expensive, not effective and time consuming. WHO 2018).

The sensitivity of my Xpert MTB/RIF study was 100%, whereby the sensitivity observed in Lima and Pero studies was as high as 96.7% and 100 % in New-Zealand and which was consistent with the findings of this study (Grriquiry *et al.,* 2012 ,Williamson *et al.,* 2012)

The specificity of X pert MTB/RIF assay found in our study was 94.29%. The result of specificity is comparable with other studies conducted in different places which have shown a specificity ranging from 94.1 to 100 (Balcha *et al.,* 2014 Ausse *et al.,* 2011).

4. CONCLUSIONS

Gene x pert is entirely conclusive and informative comparable to sputum AFB microscopy also the former is sensitive and specific for rifampicin resistance in our lab.

Personnel can obtain minimal training on its utility and secondly the imprudent use of anti-tuberculosis drugs is withheld. Industrialised and non-industrialised communities hailed the early identification of MDR/RIF T.B has dramatically decreased the erroneous results and achieved higher survival among TB patients and eradicates the sources of TB transmission by implementing excellent diagnosis and cure.

Conflict of Interest

There is no conflict of interest.

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Water quality monitoring of Duhok Dam (Kurdistan Region of Iraq)

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ABSTRACT

Surface water quality is a crucial factor that contributes for drinking water demand and agriculture use. In the light of progressive depletion of water quality of the Duhok dam, an investigation of major dissolved ions was performed. The main objectives were to detect the water quality condition for drinking and agriculture purposes and temporal variations of studied parameters. The water quality analysis of Duhok Dam was assessed based on 14 water quality parameters (turbidity, total dissolved solids, pH, electrical conductivity, total alkalinity, total hardness, calcium, magnesium, potassium, sodium, bicarbonate, sulfate, chloride, nitrate) which were collected monthly from two locations (91 samples from each location) for a period of 8 years between 2009 and 2017. The water quality analysis of Duhok Dam was assessed based on 14 water quality parameters. The water of study area was found to be characterized by the dominance ion of calcium and sulfate based on Piper trilinear diagram. The observed results indicated to high concentrations of electrical conductivity and total dissolved solids, sulfate and total hardness in all water samples and some concentrations exceed the guidelines prescribed by WHO in 2008. In addition, temporal variations of most parameters were observed. The quality of water is believed to be controlled by both geological formation and anthropogenic activities around the dam.

1. INTRODUCTION

Dams are one of the most significant human interventions in the water cycle which supply large amount of water for variety of human uses including drinking water supply, agriculture and recreation. They also considerably decrease the risk of floods and droughts (Odhiambo *et al.*, 2015). However, increasing population, agricultural activities, urbanization expansion and industrialization have made water quality deterioration a serious problem and have shortened the accessibility of drinking water. Water quality is also affected by the natural contributions such as precipitation rate, weathering processes and soil erosion (Khatri and Tyagi, 2015; Issaka and Ashraf, 2017). Consequently, it is challenging to preserve water quality at an acceptable level for various purposes, primarily potability (Venkatesharaj *et al.*, 2010).

Duhok dam is an earth-fill embankment dam built in 1988 on Duhok River with main

purpose of providing water for irrigation of agricultural land in Duhok city and area around it. Nowadays, the dam is providing water supply for drinking purposes and recreational activities (Mustafa and Noori, 2013). Water quality in Duhok Dam has suffered from remarkable stress in terms of quantity (due to drought periods and overexploitation) and quality which certainly affected by quantity and quality of supplies coming from different sources (Toma, 2013). Water quality in the dams, therefore, needs to be monitored periodically and continuously. Water quality monitoring has obtained its significance for sustainable development and appropriate management of this valuable natural resource. Assessment of physical, chemical and biological water contamination is essential for the freshwater pollution reduction.

The aims of the present study are to determine the hydrochemistry of Duhok dam and to find out the quality status of the water with reference to drinking water quality as well as irrigational purpose. Furthermore, observe the temporal variations of selected water parameters and identify the pollution sources, if obtained, at this dam.

2. MATERIALS AND METHODS

2.1. Study area

The study has been conducted in Dohuk dam situated about 2 km north of the city of Dohuk between latitudes 36°52′35″and 36°54′21″N, and longitudes 42°59′51″ and 43°00′40″E(Fig. 1). The dam is actually a reservoir created by an earth-fill embankment dam on the Dohuk River and was completed in 1988 with the primary purpose of providing water for irrigation and for the city of Dohuk but recently used for recreational activities as well. The dam is 60 m high with the capacity of holding 52 million cubic meter of water and has a maximum discharge of 81m^3. The reservoir is about 4 km long and 1.7 km wide, the total catchment area is 135 km^2 (Shekha *et al*., 2013). There are several mineral springs (Sulphur springs) that appear on the limit in the tail of the reservoir and their discharges vary seasonally and decrease considerably during dry summer period. The geological structure of catchment consists mainly of dolomite, limestone, siltstone, clay marls, and gypsum from Eocene deposits (Mohammed, 2010).

The climate of the study area is considered semi-arid which is comparable to the Mediterranean climatic condition (hot and dry summer and rainy cold winter) and partly to the Iranian climate with an influence of the relatively high altitude of the surrounding high mountains. The mean annual precipitation was approximately 587 mm for the period 2009 to the end of 2016. Rainfall storms occur between October and May, with maximum during January, February each year whereas the other months of the year are relatively dry. Another feature characterized the precipitation in the study area is its irregular yearly distribution. The temperature during winter season is at minimum (less than 1ºC), whereas the maximum temperature is about 43ºC during summer, with the average annual temperature of about 19.2ºC (Mohammed, 2010).

2.2. Data collection

The water samples were taken at the depth of 0.2m (to avoid scums and to give accurate results) from two locations at monthly intervals for a period of eight years from 2009 to the end of 2016. A total of 182 samples were collected from both locations and each sample kept in a 1L sterilized polyethylene bottle and stored in cold ice box at 4 ºC and delivered on the same day to laboratory for analysis. The geographical location of the sampling locations is shown in Fig. 1.

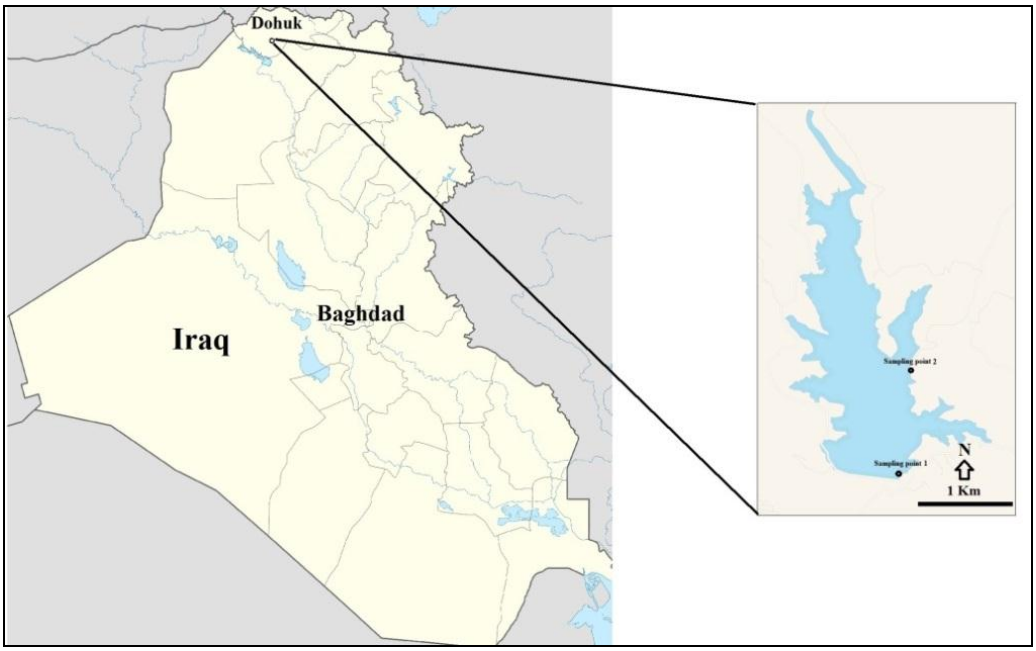

Fig. 1. The study are and sampling collection locations (latitudes 36°52′35″and 36°54′21″N, and longitudes 42°59′51″ and 43°00′40″E)

The samples were analysed for 14 parameters including turbidity (TU), total dissolved solids (TDS), pH, electrical conductivity (EC), total alkalinity (TA), total hardness (TH), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), bicarbonate (HCO_3), sulphate (SO_4), chloride (Cl), nitrate (NO_3). Electrical conductivity (µS/cm), pH, and total dissolved solids were measured in the field with a portable multi-metre (Trans ISO 9002). All other parameters were determined in the laboratory following standard protocols. Alkalinity was determined using titration with sulfuric acid carried out in the laboratory of the Directorate of Environment of Dohuk city. Other chemical analyses were carried out using spectrophotometer and flame atomic absorption spectrometer. Precipitation samples were collected based on daily interval from fixed Dohuk dam meteorological station and water level of the dam was also measured daily during study period in order to compare with temporal variations of water quality parameters. Aquacham software, Diagramme software, and Microsoft excel and word had been used to obtain tables and figures.

3. RESULTS AND DISCUSSION

3.1. Hydrochemical characteristics and temporal variation

The pH value of aquatic system is an important indicator of the water quality and the extent pollution in the watershed area. It is a measure of the acidity intensity or alkalinity conditions of a water body (Kadhem, 2013). The pH values of water samples varied from 7.68 to 8.41 with average value of 8.1 in location 1 and from 7.7 to 8.46 with average value of 8.2 in location 2. All of the water samples are alkaline due to presence high concentration of carbonates and bicarbonates and found within the limit (6.5 – 8.5) for drinking prescribed by WHO (2008).

Electrical conductivity (EC) and total dissolved solids (TDS) are two important parameters in determining salinity hazards and suitability of water for any purposes. EC values

were in the range of 814 to 1291 μS/cm with average value of 981.7 μS/cm, and 823 to 1321 μS/cm with average value of 983.7 μS/cm for sampling location 1 and 2, respectively. TDS values ranged from 520.8 to 826 mg/l with average 628.3 mg/l and from 526.7 to 845 mg/l with average of 629.7 mg/l for sampling location 1 and 2, respectively. The highest concentrations of TDS and EC were recorded when the water level in the dam was low. Fig. 2(e) shows the temporal variations of water quality parameters of sampling location 1 compared to water level of the dam and amount of rainfall. The most desirable limit of TDS for drinking water is value less than 500 mg/l and all samples exceeded this limit, nevertheless no sample exceeds the maximum permissible level of 1500 mg/l prescribed by WHO (2008). Nutrient enrichment due to intensive agricultural practices and geological condition may enhance TDS and in turn increases the EC values since these two parameters are directly related to each other (Kannan and Joseph, 2010). The higher values of EC and TDS may also be due to semi-arid type climatic condition as well as high evaporation rate (Al-Mezori and Harami, 2013; Shekha *et al*., 2017).

Water clarity is expressed by turbidity, the greater the amount of suspended particles in water the murkier it appears and as a result the turbidity value will be higher. Turbidity values ranged from 0.25 to 5.9 with average of 2.6 NTU (Nephelocetric Turbidity Unit) in location 1 and ranged from 0.25 to 6 NTU in location 2. According to drinking water standard by WHO (2008), the maximum permissible level of turbidity is 5 NTU and sampled water from Dohuk dam were acceptable except for 7 and 8 samples from both locations, respectively. This could mainly be due to the level of particulate matter include sediments during intensive rainfall events.

Alkalinity of natural water is caused by bicarbonates, carbonates and hydroxides. The alkalinity content varied between 132 and 204 with average of 165.2 mg/l for location 1 and the same range for location 2 with average value of 164.9 mg/l. The alkalinity concentrations displayed no temporal variation during study period (Fig. 2(f)). All water samples exhibit alkalinity values above permissible limit of 120 mg/l prescribed by WHO (2008). The high value of alkalinity could be due to the dissolution of limestone and other carbonate minerals in the catchment area. It may also be noted that polluted water body, other anions like PO_4 and NO_3, may contribute to higher alkalinity (Kannan and Joseph, 2010).

Total hardness (TH) is an important parameter of water use for domestic purposes. According to the general guidelines for classification of water hardness as calcium carbonate; 0 to 60 mg/L is classified as soft; 61 to 120 mg/L as moderately hard; 121 to 180 mg/L as hard and more than 180 mg/L as very hard (Ramesh and Jagadeeswari, 2012). Hence, Table 1 shows that all water samples are considered as very hard since the values of total hardness ranged between 360.5 and 495.8 for location 1 and between 365.6 and 508 mg/l for location 2. The acceptable limit for domestic use is 75 mg/l. Excess hardness is undesirable mostly for aesthetic and economic reasons (Ramesh and Jagadeeswari, 2012). The main causes of water hardness is both calcium and magnesium ions.

In all water samples, calcium ion appears to be the dominant cation. The concentration of calcium ion in water ranged from 77.3 to 107.2 with average of 91.5 mg/l for location 1 and from 75.3 to 108.8 with average of 90.7 mg/l for location 2. This indicates that all water samples were above Iraqi standard value of 50 mg/l (MOE, 1998). The content of magnesium

is comparatively less than calcium. Magnesium concentrations varied between 36.1 and 63.5 mg/l and between 35.1 and 68.8 mg/l for both locations, respectively. Of the total samples, 46% in location 1 and 44% in location 2 show concentrations outside the permissible limit of 50 mg/l. The concentrations of both Ca and Mg exhibited no temporal variation during study period (Fig. 2(a)). The high concentration of Ca and Mg is not desirable in washing, laundering and bathing. Although the sources of Ca and Mg in water body are mainly the geochemistry of the rock types, the prolonged agricultural activities prevailing in the basin may also directly or indirectly augment the concentration of both ions (Mohammed, 2010).

Sodium (Na) is one of the important naturally occurring cations and its concentration in fresh water is generally lower than calcium and magnesium. However, in the present study the average concentration of Na is comparatively higher that Mg. For aesthetic reason, the guideline value given by WHO (2008) is 200 mg/l, sodium concentrations values were found within permissible limit. Fig. 2(a) illustrates that the concentrations of Na were high during periods of low water level and concentrations reduced as water level up, possibly this could be due to the effect of dilution. Potassium concentrations were very low and vary from 4 to 8 mg/l in both locations. Parts of potassium go into clay structure and thereby its concentrations get reduced in water (Kannan and Joseph, 2010).

Bicarbonate concentrations (HCO_3^{-1}) in both sampling locations were almost the same and varied from 161.04 to 248.88 mg/l, with slightly change in average values of 201.5 and 201.2 mg/l respectively. No sample exceeded the permissible limit (250 mg/l) set by WHO (2008) for drinking water. The primary source of bicarbonate in water body is the dissolved CO_2 in rainwater and the dissolution of carbonate minerals in the study area (Mizzouri, 2007).

Sulfate ion (SO_4^{-2}) was the most dominant anion with concentrations varied from 170 to 440 mg/l with average value of 280.2 mg/l in location 1 and from 163.2 to 448 mg/l with average 282.5 mg/l in location 2. Only 4.4% of samples from sampling location 1 and 6.6% from location 2 have desired concentrations for drinking water of 200 mg/l prescribed by WHO (2008). Remarkable variation of Sulfate concentrations can be observed in Fig. 2(b), the high concentrations can be seen during low water level and the concentration becoming lesser during high water level most possibly due to the effect of dilution. Sulfate ion is released to water naturally by leaching from gypsum, other common minerals and discharge of domestic sewage tends to escalate its concentration. Limestone minerals may produce level of sulfate up to 800 mg/l (Kiely, 1997). At higher concentrations, sulfate imparts a bitter taste to water and may cause laxative effects (WHO, 2004). The high content of sulfate in the study area is mainly due to geological formation. Quaternary and cretaceous aquifers within the study area produce several sulfate springs which containing large quantities of sulfate and released to the Duhok dam (Mizzouri, 2007).

Chloride (Cl^-) is a naturally occurring anion in all types of water. The chloride content of sampling water varied from the lowest value of 49.7 mg/l (in both locations) to the highest value of 99 mg/l (location 2). In natural water bodies, the likely sources of chloride is the leaching of chloride-containing minerals (such as apatite), inland salinity and the discharge of agricultural, industrial and domestic waste water (Abbasi, 1998). The result of this study ensures that the chloride concentrations were relatively constant and did not correspond to

Table 1: Minimum, maximum, average and standard deviation values of water quality parameters at two locations of the Duhok dam reservoirs (91 water samples for each location).

Parameters	Unit	Sampling location 1				Sampling location 2			
		Min	Max	Av	STD	Min	Max	Av	STD
pH		7.68	8.41	8.1	0.2	7.7	8.46	8.2	0.2
EC	µS/cm	814	1291	981.7	116.6	823	1321	983.7	114.1
TDS	mg/l	520.8	826	628.3	73.9	526.7	845	629.7	72.7
TU	NTU	0.25	5.9	2.6	1.4	0.25	6	2.7	1.4
TA	mg/l	132	204	165.2	13.5	132	204	164.9	14.1
TH	mg/l	360.51	495.84	430.9	32.3	365.64	508.04	429.3	36.1
Ca^{+2}	mg/l	77.3	107.2	91.5	6.5	75.3	108.8	90.7	6.6
Mg^{+2}	mg/l	36.1	63.5	49.3	6.6	35.1	68.6	49.4	7.0
Na^{+}	mg/l	49	98	70.7	13.8	47	98	71.3	13.9
K^{+}	mg/l	4	8	5.8	0.8	4	8	5.9	0.8
HCO_3^{-1}	mg/l	161.04	248.88	201.5	16.5	161.04	248.88	201.2	17.2
SO_4^{-2}	mg/l	170	440	280.2	64.2	163.2	448	282.5	67.9
Cl^{-}	mg/l	49.7	96	69.9	11.3	49.7	99	69.2	11.2
NO_3^{-1}	mg/l	0.55	14.4	3.3	3.1	0.5	14.7	3.4	3.2

different sources that could change with time (Fig.2(b)).

Nitrate (NO_3^{-1}) concentrations in all the samples were found to be moderately low, with the lowest value of 0.5 mg/l and the peak value of 14.7 mg/l, but none of the samples exceeded the permissible limits of 50 mg/l for drinking water (WHO, 2008). The temporal variation of nitrate is displayed in Fig. 2(c) which indicated to the high level of nitrate during low water level in the dam and high concentration during high water level. Nitrate can reach surface water as a result of agricultural activities, from domestic waste water, and from oxidation of nitrogenous water products in human and animal excreta, including septic tank (Almasri, 2007).

3.2 Water type classification (Hydrochemical facies)

Hydrochemical facies are water masses that have diverse geochemical attributes and are useful for determining the origins and distribution of both surface water and groundwater masses (Lloyd and Heathcote, 1985). In case of a clear domination of a particular cation or anion (> 50% of total cations and anions), the facies can be identified based on dominant constituents. However, if no clear-cut domination of any ion, 33% is taken as cut-off value (Saha *et al.*, 2008).

Fig.3 clarifies the Piper trilinear diagram (Piper, 1944) for the data obtained from the chemical analysis of water samples from the study site. The diagram comprises of two lower triangles that illustrate the percentage distribution, on the milliequivalent basis, of the major cations (Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}) and major anions ($SO4^{-2}$, HCO_3^{-}, Cl^{-}, NO_3^{-1}). Only one chemical water type in the study area was recognized as Ca–Mg–Na–SO_4–HCO_3 facies. This water type may, therefore, be due to the influence associated with the geology of the study area.

3.3. Suitability of water for irrigation purposes

Water in the study area is mainly used for irrigation. The irrigation water with adequate quality is very vital for attaining maximum crop productivity. In order to assess the overall irrigational water quality of the collected samples, Sodium Adsorption Ration (SAR) computed water quality parameter has been

used which is most widely applied to evaluate suitability of irrigation water. SAR is a measure of the sodicity of the soil determined via quantitative chemical analysis of water in contact with it. An excess HCO_3^- ion in water reacts with Na^+ in soil resulting in a sodium hazard which deteriorates the soil properties by reducing permeability (Subramani *et al.*, 2005). SAR values were plotted against EC values (µS/cm) over the diagram to categorize analyzed water samples according to their irrigational suitability proportion. The SAR was calculated using the following equation:

$$SAR = Na+ /\sqrt{(Ca^{+2} + Mg^{+2}/2)} \ldots\ldots\ldots (1)$$

Where, concentrations of all ions were expressed in meq/l. Based on the sodicity diagram the water samples were classified and shown in Fig. 4, and it is clear that all samples fall in group C3-S1 which indicated to high salinity water and low SAR. Hence, the water samples of the study site can be considered moderately suitable for irrigation.

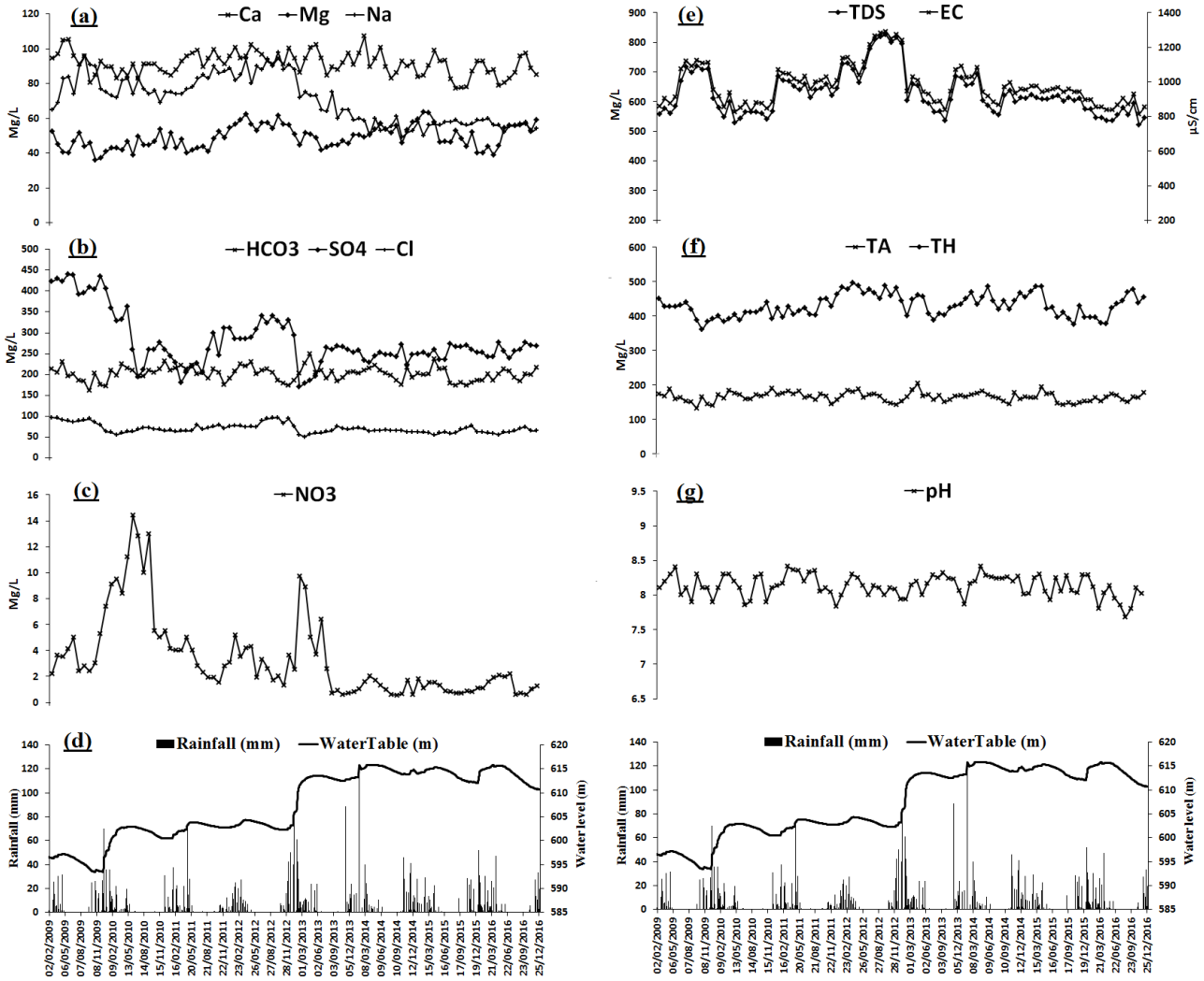

Fig. 2(a, b, c, d, e, f, g).Temporal variation of water quality parameters for sampling location 1.

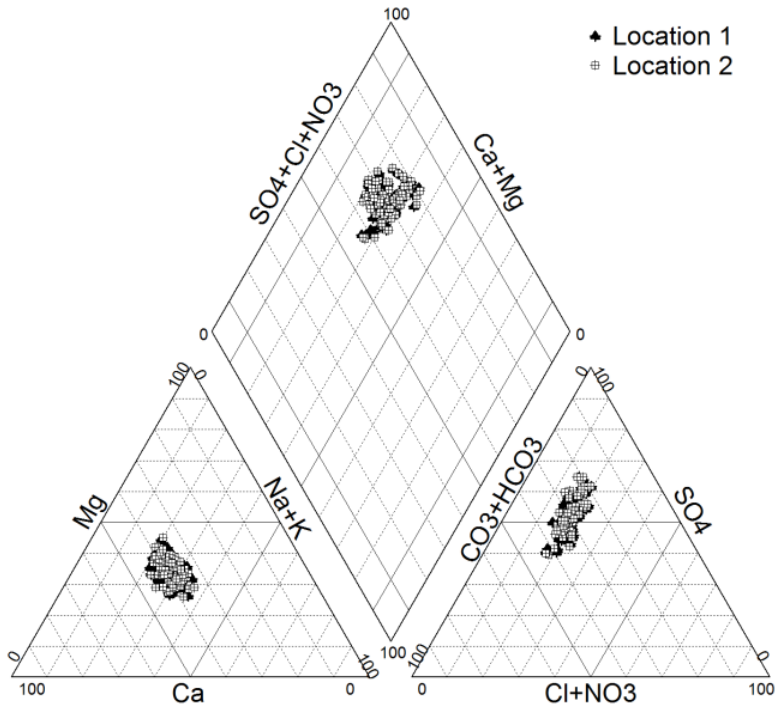

Fig. 3. The Piper diagram for the water samples from the Dohuk dam

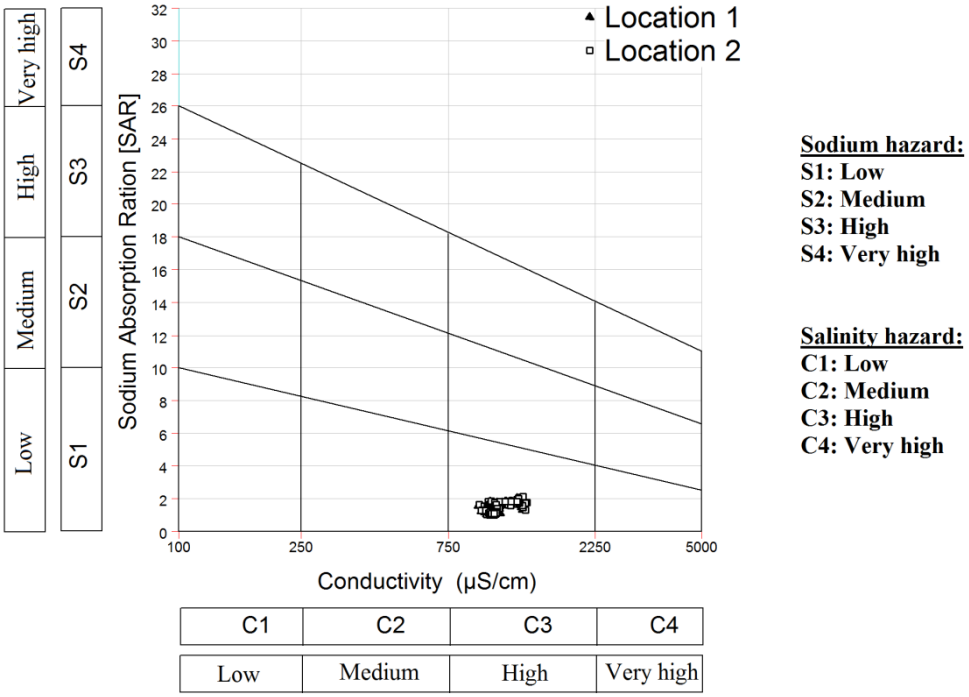

Fig. 4. Salinity diagram for water samples of two locations

4. CONCLUSIONS

The water quality of Duhok Dam has been assessed for its domestic suitability and irrigational purposes as well as temporal variation. Analysis of hydrochemical study has revealed that the water was in alkaline nature. The electrical conductivity and total dissolved concentrations of water samples were all found above permissible level of drinking purposes, however, were within the maximum desirable limits prescribed by WHO. All of the water samples were found to be very hard types in nature. Most of the water samples have been found unfit for drinking purposes due to high sulfate concentrations. The temporal variation of hydrochemical parameters was linked highly to fluctuations of water level and could also be to the climatic conditions such as rainfall. The results of chemical analysis reflect that the dominant cation in the study area was calcium and the dominant anion was sulfate that corresponds to the chemical facies of Ca–Mg–Na–SO_4–HCO_3. Based on the water quality parameters analyzed by SAR, the suitability of water for irrigation was moderate. Hence, the quality of the Duhok dam water was affected by changes in water level and anthropogenic activities, mainly agriculture surrounding the dam.

Complete and detailed quality assessment program including detection of the heavy metals (toxic elements) could be examined. Furthermore, microbiological examination of water samples should be carried out to monitor possible contamination from the waste water disposal of surrounding villages. Waste water disposal from nearby villages should be prohibited and replaced by properly designed septic tanks.

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Molecular Identification of *Acinetobacter baumanii* and *Acinetobacter* genomic species 13TU Using PCR

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A B S T R A C T

Acinetobacter baumannii is well known to be multi-drug resistant and associated with many infectins. Manual and automated identification systems, can not differentiate between both species *A. baumannii* and *Acinetobacter genomic species 13TU*. The reason is the differences in nucleotide sequences in *gyrB* of both species, therefore, specific genotypic test required for differentiating them. The aim of this study is to differentiate between two species of Acinetobacter based on *gyrB* gene nucleotide sequence differences and their antibiotic resistance profile in Erbil city. Six hundred thirty two clinical specimens were collected during the period of March to August 2016. Colony, cultural morphology and VITEK2 system were used, in which 105 of them were *A. baumannii*. PCR was then used to differentiate between *A. baumannii* and Acinetobacter genomic species 13TU. Out of 632 clinical specimens, 105 were phenotypically identified as *Acinetobacter baumannii* using VITEK2 system. PCR results showed that *Acinetobacter* genomic species 13TU gave an amplicon of 294 bp as one band, but *A. baumannii* gave a second amplicon of 490 bp. The PCR method correctly identified 43 *A. baumannii* and 62 Acinetobacter genomic 13TU. Carbapenem resistance was observed 60% (n=40), infection among male 69.53% (n=73) was higher than female. PCR detection of Acinetobacter species showed high sensitivity and accuracy as compared to other phynotypic methods. To the best of our knowledge this is the first study performed to differentiate two important species of Acinetobacter in Kurdistan region.

1. INTRODUCTION

Acinetobacter baumanii is one of the most established pathogen involved several nosocomial infections. These infections, such as pneumonia, bacteremia, surgical wound infection, urinary tract infection, and meningitis. The long period persistence of this bacterium in hospital environment as well as its ability of resistance to a wide range of antibiotics like aminoglycosides, quinolones, broad-spectrum β-lactams and cephalosporins (Maleki *et al.*, 2017). Moreover significant changes in the antibiotic resistance can be detected between *A. baumanii* and the other species (Van Looveren *et al.*, 2004). However shared with the capacity to growth at various temperatures, PH conditions and its resistance to dry or moist environment in the hospital, could help bacteria in easy transmission and

infection epidemic (Abbo *et al.,* 2005). *A. baumanii* is associated to a greater range as the causative organism of infections than Acinetobacter genomic spp. 3 and 13TU, as a significance of being the most resistant one from the genus (Visca *et al.,* 2011). Together with the environmental organism *A. calcoaceticus* which is not known to cause serious infections, are classified in the *A. calcoaceticus–A. baumannii complex*. For the reason that they are genetically related and therefore difficult to differentiate them from each other based on phenotypic methods. On one hand manual and automated identification systems, e.g., API and VITEK 2 do not differentiate between these species which leads to misdiagnosis of up to 25% Acinetobacter members belong to the complex as *A. baumanii*. As a result it is important to classify *A. baumanii* correctly for epidemiologic, clinical purposes and treatment. On the other hand investigations indicate that molecular techniques like PCR methods have been shown to be reliable, simple, effective and specific to characterize *A. baumanii* and Acinetobacter species 13 TU correctly (Higgins *et al.,* 2010 and Higgins et al., 2007). Due to deficient phenotypic identification of *Acinetobacter* isolates to the species level, several genotypic methods have been developed for this determination (Chang et al., 2005, Dijkshoorn *et al.,* 2007 and Dijkshoorn *et al.,* 1998). As a result, nucleotide differences in *gyrB* gene of both species can be detected by PCR method. As peer our knowledge this is the first study performed to differentiate two important species of Acinetobacter in Kurdistan region. Therefore a study is essential to determine the taxonomic position of Acinetobacter genomic species.

Aims and Objectives:

- Identification of *Acinetobacter baumanii* and *Acinetobacter* genomic species 13TU based on *gyrB* gene nucleotide sequence differences.
- To find the prevalence of Acinetobacter spp. In Erbil city.
- To determine the antibiotic resistance profiles of Acinetobacter species isolates.

2. METHODS 2. METHODS

2.1. Isolation and Identification

In total six hundred thirty two samples were collected between 7th of March to 17th of August 2016 from patients admitted to the Western Emergency Hospital-Erbil. Samples were collected from different clinical specimens such as urine, tracheal aspirate, vaginal swabs, wound swab and blood. The specimens were obtained by the clinical microbiology laboratory at hospital as part of routine diagnostic activities. Isolates were identified using standard microbiological methods. The identified *A. baumannii* isolates were then further subjected to automated VITEK 2 compact system for susceptibility testing and confirmation. All isolates were stored at -70°C in glycerol broth until tested. This project was approved at the first site by the Scientific and Research Ethics Committee at College of Health sciences/ Hawler Medical University. The practical part of the study was carried out in Medical Research Center, MRC/ Hawler Medical University.

2. 2. PCR Amplification of *gyrB* gene

PCR was then used to differentiate between both A. baumannii and Acinetobacter genomic species 13TU. For this, isolates were sub-cultured from frozen stock using blood agar media. Cells were grown on blood agar plates for 24 h at 37°C, and Template DNA for PCR was isolated by using the (AccuPrep® Plasmid Mini Extraction Kit Bioneer). DNA detection test was carried out using the PCR assay

according to the manufacturer's instructions GoTaq® Green Master Mix (Promega M7122, USA). For the PCR amplification, 2µl of DNA template were added to the reaction volume equal to 25 µl containing 2.5 µl PCR buffer (10 mM Tris-HCL, 50 mM KCL), 1 µl MgCl2, 1 µl dNTPs mix, 2 µl for each primer, 0.25 µl Taq polymerase (5000 U/ml) and 14.25 µl of H2O (nuclease free).

Three primers for *gyrB* gene were used as previously been used (Higgins et al., 2007), two were universal to both species, sp4F (5'CACGCCGTAAGAGTGCATTA) and sp4R (5' AACGGAGCTTGTCAGGGTTA), and one differed at the 3'end from genomic sp. 13TU, sp2F (5'-GTTCCTGATCCGAAATTCTCG). Thus, in a PCR with all three primers, both *A. baumannii* and genomic sp. 13TU would yield an amplicon of 294 bp (sp4F to sp4R) but only *A. baumannii* would yield a second amplicon of 490 bp (sp2F to sp4R). A negative control reaction in each test was set up having all components of the reaction except DNA template. The amplification conditions were: 94°C for 3 minutes followed by 30 cycles (94°C for 30 sec, 50°C for 30sec and 72°C for 50sec) and a final extension at 72°C for 10 minutes. PCR products were visualized by running on agarose 1.2 % in TBE (1X) buffer at 5 v/cm for 1 hour. A 100-1000 bp DNA marker (Norgen Biotech Corp Cat. No. 11600) was used to measure the bands of amplified products.

3. RESULTS AND DISCUSSION

3. 1. Demographic Characteristics

The patients were grouped into two categories according to age and gender. Out of the 105 samples that were identified as *A. baumanii*, the highest rate of infection was noticed in the age group (21-30 years) with 34.28% (n=36). The lowest rate of infection was observed in the age group (51-60 years) with 10.47% (n=11). Infection was higher among male than female with 69.53% (n=73) as shown in table (1).

3. 2. Antibiotic Resistance Profile

Bacterial isolates were showed highest rate of antibiotic resistance to cefepime (98.50%) followed by aztroenam (97.70%) and ceftazidime (97.20%), while all *A. baumanii* isolates were sensitive to both antibiotics minocycline and tigercyclin, as shown in table (2).

3. 3. PCR Amplification of *gyrB* gene

PCR results were identified 47 *A. baumannii* isolates correctly. In every case, two clear bands were visible on agarose gels, while all 41 genomic sp. 13TU isolates yielded only the lower 294-bp band. The remaining 17 Acinetobacter isolates failed to produce any PCR products, as shown in figure (1). Results were obtained in <2.5 h from an agar plate to a finished gels.

3. 4. Discussions

Several studies have been performed on typing of *A. baumannii* strain by different genotypic and phenotypic methods, and different genetic profiles have been reported in various countries. (Lee et al., 2007) In a study performed on 232 cases of Acinetobacter species, the most prevalent species in their study was *A. baumannii* (61.2%), followed by Acinetobacter genomic species 13TU (25.9%). *Acinetobacter* genomic species 13TU can normally be found on skin and moist habits. However, the tendency of the *Acinetobacter* genomic species 13TU for resistance development and spread is less known and studied than that of *A. baumannii* (Dijkshoorn *et al.,* 2007 and Horrevorts *et al.,* 1995). For

epidemiological and clinical concerns, it is highly recommended to differentiate among these species for a better patient management and outcome (Higgins *et al.,* 2010). In this study 105 *A. spp.* were obtained from patients during 5 months, in which the highest rate of infected groups were (11 - 40 years), which was 43% compared to other age groups. Others reported very similar age groups infected with the pathogen with average age of patients in their study of 54 years (2 – 101 years) with predominance of over 17 years. These age groups of patients was indicated as an independent risk factor for these type of infections (Turkoglu et al., 2011). Results in our study showed that the infection rate among male 69.53% was higher than female 30.47%. Similar results in terms of sex differences was reported by Abbo *et al.,* (2005), who reported 60% male among cases in their study. They stated that hormonal or other sex differences could be one of the reasons for the higher rates within male patients, which might predispose a patient for colonization and infection (Abbo *et al.,* 2005 and Uwingabiye et al., 2016).

Recently the emergence of carbapenem-resistance *A. baumannii* has great concern to health services internationally. This investigation indicated that the concern carbapenem-resistant *A. baumannii* in clinical samples of Erbil city is alarming. In our study a total of 40 *A. baumannii* isolates (60%) showed resistance to imipenem, similar results have been described by other studies (Higgins *et al.,* 2009 and Karlowsky *et al.,* 2003). Carbapenems is one of the antibiotics that have been increasingly used for treating serious infections caused by multidrug-resistant *A. baumannii*; however, the reistance to carbapenems increased in many parts of the world (Zarrilli *et al.,* 2009). Studies reported that carbapeneme resistance in A. baumannii is due to the presence of carbapenemases. Carbapenem resistance could be due to the acquisition of an insertion sequence (ISAba1) (Pourabbas et al., 2016). Data presented here, showed highest resistance to cefepime (98.5%), aztreonam (97.7%), ceftazidime (97.2%), ertapenem (96.9%), cefazolin (96.5%), tobramycin (96.1%), ciprofloxacin (93.6%), gentamycin (93.3%), levofloxacin (92%), amikacin (90.9%). But minocycline and tigecyclin were very effective against *A. baumanii* isolates used in this study. Given to notice that from 105 samples, more than (90%) multidrug resistance and only less than (10%) of the antibiotics were sensitive.

Because classical methods and phenotypic identification of *Acinetobacter* isolates at species level has shown to be insufficient, several molecular methods have been found for pathogen identification at species level (Zarrilli et al., 2009). In this study, a total of 105 isolates *A. baumanii* samples, PCR identified 43 *A. baumannii* and 62 Acinetobacter genomic 13TU. The use of *gyrB* is not new for the identification of Acinetobacter isolates to the species level. It was observed previously that amino-acid sequences and nucleotide can be performed for taxonomic purposes (Higgins *et al.,* 2007). Detection of genetic diversity among different isolates of the pathogen by DNA fingerprinting is an important method for species identification detection of the bacterial strains involved in epidemiological relationship among the bacterial isolates. Identification of genetic diversity among the isolates at species level based on nucleotide differences of gyrB gene using PCR technique, indicated that this is a powerful method for typing *A. baumannii* isolates. Moreover, as peer our knowledge this is the first study to characterize two important Acinetobacter species in Kurdistan-Iraq.

4. Conclusions

In conclusion, our data confirmed that the *gyrB* PCR method is accurate and reproducible, and

can produce the results in less than 3 hrs. The simplicity of the method could make it a powerful tool to be used readily in most labs. This should contribute to a better understanding of the infections and epidemiology of these two important species of Acinetobacter.

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Taxonomic Study for the New Record *Orobanche armena* Tzvelev (Orobanchaceae) in Iraq

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ABSTRACT

Orobanche armena Tzvelev is a new plant record within Orobanchaceae family in Iraq, from Qandil mountain (North-East of Erbil) within Rowanduz district (MRO). Identification and morphological study have been done, these illustrated by graphs. Pollens characters have been clarified like shapes, colors, sizes, surface ornamentation and numbers. In addition, some features of the leaf and stem anatomy have been examined.

1. INTRODUCTION

Orobanchaceae is one of the plant families in Iraq, which includes 210 species of 15 genera over the world (Simpson, 2006). In Iraq consist of 11 species within 2 genera (Al-Rawi, 1964). Gilli (1982) mentioned 36 species of the genus in Turkey, one of them is *O. armena*. Chater and Webb (1972) stated 45 species of the genus *Orobanche* in Europe. Wien (1964) pointed out that 47 species of the genus present in Iran. Rechinger (1964) in the low lands of Iraq indicated 5 species. Whilst Al-Rawi (1964), Ridda and Daood (1982), Karim (1978) and Chakravarty (1976) mentioned 10 species in Iraq. Faris (1983) and Hameed (2016) stated 4 species in Pira Magrun mountain and Hujran Basin separately. Khalaf (1980) mentioned 3 species of the genus in Sinjar mountain. Ahmad (2013) indicated 5 species in Hawraman mountains. Whilst Fatah (2003) mentioned 1 species in Haibat Sultan mountain. Ahmed, (2010) and Darwesh (2017) did not indicate any species in Darband Gomaspan and Choman region respectively. Chakravarty (1976) stated that *O. aegyptiaca* is reported to be used in diarrhea. It is also used to cure boils produced in the throat of cattle. From the similar studies that involve new plant records in Iraq the study of Al-Musawi and Majeed (2013), Haloob (2016) and Sardar (2017). In all the available references, the study did not find any species refers to *O. armena*, therefore the studied specimens regarded as a

new plant record for the Flora of Iraq. The purpose of the current study is to confirm the presence of *O. armena* in Iraq and to study the morphological characters as well as some pollens characters, with leaf and stem anatomy.

2. MATERIALS AND METHODS

For plant specimen's collection, various scientific trips were made to the different regions of northern districts (Kurdistan region) of Iraq in 2017. Identification of the specimens has been done by using the Orobanchaceae family key in Flora of Turkey, the specimens were treated by standard herbarium techniques to become formal specimens, and preserved in Herbarium of Education College - University of Salahaddin, Erbil (ESUH). Kruss dissecting microscope has been used in the examining of the collected specimens that belong to *O. armena*. Some environmental notes have been mentioned, and a map (figure 1) was used. For the pollens, anthers fixed in FAA (Formalin-glacial acetic acid-ethyl alcohol) solution, then a single anther removed and placed in a drop of water or 50% glycerol (the latter to prevent the material from drying out) and dissected with a needle to extrude the pollens; the anther wall material removed and stained with safranin. Then, a cover slide applied. (Simpson, 2006). A mobile camera (Sumsung-A5) has been used for photographing the different plant parts and the scientific terms that used in the study have been taken from Harris and Harris (2001), Hesse *et al*. (2009) and Agashe and Caulton (2009). For the leaf and stem anatomy, the procedure in Al-Mashhadani (1992) has been used and the information in Metcalfe and Chalk (1950) were utilized.

3. RESULTS AND DISCUSSION

3.1. Morphological Study

Orobanche armena Tzvelev in Fl. URSS 23: 686 (1958); Fl. Turkey, Gilli, 7: 21 (1982).

Herbs, glandular, (28-31) cm. Stem unbranched, erect, costate, brown, (90-130)x(7.5-8.5) mm. Leaves (scales) sessile, alternate-spiral, hairs on the lower surface and margin, margin entire, apex acuminate, base truncate, brown, lower cauline leaves narrowly oblong-cultrate or lanceolate-narrowly lanceolate, (14-16)x(3.3-4.0) mm, upper cauline leaves cultrate, (14-17)x(3.0-3.5) mm. Inflorescence a dense simple spike, (160-190) mm, Bracts, cultrate-narrowly oblong, margin dentate, apex acuminate, base truncate, glandular, brown, (15-20)x(4.0-5.2) mm. Flowers numerous, hermaphrodite, Calyx halves connate at base, 2-toothed, teeth narrowly ovate, glandular, brown, (21.3-25.0)x(5.5-7.0) mm, Corolla tubular-campanulate, of tube and limb, glandular-pilose, brown, tube (10-13)x(8.2-10.5) mm, limb bilabiate, lower lip 3-lobed, margin entire, apex obtuse and dentate, upper lip apex emarginate, (11.5-13.0)x(9.0-10.5) mm, Stamens 4, epipetalous, inserted above corolla base, filaments terete, densely pilose below, sparingly glandular-pilose above, brown, (6.5-9.0)x(0.4-0.6) mm, pilose hairs (0.15-0.90)x(0.04-0.06) mm, glandular-pilose hairs (0.20-0.70)x(0.03-0.06) mm, anthers narrowly oblong, brown, versatile attachment with the filaments, (2.1-2.5)x(0.8-1.2) mm. Pistil one, brown, ovary superior, narrowly ovoid-ovoid, glandular, (6.0-8.0)x(2.8-3.3) mm, style 1, terete, glandular-pilose, (2.2-3.0)x(0.4-0.6) mm, glandular-pilose hairs (0.15-0.40)x(0.03-0.05) mm, stigma 2-lobed, rough, (0.7-1.0)x(2.2-2.6) mm. Fruit simple, dry, capsule, ovoid- narrowly ovoid, brown, (8-11)x(3.2-7.0) mm. Seeds numerous, oblong, narrowly ovoid-oblong, reticulate, with small soft hairs, black, (0.35-0.50)x(0.20-0.25) mm. (Plates 1-3).

Type: [Turkey A9 Kars] Armenia turcica, ad declivia faucis Kagyzman-Darasu (nr Kagizman) prope fl. Arax (Aras), 18 vi 1886, V. Massalsky (holo. LE).

Studied samples

MRO: ESUH/ Qandil mountain (North-East of Erbil) within Rowanduz district, 2350 m, 6.8.2017, A. Sardar and S. Al-Dabagh, 7598.

Habitat

The plant was found as parasitic individuals on *Astragalus* sp., in the rocky clay soils; altitude: 2350 m; flowering: August. (figure 1).

3.2. Palynological Study

Pollens yellow, single, tricolpate, spheroidal in both equatorial and polar view, small size according to Erdtman (1971), axis (15-20) µm, tuberculate surface ornamentation, numerous. (Plate 4).

3.3. Anatomical Study

In the leaf, the epidermis is covered by the cuticle layer which is not equal in its thickness from region to region, (2.50-3.750) µm; The epidermal cells shape in the cross sections were oblong, circular, quadrate, irregular, different sizes, straight or oblique radial walls, straight or convex external and internal walls, (12.5-30.0) µm. the mesophyll of dense homogenous cells, oblong, circular, semi-circular, irregular, with different sizes, little intercellular spaces, (200-250) µm, vascular bundles (8-11), one is the midrib, semi-circular in shape, (87.5-100.0) µm.

A cross section of the middle of a flowering stem has been taken to be the material of the stem anatomy. The epidermis was a single continuous layer of elongate, circular, semi-circular or irregular cells, having different sizes, straight or convex walls; the thickness of the epidermis depends on the differences in the cell sizes, (10-20) µm. The cuticle layer was (2.5-7.5) µm.

The cortex consists of numerous layers of parenchymal tissue, the cells of different shapes and sizes, oblong, circular, semi-circular or irregular, (900-1000) µm. The vascular tissue as a ring, of xylem and phloem, xylem represented by vessels, phloem more developed than xylem, a number of vascular bundles present in the cortex, different in sizes, represented by vessels, (125-225) µm. The pith consists of parenchymal cells, polygonal, different sizes, (4-8 faces), with intercellular space, (2500-2700) µm. (Plate 5) .

The current work studied the new plant record *O. armena* from Orobanchaceae family in Iraq, and included some aspects like the morphological characters, pollen grains, leaf with stem anatomy and the environment. Within the literature review about the genus *Orobanche* in Iraq, involving the plant specimens of National Herbarium of Iraq (BAG), College of Science Herbarium, University of Salahaddin-Erbil, Iraq (ARB) and College of Education Herbarium, University of Salahaddin-Erbil, Iraq (ESUH), the study did not find any specimens belong to *O. armena*, therefore the study regarded the studied plant specimens as a new record for the Flora of Iraq from Qandil mountain.

O. armena has some characters differ from the related species which is *O. kurdica* Boiss. & Hausskn. that present in Iraq where *O. armena* has smaller corolla and connated halves of calyx at the base (Gilli, 1982), as well as, the other characters.

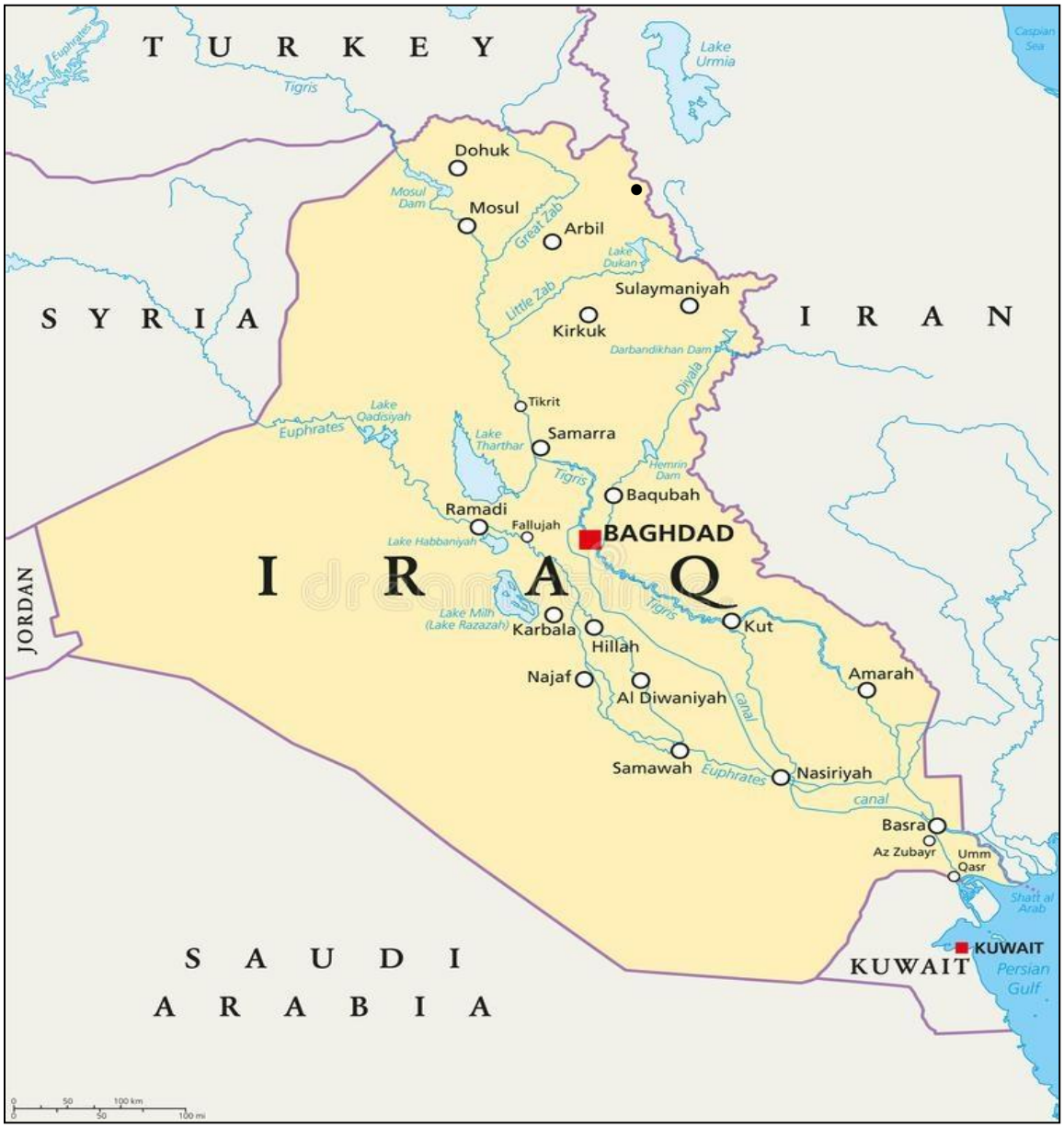


Fig (1): A map of Iraq shows the location of *O. armena* ●

Photographs of *O. armena*

Cauline leaves

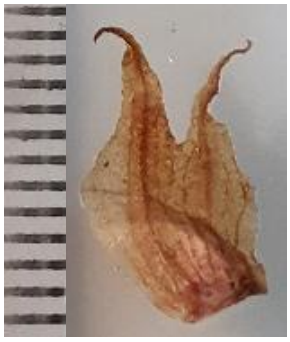
Inflorescence

Bract

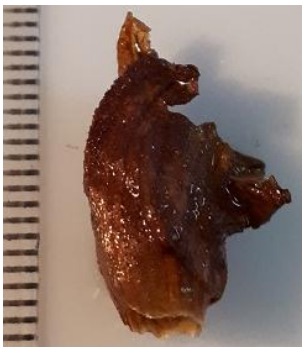
5 mm

Plate (1): *O. armena* with different plant parts

Calyx upper half

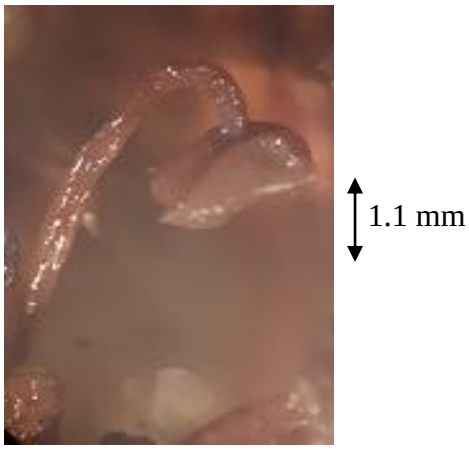


Calyx lower half

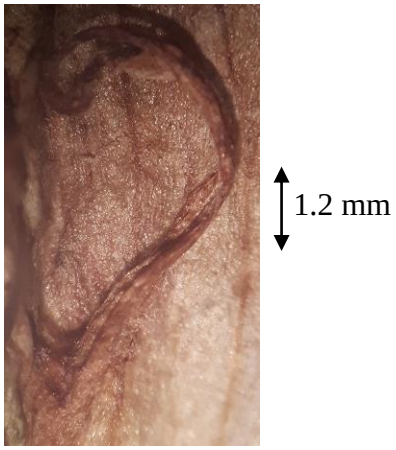


Corolla

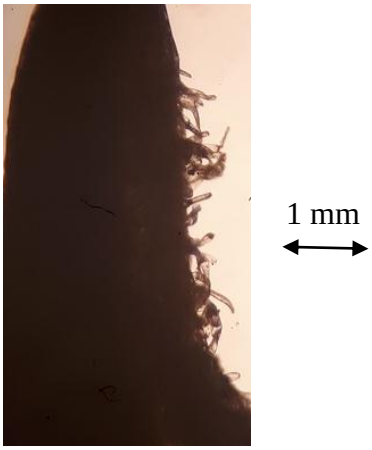
Emarginate upper lip apex



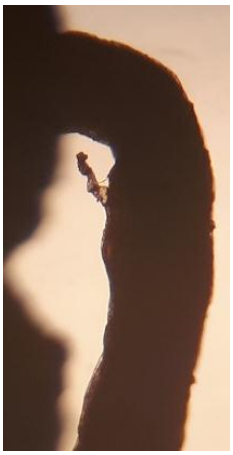
Stamen



Stamen' s insertion position

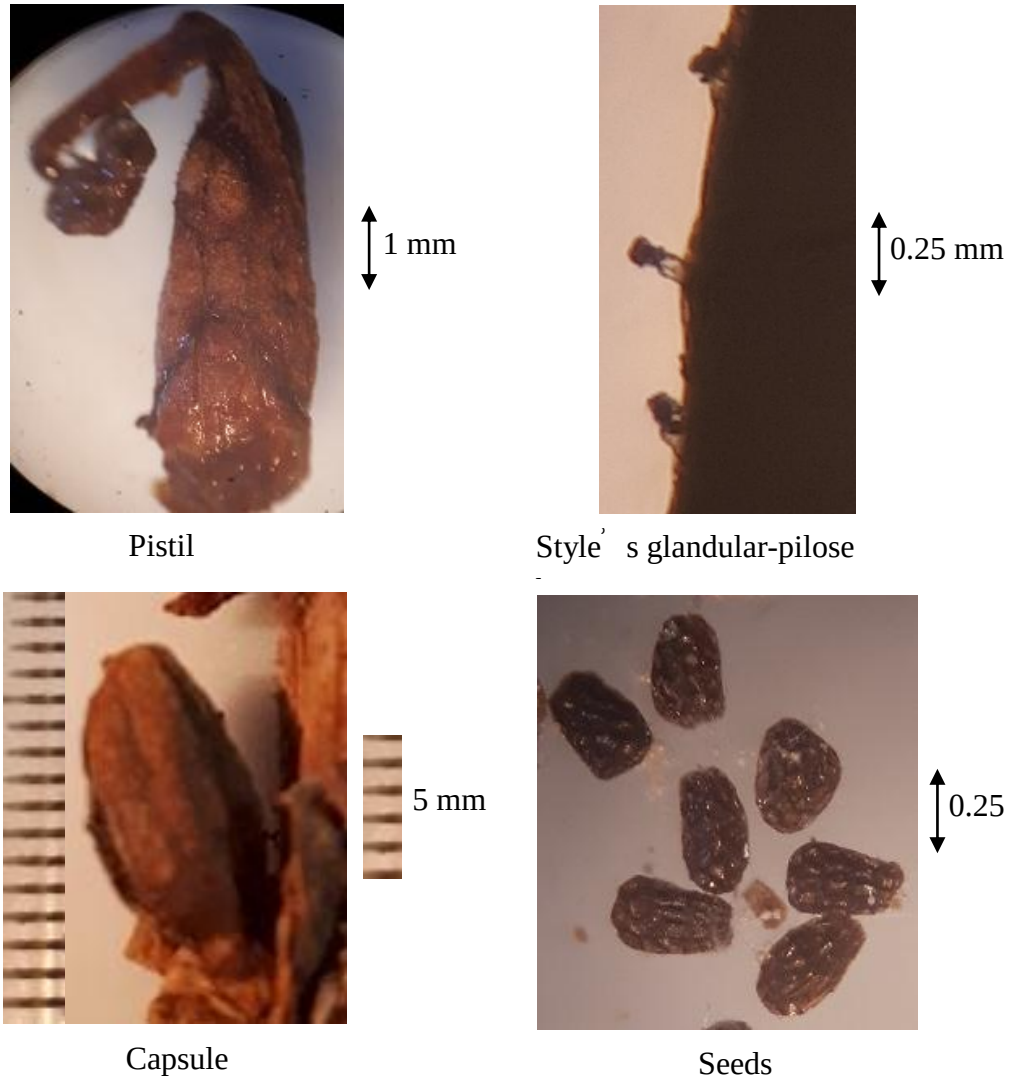


Filament' s densely pilose hairs



Filament' s sparingly glandular hairs

Plate (2): Reproductive parts of *O. armena*



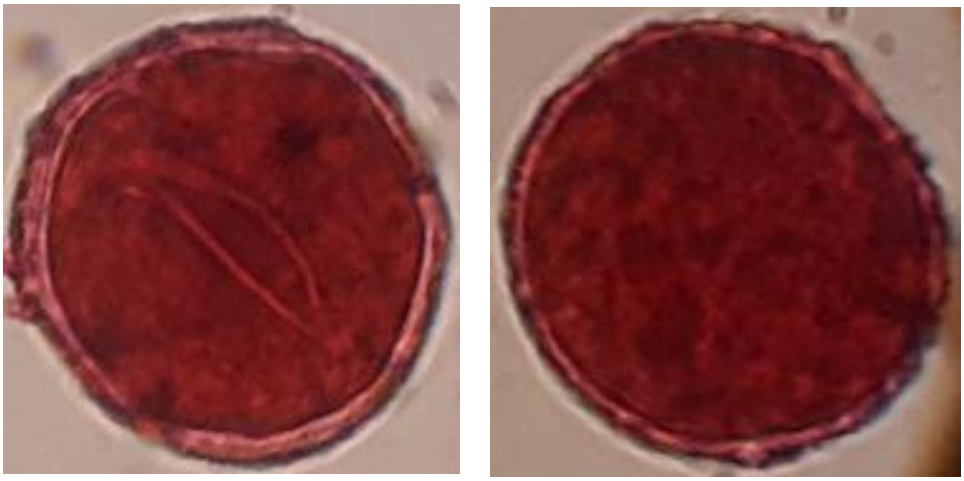
Pistil

Style's glandular-pilose

Capsule

Seeds

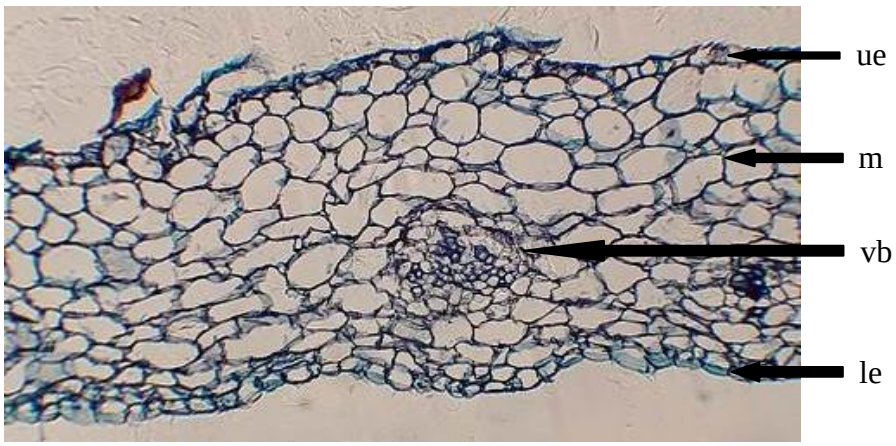
Plate (3): Reproductive parts of *O. armena*



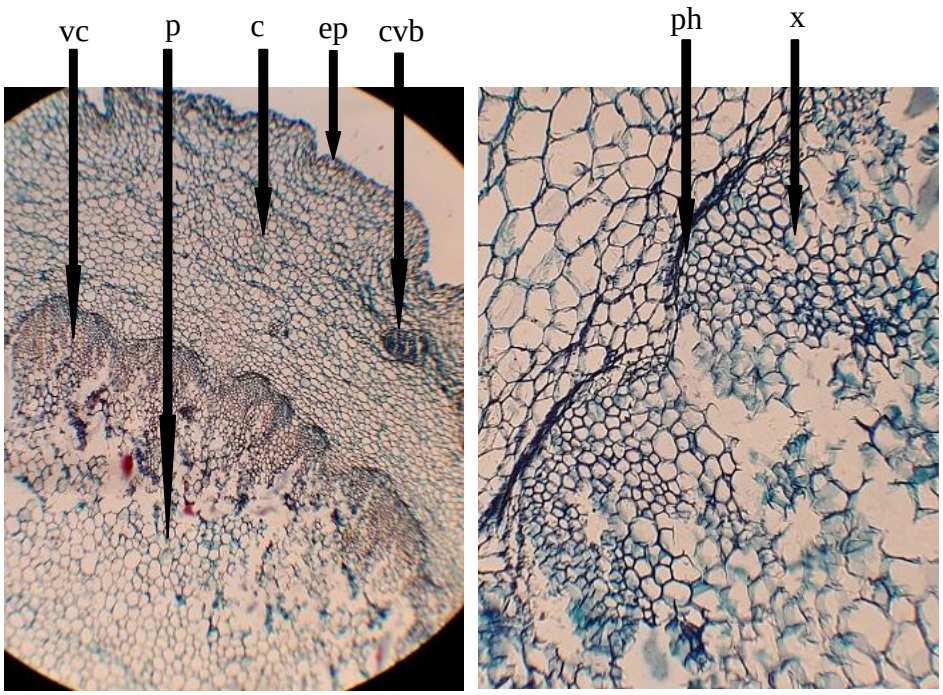
Equatorial view

Polar view

Plate (4): Pollens of *O. armena* X100



Part of Leaf X100



Part of stem X40 Enlarged part of stem X100

Plate (5): C.S. of the leaf and stem of *O. armena*: ue: upper epidermis; m: mesophyll; vb: vascular bundle; vc: vascular cylinder; le: lower epidermis; p: pith; co: cortex; ep: epidermis; cvb: cortical vascular bundle; ph: phloem; x: xylem

4. CONCLUSIONS

The present study verified the presence of the species *O. armena* from Orobanchaceae family as a new record for the Flora of Iraq which collected from Qandil mountain (North-East of Erbil), in addition, morphological, palynological and anatomical studies have been conducted for the species under study.

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Comparing Halton and Sobol Sequences in Integral Evaluation

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ABSTRACT

Halton and Sobol sequences are two of the most popular number sets used in quasi-Monte Carlo methods. These sequences are effectively used instead of pseudo random numbers in the evaluation of integrals. In this paper, the two sequences are compared in terms of the size of the number sets and dimensionality. The comparison is implemented with matlab programming for evaluating numerical integrals. The absolute error, which is the absolute difference between the exact and estimated errors, is plotted against dimensions for different functions. The practical results show that, except the first dimension, Sobol sequence is better than Halton sequence. The results also show that Sobol sequence outputs are more stable.

1. INTRODUCTION

Monte Carlo (MC) method is a numerical method for solving mathematical problems by the simulation of random numbers. The most common applications of MC method are the evaluation of integrals, mathematical finance, tree search, and simulation in several branches of science (Landau & Binder 2005) (Levy 2010).

In numerical integral application, integrals are sometimes not solved analytically. Whereas some numerical approximations can be obtained for such integrals using MC methods. The MC methods are based on computer generation sequences of pseudo-random numbers. These sequences behave as if they are truly random although they are produced by deterministic algorithms. The convergence rate of approximating an integral with MC methods

using a set of random samples of size n is $O(n^{-1/2})$ (Serre 2010).

Quasi-Monte Carlo (QMC) methods, also called low-discrepancy methods, use algorithms that reduce the discrepancy of generated random numbers. The low-discrepancy sequences cover the space better than pseudo-random sequences by reducing gaps between the points. In computer programming, the generated sequences are matrices of size n-by-s, where n is the number of points and s is the dimension of the hypercube being sampled. In Figure 1, dimension 1 is plotted against dimension 2 for both MC and QMC to show the discrepancy of generated random sequences. Quasi-random numbers appear to cover the area more uniformly than a pseudo-random numbers. The advantage of using low-discrepancy sequences is a faster rate of convergence, it achieves a convergence rate of $O(n^{-1}(\log n)^s)$ (Serre 2010). There are several high-dimensional sequences for use in QMC: Halton, Faure, and Sobol sequences. Other important sequences are Niederreiter and generalized Faure sequences (Krykova 2003).

There are several publications that compare MC and QMC methods (Owen 2008). QMC method have been successfully used for multivariate integration of high dimensions, and were significantly more efficient than MC method (Sloan & Woźniakowski 1998) (Kuo & Sloan 2013). One disadvantage of QMC integration is that its error is difficult to be estimated, unlike MC integration (Tuffin 2008) (Jank 2005). Also it is stated that the problem of QMC methods is that their convergence is not independent of dimensionality (Frey 2008). Therefore, the accuracy of high-dimensional computations will not be guaranteed to be better than the MC. Lemieux mentioned within the drawbacks of QMC that the dimension needs to be small and the number of elements needs to be large in order to enhance the efficiency of QMC over the regular MC (Lemieux 2009). Morokoff and Caflisch remark that the advantage of the QMC method is greater if the integrand is smooth, and the number of dimensions s of the integral is small (Morokoff & Caflisch 1995).

The aim of this paper is to implement a practical comparison between two of the most popular sequences used in QMC methods, Halton and Sobol sequences. The comparison concentrates on the effect of the size of random number sets and the number of dimensions on the accuracy of the integral evaluation. The approach is to use the two matlab functions, *haltonset* and *sobolset*, with some of more general parameters for generating the two sequences and apply them on the simulation of mathematical integrals. To show the contribution of the dimensions in the error of the numerical integration, 60 dimensions are used separately in evaluating one-dimensional integral functions. The integral is applied on

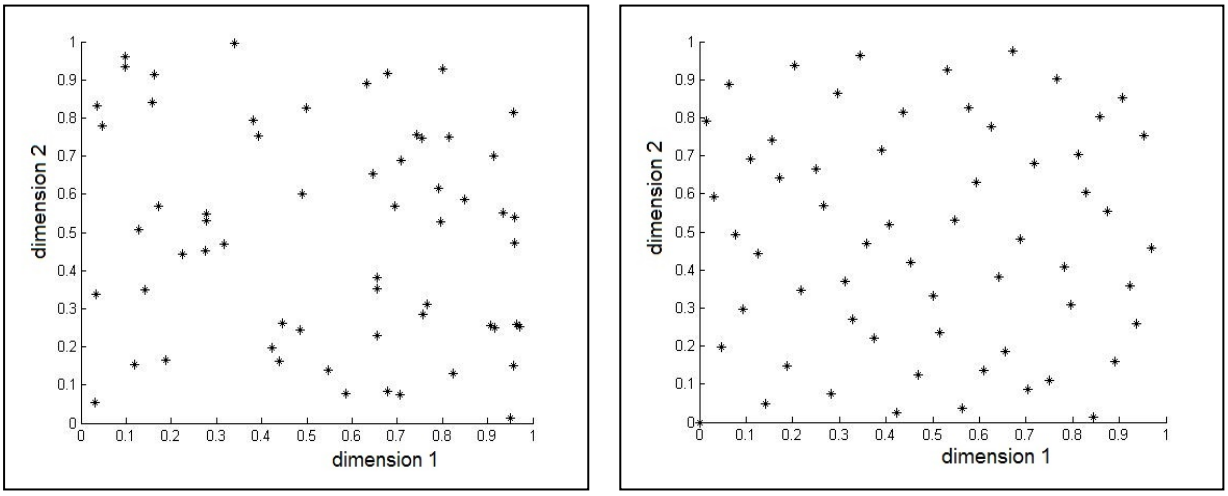

Figure 1: Two dimensional plot of pseudo-random numbers (left) and quasi-random numbers (right)

two different functions and the absolute errors are depicted to be investigated visually.

The rest of the paper is organized as follows. Section 2, and section 3 give the mathematical background for Halton and Sobol sequences. Section 4 explains the method of integral evaluation using sample technique and shows the error calculations. Section 5 presents the algorithms and programs used to achieve the goal of this paper. Section 6 presents the results and discusses the outputs of the programs. Section 6 concludes the paper.

2. HALTON SEQUENCES

The theoretical frameworks of Halton and Sobol sequences are presented in several references. The interested reader can cosult (Caflisch 1998), (Dalal et al. 2008), and (McLeish 2011). The following short description is based on (Veach 1997). Halton low-discrepancy sequences are inspired by Van der Corput sequence introduced originally for one dimension and using the binary number system. The main contribution of Van der Corput in low-discrepancy sequences is that the coefficients of the digit expansion of an increasing integer k in base b can be used to define a low-discrepancy sequence. The Halton sequence is a generalization of the one-dimensional Van der Corput sequence to higher dimensions. Each dimension is represented in a different prime base b (e.g., 2, 3, 5, 7, . . .). In one dimension, the radical inverse sequence $x_i = \emptyset_b(i)$ is obtained by first writing the base-b expansion of k:

$$k = \sum_{i=0}^{\infty} a_i b^i,$$

where $a_i \in \{0, \dots, b-1\}$. Reflecting the digits around the decimal point gives:

$$\emptyset_b(i) = \sum_{i=0}^{\infty} a_i b^{-i-1}$$

The special case when $b = 2$ is the sequence:

$$\frac{1}{2}, \frac{1}{4}, \frac{3}{4}, \frac{1}{8}, \frac{5}{8}, \frac{3}{8}, \dots$$

To obtain a low-discrepancy sequence in several dimensions, we use a different radical inverse sequence in each dimension:

$$x_i = (\emptyset_{b_1}(i), \emptyset_{b_2}(i), \dots, \emptyset_{b_s}(i)),$$

where the bases b_i are all relatively prime. To get Halton sequence, b_i are chosen to be the first s primes:

$$x_i = (\emptyset_2(i), \emptyset_3(i), \emptyset_5(i), \dots, \emptyset_{p_s}(i))$$

Even though standard Halton sequences perform very well in low dimensions, correlation problems have been noted between sequences generated from higher primes.

3. SOBOL SEQUENCES

Sobol sequences (Sobol' 1967) also inspired by Van der Corput sequence with different approach. Here, we give a short description of generating Sobol sequences based on (Dalal et al. 2008) and (Bratley & Fox 1988). To generate one sequence (i.e., one dimension) of N-bit low-discrepancy Sobol numbers, we choose positive odd integers m_i, where $i=0, 1, \dots, N-1$ and define N direction vectors c_i:

$$c_i = \frac{m_i}{2^i} = 0.c_{i1}c_{i2}c_{i3}\cdots$$

where c_{ij} denote the binary expansion of c_i. Now, construct a primitive polynomial of degree d with coefficients a_i chosen from {0, 1}:

$$p(x) = x^d + a_1 x^{d-1} + \cdots + a_{d-1}x + 1$$

These coefficients a_i are used to calculate each direction vector c_i as:

$$c_i = a_1 c_{i-1} \oplus a_2 c_{i-2} \oplus a_1 c_{i-1} \cdots \oplus a_d c_{i-d+1} \oplus c_{i-d} \oplus [c_{i-d} \gg d]$$

where $\oplus$ is bitwise exclusive-or (XOR), and the last term is c_{i-d} right-shifted by d bits.

A one-dimensional *N*-bit wide Sobol sequence $x_1, x_2, x_3, \ldots$ can be generated based on this set of direction vectors. The n^{th} term of this sequence is generated with $n = b_N b_{N-1} \ldots b_2 b_1$ in binary. Then,

$$x_n = b_1 c_1 \oplus b_2 c_2 \oplus \cdots \oplus b_{n-1} c_{N-1} \oplus b_N c_N$$

To generalize this procedure to *s* dimensions, it is sufficient to choose any *s* different primitive polynomials and calculate *s* different sets of direction vectors as explained above, and then generate each x_n using the corresponding set of direction vectors.

4. INTEGRAL EVALUATION

Monte Carlo approximation transforms the problem of integration into numerical method by calculating the average of the functions over random numbers. This is one of the MC methods for evaluating integrals which is called sample-mean method. To find the integral of a function over an *s*-dimensional unit cube, this method approximates the solution to the average of the function at a randomly set of points $x_1, \ldots, x_n$:

$$\int_{[0,1]^s} f(u)du \approx \frac{1}{n}\sum_{i=1}^{n} f(x_i),$$

where each x_i is a vector of *s* elements. In matlab, the QMC functions, *haltonset* and *sobolset*, can be used to produce quasi random sequences in the form of *n*-by-*s* matrices, where *n* is the number of points in each dimension *s* of the hypercube being sampled. Many trials are implemented for testing these functions with different integrals and different sets of *n* and *s*. To explain the results, the integrals are evaluated with *n*=10000 and *n*=100000 with each value of *s*, where *s*=1, 2, …, 60. The functions which are used for the following explanations are:

$$f(x) = \sin(2x) \qquad (1)$$

$$f(x) = e^{-x} \qquad (2)$$

The general form of the integral is

$$I = \int_a^b f(x)\,dx$$

With sample-mean method, the *n* random numbers are taken from $x \in [0,1]$ and the integral is estimated as

$$I_{est} = (b-a)\frac{1}{n}\sum_1^n f(x_i)$$

If the interval of integration is taken between a=0 and b=1, the integral is simplified to

$$I_{est} = \frac{1}{n}\sum_1^n f(x_i)$$

To illustrate the results of evaluating the integrals, matlab programming is used to simulate the MC sample-mean method. The two functions, *haltonset* and *sobolset*, are used to generate 60 dimensions used to evaluate the one-dimensional integrals of functions (1) and (2) in the interval [0, 1]. With each dimension, the one-dimensional integral is evaluated and the absolute error is found for the sake of accuracy comparison. The absolute error is the absolute difference between the exact solution of the integral I_{exc}, and I_{est}. The integral evaluation is implemented for *n*=10000 and *n*=100000 to show the effect of changing the size of random number sets on the results of the integral evaluation.

The mean absolute error (MAE) is used to illustrate the effect of increasing the number of samples used for evaluating the integrals on decreasing the error of evaluation. The mean absolute error is the average of the absolute differences between the exact and estimated integrals. It is given by:

$$MAE = \frac{1}{n}\sum_1^n |I_{est} - I_{exc}|$$

5. ALGORITHMS AND PROGRAMS

Algorithm 1 shows a general method for estimating the absolute error for the function f(x) in the interval [a, b] for any of Halton or Sobol sequences with different values of dimensions. Program 1 uses the a matlab program to plot the relation between the absolute error and the dimensions for the function *sin(2x)* in the interval [0, 1]. Algorithm 2 lists the steps of calculating MAE for different values of samples for a selected dimension. Program 2 applies the algorithm to plot the relation between the number of samples and the MAE for the function *exp(-x)* in the interval [0, 1]

Alogorithm 1: Absolute error for interval [a,b]

1- SET n to the number of samples
2- SET s to number of dimensions
3- SET I_{exc} to the exact solution of the integral
4- Use haltonset or sobolset to generate n×s matrix
5- FOR dim=1 to s
    FOR i=1 to n
        y[i]=f(x_i)
    END FOR
    I_{est}=(b-a)*AVERAGE(y)
    Error[dim]=| I_{est} - I_{exc}|
6- END FOR

Program 1: Plotting absolute error vs dimensions for interval=[0,1]

```
% function y=sin(2x)
% a=0, b=1, therefore interval is ignored
exact=0.70807;
dim=60;          % for 60 dimensions
n=100000;        % number of samples
rng default
h=haltonset(dim);
halt=net(h, n);
s=sobolset(dim);
sobol=net(s, n);
for d=1:dim
xHalton=halt(:, d); % get x vector
yHalton=sin(2*xHalton); % find f(x)
    xSobol=sobol(:, d);% get x vector
    ySobol=sin(2*xSobol); % find f(x)
    yyHalton(d)=mean(yHalton);
    yySobol(d)=mean(ySobol);
end
errorHalton=abs(yyHalton-exact); % absolute
                % error for Halton sequence
errorSobol=abs(yySobol-exact); % absolute
                % error for Sobol sequence
plot(1:dim, errorHalton, ':.k','linewidth',1)
hold all
plot(1:dim, errorSobol, '-k','linewidth',1)
axis([0 dim 0 0.0004])
grid ON
xlabel('Dimension')
ylabel('Absolute Error')
```

Alogorithm 2: Mean absolute error for interval [a,b]

1- SET s to a selected dimension
2- SET I_{exc} to the exact solution of the integral
3- SET k=1
4- FOR n=10000 to 100000, steps of 10000
    Use haltonset or sobolset to generate n×s matrix
    FOR d=1 to s
        FOR i=1 to n
            y[i]=f(x_i)
        END FOR
        yy[d]=AVERAGE(y)
    END FOR
    I_{est}=(b-a)*AVERAGE(yy)
    Error[k]=| I_{est} - I_{exc}|
    k=k+1
5- END FOR

Program 2: Plotting MAE vs number of samples for interval [0,1]

```
% function y=exp(-x)
% a=0, b=1, therefore interval is ignored
exact=0.63212;  % exact solution of the function
dim=3;  % dimension=3
rng default
for n=10000:10000:100000  % samples
    h=haltonset(dim);
    halt=net(h, n);
    s=sobolset(dim);
    sobol=net(s, n);
    for d=1:dim
        xHalton=halt(:, d);   % get x vector
        xSobol=sobol(:, d);  % get x vector
        yHalton=exp(-xHalton);  % find f(x)
        ySobol=exp(-xSobol);  % find f(x)
        yyHalton(d)=mean(yHalton);
        yySobol(d)=mean(ySobol);
  end
```

```
maeHalton(n/10000)=mean(abs(yyHalton-
exact));  % MAE for Halton sequence
maeSobol(n/10000)=mean(abs(yySobol-exact));
        % MAE for Sobol sequence
 end
 plot(1:10,maeHalton, ':k', 'linewidth', 2)
 hold all
 plot(1:10, maeSobol, '-r', 'linewidth', 1)
 axis([1 10 0 0.001])
 grid ON
 xlabel('Nx1000')
 ylabel('MAE')
```

6. RESULTS AND DISCUSSION

The results of evaluating integrals of functions (1) and (2) are shown in Figure 2 and Figure 3 respectively. The figures show the plot of absolute error of estimated integrals using Halton and Sobol sequences as a function to the dimension of the sequences.

Visual inspection of the outputs shows that the absolute errors are same when s=1 for the two sequences. For s>1, the performance of Sobol sequence is better than that of Halton sequence for all the values of the dimensions. The outputs show that the average of the absolute error increases with the increasing of the dimension for Halton sequence, while it is stable with Sobol sequence. The outputs also show that when n is changed from 10000 to 100000, the absolute error is decreased in both sequences.

The number of samples used for the evaluation affects the accuracy of integral. To show that, Program 2, listed in the previous section, is executed with different values of samples n. The values of n are taken as 10000,

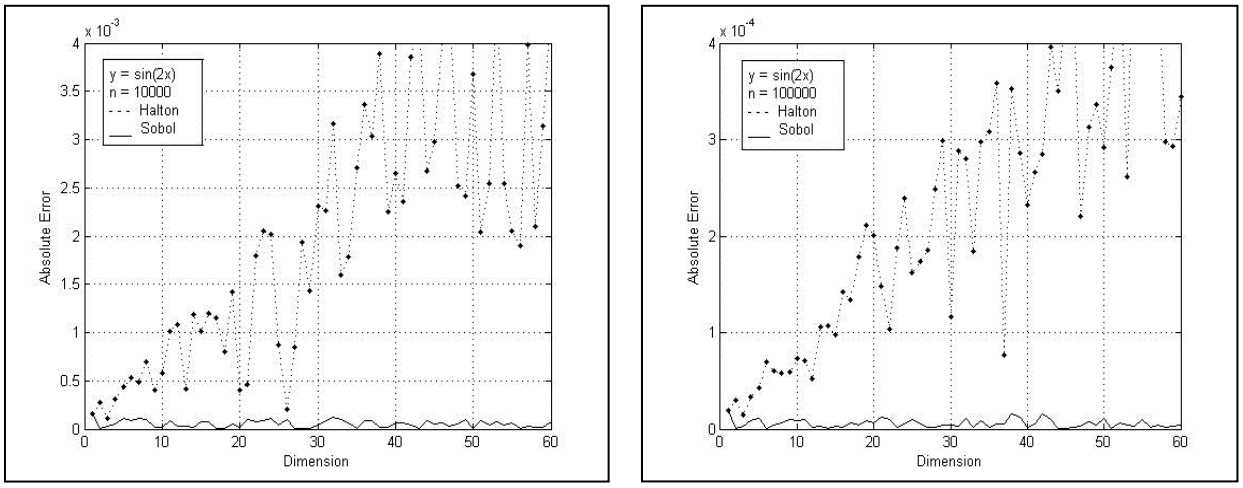

Figure 2: Absolute error of integral (1) with n=10000 (left) and n=100000 (right)

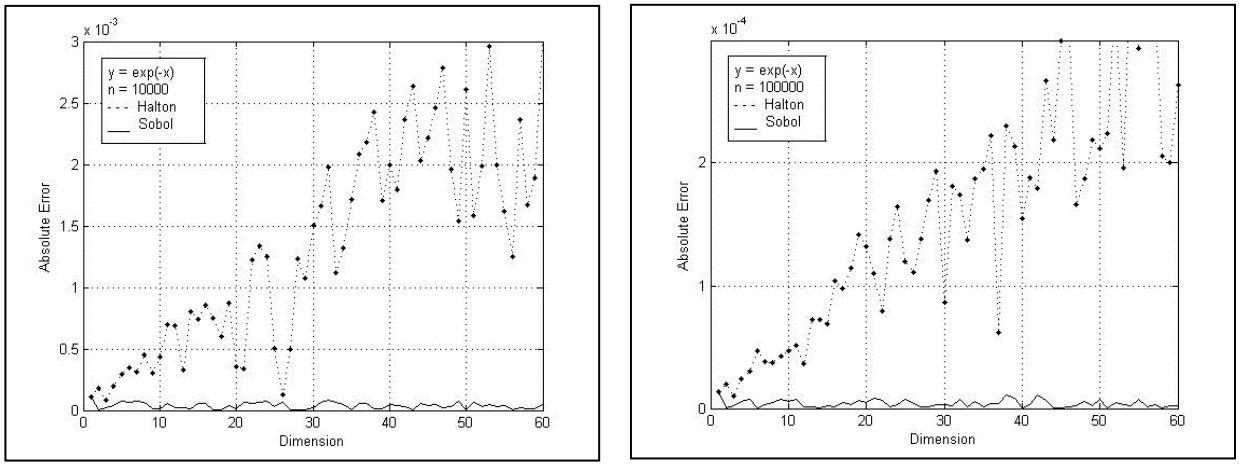

Figure 3: Absolute error of integral (2) with n=10000 (left) and n=100000 (right)

20000, …, 100000 with two values of the dimension, 3 and 60. These values are sufficient for revealing the effect of increasing n on the error with small and large values of the dimension. Figure 4 is the output of the program for integral (2) and it shows that the absolute error is decreased in both sequences with increasing n. The figure also shows that when d=3, the difference of MAE in the two sequences is much less than that when d is increased to 60. The MAE of Halton sequence produces very bad performance with large values of d and low values of n.

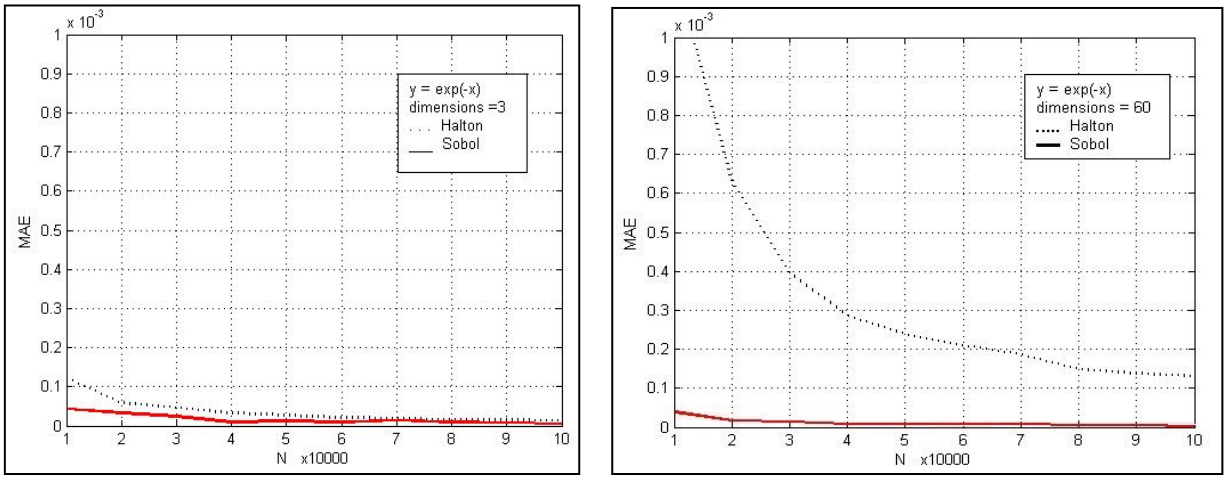

Figure 4: Mean absolute error for d=3 (left) and d=60 (right)

To find the reasons of the difference in accuracy of the results, it is important to see how the discrepancy is changed with changing dimension values in both sequences. To do so, the sequence numbers of several pairs of dimensions are plotted and investigated. All the testing of outputs show that Sobol sequence fill the space better than Halton sequence, leading to improve the results of integral estimation. Figure 5 shows examples of the relation between pair of dimensions of the two sequences. In high dimensions, Halton sequence tends to be uniform in diagonal arrangement leaving large spaces in the square unit. This bad distribution will increase the error in estimating the value of the integral. Sobol sequences maintains good distribution of the random number on all dimensions.

It is worth mentioning that there are other

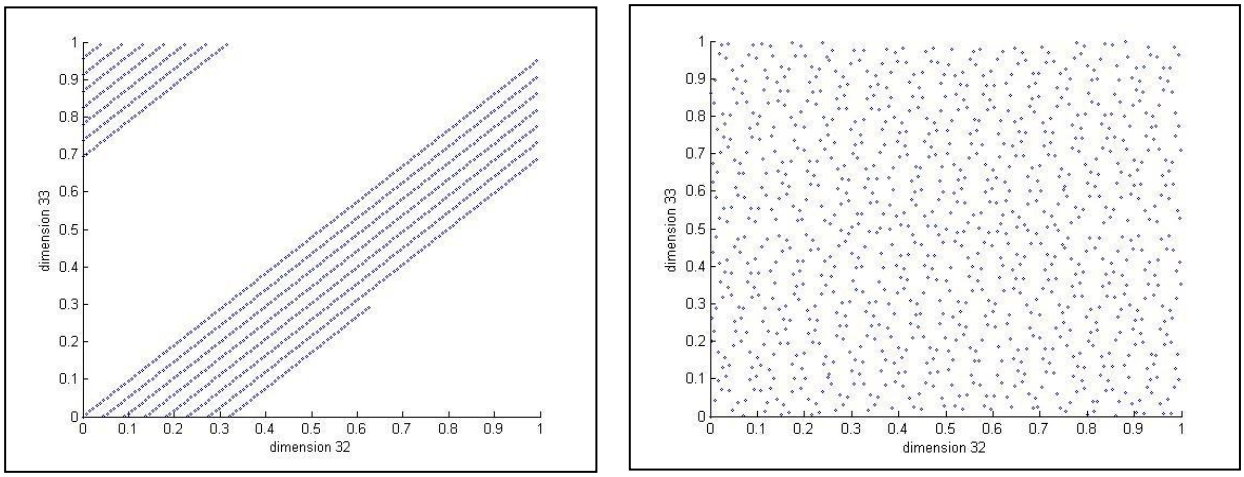

Figure 5: Discrepancy plot of Halton sequences (left) and Sobol sequences (right)

parameters, such as '*leap'* and '*skip'*, which can be used with *haltonset* and *sobolset* in matlab to change the properties of the produced sets of the numbers (Kocis & Whiten 1997). These parameters are not used in this work.

7. CONCLUSIONS

Halton and Sobol sets are used in matlab for the generation of quasi-Monte Carlo sequences. These sequences are compared with different size of set numbers and different dimensions. The comparison is based on evaluating numerical integrals for one-dimensional functions by matlab programming. The results show that the performance of Sobol sequence is better and more stable than Halton sequence. The results also show that Sobol sequence maintains the feature of low-discrepancy while this feature is deteriorated with Halton sequence when the dimension value is increased. These differences in discrepancy affect the results of integral evaluations by increasing the absolute error with increasing d of Halton sequence. In the future work, the properties that change the sequences, such as *'skip'* and *'leap'*, which are available in matlab, can be used for further analysis.

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Effect of occupational exposure to chemicals on some biochemical parameters

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ABSTRACT

Chemical and biological hazards may have serious implications on the exposed individuals and a wealth of quality-ensured guidance and information for health care workers is available on the internet. Harmful chemicals can get into the body if you breathe, eat, or drink them or if they are absorbed through the skin. Study group was 20-person Exposure to Occupational Chemicals in chemistry department at Koya university for more than 5 years. In addition, the study includes 20 subject as control group in this study. The blood was drawn into test tubes to estimation of lipid profile, liver parameters, kidney function tests and heavy metal. Full automatic chemical analyzer (Cobas C311) was used to determine the biochemical parameters and atomic absorption was used to estimate the heavy metals. The results showed significant decrease in ALT, AST, albumin and triglyceride, and non-significant difference in total protein, total bilirubin, cholesterol, urea, creatinine, uric acid, Hg and Pb in case group as compared to control group.

1. INTRODUCTION

Chemical and biological hazards may have serious complications for the individuals exposed and a wealth of quality-ensured guidance and information for health care workers is available on the internet. These hazards may affect both physical and psychosocial health (Cox, et al., 2000). Although some chemical exposures are safe, others are not. Harmful chemicals can get into the body during breathe, eat, or drink them or if they are absorbed through the skin. Liver is the main organ responsible for drugs and toxic chemicals metabolism, so it is the primary target organ for many organic solvents. Some studies suggest that organic solvents can lead to liver toxicity. Presently, organic solvents are widely used in different chemical laboratory and industries including printing, paint and adhesives industries, rubber production, photographic films and toy manufacturing (Fiedler and Lerman, 2007). Some occupational liver diseases can represent with acute symptoms such as exposure to carbon tetrachloride and chloroform that lead to severe liver toxicity, but others can reveal chronic manifestations such as prolonged exposure to aromatic and aliphatic solvents which can result in mild liver toxicity (Ladou, and Harrison., 2014; Rosenstock, et al., 2005).

In the study of Malaguarnera, et al., some solvents including dimethylformamide (DMF), trichloroethylene (TCE), toluene, xylene, carbon tetrachloride, and chloroform were proposed as factors involved in hepatotoxicity (Malaguarnera et al., 2012). Environmental exposure to chemical substances is an important risk factor that effect on lipid metabolism in human's body (Santos-Gallego and Jialal, 2016). Lead is divalent metal is a ubiquitous pollutant of the eco-system. It find naturally in the earth crust as inorganic or organic compound (World Health Organization, 2001). It is soft malleable and grey in color. Lead has been persistently used for various purposes because of its peculiar chemical properties coupled with its poor ability to conduct heat and electricity (Xinteras, 1992). Lead is used in water pipes, cosmetic industries and as anti-knock in petroleum (Goyer, 1993). The metal enters the environment through soil, food, water and air (Enuneku, 2010; Haji and Amir 2012). Lead poisoning is a global health problem but it is unrecognized as such in a number of African countries. Lead poisoning as indicated by elevated blood lead levels have been observed in the general population in some parts of Nigeria (Santos-Gallego and Jialal, 2016; Xinteras, 1992). In adults, occupational exposure to lead is the most common cause of lead poisoning. This has been associated with adverse effect on the kidney, cardiovascular, haemopoietic and hepatic systems in humans (Onyeneke and Omokaro, 2016; Bartemaeus and Jacobs, 2002; Paul, et al., 1999). The liver is a vital organ in the human body which is involved in detoxification and excretion of and product of metabolism glycogenesis etc. Occupational exposure to lead has been associated with abnormal liver function. There is therefore need to assess the blood lead levels in occupationally exposed individuals and also to evaluate the effect of such blood lead levels on the liver.

2. MATERIALS AND METHODS

2.1. Study subjects

Study group was included 20-person Exposure to occupational chemicals in chemistry department at Koya university for more than 5 years. In addition, the study included 20 subject as control group in this study. The blood was drawn into test tubes for estimation lipid profile, liver parameters, kidney function tests and heavy metals. Full automatic chemical analyzer (Cobas C311) was used to determine the biochemical parameters while flame atomic absorption was used to estimate the heavy metals.

2.2. Statistical analysis

The results express as mean ± S.E.M (stander error of mean). The results obtained were analyzed using student "t" test. Probably level of P value ($P<0.05$) was considered as statistically significant.

3. RESULTS AND DISCUSSION

Chemical occupational workers are regularly exposed to many hazardous toxins and noxious vapors. Which they can cause abnormal alterations in the functioning of many vital organs (Ali Khan et al., 2013).

The current study was conduct at Koya university, Kurdistan Region which include chemistry teaching staff that are worked in chemistry laboratory 2 days per week at least 4 hours per day.

Table 1, show the results obtained in this study.

Table 1: biochemical parameters and heavy metal in case and control group

Parameters	Control group	Case group	P value
ALT (U/L)	23.41±0.532	20.08±1.330	0.028
AST (U/L)	29.75±0.866	24.50±1.308	0.001
Albumin (g/dl)	4.569±0.101	3.813±0.133	0.001
Total protein (g/dl)	7.429±0.134	7.489±0.222	0.073
Total bilirubin (mg/dl)	0.554±0.035	0.588±0.045	0.561
Cholesterol (mg/dl)	140.6±12.12	151.8±6.051	0.124
Triglyceride (mg/dl)	116.5±5.852	94.40±8.438	0.043
Creatinine (mg/dl)	0.657±0.024	0.698±0.025	0.273
Urea (mg/dl)	28.47±4.383	32.62±5.342	0.090
Uric acid (mg/dl)	4.450±0.178	4.735±0.324	0.422
Hg (mg/l)	0.043±0.004	0.040±0.004	0.081
Pb (mg/l)	0.014±0.001	0.014±0.001	0.804

Results expressed as Mean ±SEM

The table 1 show that subjects who were occupationally exposed to chemical substance showed that significant decrease in serum ALT and AST (20.08±1.330 U/L and 24.50±1.308 U/L respectively) as compared to its level in control group (23.41±0.532 U/L and 29.75±0.866 U/L respectively) as in (figure 1 and 2), while a significant decrease were found in mean level of Albumin (3.813±0.133 mg/dl) as compared to control (4.569±0.101 mg/dl), and non-significant difference in total protein and total bilirubin (7.489±0.222 mg/dl and 0.588±0.045 mg/dl respectively) as compared to its level in control group (7.429±0.134 mg/dl and 0.554±0.035 mg/dl respectively) as shown in figure (3, 4 and 5).

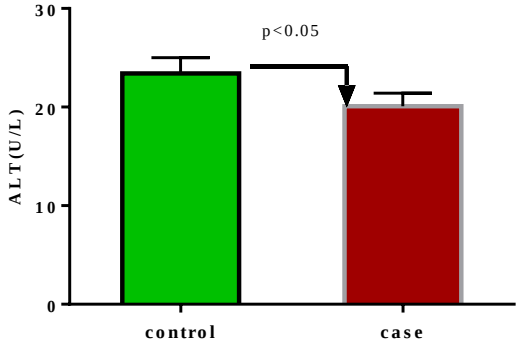

Figure (1): ALT level in control and case group.

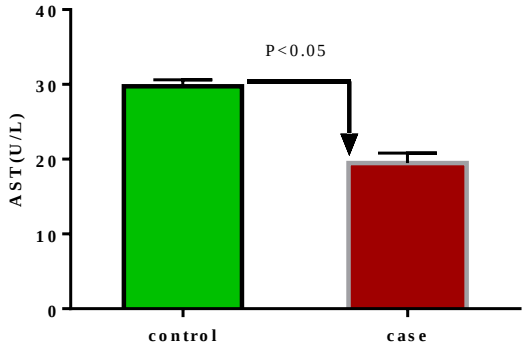

Figure (2): AST level in control and case group.

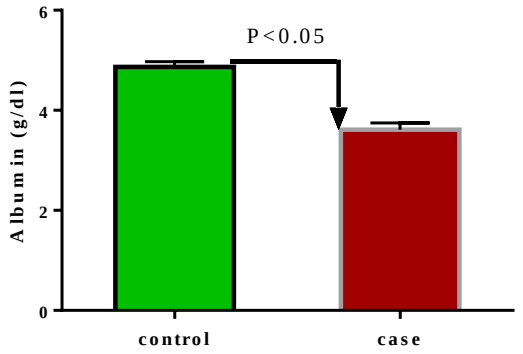

Figure (3): Albumin level in control and case group.

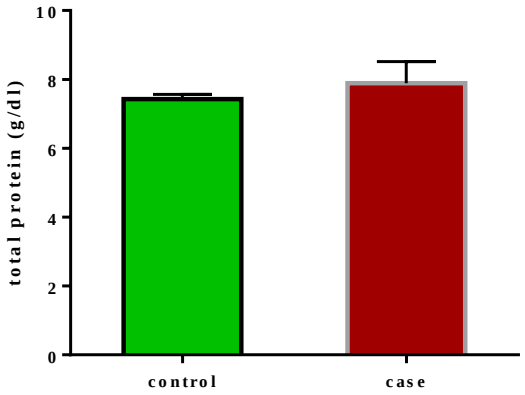

Figure (4): total protein level in control and case group.

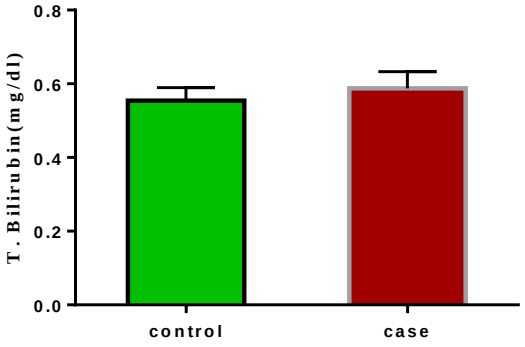

Figure (5): total Bilirubin level in control and case group.

There was significant increase in blood triglyceride levels of study group (94.40±8.438) as compared to control (116.5±5.852) as shown in (figure 6), and non-significant difference in cholesterol in case group (151.8±6.051) as compared to control group (140.6±12.12) as shown in (figure 7). The same results were found by Dere et al (Dere, et al., 2003).

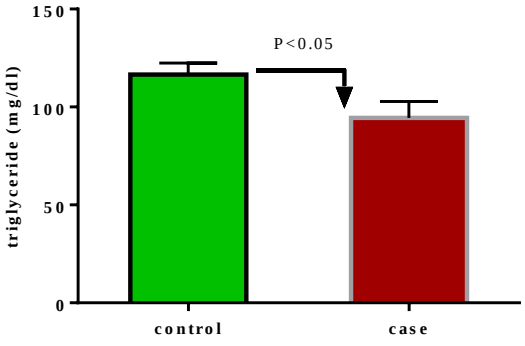

Figure (6): triglyceride level in control and case group.

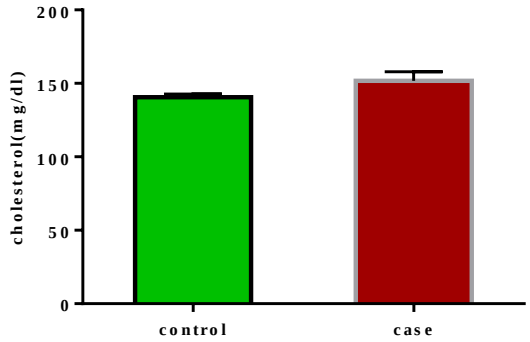

Figure (7): cholesterol level in control and case group.

Serum creatinine was within the normal ranges and had no significant differences in case group (0.698±0.025) with those of controls (0.657±0.024) as in figure 8. Serum levels of urea (32.62±5.342 mg/dl) was shown to be non-significantly elevated in case group, more than comparison group (28.47±4.383 mg/dl), though these values are still within the accepted normal ranges as in shown in (figure 9).

In the present study serum uric acid (4.735±0.324 mg/dl) was non-significantly higher among the workers as compared to control (4.450±0.178 mg/dl) as shown in figure 10. This finding is consistent with other which may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either over production or inability of excretion (Abdel Aziz, et al., 2006). Whereas, serum creatinine (0.698±0.025 mg/dl) was within the normal ranges and had no significant differences with those of controls (0.657±0.024 mg/dl), this results are accepted with previous study (Abou-ElWafa, et al., 2015).

Most of chemical substances are toxic to many organ systems including the kidney (W. Qin, et al., 2012), which may be attributed to an increase in liberating toxic metabolites including reactive oxygen species. While experiments with rats indicate that exposure by

inhalation to the aromatic hydrocarbons toluene, styrene, and xylene was nephrotoxic (Rankin, et al., 2008), this effect has not been confirmed in man (Melnick, 1992). Both human and experimental studies suggest that many chemicals can affect the kidney (Pfaller and Gstraunthaler, 1998). The results obtained show that occupationally exposed subjects had non-significantly difference in Hg (0.035±0.004 mg/l) and Pb (0.014±0.001mg/l) compared to its level in controls (0.043±0.004 mg/land 0.014±0.001 mg/l respectively).

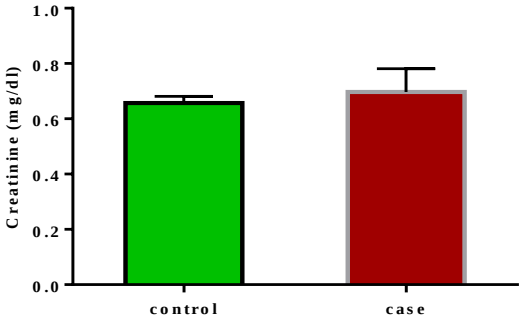

Figure (8): Creatinine level in control and case group.

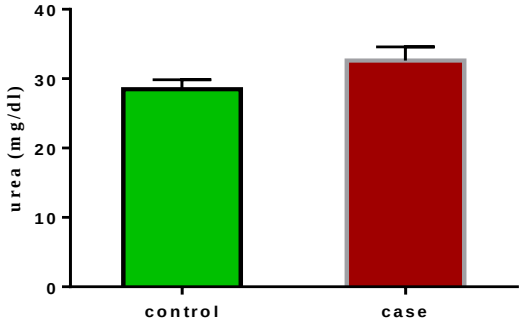

Figure (9): Urea level in control and case group.

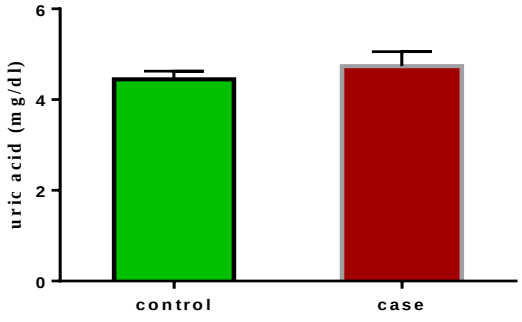

Figure (10): Uric acid level in control and case group.

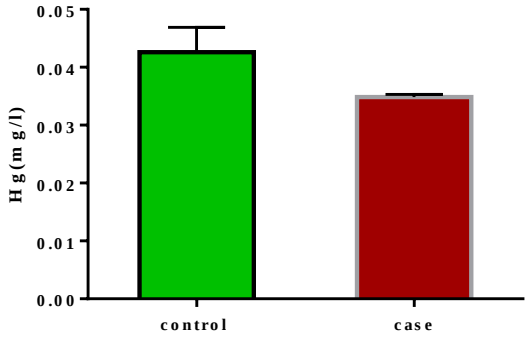

Figure (11): Hg level in control and case group.

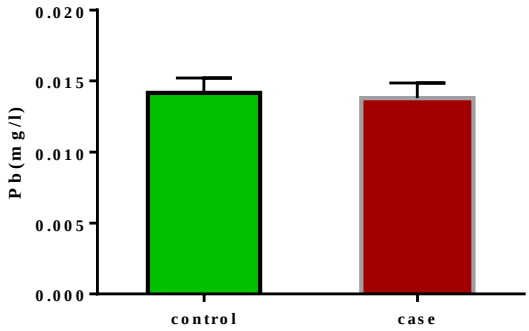

Figure (12): Pb level in control and case group.

4. CONCLUSIONS

The concentrations of serum ALT, AST, Albumin and Triglyceride were significantly lower among the workers. Whereas, normal levels of the serum total protein, total bilirubin, total cholesterol, creatinine, urea, uric acid, Hg and Pb were found among them. From these results, the Chemical exposure have significant effect on liver parameters, and non-significant effect on lipid profiles and kidney function test.

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Phenotypic and Molecular Detection of Metallo-β –Lactamase Producing *Pseudomonas aeruginosa* Isolates From Different Clinical Infections in Erbil

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ABSTRACT

Metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* has been documented to be a critical nosocomial infection. Itwas continuous intrinsic and acquired resistance to a various group of antimicrobial agents and its resistance ability to develop multidrug resistance lead to a severe therapeutic problem. The study aimed to identify the molecular characterisation of clinical isolates of Metallo-β -lactamase *P. aeruginosa*in Erbil hospitals. This study was carried out during the period from October 2017 to March 2018. A total of 300 clinical specimens were collected from patients (urine 124, wound 80, burns 40, bronchial wash 30, and sputum 26) aged 15-65 years attending Rizgary, West emergency, Erbil teaching hospitals. Out of 300 specimens, 50 isolates of *P. aeruginosa*were recovered and accounted for 1٦% of hospitalised infection isolates, the diagnosis of *P. aeruginosa*isolates was confirmed phenotypically and genotypically via the amplification of *16SrRNA*geneby using PCR technique. All isolates were tested toward the different class of antimicrobials by using agar diffusion method and VITEK 2 system. Levofloxacin, Norfloxacin, and Imipenem was the most effective antimicrobial, and most of the isolates were high resistance to (P, L,V, PI, R, CHL, E, B, A, N, TE, G, MEM, CEF, CTX, ATM).The lowest resistance was to IMP, LEV and NOR. Out of 50 of isolates,14 (28%) were found to produce MBL.*16SrRNA*were used to confirm *P. aeruginosa* and *bla*$_{VIM}$, *bla*$_{IMP}$ used to detect the MBL. All isolates were positive for *16SrRNA*, while 12 (85%) and 8 (57%) were positive for *bla*$_{VIM}$ and *bla*$_{IMP}$ genes. In conclusion, the present study proved thatMetallo-β-lactamase is producing *P. aeruginosa*isolated had phenotypic characterisation which strongly correlated with according to genotypic characterisation. To our knowledge, this is the first attempt in Erbil city.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram's negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; considered multi-resistant infections in both community and hospital settings. It causes infections in cancer, burn, urinary tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and intensive care unit (Arciola *et al.*, 2001).

Pseudomonas aeruginosa producing Metallo β-lactamase (MBL) was first ducomented in Jaan in 1991 and since then has been reported from different countries of the world including Asis, Europe, Australia, South America, and North America (Arciola *et al.*, 2001). Ambler classification[1] that divides β-lactamases into four classes (A, B, C and D) based upon their amino acid sequences. Ambler originally specified two classes: class A, the active-site serine β-lactamases; and class B, the metallo-β-lactamases that require a bivalent metal ion usually Zn^{2+} for activity.
Metallo β-lactamases belong to Ambler class B and have the ability to hydrolyse a wide group of β-lactam agents, such as Penicillins, Cdephalosporins, and Carbapenems and it consist of four enzyme groups known as VIM, IMP, SME, and NDM. These enzymes are required Zinc for their catalytic activity and is inhibited by metal chelators, like EDTA and thiol-based compounds (Asmaa *et al.*, 2001). Due to its an extremely persistence organism and usually found in soil, plants, water, humans, animals, and in hospital settings, *P. aeruginosa* is a common pathogen in hospitals generally in intesive care unit (ICU) although has the ability to colonize helthy sunjects, in addition to, exposure to variuos calss of antimicrobials may potentially increase in resistance and subsequence lead to certain mutation and may alter the bacterial genes which encode the drug targets (Azhar, 2017).

It has been understood that the resistance development in *P. aeruginosa* is multifactorial with mutations in genes encoding porins, efflux pump, penicillin-binding proteins, and chromosomal β-lactamases, all contributing to resistance to β-lactamases, carbapenems, aminoglycoside, and quinolones (Azhar, 2017). *P. aeruginosa* is an important pathogen in hospitalised patients usually their morbidity and mortality are due to its multiple resistance mechanisms. Therefore, as the therapeutic option becomes restricted, the search for a new agent is the priority (Ryan *et al.*, 2011).

P. aeruginosa is responsible for about 10% - 20% of nosocomial infections as bacteremia and sepsis in ICU, cystic fibrosis, pneumonia, urinary tract infection and wound infection. Multidrug resistance (MDR), P. aeruginosa phenotype, is defined as resistance to one antibiotic in three or more anti-pseudomonas, antimicrobial classes (carbapenems, aminoglycoside, fluoroquinolones, and cephalosporin) (Mohammed, 2015). The phenotypic method such as biotyping, serotyping, and molecular methods such as plasmid profile analysis and PCR were used for an epidemiological purpose (Shukriyah, 2013).

Genes responsible for the MBL productions in *P. aeruginosa* are typically part of an integron structure and are carried on transferable plasmids or transposons, but also might be a part of chromosomes. Accordingly, due to its integron-associated gene cassettes, *P. aeruginosa* isolates producing MBL are capable for resistant to the different broad variety groups of antimicrobial agents which further can be transferred to various types of bacteria (Alla *et al.*, 2014). MBL-producing organisms infections is linked with greater rates of mortality, morbidity, and healthcare costs. The international epidemiology of MBL-producing *P. aeruginosa* is still unknown in most countries (Sunite *et al.*, 2016), which at least due to partly lack of proper screening and

recommendations. In some countries, such as Egypt, Brazil, and Korea the proportion of MBL-producers among imipenem-resistant *P. aeruginosa* has been estimated to 27%, 20%, and 11.4, respectively (Clare *et al.,* 2006).

Nosocomial infection involving multi-resistant Metallo-β-lactamase producing *Pseudomonas aeruginosa* is a growing problem worldwide (David *et al.,* 1990). Accordingly, this study focused was designed according to the proposed project of the Ministry of Higher Education and Scientific Research for trying to solve the problem in hospital and health care centres. On the causes of the continuous antibiotic resistance and MBL producing by *P. aeruginosa* that prevalent in hospitals especially that caused the hospitalised infections based on the identify the gene responsible between the isolates collected from different clinical samples by using the Polymerase chain reaction (PCR) and genotypic characterisation for MBL. The current study aimed to detect the presence of MBL-producing P. aeruginosa isolates from the different clinical infections and genotypic method.

2. MATERIALS AND METHODS

2.1. Patients and sample collection

A total of 300 clinical samples from both gender (140 males and 160 females) with different age, whom are suffering from different clinical infections **(**Urine 124, Wound swab 80, Burn swab 40, lower respiratory tract 30, and sputum 26) from Erbil hospitals during the period from October 2017 to March 2018 were enrolled in this study. Depending on clinical criteria that diagnosed by physician and laboratory criteria of infections based on diagnosis microbiology (Hussam, 2016). All specimens were collected by clean sterilised cotton swabs or containers under the supervision of clinical consultant physicians. Each collected swab was collected and placed in a sterile tube with transport media till reaching the laboratory to be inoculated on culture media then inoculated into blood agar, MacConkey agar, and cetrimide agar by streaking method and incubated at 37 °C for 24 hours (Asmaa *et al.,* 2015).

2.2. Phenotypic identification of bacteria

Phenotypical identification of *P. aeruginosa* isolates was made by Gram staining. Colony morphologies on MacConkey's agar, Cetrimide agar for pigment production, growth at 42∘C. The biochemical tests included by using API 20 NE identification system for non-enteric Gram-negative rods and VITEK2 automatic system (BioMerieux) was used with the ID-GNB card for identification of Gram-negative bacilli for phenotypical confirmation by using Gram-negative card (Yoshichika *et al.,* 2000).

2.3. Antimicrobial sensitivity test

The susceptibility of the isolates to the following antibacterial agents was tested by the disc diffusion method, also known as the Kirby-Bauer disc diffusion method was carried out by using disks (Bioanalyse/Turkey) on Mueller Hinton agar interpreted according to the clinical laboratory standard institute guidelines CLSI guidelines (CLSI, 2017): Penicillin (P,10ug**),** Amoxicillin/ Clavulanic acid (AMC, 20/10 ug), Amikacin (AK, 10 ug), Neomycin (N, 10 ug), Lincomycin (L, 10 ug), Levofloxacin (LEV, 5ug), Vancomycin (V, 30 ug), Bacitracin (B, 10 ug), Norfloxacin (NOR, 10 ug), Erythromycin (E, 10 ug), Piperacillin (PI, 10 ug), Tetracycline (TE, 30 ug), Imipenem (IMP, 10 ug), Meropenem (MEM, 10 ug), Cefotaxime (CTX, 30 ug), Ceftazidime (CEF, 30 ug), Aztreonam (ATM, 10 ug), Chloramphenicol (CHL, 30 ug), Rifampicin (R, 5 ug). Also, the isolates were to awarded to the different class of antimicrobials by VITEK2 automatic system for confirmatory susceptibility tests of isolates (CLSI, 2017).

2.4. Phenotypic detection of MBL

The Imipenem-EDTA combined disc test was performed, test organisms were inoculated on to plates of (Firdous *et al.,* 2011). Two (10 μg/ml) Imipenem discs were placed on the plate in distance 30mm and appropriate amounts of 4 μl of EDTA solution were added to one of them to obtain the desired concentration of (750 μg/ml). The inhibition zone of the Imipenem and Imipenem-EDTA discs were compared after 16-18 hours of incubation at 37 ˚C. The result of combined disc test, IMP-EDTA disc, increase in inhibition zone by ≥7 mm than the IMP disc alone was considered as MBL positive (Mariappan *et al.,* 2014). A Solution of a 0.5 m (Molarity) EDTA was prepared by dissolving 16.8 gm of EDTA in 100 of distilled water and its pH was adjusted to 8.0 by using NaOH and sterilized by autoclaving (Agnieszka *et al.,* 2017).

2.5. DNA extraction and Purification Kit

DNA templates were prepared according to the previously described method (Smet *et al.,* 2009). The extraction kit was provided by (GeNet Bio, Korea) as follow steps: The bacterial cells harvested from overnight culture cell 100-200 µl by centrifuge at 13000 rpm for 30 seconds then discard the supernatant followed by adding 200µl of resuspension buffer for resuspend the cell pellet. Then adding of 20 µl of Proteinase K solution for lyses the bacterial cells then mixed by vertexing and incubated at 56˚C until the cell is completely lyses followed by spin down briefly the tube for removing the reaming drops. Adding of 200 µl of binding buffer to the sample then mix well by pulse-vertexing for 15 sec then incubated at 56˚C for 10 minutes, then adding 200 µl of Ethanol and mixed well by vertexing for 15 sec after that spin down to get remaining drops from clinging under the lid. Transfer the lysate into spin tube then centrifuge 13000 rpm for 1 min followed by transferring the lysate to the new collection tube for filtration then adding of washing buffers and discarding of flow through two times then centrifuge once more to completely removing of remain ethanol. Then the spin column transfer to collection tube then adding 200 µl of elution buffer then stored at -40 ˚C.

2.6. Estimation of DNA yield and purity

The extracted genomic DNA was checked by using Nanodrop spectrophotometer to estimate the concentration and purity of extracted DNA through reading the absorbance at (260 /280 nm) (Mai *et al.,* 2014).

2.7. Preparing the Primers

Lyophilised forward and reverse primers of 16SrRNA gene (F-GGG GGA TCT TCG GAC CTC A, R- TCC TTA GAG TGC CCA CCC G) expected product size 900bp, MBL genes *bla*$_{\text{VIM}}$ (F-GTT TGG TCG CAT ATC GCA AC, R- AAT GCG CAG CAC CAG GAT AG) expected product size 380bp, *bla*$_{\text{IMP}}$ (F- GAA GGC GTT TAT GTT CAT AC, R- GTA TGT TTC AAG AGT GAT GC) expected product size 600bp were prepared depending on manufacturer's instruction by dissolving the lyophilised sample with nuclease-free water to give a final concentration of (100 pM/µl) (as a stock solution) then rotating down briefly. To prepare 10µM of working primer (working aliquot) re-suspended 10 pM/µl of stock primer in 90µl of deionised water to reach a final concentration 10µM.These primers synthesized by GeNet Bio company (Korea).

2.8. Amplification

PCR was done according to manufacturer protocol for amplification of target genes in a final volume 20µl. The amplification was performed

using hot start Master Mix 2x (GeNet Bio), specific primers for target genes (1µl forward and 1µl reverse), 8µl of *P. aeruginosa* DNA extract as a template. Sterile distilled water was used instead of DNA template to ensure the absence of contaminants in the reaction preparations as a negative control. The PCR conditions started with thermocycler program according to showed in the (Table 2).

Table 1- The PCR thermocycler program for *P. aeruginosa* target genes

Steps	Temperature	Time	Cycle
Initial Denaturation	**95ºC**	**5 min**	**1**
Denaturation **Annealing** **Elongation**	**95ºC** **50-60ºC** **72 ºC**	**30 secs** **30 secs** **30-60 secs**	**30-45**
Final extension	**72 ºC**	**5 min**	**1**

3. RESULTS AND DISCUSSION

A total of (300) three hundred were collected from different clinical specimens (urine 124, wound swab 80, burn swabs 40, bronchial wash 30, and sputum 26) from patients attending Hawler teaching hospital, Rizgary hospital, and West Emergency hospital in Erbil city during the period from October 2017 to March.

3.1. Collection and identification of *P. aeruginosa* isolates

The obtained *P. aeruginosa* yields and their frequencies from total studied isolates were 5(4%), 14(17.5%), 16(40%), 8 (26.6%) and 7(26.9%) for each of 124 urine, 80 wound, 40 burn, 30 bronchial, and 26 sputum specimens respectively. The highest *P. aeruginosa* frequency occurred in the burn followed by wound infections, and the lowest frequency was found among urine specimen as shown in the (Table 3) and (Fig. 1 &2).

In Brazil (Milena *et al*,.2012) found that the percent of *P. aeruginosa* was15% from the burn which is lower than our results. (Nasih *et al*,.2014) maintained 17% *P. aeruginosa* from the patients. Alla *et al*, (2014) obtained 18% from the burn. (Hussam, 2016) Who collected 30% isolates from 142 clinical swabs from patients in Baghdad hospitals which is by our results. Also, our results agreed with (Nasih *et al*,.2014) who collected 30% from 985 burn samples in Sulaymaniyah hospitalized patients. Asmaa *et al.*, (2015) mentioned that 38% *P. aeruginosa* isolates obtained among 100 samples of burn-in Baghdad hospitals. Sunite (2016) reported that the percent of *P. aeruginosa* was 39% from burn patient. *Pseudomonas aeruginosa*, are among the most common causes of nosocomial as well as community-acquired infection. Burn wounds are a suitable site for multiplication of bacteria and are a more persistent more abundant source of infection than other types of wounds, mainly due to the larger area are included, and longer time of patient stay in the hospital (Delissable and Aibile, 2004). Ali (2016) found that among 60 isolates 81% of isolates can produce pigments among patients in Baghdad hospitals. Another study by (Shukriyah, 2013) reported that among 100 isolates 96% of isolates could produce pigments in Arbil city which is an excellent agreement with our results.

Table 2- Distribution of clinical samples and *P. aeruginosa* isolates

Source of sample	No. of the collected sample	No. of positive culture	Percen. %
Urine	124	5	10%
Wound	80	14	28%

Burn	40	16	32%
Bronchial wash	30	8	16%
Sputum	26	7	14%
Total	300	50	16.66%

3.2. Antibacterial sensitivity of *P. aeruginosa* isolates

Fifty *P. aeruginosa* isolates were screened for their resistance to nineteen different types of antibacterial agents which include (Penicillin, Amikacin, Neomycin, Lincomycin, Gentamycin, Vancomycin, Levofloxacin, Norfloxacin, Bacitracin, Erythromycin, Piperacillin, Tetracycline, Ceftazidime, Aztreonam, Cefotaxime, Chloramphenicol, Rifampicin, Imipenem, and Meropenem). Table (4) illustrates that the isolates were vary in their response to the use of antimicrobial agents and the highest resistance percentage was100% to Penicillin, Lincomycin, Vancomycin, Piperacillin, Rifampicin, and Chloramphenicol, while for Erythromycin was 96%, Bacitracin 82%, Amikacin 78%, Neomycin 78%, Tetracycline 24%, Gentamycin 20%, Meropenem 20% Ceftazidime 16%, Cefotaxime 16%, Aztreonam 16%, and lowest resistance percentage was for Imipenem 4%, Levofloxacin, and Norfloxacin 2%. The isolated bacteria were grouped according to their resistance to the used antimicrobial agents as shown in the (Table 4).

P. aeruginosa isolates across countries are increasingly resistant to a higher number of antimicrobial agents. Ali (2016) described that among 60 isolates of *P. aeruginosa*, 30% resist to 14 different antibacterial agents but all isolates were resistant to G, L, CEF, ATM, PI, and CTX. On the other hand, Produção *et al.*, (2006) demonstrated that the pattern of antibacterial resistance of pathogenic bacterium of *P. aeruginosa*, usually variable from one clinical location to another. Robert (1998) reported that the *P. aeruginosa* is inherent resistance to many antibacterial agents owing to impermeability multidrug efflux pump and a chromosomal AmpC *B. lactamase* and useful activity is seen among Aminopenicillin, 1st to the 4th generation of Cephalosporin monobactam, Carbapenem, amino Glycosides, and Fluoroquinolones. Johann *et al.,* (2005) reported that 20% of *P. aeruginosa* isolates from clinical samples obtained from the surgical units of Ahmadu Bello university teaching hospital in Nigeria were sensitive to imipenem which is in a good agreement with our results that among 50 isolates 28% were resistant to Imipenem and Meropenem. Amina (2013) reported that the *P. aeruginosa* have multidrug resistance gene against various antibacterial agents which was located on the chromosomal DNA. Also, our results showed that all the isolates were highly resistant to Chloramphenicol and Vancomycin. These results agreed with those reported by (Mai *et al.,* 2014) who found that the resistant to Chloramphenicol and Vancomycin were 100% and 100% respectively. Asma *et al.,* (2015) pointed that more than 50 isolates of *P. aeruginosa* among different clinical specimen showed 96% resistance to Amikacin, 96% to Lincomycin, 92% to Erythromycin, 86% to Chloramphenicol, 75% to Amikacin, Neomycin, and 70% to Bacitracin respectively. Hussam (2016) reported that *P. aeruginosa* isolates from burn cases were entirely resist (100%) for each of the following antibacterial agents Penicillin, Lincomycin, Vancomycin, and Piperacillin while exhibited moderate resistance rate (80%) to Amikacin, Rifampicin, Chloramphenicol, and Erythromycin, and were

sensitive to Imipenem, Meropenem, and tazobactam in a percentage rate (10%) for each antibacterial. Resistance mediated by *P. aeruginosa* can be attributed both to an inducible, chromosomally mediated beta-lactamases that can render broad-spectrum cephalosporin indicative and to a plasmid-mediated beta-lactamase that can lead to resistance to several Penicillin and Cephalosporin (Hussen, 2010). The mechanisms of bacterial resistance to aminoglycoside antibacterial in clinical isolates are usually controlled by enzymatic inactivation of the antibacterial, since nine different enzymes that catalyse the phosphorylation, acetylation, re-adenylation of aminoglycosides have now been identified in bacteria (Eigner *et al.,* 2005). The evolution of multi-resistant *P. aeruginosa* and its mechanisms of antibiotic resistance mechanisms include reduced cell permeability, efflux pump, changes in the target enzymes and inactivation of the antibiotics (Drieux *et al.*, 2008). *P. aeruginosa* is a versatile opportunistic pathogen. It is the predominant cause of wound infection in pre-antibiotic and persists as critical pathogen, strongly studies as a significant cause of nosocomial infections. In recent years, a marked increase in the number of hospitals acquired infections due to multidrug resistance has been reported from many countries (Ekrem and Rokan, 2014).

Table 3- Resistance of *P. aeruginosa* to understudy to different antimicrobial agents

Antimicrobial agent	No. of Isolates	% of Resistant
Penicillin	50	100%
Amikacin	37	74%
Neomycin	37	74%
Lincomycin	50	100%
Gentamycin	10	20%
Vancomycin	50	100%
Levofloxacin	2	4%
Norfloxacin	2	4%
Bacitracin	40	80%
Erythromycin	48	96%
Piperacillin	50	100%
Tetracycline	12	24%
Ceftazidime	8	16%
Aztreonam	8	16%
Cefotaxime	8	16%
Chloramphenicol	50	100%
Rifampicin	50	100%
Imipenem	2	4%
Meropenem	10	20%

3.4. Metallo β-lactamase production in *P. aeruginosa*

Combined Imipenem-EDTA disc method performed for detection of the ability of *P. aeruginosa* isolates to produce MBL. That is responsible for their resistance to beta-lactam antimicrobial agents like Imipenem and Meropenem. Among 50 isolates of *P. aeruginosa* 14 (28%), of the isolates were MBL producers, the inhibition zone with Imipenem-EDTA is more than 7 mm than the IMP disc alone. The remaining *P. aeruginosa* isolates 36 (72%) were non-MBL producers (Fig. 1&2).

In the present study, 28% of isolates have Metallo β-lactamase, and this result agreed with (Ami *et al.*, 2008) who found that 21% of *P. aeruginosa* isolates from ICU patients have MBL enzyme, also agreed with (Tanzinah *et*

al., 2010) who reported that 25% of the isolates have MBL enzyme. In contrast, these results do not agree with (Johann *et al.,* 2005) who documented that 66.66% of the isolates have MBL enzyme, and also didn't agree with (Shukriyah, 2013) who demonstrate 69% of isolates have MBL enzyme. Our result also compatible with (Hallem *et al.,* 2011) who detected that 28% of *P. aeruginosa* isolates in Tehran, Iran was MBL producers. Johann *et al.,* (2005) who detected that 30% of *P. aeruginosa* isolates in largely centralised laboratory Canada was MBL producers by using combined Imipenem-EDTA disc method. The present study indicates the high prevalence of MBL of diverse mechanisms. To confilct these problems, epidemiological studies should be done in hospital settings to determine the source of infection. Early detection of these MBL producing isolate in a routine laboratory could help to avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility testing. Furthermore, strict antibiotic policies and measure to limit the indiscriminative use of Imipenem and Meropenem in the hospital sittings should be undertaken to minimize the emergence of this great multiple MBL producing pathogen, and to prevent spreads would leave no other option to treat Gram-negative nosocomial infections (Al-Haidary, 2010).

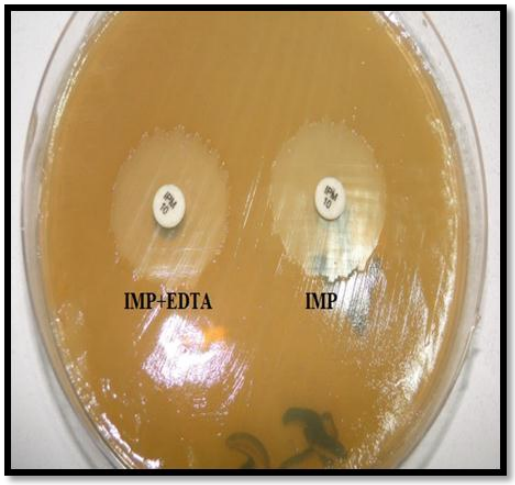

Figure (1): Combined disc diffusion test MBL negative

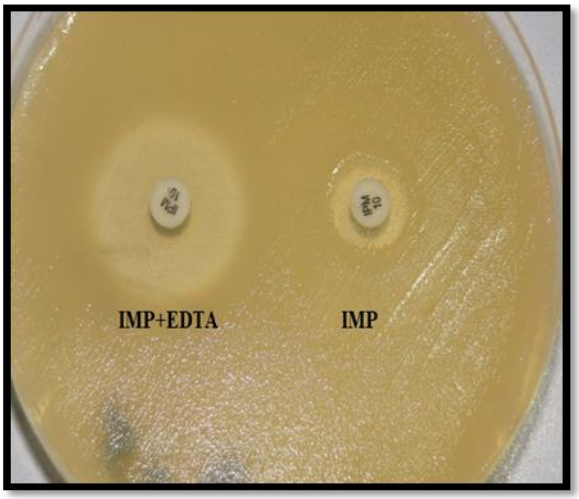

Figure (2): Combined disc diffusion test MBL positive

3.5. Molecular aspect of *16SrRNA* and MBL genes bla_{VIM}, bla_{IMP} of *P. aeruginosa* isolates

The outcome of PCR amplification among 50 isolates, 14 (28%) was found to be MBL producers by Combined Imipenem-EDTA disc method (phenotypic method) and was genotypically confirmed by PCR amplification of the *16SrRNA* (Fig. 3). The amplification of the *16SrRNA* gene showed that among fourteen MBL isolates all of them carried this gene.

Among 14 phenotypic Metallo β-lactamase isolates the results achieved by using PCR revealed that 12 (85%) isolates have bla_{VIM} genes (Fig. 4), while 8 (57%) isolates carried bla_{IMP} genes (Fig. 5).

MBLs genes were reported by (Mai *et al.,* 2014) which among 122 clinical samples in Cairo Egypt 85% were carried bla_{VIM} genes and 54% were positive for bla_{IMP} genes which strongly agreed with our findings. However, our finding agreed with (Alla *et al.,* 2014) among 75 isolates in Baghdad hospitals 70% was carried bla_{VIM} genes, 40% was carried bla_{IMP} genes. This finding was supported by results of previous studies demonstrating

MBLs genes. Amina (2013) mentioned that 100 *P. aeruginosa* isolates 60 (60%) carried bla_{VIM} genes and 40% carried bla_{IMP} genes. In worldwide nosocomial outbreaks, the VIM is the most dominant MBL gene associated with outbreaks due to MBL producing *P. aeruginosa*. In our study, 71% of MBL isolates carried MBL genes this is agreed with (Ali, 2016) who found that 68% of isolates carried MBL genes. Most of the phenotypic methods were used to detect MBL in *P. aeruginosa* and are very useful for laboratory detection and research purposes, but the confirmation of the results nowadays become a strong point in medicine and to ensure the quality of research and feature of antimicrobials uses, however, the results sometimes maybe differ due to sensitivity and specify of certain genes.

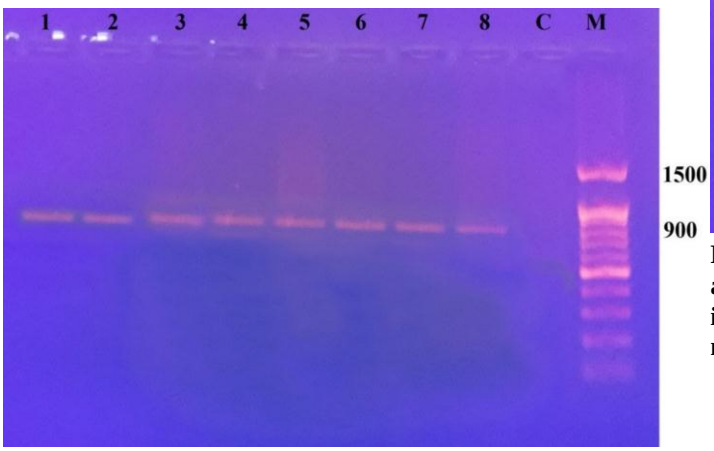

Figure (3): PCR products of *16SrRNA* gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

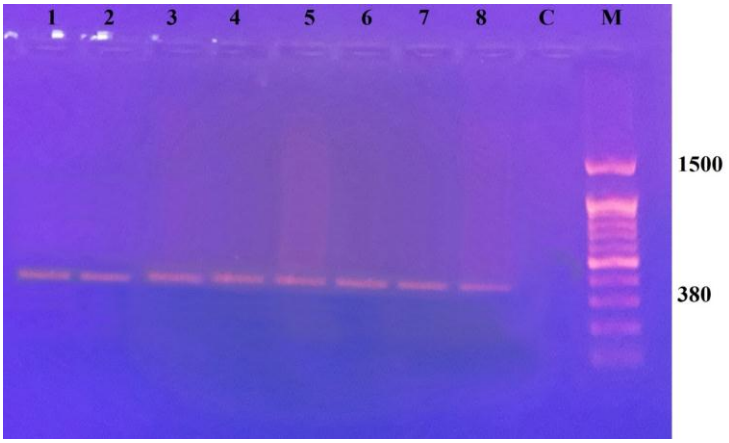

Figure 4: PCR products of a bla_{VIM} gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

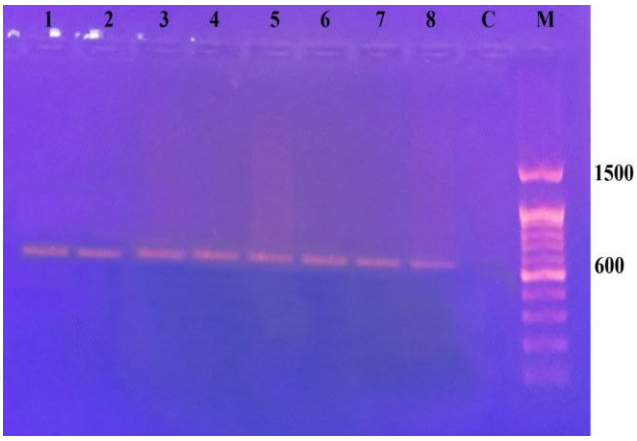

Figure 5: PCR products of a bla_{IMP} gene run on 1% agarose gel (30 min /70 vol), lane 1-8 *P. aeruginosa* isolates; lane C negative control; lane M: 100bp size marker.

4. CONCLUSIONS

We can conclude that threats MBL *P. aeruginosa*infection becomes a significant concern often life-threatening to patients and its increasingly growing resistance to various antibacterials are the massive problem in such infection. Majority of studies isolates were multi-resistant to antimicrobial agents which may indicate that they were subject to frequent modifications in their genetic material. Levofloxacin, Norfloxacin, and Imipenem are the most effective antibacterial against *P. aeruginosa*while the most isolates seen to be highly resistant to Penicillin, Lincomycin, Vancomycin, Piperacillin, Rifampicin, and Chloramphenicol. A novel rate isolate is MBL producers. Molecular detection of *16SrRNA* gene is beneficial for *pseudomonas* diagnosis confirmation. Polymerase chain reaction for genotypic characterisation showed that the

majority of MBL isolates are carrying *bla*$_{VIM}$, *bla*$_{IMP}$ genes.

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Efficiency of different coloured pan traps for collecting flower visiting insects from floricultural plants in Erbil province-Kurdistan region - Iraq.

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A B S T R A C T

. The abundance of flower visiting insects from floricultural plants were investigated at two different sites within Erbil Province- Kurdistan region-Iraq using four different colored pan traps (yellow, white, blue and red). Five insect orders were reported with sixteen insect families. The order Hymenoptera was the most abundant insect order recovered followed by the orders Coleoptera, Diptera, Homoptera and Orthoptera respectively. The color of pant trap affected the abundance of flower visiting insects at both study sites mainly the family Apidae. The highest number of flower visiting insects was observed in yellow traps followed by white, blue and red traps respectively suggesting that the majority of flower visiting insects are attracted to yellow pan traps which could be an effective method for sampling and monitoring flower visiting insects mainly Apidaeinthis type of habitat.

1. INTRODUCTION

Floriculture which is a branch of ornamental horticulture concerned with growing and marketing flowers and ornamental plants, as well as with flower arrangement. (Xia et al 2006). Because flowers and potted plants are largely produced in plant-growing structures in temperate climates, floriculture is largely thought of as a greenhouse industry; however, many flowers are cultivated

outdoors(Kamenetsky, 2004). Both the production of bedding plants and the production of cuttings to be grown in greenhouses or for indoor use (foliage plants) are usually considered part of floriculture (Britannica, 2016).

Floriculture production includes variety of plants which are mainly cut flowers, pot plants, cut foilage, seeds bulbs, tubers, rooted cuttings and dried flowers or leaves. The important floricultural crops in the international cut flower trade are rose, carnation, chrysanthemum, gargera, gladiolus, gypsophila, liastris, nerine, orchids, archilea, anthuriu, tulip, and lilies. Floriculture crops like gerberas and carnation are grown in green houses. The open field crops are chrysanthemum, roses, gaillardia, lily marygold, aster, tuberose (Apeda, 2016). Flower-visiting insects are of major significance for the functioning of natural ecosystems and for the services they provide for agriculture, such as crop pollination and pest control (Kremer and Chaplin-Kramer, 2007). The majority of flower-visiting adult insects belong to the bees, wasps, flies, butterflies, moths, .. certain beetle oreover (Schuh and Mote 1948) explained that The strawberry spittle bug is a very common pest of many plants in Oregon; in fact there are few plants on which it is not found in the Willamette Valley. Its attacks are most severe onperennial plants. Occasionally it injures annuals, shrubs, and other woody plants. (Ramadevi et al 2005) indicated that there are two families of orthoptera were found in the nursery seedlings and reported as a harmfull insects which can cause damage to leaves and shoots of these seedlins . (Ojiako , et al 2012) reported that the variegated grasshoppers (Zonocerus variegatus L.), houseflies (Musca domestica L.) and the red wood ants (Formica rufa L.) were the major insects in the nursery. They obtain nutrition from floral nectar and pollen and a few from petal tissue(Nicolson, 2007.).. Occasionally, adults from other orders also feed on floral food. In many instances they ensure the pollination of the plants they visit. In addition to food, flowers are frequented to collect fragrances, to seek shelter, prey, larval hosts and mates (Krenn et al., 2005). It is unfortunate that many flower visiting insects are exposed to harm or danger and their populations are declining due to several factors such as habitat destruction and the fragmentation of the natural and semi-natural landscapes which are main threats to biodiversity decline of flower visiting insects. Although some flower visiting insects are remarkably mobile, there are strong indications that habitat fragmentation is one of the most important factors affecting their decline (Rathcke, 1993). (Kleijn and Van Langevelde, 2006,) (Steffan-Dewenter *et al*., 2002,) The decline in the abundance of flower-visiting insects is a threat to ecological processes and to

the services these insects provide (Noordijk*et al*., 2009).

Colour is an important attractant for many flower-visiting insects. Consequently, coloured pan trapping is an efficient technique that can be easily and cost-effectively used to quantitatively sample assemblages of flower-visiting insects(Vrdoljak and Samways, 2012). It has been used for sampling and collecting flower visiting insects in various ecosystems such as forest ecosystems (Campbell and Hanula, 2007), desert landscape (Wilson *et al.*,2008), flowering crop canopies(Tuell and Isaacs, 2009) and potato field crops (Boiteau, 1990).

With these issues in mind, the aims and objectives of the current study were to:

With these issues in mind, the aims and objectives of the current study were to:

1- Identify the flower visiting insects available on floricultural plants within Erbil province at the family level And Examine the effects of colour on pan trap catches and determine which colour might provide better estimates of flower visiting insects in our region when sampling with multi-colour sets of pan traps.

The purpose of these documents is to provide information a standard set of materials to teach the taxomony of orders and common families of insects. They were originally developed for an insect systematics in the artificial nurseries. The major events of each of these episodes are briefly summarized and some of the more notable researchers highlighted, along with their influence on our current understanding of nursery insect relationships with aforementioned plants in the nurseries

2- Examine the effects of colour on pan trap catches and determine which colour might provide better estimates of flower visiting insects in our region when sampling with multi-colour sets of pan traps.

2. MATERIALS AND METHODS

The survey was initiated at Erbil Nurseries (Floricultures) from the beginning of May 2016 to the end of July 2016 using pan traps. Two different Nurseries were selected as a study area; one at the city center and the last bout 5 kilometres away from the city centre each covering an area of approximately 2500^{m2} (because only artificial nurseries were found in the centre of Erbil city centre which is provided with many types of forest trees) . The sampling station was selected using Randomized Complete Block Design (RCBD) because it is Complete flexibility. Can have any number of treatments and blocks. Provides more accurate results in the forested ecosystem due to grouping , relatively easy statistical analysis even with missing data. And allows calculation of unbiased error for specific treatment (Fleiss , 2011)and at each study site, four different coloured pan traps (Yellow, White, Blue and Red) and (the control group is not find because the study depends on colures

which each colour has its own impact that can compere individually)were installed each with three replicates (blocks) to provide an even coverage of the studied area. In each block the four different coloured pan traps were installed, these comprised of plastic bowls set side by side with a distance of 5 meters between them and at the upper margin of each trap,a small hole was made in order to prevent flooding of the traps by the irrigation system. The traps were half full of water with the addition of some alcohol (ethanol 25%) which acts as a bactericide and thus prevents decay of the catch. It is also nontoxic to mammals and thus was not a hazard to visitors. The pan traps were emptied every 7 days (it is weekly based data) over the study period. The collected samples were kept in 70% ethanol and then transported and kept in laboratory for identification. Insects were identified to

family level using the keys available and online information.

https://www.royensoc.co.uk/publications/index.htm

the majority of the ornamental plants which were cultivated at both study sites are shown in table 1.

Table 1: The common name, order , family and scientific name of the ornamental plants at both study sites in which the different coloured traps were installed.

3. RESULTS AND DISCUSSION

In total, 214 insects approach provides a better estimate of the scale of the problem, even if it doesn't show the specific details. For example, whether the insects are getting smaller or fewer in number, the decline would have just as big an impact on some forests or fields products . Previous studies looked mostly at one particular group of insects, and an argument about why a particular group was at risk overshadows the bigger question of whether this is a general problem. The new study suggests there is a very large, general problem (Kover , 2017) insects were collected at both study sites (96 site 1 and 118 site 2) belonging to five Insect orders and sixteen insect families .

The colour of pan trap affected the abundance of each insect family throughout the study period at both study sites.In general, the greatest insect number was observed in yellow pan traps followed by white, blue and red traps respectively (Figure 1).The family Apidae was the most abundant insect family collected in yellow pan traps at both study sites followed by the family Gryllidae, Cicadellidae, Formicidae at site 1

Table 1: The common name, order , family and scientific name of the ornamental plants at both study sites in which the different coloured traps were installed.

No	Common name	Order	Family	Scientific name
1	**Sweet Basil**	**Lamiales**	**Lamiaceae**	***Ocimum basilicum***
2	**Roses**	**Rosales**	**Rosaceae**	***Rosa sp***
3	**Marigolds**	**Asterales**	**Asteraceae**	***Tagetes***
4	**Mexican Washingtonia**	**Arecales**	**Arecaceae**	***Washingtonia robusta***
5	**Conifers**	**Pinales**	**Pinaceae**	***Pinus sp***
6	**Cedar**	**Pinales**	**Cupressaceae**	***Cedrus sp***
7	**Weeping Fig or Ficus**	**Rosales**	**Moraceae**	***Ficus benjamina***
8	**Paper flower**	**Caryophyllales**	**Nyctaginaceae**	***Bougainvillea glabra***
9	**Grape Vine**	**Vitales**	**Vitaceae**	***Vitis vinifera***
10	**Fruitless Mulberry Tree**	**Rosales**	**Moraceae**	***Morus alba***
11	**Carnations and Pinks**	**Caryophyllales**	**Caryophyllaceae**	***Silene dioica***
12	**Garden Pansy**	**Malpighiales**	**Violaceae**	***Viola tricolor***
13	**Christmas fern**	**Polypodiales**	**Dryopteridaceae**	***Polystichum acrostichoides***
14	**Miaruys**	**Ericales**	**Polemoniaceae**	***Acanthogilia gloriosa***
15	**Dodonaea**	**Sapindales**	**Sapindaceae**	***Melaleuca viminalis***
16	**Weeping Bottlebrush**	**Myrtales**	**Melaleuceae**	*Melaleuca viminalis*
17	**Willow**	**Malpighiales**	**Salicaceae**	***Salix alba***
18	**Chinaberry Tree**	**Sapindales**	**Meliaceae**	**Melia azedarach**
19	**Soybean**	**Fabales**	**Fabaceae**	**Glycine max**

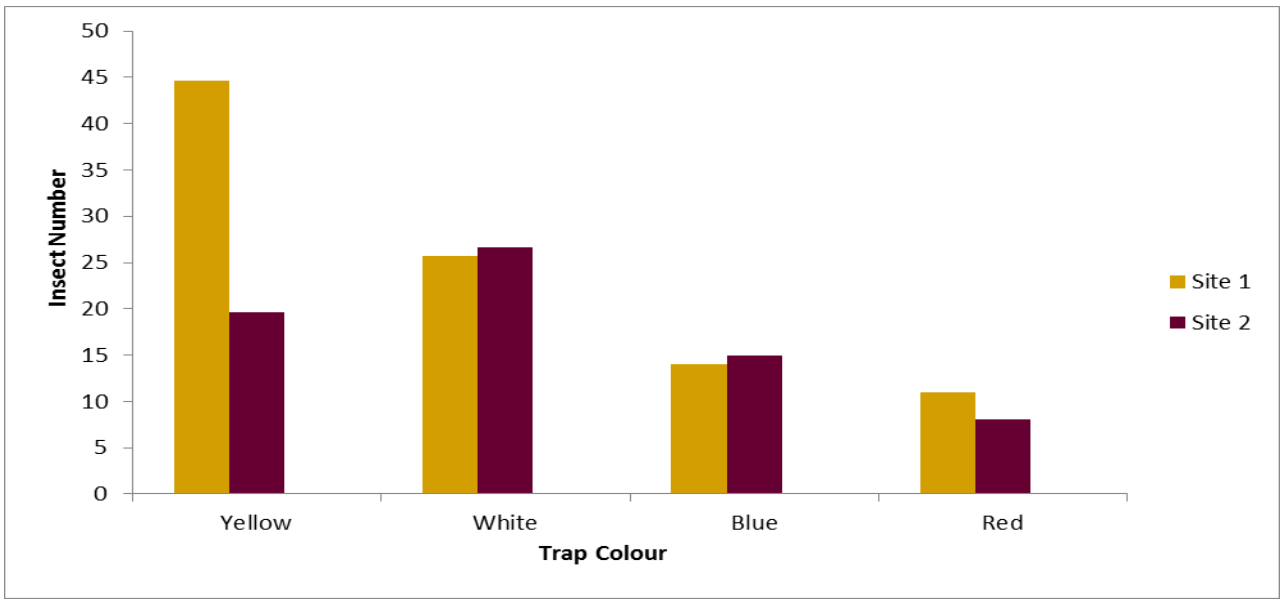

Figure 1: Total number of insects collected for each trap colour at both study sites.

The order Hymenoptera was the most abundant insect taxa collected at both study sites comprising the families Apidae, Ctenoplectridae, Formicidae, Vespidae and Megachilidae whereas the composition of other insect family were lower for the orders Coleoptera, Dipera, Homoptera and Orthoptera respectively (Figure 2 a and b). The order Hymenoptera was dominated by the family Apidae and Formicidae, Coleoptera: Chrysomelidae, Diptera:Syrphidae, Homoptera,Cicadellidae and Orthoptera: Gryllidae.

individuals were found inside the traps which they fall inside the colour traps to obtain the nutrition's and food sources (Khan et al 2016) Syrphidae and Formicidae and Cicadellidae at Site 2 (Figures 2 a and b).Although the highest insect number was found primarily at yellow pan traps, however; there were variations in the response of each insect family to trap colours as well as differences in the study sites (Figure 2 a and b).

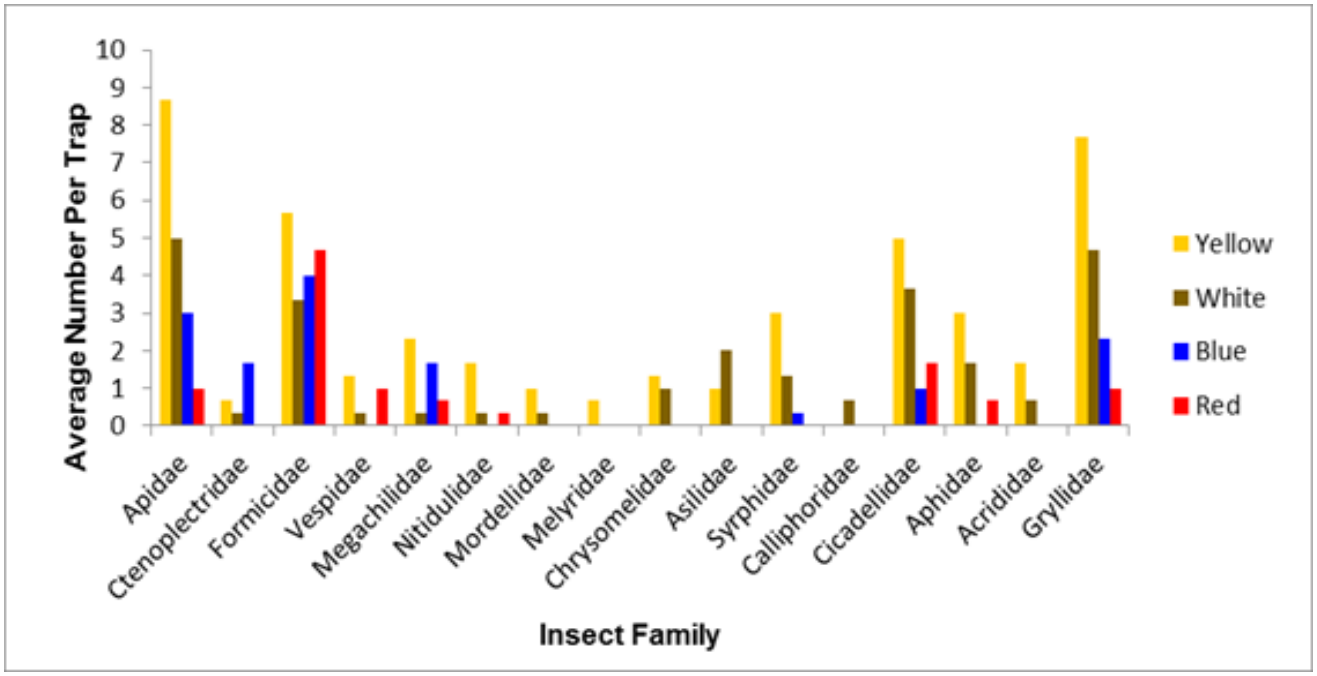

Figure 2a: The average number of each insect family collected per trap colour at site one.

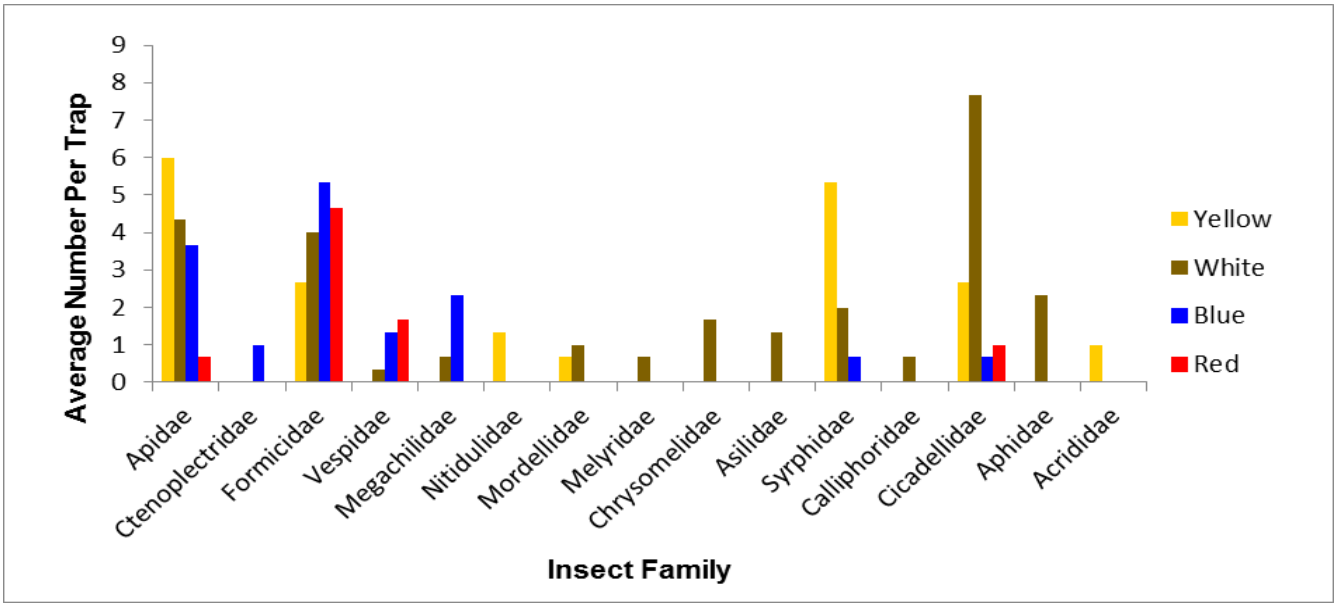

Figure 2b: The average number of each insect family collected per trap colour at site two

Overall, the families Apidae, Nitidulidae, Megachilidae, Syrphidae and Aphidae were more attractive to yellow pan traps at both study sites while the response of other insect families changed depending on each study site. For example, no Ctenopteridae insects were found in red traps at site 1 with higher numbers at blue traps at the same site whereas at site 2, they were found only in blue traps with no numbers at other traps (Figure 2 a and b). The family Formicidae was found at higher numbers in yellow pan traps and lower in red, blue and white traps respectively. In contrast, the same family was found at higher numbers in blue traps and with lower numbers in red, white and yellow traps respectively. Similarly, the composition of other family changed depending on trap colour and study site with some insect family such as Gryllidae was not observed at site two (Figure 2 a and b).

In table (2) The results of this research shows that the highest mean number of all insect families was found in white colour in comparison with yellow blue and red color , while the highest STD number was found in yellow number followed by white blue and red colours respectively, in related with the analysis the SE number of all studied colours were less than the Mean number this is approved that the shown numbers were represented the real numbers in the study.

Table (2) representing Mean and SE number of flower visiting insects

Date			Trap Color			
	Taxa (Insect Order)	Taxa (Insect Family)	Yellow	White	Blue	Red
1-May	Hymenoptera	Apidae	5	2	0	0
	Diptera	Syrphidae	2	0	0	0
	Hymenoptera	Formicidae	3	5	3	1
	Homoptera	Cicadellidae	3	0	0	1
07-May	Hymenoptera	Formicidae	0	3	2	2
	Coleoptera	Nitidulidae	4	0	0	0
	Coleoptera	Mordellidae	2	1	0	0
	Homoptera	Cicadellidae	3	4	2	2
14-May	Hymenoptera	Apidae	6	3	4	2
	Coleoptera	flea beetle	0	2	0	0
	Hymenoptera	Formicidae	5	2	3	3
	Diptera	Syrphidae	5	1	2	0

	Homoptera	Cicadellidae	0	4	0	0
21-May	Hymenoptera	Apidae	4	2	2	0
	Homoptera	Cicellidae	0	3	0	0
	Diptera	Syrphidae	5	2	0	0
	Coleoptera	Mordellidae	0	2	0	0
28-May	Coleoptera	Myleridae	0	2	0	0
	Homoptera	Aphidide	0	7	0	0
	Homoptera	Cicellidae	2	7	0	0
04-Jun	Hymenoptera	Formicidae	0	0	5	8
	Hymenoptera	Vespidae	0	1	4	5
	Homoptera	Cicellidae	0	5	0	0
	Hymenoptera	Megachelidae	0	0	4	0
11-Jun	Diptera	Syrphidae	2	0	0	0
	Hymenoptera	Apidae	0	3	1	0
	Coleoptera	Chrysomellidae	0	2	0	0
18-Jun	Diptera	Asilidae	0	4	0	0
	Diptera	Syrphidae	2	3	0	0
	Hymenoptera	Megachelidae	0	2	1	0
	Coleoptera	Chrysomellidae	0	3	0	0
	Orthoptera	Acrididae	0	0	0	0
25-Jun	Hymenoptera	Ctenoplectridae	0	0	3	0
	Diptera	Caliphoridae	0	2	0	0
	Hymenoptera	Apidae	0	3	3	0
02-Jul	Hymenoptera	Formicidae	0	2	3	0
	Hymenoptera	Apidae	3	0	1	0
	Hymenoptera	Megachelidae	0	0	2	0
Mean			1.473684	2.157895	1.184211	0.631579
STD			1.904608	1.819077	1.499663	1.575795
SE			0.811107	0.324443	0	0

There was a considerable information gap regarding flower visiting insects in the Iraqi Kurdistan region in general and Erbil province in particular which most of these insects are known as pollinators or ecosystem services. The present survey is a contribution to knowledge of the abundance of flower visiting insects at the family level in urban areas mainly floriculture and their response to various colured pan traps. Based on the data obtained from this study, the current survey shows that the abundance of flower visiting insects could be affected by sampling methods (Saunders and Luck 2013) indicated that Coloured pan trapping is a simple and efficient method for collecting flying insects, yet there is still discussion over the most effective bowl colour to use for particular target groups (e.g.

pollinator and visitor insects) Most published pan trap studies have been conducted to investigate the effects of habitat on pan trap Since many flower visiting insects are active during the day and attractive to various colures of flowers, coloured pan traps were used an effective method of sampling Despite variations in the response of each insect family to each trap color, yellow and white pan traps collected highest number of insects in comparison with other traps with the first one being the most effective particularly for Apidae and Syrphidae (Figure 2 a and b).In the relation with family level Hoback *et al* (1999) indicated that the effects of color (yellow and blue) and placement (exposed and shaded by plants) on insect captures and diversity estimates from a Nebraska inland salt marsh community. they identified 1913 specimens from 67 insect families collected during five trapping dates in July 1996. More Cicindelidae were collected on exposed traps, and more Staphylinidae, Dolichopodidae, Cicadellidae, and Thripidae were collected on shaded traps. More Dolichopodidae were collected on yellow traps, while more Syrphidae and Thripidae were collected on blue this study also concentrated on family level as representation of insect diversity .

This is also agreement with studies for example (Vrdoljak and Samways, 2012) found that colour plays an important role in determining the species richness and composition of pan trap catches, with colour sets that included high reflectance yellow and white generally having catches with the highest species richness. Gonçalves*et al*., (2012)used Malaise and pan traps to survey bee species surveyed along an altitudinal gradient in Ubatuba, Brazil. They found that the subfamily Apinae is the most abundant and species rich with 32 species collected during their study. Ramírez-Freire et al., (2012)collected a total of 38 specimens of Abidae belonging to the genus *Xylocopa*spp with 25 females and 13males using yellow pan traps at flowers: *Caesalpiniam exicana, Daleaf ormosa , Sophorasecun diflora* (Fabaceae) and *Tecomastans* (Bignoniaceae). Gollan*et al*., (2011) compared the use of yellow and white pan traps in surveys of bee fauna in New South Wales and found that in all surveys, yellow pan traps collect significantly larger number and greater diversity of bees than white pan traps. Similarly, Laubertie et al., (2006)compared the effect of different coloured pan traps to survey Syrphid flies and found that completely yellow traps capture significantly more hoverflies

than in yellow and green traps or in completely green traps for the two main hoverfly species captured, *Melanostoma fasciatum* (Macquart) and *Melangyna novaezelandiae* (Macquart). Despite that, the addition of rose water increased the number of hoverflies caught significantly. Sadeghi Namaghi and Husseini,(2010) found that, in the yellow pan trap,

Episyrphusbalteatus was the key species representing about 45% of all specimens collected inthe agroecosystems of Neyshabur, Iran.

The other taxa (Figure 2 a and b) were found in relatively low numbers and their response to trap colours varied at both study sites. This could be related to various factors for example, the presence of the families Acrididae, Gryllidae, Cicadellidae, could refer to the fact that these insects are mostly jumping therefore, their presence in the traps are mostly by accident than being a response to trap colour. Similarly, the families Nitidulidae, Mordellidae, Melyridae, Chrysomelidae are less active flying insects than the Apidae and Syrphidae, thus their numbers in each trap colour were lower. And no hemipteran insects were found While in family Formicidae the case was different the spacemen's were found in both sides but in the side 1 the highest formicidae number was found in yellow trap but in the side 2 the highest number of formicidae was found in blue trap this is due the different directions of nurseries and different forest ecosystem and cultivated plants that is why the family formicidae was different . and the presence of other insects in the traps could refer to their predator and prey behaviour not pollination such as Asilidae, and Vespidae which are predators or mutualism between aphids and ants moreover family Formicidae individuals were found inside the traps which they fall inside the colour traps to obtain the nutrition's and food sources (Khan et al 2016) . These insects caught in the traps in their response to these factors which may have fallen from the plants by accident in to the traps not by their physical response . (Kevan 1979) cleared that The importance of measuring floral colors as insects might see them is often ignored. Ultraviolet, only one of the wavebands visible to insects that they depend on broad spectrums and wave length , is often recorded in vacuo, without reference to the insect visual spectrum, without quantification, and without reference to background coloration and ambient lighting. Such oversights may lead to serious errors in interpreting the functional significance of floral colors and color patterns. The efficiency of pan traps is still not well studied, and may be closely dependent on the local vegetation type or on abiotic factors such as water availability. In addition, pan traps can be positioned close to flowers in open areas of vegetation, possibly increasing sampling rates(Gonçalves et al., 2012).

4-CONCLUSIONS AND RECOMMENDATIONS

This study confirms that coloured pan traps is an important attractant for many flower-visiting insects (anthophiles). Consequently, coloured pan trapping is an efficient technique that can be easily and cost-effectively used to quantitatively sample assemblages of

anthophiles. However, colour preferences of anthophiles is an important source of bias that needs to be considered in pan trap surveys. By drawing sub samples comprised of different colour combinations from a database of pan trap surveys in the lowlands of the Cape Floristic Region, we examine the effects of colour on pan trap catches and determine which combinations of colours might provide better estimates of diversity when sampling with multi-colour sets of pan traps (Vrdoljak, et al 2012.) could be reliably used for sampling and monitoring flower visiting insects from floricultural plants in urban areas with the yellow pan traps being the most effective particularly for the families Apidae and Syrphidae. The combination of this method with other sampling methods could give a better result on the overall estimation of populations of flower visiting insects in urban area or even wild flowers in the field. Therefore, future research should focus of the use of these methods to better understand the populations of flower visiting in this type of habitat in our region.

CONFLICT OF INTEREST STATEMENT

This research was conducted in the absence of any commercial or financial relationships that could because not all the visitors are use full insects which can influence the pollination of plants or can cause commercial or financial damage to both nurseries and can be construed as a potential conflict of interest.

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Analyze the Tranmissivity for Pumping Well testing with Single and Observation Well

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ABSTRACT

In general, for determining aquifer parameters pumping test conducted on the production well due to lack of observation wells in Erbil catchment area. In the present study steady state pumping test method using single well was used to estimate the tranmissivity of the aquifer. For this purpose pumping test conducted on a single production pumping well at the specific area which is located at the QATAWI area near Erbil city. The results of transmissivity obtained from single well pumping test data was compared with that obtained from observation well. The value of Transmissivity calculated from the field data via pumping test on single pumping well equal to 13.05 m2/day however its value obtained from observation well equal to 197.7 m2/day. It was observed that the results of transmisivity of the aquifer using single pumping well is less than that using observation well; this is mostly due to the losses that are available within the pumping well. To get a real value of transmissivity of the aquifer the pumping test should be conducted on an observation well or adjustment of the data is required when the test is conducted on the single pumping well.

1. INTRODUCTION

In order to understand the condition of aquifer basin in Erbil area; the property of aquifer layer and its thickness are required. The only reliable aquifer property obtained from single well test is transimissivity. For determination aquifer parameters, in many cases even in ministry of water resources it is common to conduct pumping well on single well as followed in Kurdistan region). The water stored in a saturated fractured rock or sand, which can

transmit water at a rate fast enough to supply significant amounts to wells (Fetter, 1998). The most important parameters of the aquifer are transmissivity and storativity. A constant rate pumping test consists of withdrawing ground water at a constant rate from a well and

monitoring water level from another observation well nearby pumping well. While, pumping test in the case with single well the variation of water level monitored from the pumping well. There are many published researches to estimate aquifer parameters using different method. (Jacob and Cooper, 1946) determined the aquifer parameters (Transmissivity and Storativity) using straight line method for a single well discharging at steady state. (Chenini, I, et al, 2008) proposed a simple graphical method to estimate transmissivity and storativity of the aquifer which is based on Theis‘s formula. (T. P. Clement et at, 1996) presented a two well test to determine the aquifer parameters. Furthermore, (Amah E. A. and Anam G. S. 2016) conducted a pumping test using five boreholes to estimate aquifer parameters including transmissivity, the study recommended that the test duration should be long enough (2-3 hrs.) to give accurate estimation of parameters. Using single well test with the Cooper-Jacob method shows overestimate of transmissivity for unconfined aquifer while, for confined aquifer near to the known value (Keith J. Halford, et al, 2006). Moreover, (Anomohanran & serhien-Emekeme , 2014) performed a pumping test data to estimate aquifer parameters using Cooper-Jacob straight line method. They concluded that the aquifer is confined and it can effectively produce the required quantity of water for the area. (Cimen, 2015) proposed a method to estimate aquifer parameters, which is depends on the straight line through the field data of residual drawdown.

In the present study the transmissivity of a confined aquifer were analyzed using single and observation well. Jacob Cooper (1946) method was used; the tarnsmissivity of the aquifer was estimated using best fit straight line between time on ordinary scale with drawdown on logarithmic scale. Pumping test only conducted on a single production well in the field from Qatawi area at 23 August 2017. In order for comparison transmissivity for single and observation well, the existing data of pumping test on observation well 25 m away from pumping well received from (BATU, 1998).

STUDY AREA

The production well for the study area (QATAWI area), is located at south of Erbil City, Kurdistan Region of Iraq. The distance between Erbil city center to the study area is about 9.4 Km, the pumping well located at the coordinate of (0409055 – 3998851 WGS_1984_UTM_Zone 38 N) as shown in Figure 1.

Figure 1 Location of Qatawi well.

2. MATERIALS

Pumping well test required to compute aquifer parameters among them transmissivity. The following tools were used during pumping test for measuring the discharge and level of water in the pumping well:

1. Water pump
2. Container for measuring discharge (Figure 2),
3. Stop watch for recording time
4. Sounder for measuring water level in the well (Figure 3).

Figure 2 measuring discharge during pumping test

Figure 3 equipment used during pumping test

3. METHODS

To estimate aquifer parameters the generalized graphical method presented by Cooper-Jacob (1947) are used. The drawdown at a piezometer distance (r) from the pumping well is monitored over the time; this is based upon the Theis (1936) analysis:

$$s = \frac{Q}{4\pi T} W(u) = \frac{Q}{4\pi T}\left(-0.5772 - \ln u + u - \frac{u^2}{2.2!} + \frac{u^3}{3.3!} \ldots\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

The value of u decrease as the time of pumping increase and as the distance between monitoring and pumping well decrease. Thus for pumping well the drawdown can be approximated after cancelling small orders:

$$s = \frac{Q}{4\pi T}\left(-0.5772 - \ln \frac{r^2 S}{4Tt}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

The above equation with logarithms (base 10) and rearranged can be written as:

$$s = \frac{2.3Q}{4\pi T} log \frac{2.25\,T}{r^2 S} t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Equation (3) is similar to the equation of straight line, which is the relation between drawdown (s) and time (t), it can be written as

$$s = \frac{2.3Q}{4\pi T} log \frac{2.25\,T}{r^2 S} + \frac{2.3Q}{4\pi T} \log t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

$Y = C + BX$ 5

Where: C is the intercept and B is the slope of the line

The relation between drawdown (s) and time (t) on log scale are shown in figure (2), which is a straight line. To calculate transmissivity and storativity the line was extended to the time axis when the drawdown is zero and t = t_o, it gives:

$$\frac{2.25\,T t_o}{r^2 S} = 1 \quad \ldots\ldots 6$$

The slope of the straight line (per log cycle) is equal to:

$$\Delta s = \frac{2.3Q}{4\pi T}$$

Where:

s: is the draw down at any time (m)

T: is the Transmissivity of the aquifer (m^2/min)

Q: is the pumping flow rate (m^3/min)

S: is the storativity of the aquifer

r: is the radial distance (m)

t: is the pumping time (min.)

W (u): is the well function

t_o: is the initial time when the pump start to working at zero drawdown (min.)

5. RESULTS AND DISCUSSION

The Transmisivity of the aquifer were calculated for the data obtained from pumping test for single well at Qatawi area (table1) and the existing data of the observation well which is drawdown and time using Cooper-Jacob's (1946) straight line method.

Table 1 Time and Drawdown for pumping well

Time (min.)	Drawdown (m)	Discharge (m^3/min)
0	0	0
0.5	6	0.7425
1	11	0.7425
1.5	15	0.7425
2	16.5	0.7425
3	19.5	0.7425
4	21.1	0.7425
5	22	0.7425
6	22.4	0.7425
7	22.4	0.7425
8	22.6	0.7425
9	22.7	0.7425
10	22.9	0.7425
15	23.1	0.7425
20	23.3	0.7425
25	23.5	0.7425
30	23.6	0.7425
40	23.6	0.7425
50	23.6	0.7425
60	23.6	0.7425

The drawdown and time is plotted on a semi-logarithmic paper, and then the difference in drawdown for one cycle of time (ΔS) was determined. The plot of drawdown versus time for pumping well testing data and observation well are shown in figures 4 and 5 respectively.

$$T = \frac{2.3Q}{4\pi\Delta s} = \frac{2.3 * 0.7425}{4\pi * 16} = 0.00906\frac{m^2}{min} = 13.05\frac{m^2}{day} \text{ for single well}$$

The Tranmissivity of the aquifer using observation well which is 25 m away from the pumping well is:

$$T = \frac{2.3Q}{4\pi\Delta s} = \frac{2.3 * 0.375}{4\pi * 0.5} = 0.137\frac{m^2}{min} = 197.7\frac{m^2}{day} \text{ for observation well}$$

Also AQTESOLV software was used for calculation the transmissivity of the aquifer for single at Qatawi area and observation well the results are shown in figures (6 & 7). The results of AQTESOLV software and spread excel sheet are very close to each other. According to the values of Transmissivity for single well and observation well it is clear that there is a difference between the value of Transmissivity obtained from single at Qatawi area and

existing data of observation well. This is mostly due to the fact that in the pumping well there are extra losses due to well screen. According to the results it is clear that the transmission rate of the ground water in the aquifer is low.

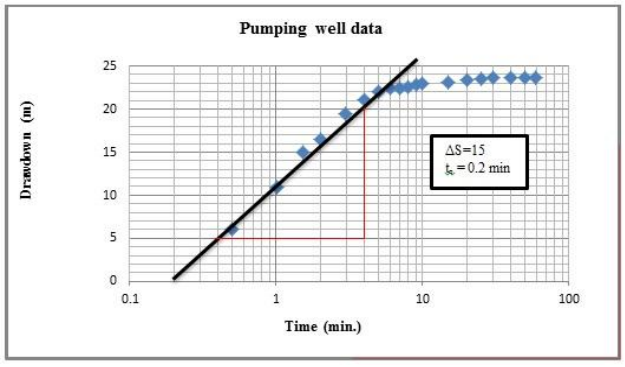

Figure 4 Jacob method for single well method

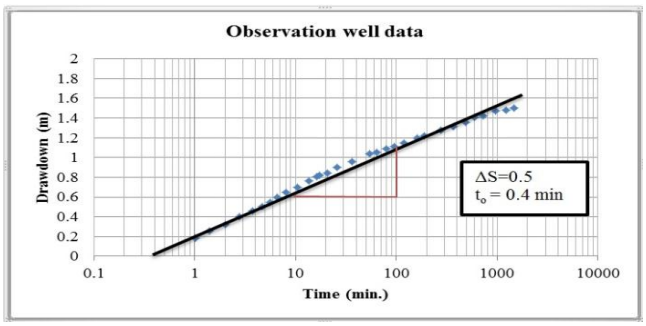

Figure 5 Jacob method for observation well method

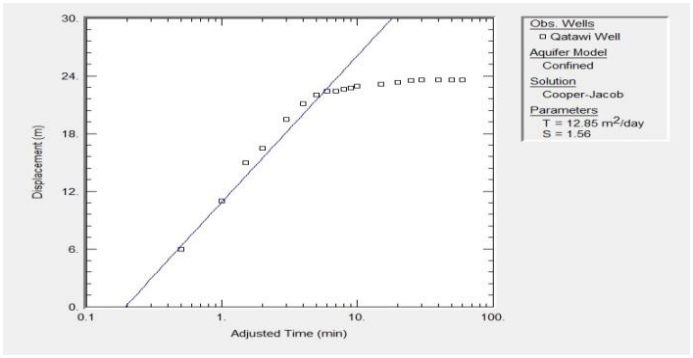

Figure 6 Jacob method for single well method using AQTESOLV software

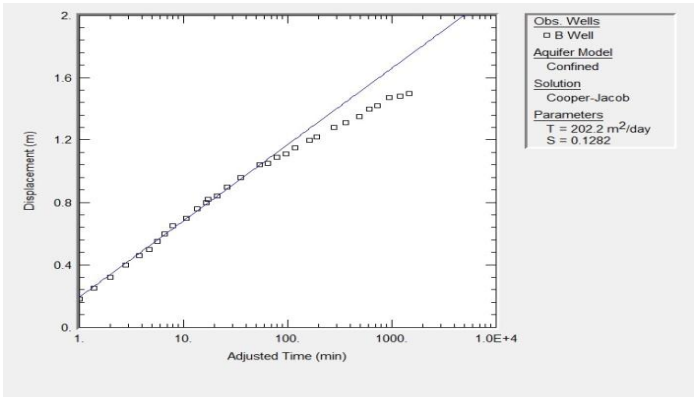

Figure 7 Jacob method for single well method using AQTESOLV software

6. CONCLUSION

The present study deals with the estimation of the transmissivity of the aquifer using approximate straight line proposed by Cooper – Jacob (1946). In addition with the excel spreadsheet the AQTESOLV software was provided for calculation transmissivity of aquifer. The results for the single at Qatawi area and observation well are compared to each other, the following conclusion were obtained:

1. The transmissivity of the aquifer calculated from single well at Qatawi area is equal to (13.05 m^2/day), while from observation well equal to (197.7 m^2/day).
2. The results of transmissivity of the aquifer that obtained from single well pumping test in many cases is not a real value, since it affected by many factors such well losses and aquifer losses. Well losses consists linear component which include losses in gravel pack and losses due to screen entry velocity, non-linear component include head loss due to turbulent flow in well casing.
3. In order to obtain a reasonable value of transmissivity a pumping test should be conducted on the observation well rather than pumping well or adjustment of the

.

data after each single well testing. In that case since it has no any screen the results of the transmissivity of the aquifer are more accurate. The adjustment includes quantifying the aquifer and well losses, since the total draw down consists both aquifer (BQ) and well losses (CQ^2) as it is clear from (Figure 8).

B and C are aquifer and well losses coefficient respectively.

The aquifer and well loss coefficient can be estimated using step drawdown test as shown in figure 9

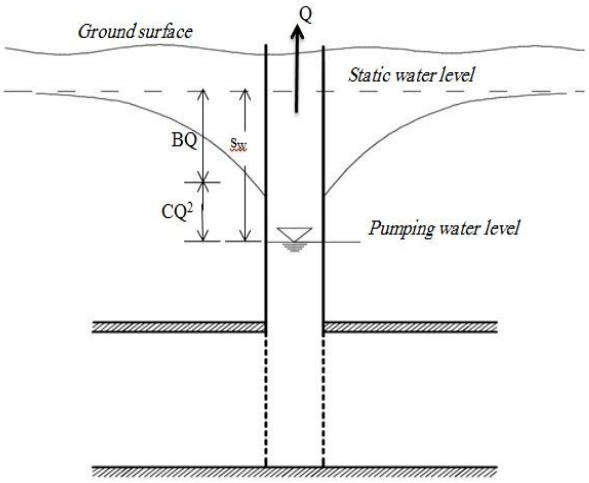

Figure 8 Well and aquifer losses

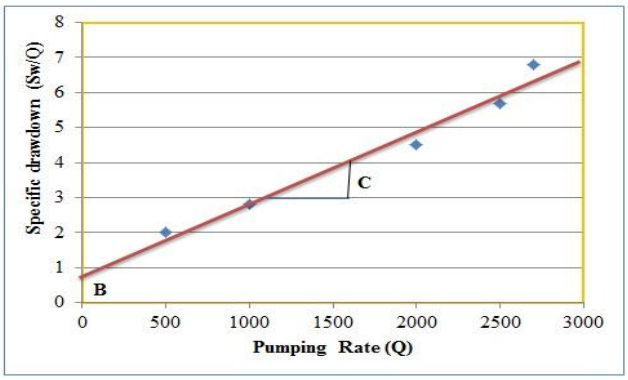

Figure 9 Estimation of aquifer and well losses coefficient

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Analysis of Electron Transport Coefficients in SiH_4 Gas Using Boltzmann Equation in the Presence of Applied Electric Field

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ABSTRACT

Monosilane (SiH_4) plasma has numerous applications in plasma processing, transport coefficients are better to understanding and modeling of these gas discharge processes. The electron swarms in a monosilane gas under influence of uniform electric field can be calculated using two term approximation of Boltzmann equation for the range $1 \leq E/N \leq 1000$ Td (1 Td= $1x10^{-17}$ V.cm^2). The effective ionization coefficient's $(\alpha-\eta)/N$ and electron swarm parameters are calculated and compared with experimental and theoretical values of drift velocity, characteristic energy, mean electron energy and ionization coefficient. The critical field strength $(E/N)_{cr}$.is calculated from the effective ionization curves. A set of electron molecule collisions has been assembled for monsilane gas which gave a good fit between the calculated and experimental values over the range of E/N investigated. The calculated distribution functions (EEDF) are found to be non-Maxwellian, having energy variations which reflect the important electron / molecule energy exchange processes. In addition, the percentages of energy lost by different types of elastic and inelastic collisions are given as a function of electric field strength E/N.

1. INTRODUCTION

In the present paper, the behavior of electron swarms in monosilane (SiH_4) gas is studied for E/N from 1 to 1000 Td by two term approximation of Boltzmann equation. Where, E is the electric field and N is the gas number density.

Monosilane, silane (SiH_4) gas is a colorless, extremely flammable, pyrophoric in air , very toxic gas and it is lighter than air, silanes refers to many compounds including organosilicon compound like, triethoxysilane (Yoshida et al., 2011), tetramethylsilane (TMS) and tetraethoxysilane (TEOS) (Kawaguchi et al., 2017). Silane has been widely used not only in the form of pure but also in mixtures with other gases for both in semiconductor industry and in thin film technologies (Shimozuma et al., 1986, Vasenko, 1999, Yamaguchi et al., 1989, Peck, 2014, Kovalgin et al., 2009, and Xi-Feng et al., 2017). The weakly ionized gas plasmas of different discharge types (DC, rf, microwave) in pure monosilane and mixtures with other gas (Haq,

2005, Nagpaland Garscadden, 1994, Shimada et al., 2003, Lisovskiy et al., 2007, Wen- Zhu et al., 2017, and Lyka et al., 2006) have been used to fabricate hydrogenated amorphous silicon (a-Si:H) film in plasma CVD (chemical vapor deposition) processing for use in solar cells (Xiaojiao, et al., 2016, Matsui et al., 2016 and Tachibana et al., 1982), and the mixture of monosilane and nitrogen have been used for deposition of a silicon nitrite thin film for interlayer insulation of integrated circuits and semiconductor device surface passivation (Sueoka et al., 1994, Tachibana et al., 1982). The properties of these films can be influenced by changing the plasma process parameters such as RF power, pressure, RF frequency and gas mixture. Study of the complicated chemistry in a SiH_4/H_2 discharge is of importance to optimize the material properties (Akdim and Goedheer, 2003).

The electron energy distribution function (EEDF) plays a central role in defining the physical properties of plasma (Boogaard et al., 2007). Transport coefficients of electron are dependent on EEDF, theoretically can be obtained by solving Boltzmann equation (Nighan, 1970). Because of its physical and industrial importance, a reliable set of electron collision cross sections for the SiH_4 molecule (Verma et al., 2017) and electron transport coefficients in binary mixtures of the SiH_4 molecule with buffer gases are necessary for understanding discharges plasma (Sto et al., 1989, Pfau and Winkler., 1990). Therefore, electron collision cross sections and swarm parameters in monosilane may be required to make clear the properties of monosilane plasmas on which the electric and optoelectronic properties of the deposited films depend. In addition, in SiH_4 the details of electron collision cross-section are important to understand the behavior of mono-silane plasma, since the properties of amorphous silicone film prepared in plasma vapor deposition (PVD) are sensitive to the plasma excitation conditions (Kurachi and Nakamura, 1989, Nakamura, 2013).

Several experimental and theoretical studies have been carried out on electron swarm parameters in monosilane, and determined sets of cross sections for the molecule. (Shimozuma and Tagashira, 1989) measured the ionization and attachment coefficients, (Cottrell and Walker, 1965) determined the electron drift velocity in the range $E/P_o < 10$ Vcm^{-1} $Torr^{-1}$. (Pollock, 1968) measured the electro drift velocity and characteristic energy in the range $0.06 \leq E/P_o \leq 26$ Vcm^{-1} $Torr^{-1}$, and measured electron collision cross sections for electron energies greater than 5 eV. Townsend first ionization coefficient and electron attachment coefficient for the range $80 \leq E/P_o$ 300 V cm^{-1} $Torr^{-1}$ measured by (Shimozuma et a1., 1983) using steady-state Townsend method. For the theoretical approach, (Ohmori et a1., 1986) who have estimated electron swarm parameters by the Boltzmann equation method. A set of electron collision cross section is determined

by fitting the calculated values of the electron drift velocity, characteristic energy and effective ionization coefficient to experimental values. (Garscadden et al., 1983) have calculated dissociation rates for monosilane-argon mixtures at low monosilane concentration which agreed well with experimental values of (Nolet, 1975). However, the other swarm parameters, e.g. electron drift velocity and longitudinal diffusion coefficients in SiH_4-Ar mixtures measured by (kurachi and Nakamura, 1991). A sets of electron collision cross sections of monosilane derived by electron swarm parameters from previous study were reported by (Hayashi, 1987). (Kurachi and Nakamura, 1988) they have measured the electron swarm parameters (the drift velocity, the longitudinal diffusion coefficient) in SiH_4-Ar mixtures due to the energy dependence of the vibrational excitation cross sections for monosilane molecules, and the ionization coefficient in SiH_4-Kr mixtures containing 5.15 and 9.65% monosilane over a wide range of electric field strength E/N. Because the momentum transfer cross section of the krypton atom is similar to that of the argon atom, and the metastable krypton atom cannot ionize a vibrational excited monosilane molecule, then krypton gas, was use instead of argon to study EEDF and electron swarm parameters in SiH_4-Kr mixtures using two term approximation Boltzmann equation (Othman, 2011) in the range of $1x10^{-18} \leq E/N \leq 1x10^{-14}$ V.cm^2. In recent years electron kinetic quantities have been studied in dc plasmas (Pham et al., 2013) by solving Boltzmann equation for pure silane and BF_3-SiH_4 mixture varying the mixture composition from pure SiH_4, up to pure BF_3. By such a study electron collision cross sections for BF_3 molecule and electron transport coefficients were calculated.

Finally, The binary mixtures of TEOS-N_2 have been also used instead of SiH_4-based gas mixtures to reduce the dangerous of explosion and toxicity while improving quality of the deposition of SiO_2 by plasma-enhanced chemical vapor deposition (PECVD) in remote microwave oxygen plasma reactors because the deposited films show good step coverage compared with films deposited from SiH_4 (Tochitani, 1993).

2. TRANSPORT PARAMETERS

The transport coefficient calculated for pure gas and mixtures were obtained by the method of (Frost and Phelps, 1962, 1964), where the Boltzmann transport (Nighan, 1970, Morgan and Penetrante, 1990) equation was solved to obtain the distribution function for electrons as a function of energy. The Boltzmann equation can be described as,

$$\frac{E^2}{3}\frac{\partial}{\partial u}\left(\frac{u}{NQ_m}\frac{\partial f}{\partial u}\right)+\frac{2m}{M}\frac{\partial}{\partial u}\left(u^2 NQ_m f\right)+\frac{2mK_B T}{Me}\frac{\partial}{\partial u}\left(u^2 NQ_m \frac{\partial f}{\partial u}\right)$$

$$+$$

$$\sum_j (u+u_j) f(u+u_j) NQ_j(u+u_j) - uf(u) N \sum_j Q_j(u) +$$

$$\sum_j (u - u_j) f(u - u_j) N Q_{-j}(u - u_j) - uf(u) N \sum_j Q_{-j}(u) = 0$$

(1)

Here e and m are charge and mass of electron, M is the mass of a molecule, KB is a Boltzmann's constant, *N* is the gas density and u is the electron energy in volts. Thus, $u = mv^2/2e$. Where *v* is the velocity of electron. Q_m is the elastic cross section and Q_j and Q_{-j} are defined as the cross-section for a collision in which the electron loss and gain energy u_j, respectively from j^{th} inelastic process. The electron energy distribution function (EEDF) $f(u)$ is obtained by solving the Boltzmann equation using all the collision cross sections. By using electron energy distribution function and collision cross section the swarm parameters are defined as follows:

The relation between drift velocity *Vd* and electron energy distribution function is (Smith and Thomson, 1978):

$$V_d = -\frac{1}{3}\left(\frac{2e}{m}\right)^{1/2} \frac{E}{N} \int_0^\infty u \left[\sum_s \delta_s Q_{sm}(u)\right]^{-1} \frac{df(u)}{du} du$$

(2)

Where, $\delta_s = \frac{N_s^j}{N_s}$ represents the fractional concentration of the (s) species and N_s^j is the number of molecules of species (s) in the excited state (j).

Here, $Q_{sm} = Q_m(u) + Q_v(u) + Q_{ex}(u) + Q_i(u) + Q_a(u)$, denotes the total collision cross section, where, Q_m, Q_v, Q_e, Q_i, and Q_a are the electron cross sections of momentum transfer, vibration, excitation, ionization, and attachment, respectively.

The electron energy distribution function is normalized by,

$$\int_0^\infty u^{1/2} f_o(u)\, du = 1 \qquad (3)$$

From the computed drift velocity, the electron mobility μ is calculated by the relation,

$$\mu = v_d E \qquad (4)$$

The diffusion coefficient D is given by (Al-Amin and Lucas, 1988):

$$D = \frac{1}{3}\left(\frac{2e}{m}\right)^{1/2} \frac{1}{N} \int_0^\infty \frac{u}{\sum_s \delta_s Q_{sm}(u)} f(u)\, du \qquad (5)$$

If the distribution is non Maxwellian, the Einstein relationship is useful for defining characteristic energy (Morgan and Penetrante, 1990),

$$\varepsilon_k = \frac{eD}{\mu} \qquad (6)$$

Using the distribution function one can compute the electron mean energy,

$$\bar{\varepsilon} = \int_0^\infty f(u) u^{3/2} du \qquad (7)$$

For Maxwellian energy distribution $\frac{2}{3}\langle\varepsilon\rangle = \frac{D}{\mu}$

Therefore, obtaining the EEDF, the reduced ionization α/N and η/N attachment coefficient are calculated according to the following definitions (Yasumori, 2004).

$$\frac{\alpha}{N} = \frac{1}{v_d}\left(\frac{2e}{m}\right)^{1/2} \sum_k \int_i^{\infty} \frac{N_k}{N} Q_i(u)\, f(u) u \quad du \tag{8}$$

$$\frac{\eta}{N} = \frac{1}{v_d}\left(\frac{2e}{m}\right)^{1/2} \sum_k \int_I^{\infty} \frac{N_k}{N} Q_a\ (u)\, f(u) u \quad du \tag{9}$$

Where, N_k/N is the relative concentration of the k- component, v_d is drift velocity given by equation (4), (i) is the ionization onset energy for SiH_4 equal to 11.6 eV, and $Q_i(u)$, $Q_a(u)$ are ionization and attachment cross-section respectively. An electron avalanche can occur when the effective ionization coefficient $(\alpha - \eta)/N > 0$. A zero effective ionization coefficient is therefore a critical value for electrical breakdown phenomena of gases. which give $(\alpha - \eta)/N = 0$, then the reduced critical electric field strength $(E/N)_{cr.}$ is therefore determined when the formation and loss electrons reach a balance, this mean that the effective ionization coefficient equal to zero (Xingwen Li., 2012).

$$\frac{\alpha}{N} - \frac{\eta}{N} = 0 \quad \text{or} \quad \frac{\alpha - \eta}{N} = 0 \tag{10}$$

Since both drift velocity and characteristic energy are sensitive to changes in the elastic (momentum transfer) and inelastic cross-sections, the momentum transfer and energy exchange collision frequencies ν_m/N and ν_u/N respectively are introduced to separate the elastic and inelastic collisions. The definition of effective elastic collision frequency is (Engelhard and Phelps., 1963),

$$\frac{\nu_M}{N} = \frac{e}{m}\left[\frac{E}{N}\frac{1}{V_d}\right] \tag{11}$$

and an energy exchange collision frequency ν_u defined by the equation,

$$\frac{\nu_u}{N} = eV_d\left(\frac{E}{N}\right)\left(\frac{1}{(\varepsilon_k - kT)}\right) \tag{12}$$

Where m is the electron mass, V_d is electron drift velocity, $eV_d(E/N)$ is the power input per electron due to electric field and ($\varepsilon_k - KT$) is excess of electron energy.. The effective elastic collision frequency $\nu_{m/N}$ is sensitive to changes in the elastic collision cross section and slightly affected by the inelastic cross section, while ν_u/N, being closely related to the fractional energy loss, is is most sensitive on changes in the inelastic cross-section (Frost and Phelps, 1962).

However, energy loss also occurs due to inelastic collisions, collision frequency coefficient for l^{th} inelastic process (Engelhard and Phelps, 1963) define by,

$$\frac{\nu_l}{N} = \left(\frac{2}{m}\right)^{1/2} \int_0^{\infty} uf\ (u) Q_{sm}(u)\, du \tag{13}$$

The mean rate R_j of collision for the j_{th} inelastic collision process may be calculated from knowledge of the cross-sections and the electron energy distribution as follows (Nakamura and Lucas, 1978),

$$R_j = \left(\frac{2e}{m}\right)^{1/2} \int_0^{x} uNQ_{s_j} f(u)\, du \tag{14}$$

Where Q_{Sj} is the cross-section of excitation of level *j* in species s, and the fractional energy loss F_j by the ^{jth} inelastic process is,

$$F_j = \frac{u_j R_{sj}}{eEV_d} \quad (15)$$

Where u_j is the onset energy for the excitation.

3. COLLISION CROSS SECTIONS IN MONOSILANE (SiH_4)

Silane is taken as a sample problem in order to calculate the electron distribution function and the transport parameters. Four types of cross sections are used for the analysis, momentum transfer, vibration, electronic excitation, ionization and attachment. During past 30 years theoretical calculations and experimental techniques were used for calculating collision cross sections from electron swarm parameters. In the analysis of the electron swarm data according to (Pollock, 1968), and (Ohmori et al., 1986) the maximum momentum transfer cross-sections were observed at 4-4.5 eV which has a Ramsauer-Townsend minimum around 0.35 eV. (Chatham et al., 1984) measured the total and partial ionization cross sections by using electron beam technique. (Garscadden et al., 1983) has derived cross-sections for the mono-silane molecule using two-term method up to 20 eV. In years 1986-1994, (Ohmori et al., 1986 , Hayashi, 1987, Mathieson et al., 1987 and Nagpal and Garscadden, 1994) have calculated cross-sections from electron swarm parameters in pure mono-silane using Boltzmann equation analysis and Monte Carlo simulation.

The monosilane (Silane) molecule has two vibrational modes, the bending mode (v_2, v_4) and the stretching mode (v_1, v_3), which been reported by (Ohmori et al., 1986) used in the present calculation. The onset energies for Q_{v24} and Q_{v13} are 0.113 eV and 0.271eV, respectively, the other cross sections electronic excitation Q_{ex} and attachment Q_a compiled by (Hayashi, 1987), momentum transfer cross section derived by (Kurachi and Nakamura, 1988) and the ionization cross section Q_i obtained by (Chatham et al., 1984) were used in the present study.

4. RESULT AND DISSCUSION

The numerical solution of the two term approximation Boltzmann equation is used for calculating electron swarm parameters in DC uniform fields in the range $1Td \leq E/N \leq 1000$ Td. One of the most important parameters for gas discharge phenomena is the electron energy distribution (EEDF). The Boltzmann equation used to calculate the EEDF, using these parameters (EEDF) other swarm parameters are obtained by appropriate integration. The Equation (1) was solved numerically for wide range of E/N values, the E/N values were chosen to yield mean electron energies in the range 0.05 eV- 6.5 eV. The calculation of electron distribution function in SiH_4 is shown in figure (1), the distribution function is normalized by equation (2), at

E/N=10 Td Maxwellian distributions would

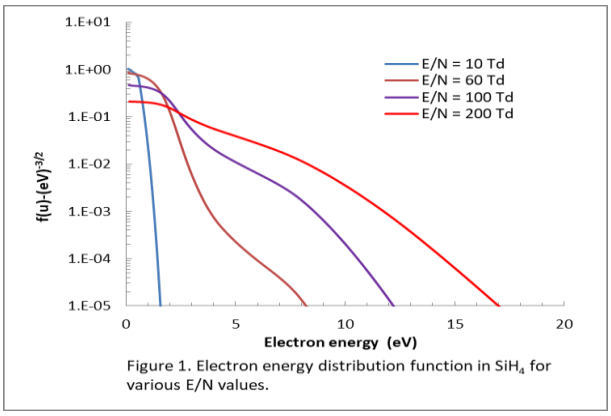

Figure 1. Electron energy distribution function in SiH_4 for various E/N values.

appear as straight lines. The calculated electron energy distribution functions are non-Maxwellian having the dominant electron-molecule energy exchange processes. For example, at E/N values approximately 10 Td the distribution function in SiH_4 is characterized by the absence of electrons with energy greater than 1.6 eV.

As E/N increases, the energy input to the electron gas increases, therefore, for mean electron energy near 2.0 eV and above, has substantially more high-energy electrons than the corresponding Maxwellian function.As seen in figure (1), $f(u)$ will tend toward zero at high energy for E/N value such that the high-energy portion of the distribution function is cutoff by the large cross section for vibrational excitation. The electron energy distribution function (EEDF) is strongly affected by changing the electric field strength (E/N) value.

Using EEDF electron swarm parameters have been calculated throughout the whole range of E/N values, the calculated electron drift velocity as a function of E/N is shown in figure (2) with comparison theoretical and experimental values. The agreement between the present calculation and other studied values (Shimada etal., 2003, Cottreland Walker, 1965, Pollock, 1968, Ohmori et al., 1986 and Pham et al., 2013) are excellent. The experimental results of (Millicant and Walker, 1987) in the range E/N ≤ 10 Td is less than present results and experimental results of (Lisovskiy et al., 2007) in the E/N ≥ 420Td greater than present calculation.

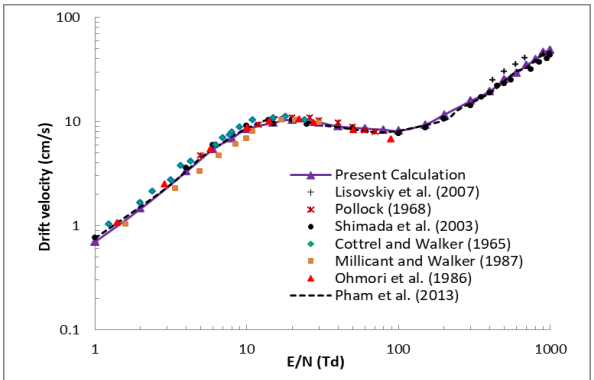

Figure 2. Electron drift velocity of SiH_4 as a function of E/N.

The electron drift velocity v_d in pure monosilane molecule is linear up to values of E/N=10 Td, and then exhibits a maximum at E/N ~15Td. In the linear mode, the EEDF is linear independent on the field. Using equation (2) the electron drift velocity in SiH_4 with low fields, from which it follows that the drift velocity is defined by the integral over energies of the elastic cross section $Q_m(u)$.

This mode of linear dependence v_d (E/N) is disturbed at value of E/N exceeding the upper limit of the range reassigned by conditions $e^{-1}u(3Q_m(u)Q_{v24})^{1/2} \le E/N \le e^{-1}u \quad (3\delta Q_m(u))$, where u_i is threshold energy, $\delta = \frac{2m}{M_{siH_4}}$ is the parameter of elastic loss of electron energy,

Q_v vibrational cross-section. At u =0.3 eV then $Q_m(u) \approx Q_{v13} \approx 10^{-16}cm^2$, this is to be expected for E/N above 15 Td , when the average energy of electrons exceeds the inelastic process threshold (u). As it is demonstrated by the results of experiments and numerical calculation, a maximum value appears on this dependence curve (figure 2) one will focus on the maximal value of the drift velocity in SiH_4, via this mode, the electron energy balance is defined by the heating in the field and by the loss during inelastic collision with a frequency, when the energy is lost in each collision.

The characteristic energy was calculated for the same purpose, which was to confirm the validity of Q_m and Q_v. Figure (3) shows the value of characteristic energy (D/μ), where (D) is diffusion coefficient and (μ) is the electron mobility defined as ($\mu = \nu_d/E$), both of them are obtained by the two-term approximation Boltzmann analysis. It has been pointed out that the diffusion coefficient obtained by the conventional two-term approximation can be inaccurated (e.g Kitamori et al., 1980) even if the effect of ionization is considered. Therefore, the two-term results were used here to make a rough estimate of (D/μ), and then the values were employed for comparison with experimental data. The present calculations are in good agreement with the theoretical values of (Haq, 2005) and (Ohmori et al., 1986). However, above 1 Td the situation is not so satisfactory and there are significant difference between the present calculation and the various experimental data of (Pollock,1968, Millicant and Walker, 1987 and Garscadden et al., 1983).

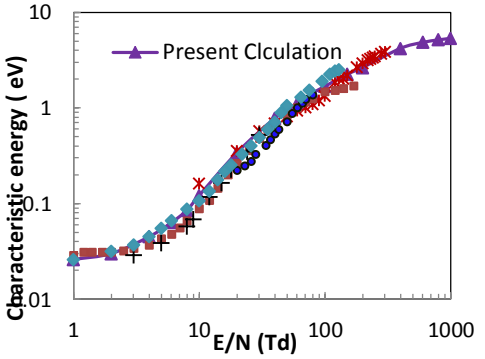

Figure 3. The characteristic energy of SiH_4 as a function of E/N.

Figure (4) shows mean electron energy, which increases with increasing E/N value, it is seen that the present calculation agree well with the experimental values of (Lisovskiy et al., 2007) and theoretical values of (Ohmori et al., 1986).

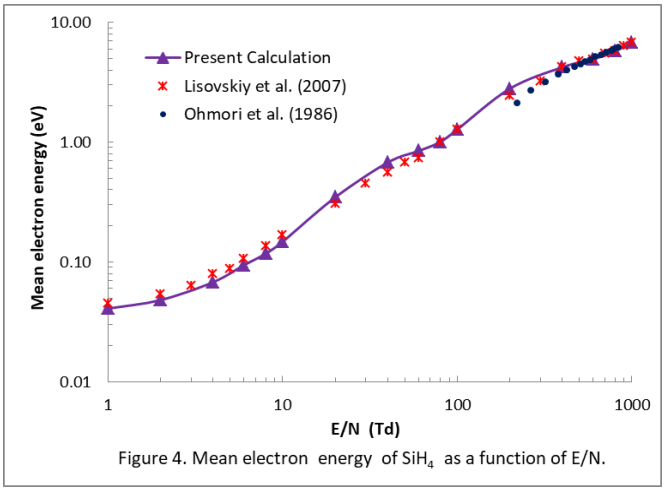

Figure 4. Mean electron energy of SiH_4 as a function of E/N.

The Townsend ionization coefficient, α/N and effective ionization coefficient, has been calculated for the range (170Td≤ E/N≤1000Td),

where (1Td =1x1^{-17}V.cm^2), using Equations (8 and 10) respectively. The results for ionization coefficients are shown in figure (5), good agreement has been obtained with the theoretical data of (Pham et al., 2013) and experimental data of (Shimozuma et al., 1986) for ionization coefficient. An electron avalanche can occur when the effective ionization coefficient (α-η)/N is positive.

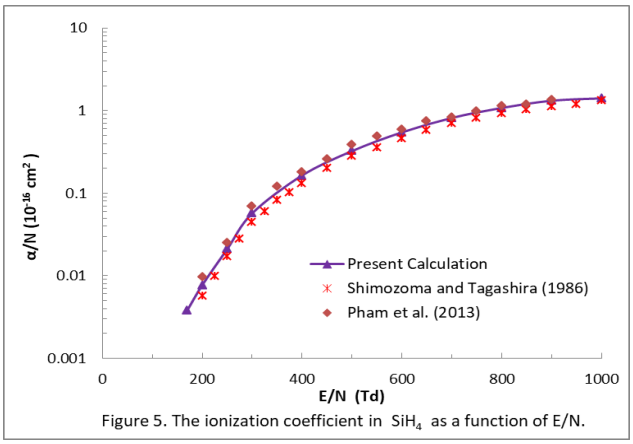

Figure 5. The ionization coefficient in SiH_4 as a function of E/N.

A zero effective ionization coefficient is therefore a critical value for electrical breakdown phenomena of gases. Critical electric field strengths, which gives [(α-η)/N] = 0. Figure (6) shows the density normalized effective ionization coefficient (α-η)/N in SiH_4 calculated over a range of E/N from 170-800 Td by using two- term solution of Boltzmann equation. The calculated limiting E/N =181 Td corresponds closely to the theoretically (Nagpal and garscadden, 1994) and experimentally (Shimozumat and Tagashirae, 1986) determined value of E/N=180 Td and E/N=183.7 Td, respectively.

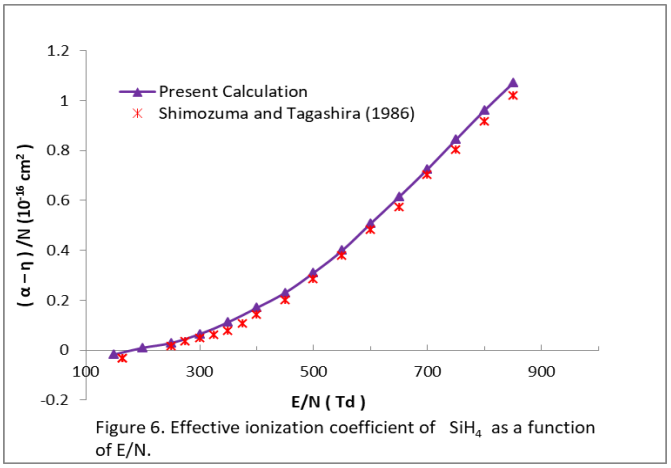

Figure 6. Effective ionization coefficient of SiH_4 as a function of E/N.

The effective ionization coefficient is almost zero, since ionizing collisions are balanced by attaching collisions, and for E/N values smaller than the $(E/N)_{cr.}$ attachment processes that becomes dominant, yielding negative values for the effective ionization coefficient as E/N is decreased and, on the other hand, for E/N values above the $(E/N)_{cr.}$, the effective ionization coefficient increases with increasing E/N where the ionization collisions become dominant whereas the effect of the attachment processes is not significant. The effective ionization coefficient (α-η)/N values for the range 150≤E/N≤850 Td may be represented by the equation:

$$\frac{\alpha-\eta}{N} = 2x10^{-6}\left(\frac{E}{N}\right)^2 - 2x10^{-4}\left(\frac{E}{N}\right) - 0.036$$

Collision frequency is a parameter closely related to drift velocity ν_d, figure (7) shows the effective collision frequency ν/N as a function of E/N. The influence of the Ramsauer–Townsend minimum below 0.5eV and abroad maximum in the range 2.5eV on the elastic collision frequency which has been observed near E/N ~10 Td, the hump in the inelastic

collision frequency is very sensitive to the position of the higher energy peak of the vibrational excitation cross sections. The inelastic collisions are predominantly due to excitation as shown figure (7).

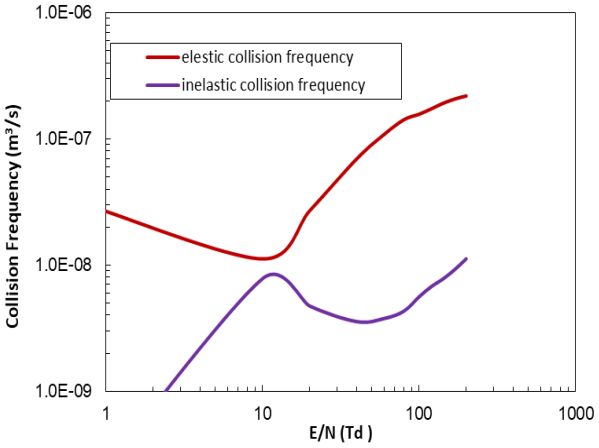

Figure 7. Elastic and inelastic collision frequency of SiH_4 as a function of E/N.

Figure (8) shows the percentage energy losses by different processes (elastic and inelastic) as a function of E/N, for low values of E/N vibrational excitation is the only loss mechanism. For example at E/N = 1 Td , 5% of the energy is lost through elastic collision and in the range 2 Td ≤E/N≤60 Td, 99% of the energy is lost through vibration excitation. For 80 Td≤E/N≤1000 Td, i.e. the mean electron energy 1.5ev < $\bar{\varepsilon}$ <10eV, there is a competition between vibrational, electronic excitation, ionization and attachment fraction. Figure (8) shows that for an E/N of approximately 200 Td, nearly 16 % of the electron energy goes into the vibrational excitation, where 78% into electronic excitation and 5.5% into ionization, the energy transfer to attachment is very small at higher E/N only electronic and ionization fraction dominated.

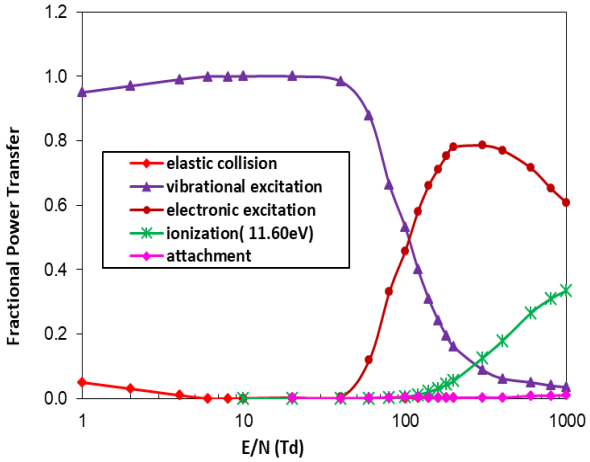

Figure 8. Fractional power transfer in SiH_4 as a function of E/N.

5. CONCLUSION

In the present work, we have attempted to use the two term solution of Boltzmann equation method to calculate the electron swarm parameters of SiH_4 gas for the range 1≤E/N≤1000 Td, which the effect of ionization coefficient may be considered. The calculated swarm parameters, that is drift velocity, characteristic energy, mean electron energy and ionization coefficient are agree well with experimental and theoretical results. The electron energy distribution functions and represented of energy losses by different types of elastic and inelastic collisions have been explained. The critical field strength has been determined from the effective ionization curves.

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