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Modified Deterministic Parameter Controlled Harmony Search Algorithm to Solve 4-Color Mapping Problem

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ABSTRACT

Harmony Search Algorithm (HSA) is one of the optimization algorithms which is imitating the behavior of musicians when composing melodies. This algorithm which consists of three phases; initialization, improvisation, and selection has been applied in this paper with some variations to solve the 4-Color Mapping Problem.

In this study, two approaches have been applied together and introduced to enhance the performance of HSA, in solving the 4-Color Mapping Problem. The first modification has been applied to the initialization section of the algorithm. And the second approach included using a number of deterministic parameter control rules to fine-tune these parameters individually and dynamically, turning harmony search into a more dynamic algorithm.

Hence, by applying both of them, better results were obtained in terms of higher performance of the improvisation process, and consequently, reducing the time and number of cycles taken to solve the 4-color mapping problem compared to the original HSA. In this paper, maps with different numbers of regions have been taken as case studies, using HSA, Modified Harmony Search

Algorithm (MHSA), Deterministic Parameter Controlled Harmony Search Algorithm (PCHSA), and Modified Deterministic Parameter Controlled Harmony Search Algorithm (MPCHSA). The experimental results revealed that MPCHSA has better outcomes compared to HSA, MHSA, and PCHSA.

1. INTRODUCTION

Optimisation in some of its aspects refers to a process of finding then selecting the best element from a set of available alternatives. The objective of a formulated optimization problem can be the minimization of time, cost, and risk or the maximisation of quality and efficiency (Parikshit et al., 2012).

The harmony search algorithm can be considered as one of the recently developed optimization algorithms works by generating a new vector that represents a candidate solution, after considering all of the existing vectors. HSA increases the robustness and flexibility of the underlying search mechanism hence it ensures better solutions.

As a matter of fact, HSA is developed in the year of 2001 then it is modified and hybridized by other researcher. Zong Woo Geem et al. [Zong, 2001] developed harmony search meta-heuristic algorithm that was conceptualized using musical process of searching for a perfect state of harmony. Kang Seok Lee and Zong Woo Geem (Kang and Zong, 2005) described a new harmony search meta-heuristic algorithm-based approach for engineering optimization problems with continuous design variables. Mohammed S. Ibrahim, Ahmed T. Sadiq, and Ali M. Sagheer (Mohammed et al., 2012) hybridized scatter search algorithm with simulated annealing algorithm then the hybrid and original scatter search algorithm have been tested by 4-Color Mapping problem. 6 local instances for Middle East maps have been tested and reported in tables. Romie B. Horca and John Paul T. Yusiong (Romie and John, 2012) used harmony search algorithm in solving the Four Color Map Problem. Sachin A. Patil and D. A. Patel (Sachin and D. A. Patel, 2013) tried to inform the readers about Harmony search algorithm, improved harmony search algorithm and applications of improved harmony search algorithm works in engineering. Ali Kattan and Reem A. Alrawi (Kattan and Alrawi, 2014) proposed the dynamic self-adaptive harmony search algorithm to solve some continuous and constrained problems having continuous value variables. The main merit of the proposed method was in its ability to dynamically set the optimization parameters based on a quality measure that is computed in every optimization cycle. Parikshit Yadav, Rajesh Kumar, S.K. Panda, and C.S. Chang (Parikshit et al., 2012) proposed a new variant of the harmony search algorithm that maintain a proper balance between diversification and intensification throughout the search process by automatically selecting parameters.

The nature of pre-defined constant parameters limits the exploitation of the algorithm, while deterministic parameter control approach uses predefined parameter alteration strategies to modify parameters deterministically (Parikshit et al., 2012; Ren and Qiang, 2013). In this paper, the pitch adjusting rate (PAR) and harmony memory consideration rate (HMCR) parameters of HSA have been modified using some of deterministic rules to adjust the parameters making HSA more dynamic and efficient algorithm in terms of finding better solutions. All of the previous was joined with a modification in the process of harmonies generation in HSA. After the generation of the harmonies, four different colors are randomly assigned and fixed – out of improvisation process - to the first four regions. The result is saving time and cycles in solving the 4-Color Mapping Problem.

4-Color mapping in turn is one of the known optimization problems that was first conjectured in 1852 by Francis Guthrie

(Mohammed et al., 2012). The 4-Color Mapping can be said that the regions of any map can be colored with only four colors, in a pattern that any two adjacent regions must not have the same colors (Georges, 2008). Two regions are considered adjacent if and only if they share a common boundary, but not just a common point (Romie and John, 2012). Figure 1 illustrates regions colored with four colors and no adjacent regions have the same color.

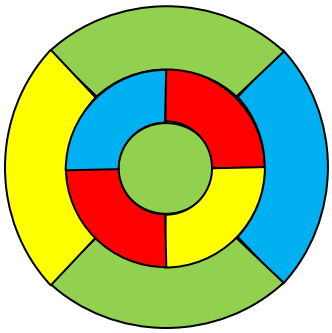

Figure 1. 4-Color Mapping Example

In Section 2 HSA is overviewed, whereas section 3 describes MPCHSA. Then solving the 4-Color Mapping Problem using MPCHSA is described in section 4. And in section 5, experimental results are presented. Finally, some concluding remarks are presented in Section 6.

2. HARMONY SEARCH ALGORITHM

Harmony Search algorithm is a meta-heuristic algorithm that mimics musicians' approach to finding harmony while playing music. When musicians go to compose music, they probably use one or a combination of three possible methods for music improvisation which are: (1) playing the original melody, (2) playing in a way similar to the original, and (3) generating a melody through random notes (Romie and John, 2012).

Zong Woo Geem et al. (Zong, 2001), discovered the similarity of this behavior in getting the optimal solution of a problem. In 2001, he proposed three corresponding methods which are (1) random selection, (2) memory consideration, (3) and pitch adjustment. These became the steps for the newly developed meta-heuristic optimization algorithm; the HS algorithm (Romie and John, 2012).

The design of a basic HSA is mathematically based on the following steps (Parikshit et al., 2012; Sachin and D. A. Patel, 2013):

Step 1: Initialize the problem and algorithm parameters.
Step 2: Initialize the Harmony Memory (HM).
Step 3: Improvise a New Harmony memory.
Step 4: Update the Harmony memory.
Step 5: Check the stopping criterion.

In this study, the feasibility of the mentioned algorithm with deterministic parameter control and the modified improvisation is being tested while solving 4-Color Mapping Problem.

3. MODIFIED DETERMINISTIC PARAMETER CONTROLLED HARMONY SEARCH ALGORITHM (MPCHSA)

A combination of two approaches has been applied and presented in this paper as an attempt to boost the performance and increase the efficiency of HSA. First, a modification has been applied to the initialization phase of the algorithm. The first four elements of each harmony will be excluded from the improvisation process.

After generating the candidate solutions, the first four elements in each vector will be randomly given four different values fixed per each solution. Therefore, the improvisation section will process (n-4) elements, where n is the number of the regions in the considered map. Those values should be within the range of the four colors to guarantee the condition of the 4-Color Mapping Problem.

Traditional HSA uses pre-defined values for each of HMCR and PAR parameters throughout the search process, making it

difficult to find out a good setting within a few numbers of trial runs. Then the second approach comes and is applied using a number of deterministic parameter control rules to fine-tune HSA parameters dynamically (Ren and Qiang, 2013). The goal will be producing a better searching result, and maximizing the utilization of each iteration.

Parameter controlled harmony search PCHSA uses variable values of HMCR and PAR in improvisation step (Step 3) changing dynamically in every iteration, ranging from 0 to 1 for each. The mentioned parameters are expressed as shown in equation (1 & 2) (Ren and Qiang, 2013; Wan-li et al., 2014).

$$HMCR(iter) = HMCR_{min} + (((HMCR_{max} - HMCR_{min}) / maxiter) * iter) \quad (1)$$

$$PAR(iter) = PAR_{min} + (((PAR_{max} - PAR_{min}) / maxiter) * iter) \quad (2)$$

where,

HMCR(iter) = harmony memory consideration rate for each generation

$HMCR_{min}$ = minimum harmony memory consideration rate

$HMCR_{max}$ = maximum harmony memory consideration rate

PAR(iter) = pitch adjustment rate for each generation

PAR_{min} = minimum pitch adjustment rate

PAR_{max} = maximum pitch adjustment rate

iter = current iteration

maxiter = maximum number of iterations

4 SOLVING THE 4-COLOR MAPPING PROBLEM USING MPCHSA

Here in this section, the two applied approaches are going to be presented. Arrays of integer variables are generated as candidate solutions for the problem. The number of variables per each solution is the same as the number of the regions for the target map. Also the value of each variable or element in the solutions is ranging from 1 to 4 representing the four used colors as shown as an example in Table 1.

Table 1. Colors codes

Number	Corresponded Color
1	Red
2	Green
3	Blue
4	Yellow

Then the equation for random solutions generation in the harmony memory is:

$$r = \{R_1, R_2, R_3, \ldots\ldots, R_n\} \quad (3)$$

Where

R = Regions with values 1, 2, 3, or 4

n = Number of regions in the map

The process in few lines consists of setting up the harmony memory, and then the improvisation process comes. The generated solutions are then tested with respect to the objective value. The optimization process terminates when an optimal solution is earned or the maximum trials are reached for improvisation. MPCHSA involves the steps below:

MPCHSA Parameter Initialization Step: In which five parameters are set; the number of decision variables, number of allowed iterations, and Harmony Memory Size. Then the Dynamic Harmony Memory Consideration Rate HMCR (Equation 1) is tuned to determine whether the harmony solution must be chosen from the harmony memory, or randomly generated from the range of all possible values between 0 and 1, i.e. high HMCR value makes the harmony more affected by the historical values, and vice versa. Finally, Dynamic Pitch Adjustment Rate Parameter PAR (Equation 2) is set to mostly affect the rate of finding and converging to the optimal solution as illustrated in Table 2.

Table 2. Parameter Values

Parameters	Values
No. of Variables	No. of Regions in the map

Cycles	1000000
HMS	10
HMCR(min – max)	0.1 – 0.9
PAR(min – max)	0.1 – 0.9

MPCHM Initialization Step: The modification approach is applied here where the initial MPCHMA is normally generated then the first four regions in each vector are randomly given the four different colors as shown below. Therefore, random selection, memory consideration, and pitch adjustment will start from the element of region five and upwards.

r = { 2 , 3 , 1 , 4 , 1 , 3 , 2 , 4 , 2 }

Randomly Generated and Fixed

Random Selection Step: Now the variables in each harmony are assigned random values; namely, a random color out of the available four.

Memory Consideration Step: The elements of the harmony memory are given a chance to be selected from the available colors.

Pitch Adjustment Step: To apply a variation in the candidate solutions, each element in the memory can be altered through pitch adjustment.

Objective Function (Fitness) Step: In this process the termination of the algorithm is decided based on the outcome of the fitness function or the number of iterations. The result returned by the function reflects the number of errors existing in the solution. Therefore, the solution is considered as optimal as it getting closer to zero. Whereas zero result means that there are no color conflicts in the map at all.

As an example let's color the sectors in Figure 2. Assuming that our harmony memory HM=2 so we need to calculate r^1 and r^2. Half of the r matrices were considered in calculations because they are symmetric. Note that two regions are said to be adjacent if and only if a common boundary is shared, not just a common point.

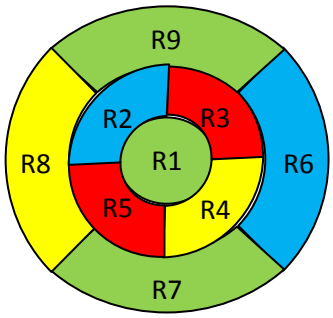

Figure 2. Colored 9-Region Shape

r^1 = {1, 2, 3, 4, 1, 4, 3, 2, 1}
r^1 =
0*0 + 1*0 + 1*0 + 1*0 + 1*1 + 0*0 + 0*0 + 0*0 + 0*1
1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*0 + 0*0 + 1*1 +1*0
1*0 + 1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*1 + 0*0 + 1*0
1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 1*1 + 1*0 + 0*0 + 0*0
1*1 + 1*0 + 0*0 + 1*0 + 0*0 + 0*0 + 1*0 + 1*0 + 0*1
0*0 + 0*0 + 1*0 + 1*1 + 0*0 + 0*0 + 1*0 + 0*0 + 1*0
0*0 + 0*0 + 0*1 + 1*0 + 1*0 + 1*0 + 0*0 + 1*0 + 0*0
0*0 + 1*1 + 0*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*0 + 1*0
r^1 = 6/2 = 3
[1, 5] [5, 1]
[2, 8] [8, 2]
[4, 6] [6, 4]

r^2 = {2, 3, 1, 4, 1, 3, 2, 4, 2}
r^2 =
0*0 + 1*0 + 1*0 + 1*0 + 1*0 + 0*0 + 0*1 + 0*0 + 0*1
1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*1 + 0*0 + 1*0 + 1*0
1*0 + 1*0 + 0*0 + 1*0 + 0*1 + 1*0 + 0*0 + 0*0 + 1*0
1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*0 + 1*0 + 0*1 + 0*0
1*0 + 1*0 + 0*1 + 1*0 + 0*0 + 0*0 + 1*0 + 1*0 + 0*0
0*0 + 0*1 + 1*0 + 1*0 + 0*0 + 0*0 + 1*0 + 0*0 + 1*0
0*1 + 0*0 + 0*0 + 1*0 + 1*0 + 1*0 + 0*0 + 1*0 + 0*1
0*0 + 1*0 + 0*0 + 0*1 + 1*0 + 0*0 + 1*0 + 0*0 + 1*0
0*1 + 1*0 + 1*0 + 0*0 + 0*0 + 1*0 + 0*1 + 1*0 + 0*0
r^2 = 0
Stop

5. TESTS AND RESULTS

Microsoft Visual C# has been used to implement the algorithms HSA, MHSA, PCHSA, and MPCHSA. To figure out the performance and efficiency of the new approach, tests and comparisons among the

algorithms have been carried out using seven different map samples; 7-region Australia map, 10-region Austria map, 18-region Iraqi map, 29-region Kurdistan-Iraq map, 32-region China map, 48-region USA map, and finally 50-region Africa continent map. Per each case around fifteen runs have been done, then the standard deviation of time and cycles required to get to the optimal solution have been stated in Table 3 (a - g) respectively.

Table 3.a HSA, MHSA, PCHSA, and MPCHSA performance in solving 7-Region Australia Map

Algorithms	7-Region Australia Map	
	Time (Seconds)	Cycles
HAS	0.007656975	10
MHSA	0.005310974	2
PCHSA	0.004690345	6
MPCHSA	0.00161192	2

Table 3.b HSA, MHSA, PCHSA, and MPCHSA performance in solving 10-Region Austria Map

Algorithms	10-Region Austria Map	
	Time (Seconds)	Cycles
HAS	0.006988317	100
MHSA	0.006027705	40
PCHSA	0.006958062	18
MPCHSA	0.001361214	9

Table 3.c HSA, MHSA, PCHSA, and MPCHSA performance in solving 18-Region Iraqi Map

Algorithms	18-Region Iraqi Map	
	Time (Seconds)	Cycles
HAS	0.173869271	1940
MHSA	0.086737074	1036
PCHSA	0.068198587	870
MPCHSA	0.047055263	636

Table 3.d HSA, MHSA, PCHSA, and MPCHSA performance in solving 29-Region Kurdistan-Iraq Map

Algorithms	29-Region Kurdistan-Iraq Map	
	Time (Seconds)	Cycles
HAS	0.889333565	7042
MHSA	0.316231162	2047
PCHSA	0.470241322	3604
MPCHSA	0.170433108	1350

Table 3.e HSA, MHSA, PCHSA, and MPCHSA performance in solving 32-Region China Map

Algorithms	32-Region China Map	
	Time (Seconds)	Cycles
HAS	1.073332541	7576
MHSA	0.616941191	3332
PCHSA	0.747933408	5991
MPCHSA	0.28596675	2108

Table 3.f HSA, MHSA, PCHSA, and MPCHSA algorithms performance in solving 48-Region USA Map

Algorithms	48-Region USA Map	
	Time (Seconds)	Cycles
HSA	6.400353566	21704
MHSA	6.022102219	21212
PCHSA	3.609366029	16649
MPCHSA	3.3849213	13635

Table 3.g HSA, MHSA, PCHSA, and MPCHSA performance in solving 50-Region Africa Map

Algorithms	50-Region Africa Map	
	Time (Seconds)	Cycles
HSA	7.961991411	26455
MHSA	7.513218207	23564
PCHSA	5.038387111	19907
MPCHSA	4.178034252	15701

As noticed from the tables above, the time and number of iterations needed to reach the optimum result for each map were the basis of the comparison that has been accomplished among HSA, MHSA, PCHSA, and MPCHSA.

Figures 3 and 4 are illustrating the variances in performance among HSA, MHSA, PCHSA, and MPCHSA, and how efficient is the combination of the two approaches (MPCHSA) in obtaining the optimal solution in terms of time and number of cycles respectively.

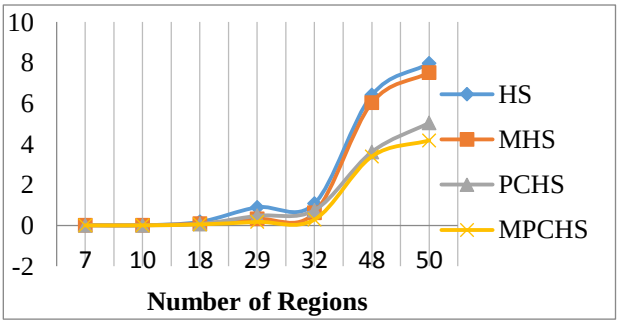

Figure 3. HSA, MHSA, PCHSA, and MPCHSA performance in terms of time in solving different number of maps

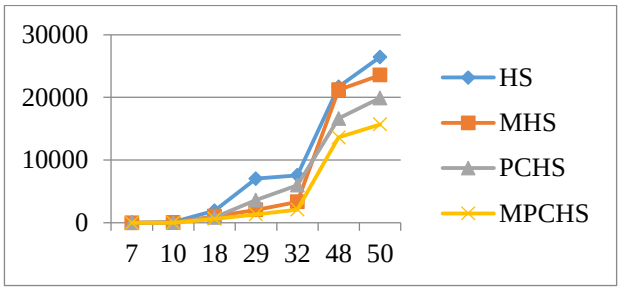

Figure 4. HSA, MHSA, PCHSA, and MPCHSA performance in terms of cycles in solving different number of maps

6. CONCLUSIONS

- Despite that PCHSA and MHSA have recorded better performance than the original HSA, the MPCHSA which combines the first two has proved and recorded even better results than its ancestors.
- The efficiency of tuning up the parameters in HSA has been tested and presented in this study. The improvements could be seen in a better exploration for the solution space, higher utilization of all iterations, and finer tuning to reach the optimal solution.
- The modification in the improvisation process resulted in a clear reduction of the required time to reach the optimum solution through shortening the way to the desired harmonies.

From the many tests that were applied on different maps, it is noticed that reaching target results is in need of more time and trials as the number of regions per map increases, since longer vectors should be created which means in turn much more probabilities.

7. REFERENCES

Ali Kattan and Reem A. Alrawi (2014) 'Pressure Vessel Design Using the Dynamic Self-adaptive Harmony Search Algorithm', *Conference Paper*.

Georges Gonthier (2008) 'Formal Proof—The Four-Color Theorem', *Notices of the AMS*, Volume 55, Number 11, pp. 1382-1393.

Kang Seok Lee and Zong Woo Geem (2005) 'A new meta-heuristic algorithm for continuous engineering optimization: harmony search theory and practice', Computer Methods in Applied Mechanics and Engineering, vol. 194, pp. 3902-3933.

Mohammed S. Ibrahim, Ahmed T. Sadiq, and Ali M. Sagheer (2012) 'Hybrid Scatter Search Algorithm for 4-Color Mapping Problem', *the 13th International Arab Conference on Information Technology (ACIT)*, ISSN 1812-0857.

Parikshit Yadav, Rajesh Kumar, S.K. Panda, and C.S. Chang (2012) 'An Intelligent Tuned Harmony Search Algorithm for Optimisation', *Department of Electrical and Computer Engineering*, National University of Singapore, Information Sciences 196, pp. 47-72.

Ren Diao and Qiang Shen (2013) 'Deterministic Parameter Control in Harmony Search', *Department of Computer Science*, Aberystwyth University, UK.

Romie B. Horca and John Paul T. Yusiong (2012) 'Using Harmony Search Algorithm to Solve the N-Region Four Color Map Problem', *Journal of Applied Computer Science & Mathematics*, Computer Science Section, no. 12 (36).

Sachin A. Patil and D. A. Patel (2013) 'an Overview: Improved Harmony Search Algorithm and Its Applications in Mechanical Engineering', *International Journal of Engineering Science and Innovative Technology (IJESIT)*, Volume 2, Issue 1, pp. 433-444.

Wan-li Xiang, Mei-qing An, Yin-zhen Li, Rui-chun He, and Jing-fang Zhang (2014) 'An Improved Global-Best Harmony Search Algorithm for Faster Optimization', Expert Systems with Applications 41, pp. 5788–5803.

Zong Woo Geem, Joong Hoon Kim, and Loganathan G. V. (2001) 'A New Heuristic Optimization Algorithm: Harmony Search', SIMULATION 76:2, 60-68.

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Evaluating Quality of City Square, A practical Survey on Neshtiman park / Erbil City

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A B S T R A C T

Nowadays there is a huge understanding to the importance of the public open spaces and green spaces infrastructure which provides a vital environmental services for their users, which considered as the main attraction point in every city. One of these significant spaces is; squares which functions as one of the most important open spaces where people can exhibit many activities and events in the city, the 'life' of squares evolves and changes according to the contemporary demands of its users and city cause ,each square has its own characteristics that recognized from other ones due to its relation to the local culture identity, all have to connect strongly within the evolution of Nishteman Square, throw analyzing and explaining the most important diversity factors that could effect on the usability of these spaces, how its designs effect the accommodation (to stay) for users, the study employs spatial analysis to show the assessment of square's physical design characteristics with people's different activities, which effect the social side of the users. This study will focus on one of the important spaces in Erbil city due to its location to one of the most important significant value (the Citadel), gives the square a special identity and relative design factors that should be respected.

The findings reveal that square in order to be a successful open space it have to work on applying diversity of service which have a great impact on attracting people in, that constantly and permanently used, safety within a good looking landscape and daily maintenance would insist the impression to people to stay there, diversity of functions on the edges around the square gives a very high level of vitality to both the square itself as well as the people who visit this important liveliness space.

1.RESEARCH QUESTION

Can Squares design guidelines standards and factors applied to Erbil City Square?

1.2. Research Problem

City square works as an attractive, historical, inclusiveness socialize space for people and other foreign out of the city, to be one of the most important places which they attend during their daily life. Neshtiman Square and functions around it located in the center of Erbil city with a significant historical land mark, Erbil Citadel, the research will try to apply square guidelines for evaluating and indicating the most effective standards for such spaces, the main research problem will be; (obvious data and design criteria that associated with the formation of this dynamic space in terms of Architectural design factors throw the social quality of life in Erbil City Square), due to that there are some other related problems;

1) There aren't any clear design guidelines for squares in Erbil city such (Identity, connectivity, appropriateness, landscaping issues, clarity, attractiveness, availability, spatial design issues, and maintenance).

2) Neglecting the social life Quality criteria in square in term of (safety, comfort, social space, and place for event, historical value, diversity, accessible, noise, vitality, popular, interesting, visible, interaction with environment, homogeneity, and commercializing).

1.3. Research Goals

The research main goal is (to Match the design criteria of the squares with the main standards within the social framework of the city reflecting its identity, as well as evaluating Erbil city square quality through assessing Architectural design factors and social life quality).

1.4. Research Limitation

The research will focus on two main side the Architectural design factors and social factors for social life only.

1.5. Research Hypothesis

To reach the research goal, the statement below should be realized: -

1) It is necessary to assess the Architectural design factors for the existing Square.

2) It is necessary to assess the Quality of social life.

1.6. Research Methodology

This research summarized the guidelines for successful city squares, discussing the factors that affect the quality of social life in a theoretical framework, explaining the history of Erbil city square and clarifying the space context for the square.

Data collection has been done in three unified procedures:

First: - (Site analysis) involves in analyzing and clarifying Architectural design factors related to city square, have been analyzed and measured by AutoCAD, and on the master plan.

Second: The evaluation data collection refers to collecting data for assessing and measuring purposes: -

- Questionnaire to Selected people which are (Architect, urban designer, and planner), to assess Architectural design factors.
- Questionnaire to public people to assess the quality of social life.

2. INTRODUCTION

The outdoor open spaces specially these squares have a very great effect on raising the

well-being for users within enhancing the community sense. **(**Project for Public Spaces. (2014). Squares). the roles of urban squares as a social space till nowadays suggested to be an important space as it in the past, it's importance comes from its relation with the local context, physically and socially. different characteristics will build the existing form of square by questioning how the design revolutions of a square modify its sociability? (Khalilah Z., Nor Z., H., Mazlina, M., 2014, P.2), these spaces need to use according to several factors such as; clean, safe, suitable, which effects the creation of sociality inside these spaces. (Capital Spaces 2012. P.2). Elements in these spaces such as an old historical building will affect positively on square attraction as well as enhancing its design procedure, squares will provide an opportunity to be more occupied with different activities than other spaces. (Hossein S., and, Fataneh F., M., 2017., P.4), square is designed to be easily accessible by all people, and it is usually tending to be spaces with a definite edge, identified with buildings, characterized with; safety, accessible, and welcoming. (Urban Squares, 2009, P.1), and the importance of such spaces in providing a great social platform for the people and gives good chances to enjoy, set, rest, and get socialized.

3. HISTORICAL REVIEW

Historically squares referred to open central space, centered in the old city occupied with different amenities, meeting places, trade, and celebrate, such spaces will give a great essential character to city, and it isn't a leftover space between the buildings. (Larry D., 1992, P.3).

Many studies have been done and specified the importance of city square, using different approaches to explain how it works presenting more than one factor for such space, must of these spaces related to two sides; design factors which if it is satisfying will lead to the other part of factors the social factors. This research noticed that many previous studies didn't takes in consideration all the factors that create this space, so this research will try to expose all the effective factors that create this square basically as a standard effecting its user socially.

M. Zawidzki, explained that, smallness, enclosure, compactness, and regularity presents four geometrical properties which is as a whole identified as geometrical evaluation proposed to support design tool for any square throw using questionnaire related to several human factors based on the geometrical properties for the square's plan, smallness, enclosure, and regularity as well as compactness. (M. Zawidzki,2016).

Nor and others (2014) explained different important factors within squares connected to social sustainability, and the most important ones, is the availability of public spaces with its articulated physical factors, such spaces have a multiple meaning with an excessive cause to encourage using squares. (Nor Zalina Harun & others.,2014).

Jack L. Nasar and Saleheh Bokharai, have been explained using factor for squares at night throw the availability of light, showing that uniform, bright, and overhead more closely estimates daylight conditions and offers clear views of what is around and inside this space, showing that inclination related with uniform, bright, and overhead may rise in comfort and safety. (Jack L. N., Saleheh B., 2017).

Halprin (1981) says that experience of the city is shaped by open spaces where people have the chance to involve with the communal city life. (Halprin, L.,1981).

While Khalilah Z., Nor Z. H., Mazlina M. (2014), presented the evolution of a square design and how the changes transformed people's use, from exposing the physical squares characteristics and analyzing spatial changes modify activities. (Khalilah Z., Nor Z., H., Mazlina, M., 2014).

(Achmad D., N., and Wahyuni., Z., clarified that quality of squares will affect the spaces uses and its activities, which it could be achieved throw the well manageable designed

to promote life quality. (Achmad D., N., and Wahyuni., Z., 2012).

Whilst **(**Capital Spaces 2012., A Design Guide for London's Public Spaces), in their guidelines introduced factors effects on creating a corporate space, which explained a commonly community square through increasing its flexibility to environment with a diversity of function to be an attractive space. **(**Capital Spaces, 2012).

Hossein S., and, Fataneh F., M; used another way in describing squares through the relation between visibility and connectivity, if there is a low level it will lead to low static activities, and the accessibility and sociability design for squares based on static activities, they depend on different relative factors such as; pathways, visitors, gender, races, and movement to explain these characteristics. (Hossein S., and, Fataneh F., M. 2017). Juliana F., B. and others has been clarified squares practices managements and the role of the green spaces, and how is some factors such (cleaning) spaces affects family's motivation to spend a lot of time, such factor responsible about maintains. (Juliana., F., B. ,2017), most of previous studies presents different factors for this important socialize space, explains that many different factors will create and effect the design of this space to be as social interactive for relaxation.

4. PUBLIC OPEN SPACE

Nowadays public open spaces considered as one of the main aspects of our environment, essential to our life, should be existis in any livable network to enhance our life value. (Boris J.2008, P.7). It is outdoor spaces with free access where the availability of different activities such as; cafes, retail, market, greens, streets and paths to maintain its sociality, to be a successful space while it becomes an encouraging one for social interaction between users. (Madanipour, 1999, P. 879), squares will be as a platform where people can be a part of the public land as a public space where people can physically become a part of the larger community (Khalilah Z., Nor Z., H., Mazlina, M., 2014, P.2). The importance of open space commonly associated with social and economic life of communities, new kinds of public spaces and meeting places are now being created in cities, as well as to be an important social resource for users. (Nor Zalina Harun & others.,2014, P.2), it is considered as one of the important urban environment elements which gives an excessive positive influence to life quality. (Achmad D., N., and W., Z., 2012., P.1), and this system of open spaces will introduce a presence image for 'energetic balance' in the human race. In our communal life there is an active balance between public and private activities, with a big awareness towards urban development which can be realized through different usable amenities by people in public spaces, square will be the awareness result where such spaces can be recognized as 'city lung' introducing fresh air and sunlight with providing a great chance of relax and enjoying. (Nor Zalina Harun & others.,2014, P.2). Growing and needing for open spaces resulted from the growing of public support for taking care of these spaces in our public daily life, awareness and support from, architects, planners, and landscapers, is needed. (Nor Zalina Harun & others.,2014, P.3). such spaces have an important value in many sides such as social, economic, and environmental scope, due to this diversity these spaces will attract users and be the site for different social actions, such as; meeting, festivals, and some other community gathering. (Pugalis, 2009., P.31), squares would be the public open space for gathering as well as attractive point in the city.

4.1. City Squares

City square has too many different synonyms, they also known as, plaza, civic center, urban square, market square, public square, or piazza, all will be an open space of an important and specific issues in urban design. (M. Zawidzki.,

2016., P1). city squares have more than one meaning to their users through encouraging the locals to use them within containing main important features like, a place to socialize, stress relief and relaxation, events and significant landmark for the city. (Nor Zalina Harun & others.,2014, P.8), such squares play a major role in providing a lot of benefits to population such as; integration, leisure and recreation. (Mariani, et. al., 2016., 569.), it is a collective open spaces where anyone can use, usually its main purpose for entertainment. (McColl, L., 2002. P.21), and would be a part of the urban green spaces. (Juliana., F., B. and others, 2017., P.2).

4.2. Definitions

city squares play an essential role in enhancing urban life quality, it will be the center of city events, social node, and as a physical void that offer breathing among the buildings.

To be a sociable place, this doesn't mean that strangers will always socialize with others, but it will indicate that people are comfortable to sit, or eat at this livable square. (Khalilah Z., Nor Z. H., Mazlina M., 2014. P1.). City Square has increased as a more intimate, active and flexible public space, at the same time as a green space (Khalilah Z., Nor Z. H., Mazlina M., 2014. P10.), such spaces provide an active platform within entertainment sense for its users, to reach this level such spaces need too many articulated factors to introduce leisure, to be the gathering space, safety place, and for sport practicing. (Juliana., F., B. and others, 2017., P.5,6), moreover they are public open spaces with a lively atmosphere serve people as well as their city with different commonly activities and practices, such space usually connected to the surrounding streets with a high visibility and flexibility. (Report On [Pospd].,. 2009, P.44), squares are open spaces designed for public use, and to be expressed through its surrounding buildings and streets, its main function giving different opportunities for social interaction within safety and useful outdoor spaces. (Larry D.,1992, P.3). Generally, City square is for a public use where interaction besides gathering between users have been introduced with an urban furniture, to reach its main function which is, social gathering and entertaining users.

4.3. Squares Influence in The City

The Industrial revolution have been introduced a new theoretical origin of garden city tradition changed by Ebenezer Howard in 1898, besides he introduced ways to protect the natural sites and urban green area, putting in mind the importance of the visual and functional aspects for visitors, without neglecting the environment influences, environmental psychology and the behaviors. (Nor Zalina Harun & others.,2014, P.3). Each city need an open area to be a breathing nodes for users, so square must promote the use of such spaces as a different attractive features encouraging users to connect within the space as well as people, giving a special identified character for the square, different activities play a major role in feeling the essence of such spaces as; café, restaurants, seating area, welcoming entrances and pathways. (Urban Design Principles and Development Guidelines., 2004., P.21). These spaces will affect people lives and suggest all user categories to corporate with these spaces within variety of opportunities; community spaces, and civic elements, to reflect the 'dominant' activity and contain both new and historic identity. **(**Capital Spaces2012., P.5), squares have a very great influence in supporting social activities in any city, to be the place for recreational interaction where crowds are found at every intersection merging in different types of activities sitting, playing football, selling, buying, and the availability of different features such as, seating benches, toilets, food shops, that would satisfy user's needs, being a great platform for different social activities. (Nor Zalina Harun & others.,2014, P.8,9).

Squares will have a very great impact on attracting variation of people not only the locals but others who come out from the city, due to the identity and image quality for these spaces which is connected with city history, with a great character that no one will forget, it will give the spaces a positive energy for welcoming and inviting people as well as enhancing the city identity.

4.3.1. Local Influence

There is a great relationship between the physical characteristics of the City Square with its sociability and livability as an urban space, it needs to have a strong connection with its users, physically, socially and environmentally, essentially this comes from the unique role for square to its immediate context, climate and local culture, due to that number of important factors have a great effect on how people inhabit the square and how it can function probably, considering the intangible qualities that make the square what it is. Basically, square is a space for people. (Khalilah Z., Nor Z. H., Mazlina M., 2014. P10.), which would exploit on surrounding context, as well as focusing to the main pedestrian flows, allowing grouping of public spaces to form. **(**Capital Spaces.,2012., P.17), then through careful design details, it will contribute and increased the usages of this space specially if it has been applied with both day and night activates. (Urban Squares, 2009, P.4).

In order to invite people to walk in and use this space, multiple layers have to be added, the function of attraction to invite the users, to sit or talk, or to walk. (barbara G.,Darby W., 2009.P9), so each square would have a special identity and character related with local environment to be a social platform for the users.

4.3.2. Human Influence

The theory of social needs in an open space is actually based on the concepts of needs and work, it is the activities which applied to satisfy human needs by considering the connection process between society and nature focusing on human action and nature relationship, which influences the stability of natural resources.

The success of this square is still uncertain since the success of a particular public space is not solely in the hands of the architect, urban designer or town planner; it also relies on people accepting, using and managing the space, in other words, people make places, as much as places make people,to create this social space. (Nor Zalina Harun & others.,2014, P.2,3), and in order to be a social space it must have sidewalks, seating bench, some usable equipment for all ages like playing machines, playgrounds for children, (Juliana F., B. and others, 2017., P.5), which it will effect positively on using and staying.

Squares attract people as an active, exciting, and safe place, and generally all around the world and despite the cultural and local differences these squares will have the same general characteristics. (Urban Squares, 2009, P.2), and usually attractive square provide a platform for an elective and social activities, to be a welcoming and attractive space within providing; exercise, recreation and playing for different ages, children, teenager, and adults, as well as acting act as an urban meeting space. (barbara G., Darby W., 2009.P.43). finally, this space would show a positive experience for urban living, through housings a variety of activities for users like, open markets, resting, eating, and exhibition, such actions enhance diversity and people interaction inside these spaces, as well as increasing user's awareness towards these vital spaces.

4.4. Squares Success

Square success depends on user's ability to get in to these spaces, to communicate, and its ability to work as platform to support social, comfortable, and safe environment, it designed to be an open space where people sit, walk, stand or perform different social activities. (Urban Squares, 2009. P.2), it need to be active spaces through using multiple actions, like

retails, restaurant, and others which attracts pedestrian to function in it. Architectural styles and historical buildings and elements in these squares play a major role throw enhancing its successful level. (Urban Design Principles.,2004. P.32), besides these important elements and its existing, it should be well used, and the most effective connection for using spaces is; providing safety, throw using defensible spaces, good lighting, and clear views. (Larry D., 1992, P.5).

A good sign for a successful square is the always using it, and it could work through a combination of two important items;

- Number of people in the square.
- Time spend by people.

Using spaces all the day with a long period of time make this space a successful one, on the contrary it will be unsuccessful space if few people hang there or within short period of time, some effective details could make the square a successful space, as emphasis on the designing an attractive ground floor façade close to square space to be inviting and attractive. (barbara G.,Darby W.,2009.P.7, 88). Adding an Architectural element in squares would have a great effect on staying and using these spaces.

4.5. Squares Factors

The importance of squares has a great impact on raising the vitality of these spaces throw some special functional factors, such as settable space, food sellers, sculptures, and vegetation, strengthen the liveliness of these important squares. (Abdulkarim & Nasar, 2014a, b).

There are different activities that enhance the social influence in squares, it effects a lot on people gathering throw variety of activities, such as, football, volley ball, basket, wall-climbing, run, and physical exercise, even just sitting, strolling or talking with friends, will give comfort, safety, and entertainment. (Achmad D., N., and W., Z., 2012., P.5). M. Zawidzki explained three basic factors for a square design that may shape the space, give it an identity as well as affecting people;

Regularity: it is a natural quality in human perception of a shape, and even with the human ability to percept, mathematical measurement of shape's regularity seems still problematic.

Historical background: if any square had a strong historical background or have been evolved over centuries it will consider as a point of reference for urban composition related to squares quality.

Quality: if the square does have an architectural aspect, will give meaningful evaluation of a square's quality, with the architectural quality of surrounding buildings effects positively on square perception. (M. Zawidzki., 2016., P.7,9,11).

Due to that the research will introduce several factors divided to two interrelated categories, the first one connected to social factors while the other one to design, reaching by that a unified image to obtain comparative factors for integrated design of city squares.

The most effective commensurate factors related to requirements of the international standards, without overlooking the local and heritage values of our country as well as our city, so the research reveals two effective factors connected with a secondary matching factors.

4.5.1. Design factors

4.5.1.1. Image & Identity; if such spaces works as welcoming, inviting open space with different using facilitates, it will insure city image as well as the square image. **(**Capital Spaces.,2012., P.13). Usually such factor related with the local identity and held cultural features.

4.5.1.2. Connectivity; squares should be sociable and healthy enough in order to facilitate people's varied activities, squares should be well connected to other spaces rather than dividing it in to separated parts, offering people opportunities to do unplanned activities.

(Khalilah Z., Nor Z., H., Mazlina, M., 2014.P.2)

4.5.1.3. Accessibility; an approachable way from all sides and directions within availability of different civic uses, to clear accessibility, with providing an inviting and attractive entrance to the space, easy for users to enter. (Capital Spaces.,2012., P.9,17).

4.5.1.4. Visibility & views*;* linkages with eyes and feeling restful within the space, as well as clear leading streets to square will reveal its attraction, to work from two sides at the same time, as a safe place for its users and keeping connection with adjacent streets, by using walls and planting without blocking off the square, (Larry D., 1992, P.4).

4.5.1.5. Appropriateness*;* design and shape of square can be better defined if it used common types, that anyone can realize and recognized directly. (Report On [Pospd], 2009, P.8)

4.5.1.6. Functionality; the motivating energy of squares, determine as a part of the overall design practice. (Larry D., 1992, P.4), throw offering the best usable and functionally usages that users can adopt inside the space.

4.5.1.7. Flexibility; it is a unique characteristic of such space, may gain a certain uses and stable forms, have the potential to change and provide the space for surprising activities and special events, to be more active and attractive space, and to contribute to civic life it should add function for enhancing occasional events within multi-functional outdoor furniture to connect with the surrounding environment. **(**Capital Spaces.,2012., P.135,20), means square adapting to multiple different activities. (Report On [Pospd].,2009, P.9).

4.5.1.8. Usability; having a good balance between the space itself and its facilities, make it easy to realize, leading for more using. (Report On [Pospd].,2009, P.9). easy, readable, and clear.

4.5.1.9. Inclusiveness; squares design to work as a space that attract people at different ages by using irregular shapes give the sense of renewing to create a social interaction. (Report On [Pospd].,2009 P.9).

4.5.1.10. Linkage; such factor will have great impact on giving the sense of space and its identity, spaces should be well integrated to urban fabric connecting to street network and pedestrian path way. **(**Capital Spaces., 2012., P.13), as well as linked to the surrounding open spaces and to insure the pedestrian floating to increase the square usages. (Larry D., 1992, P.5).

4.5.1.11. Landscaping issues, landscaping within its different elements is a vital aspect in squares, using greens and low level planting to indicates sight lines and lining walkways, trees for giving enclose sense and other art features as an essential part for enhancing comfort feelings, **(**Capital Spaces., 2012., P.16).

Merging the green elements within squares as a part of infrastructure, gives the users a physical, perceptive and social comfort, effecting the frequency attendance for them and introducing a good reason for using these squares, merging within intended activities such as physical-kinetic, leisure and social activities. (Nor Zalina Harun & others.,2014, P.9), these greens should consist of both soft scape and hard scape with a central feature, like fountain or iconic element. (Town Centre Design Guidelines, 2011. P.20), and usually social interaction in such spaces is expected if its design enhanced with green and connected to the organic environment which have too many helpful issues such aiding pedestrian movement. (Hossein S., Fataneh F., M., 2017., P.8).

4.5.1.11.1 Major and Minor space; squares should be in a leveled area and size not too small so the users to feel uncomfortable, too clogged, not too big so they tend to use the edges and the boundaries. (Urban Squares, 2009, P.4), a good connection between the main space in the square with some other smaller ones within a clear transition to major space will affect the space of possible activates. (Report On [Pospd].,2009, P.35).

4.5.1.11.2. Enclosure*;* using short shrub, planting, low walls or fences usually

transparence will provide a great opportunity to get the feeling of an identified space.

4.5.1.11.3. lighting; one of the factors which have a great effect on the squares impression and its users at night, hence it will be usable even after the sunset (Jack L. Nasar, Saleheh Bokharae, 2017, p1), people would desire uniform, bright, and overhead lighting with focusing on lighting effect, such as illuminance, shadowy space distribution, and spatial distribution to verify too many important factors like, safety & security, clear spaces and views. (Boyce et al., 2000; Fotios, Unwin, & Farrall, 2015), giving the users better way to see around and indicates the space at all.

Lighting effect factor could vary according to its relation with many other aspects such as; sidewalks or parking lots, or having a special contexts related to, openness, neighborhood, size and type of building adjacent to square, and users number, merged with sitting space, vegetation, food retailers, to emphasis on safety and space livable. (Jack L. N., Saleheh B.,.2017, P.8).

4.5.1.11.4 Amenities*;* furnishing the square with different amenity structures will encourages the users to stay and use the space improving its liveliness through creating an attractive node within the essential component to the whole square design. (Larry D., 1992. P.11).

4.5.1.11.5. Clarity; this factor will fulfill squares, the difficulty of finding pathways causes stress for the users to be uncomfortable space, clear leading streets connected to main pathways will improve site integration to be easy used. (Hossein S., Fataneh F., M., 2017., P.8).

4.5.1.11.6. Attractiveness*;* the quality of being pleasing and have the ability to control features that brings interest to users inside square, could be proved throw introducing multiple layers of rest areas, good integrated design.

4.5.1.12. Availability; too many elements would work together in order to give the sense of space using, as a significant square. landscaping should have applied within highest quality to provide well designed locating for public activities throw design process to be planned to held different attractive public actions such as weekend market, yearly held festival, national holiday celebrations, or to be a site for a public art fitting. (Urban Design Principles and Development Guidelines., 2004. P.21).

4.5.1.13 Spatial issues; in this factor three dimensional lines will work together to give a space feeling throw identifying them with a clear edge related to square shape and its width and length. (Report On [Pospd].,2009, P.13). such related spatial issues;

4.5.1.13.1. Transparency; the adjoining uses for the street and its visual accessibility related to squares attraction will add a positive practice for users, that mean landscaping elements, features and booths or food stalls preferred to occupied with a transparence material to increase sociality. (Town Centre Design Guidelines, 2011.P.18), no sold barriers to be used and no too high walls or fences that could interrupted the sight, it has to be short planting or fences.

4.5.1.13.2. Contextual relationship of site; the immediate context relation to square played a vital role in supporting square life. Historical, religious, administrative and commercial buildings surround the city square, as a places people go and use daily, gives a great identity to such space.

The role of the square needs to complement people's activities in outdoors as a way for enhancing their life quality, hence the most important square designs are to pay close attention to the social needs of its users. (Khalilah Z., Nor Z., H., Mazlina, M., 2014.P.10), square design should respect the special local context in the site to get a high quality of spatial design issues for this space. (Report On [Pospd].,2009, P.25).

4.5.1.13.3. Location; one of the important factors that made up this space and transport activities in to this social node is the center location in the city, and when it enclosed by buildings it will generate a sense of defining

this space in its location. (Urban Squares, 2009, P.7), and squares should reflect the character of its location. (Larry D., 1992, P.3), so the importance of Neshteman location in; the center of Erbil city and to the most iconic historical building (Erbil Citadel), this have to put in consideration when applying any design concepts or elements in this space.

4.5.1.13.4. Design Integration*;* all movement directions should explain the continuous feeling design into the existing context, connecting to the urban fabric and streets to strengthen integration to whole and enhance space sense. **(**Capital Spaces.,2012., P.13).

4.5.1.13.5. Area; it is the available specified space for a specific use, that should be done through the balance between the area and activities in the space, too big spaces without controlling design and sufficient amenities will affect the human scale. (Report On [Pospd].,2009, P.35).

4.5.1.13.6. Historical landmark*;* this element among the essential characteristics in leading and connecting to the spaces, gives a very well unified approach to the local level, such physical and social aspect lead to populate people in any open space. (Hossein S., Fataneh F., M., 2017., P.4,8), such as the attractive elements close to Neshtiman Park, Erbil Citadel, and the old Bazar.

4.5.1.13.7. Martials*;* using materials in a regular pattern within a high quality will provide legibility to users, as well as providing a good design for people using these square, **(**Capital Spaces., 2012., P.9).

4.5.1.14. Maintenance*;* squares should manage its own cleaning and maintenance staff, and had a very quick response to any failure issues. **(**Capital Spaces., 2012., P.9).

All those factors summarized by researcher through depending on the most important papers in a table as shown in (table 1).

4.5.2. Social Factors

One of the important factors that enhance the psychological level for the users in squares, is to keep the green infrastructure in touch within the whole design to improving social well-being for users, (Nor Zalina Harun & others.,2014, P.9), and as Khalilah, Z. and others said "*sociability is defined as the ability for the public to become attracted to a space that allows them to conduct social and leisure activities, whether individually or as a group*". (Khalilah Z., Nor Z., H., Mazlina, M., 2014);

4.5.2.1. Safety; square should provide a secure area enough for users to use and socialize with the surrounding environment and with others, **(**Capital Spaces.,2012., P.14), and one of the important related factors for enhancing safety is that, squares should provide lighting along the main route for at night, (Larry D., 1992. P.6), that mean using squares without any threat, it needs to be safe and look safe. (Report On [Pospd].,2009. P.11).

People need to feel comfortable during day and night in order to keep using and visiting squares through producing a friendly environment as a welcoming and inviting one. (barbara G.,Darby W.,2009.P.67).

4.5.2.2. Comfort*;* comfort side connected with safety, it could be achieved throw the well manageable for squares activities that occur all the day, supporting with different amenities as shops, restaurants, and café. (Achmad D., N., and W., Z., 2012., P.5), with providing too many sufficient facilities to be installed working together to get the sense of a comfortable spaces such as; seating, landscaping, shelters, combining with the whole square design. **(**Capital Spaces.,2012., P.15).

4.5.2.3. place for socialize*;* connection as well as diversity of factors, and in order to indicates wither this factor is enhancing the square quality, it depends on what the people need, how they interact, and which facilitates make these interactions. (Kallus, R. ,2001, P.129.), it designed to be a space for interact, connect, and support different activities which

supports social and cultural life for its users. (Urban Squares,2009, P.1).

4.5.2.4. Place for event, and historical significant; connecting people to different elements around them, streets and buildings where they can gather and sit away from the city's traffic and busy streets, one of its effective carchter. (Carmona, Heath, Oc, & Tiesdell, 2003).

4.5.2.5. Variety of Activities (Diversity); the availability of different retail spaces, shops, café, and restaurants, offers a great opportunity for more site using, even more could attract people for socializing in this spaces, like providing health club, dry cleaners and even supermarket. **(**Capital Spaces.,2012., P.17), and as it offers social and cultural activities generally will effect on enhancing social interaction, and definitely public development will have increased the activity levels for squares. (Urban Squares, 2009. P.3).

researches	Design factors																										
		Connectivity		Appropriateness					Landscaping issues							Spatial Issues											
	Image & Identity	Accessibility	Visibility & views	Functionality	Fxibility	Usability	inclissivness	Linkage	Major and Minor space	Enclouser	Lighting	Amenites	Clarity	Attraction	Availability	transperency	Contextual relationship of site	Human Scale	Location	Design integration	Width & Length of the street	Defenceble space	area	historical landmark	materials	maintenance
1- M.Zawidziki		•							•	•										•						
2- Nor zalina	•																							•		
3- Abdulhakim &nasar									•		•															
4- Reed 2011		•	•		•		•	•	•		•	•												•		
5- Achmad Delianor	•	•	•					•	•			•						•		•				•	•	•
6- Pablo Marti		•					•													•						
7- project of Public Spaces (Danial maft 2005)	•	•	•		•		•	•				•		•												•
8- capital Space/Cosumption Space 2012	•	•			•		•	•	•	•		•					•			•						•
9- Hossein Safari 2017		•	•														•							•		
10- Mohammad raza 2013																								•		
11- juliana 2017		•							•																	
12- helth Space &Space		•					•		•			•														
13- Garrette 2004		•	•				•					•					•									
14- harvard Gild lines 2000		•	•				•	•	•		•	•						•		•				•	•	•
15-Plaza design Guidelines 1992		•	•				•	•	•		•	•							•	•		•		•	•	
16- Town Center Design Guild line 2011		•	•				•		•			•				•	•								•	
17-Report on consultant study on public open Space in private development		•	•	•	•	•	•	•	•	•	•	•	•	•	•					•	•		•				
18-open Space Combo	•	•			•			•	•	•	•	•					•			•				•		
19- P.o.s in new housing development						•		•				•														•
20- public open Space guidelines				•													•			•				•		•
21- guide 10	•								•	•	•	•								•						•
22- Mayor Strategies in open spaces		•		•					•	•	•	•					•			•		•	•			

(Table 1: - The most important Design factors in Successful squares).

4.5.2.6. Accessible; many elements work together for enhancing easy access, such as surface materials to accommodates with different activities holding in square to provide easy direct access. (Larry D., 1992. P.5) it means; how suitable a space could be

visited or avoided by the daily users. (Report On [Pospd]. 2009, P.8).

4.5.2.7. Noise; usually noise reduce the enjoyment of the squares specially when it is generated from high traffic and some other annoying ambient noises, somehow it is difficult to control on such factor due to its direct contact with the street, by switching to

another attracting element would have a great impact on detracting attention from noise, such as fountain. (Larry D., 1992. P.8)

4.5.2.8. popular space*;* usually related with too many factors, a historical building, diversity of activities, availability of services, and other architectural elements that gives an identity to squares to have the popular quality.

4.5.2.9. Vitality (vibrancy*);* one of the most effective factors that adapt attraction to the square, different activities and action within the space especially at night will make this

researches	Social factors														
	Safty	Comfort	place for Socialize	place for Events	historic significant	Variety of activites(diversity)	Accessible	noise	populer space	Vitality (vibrancy)	interesting	vissible	interaction With environment	Homogenization	Commrcialization
1- M.Zawidziki		●	●	●		●	●	●			●			●	
2- Nor zalina		●	●	●	●										
3- Abdulhakim &nasar										●					
4- Reed 2011				●				●	●			●			
5- Achmad Delianor	●	●	●	●			●						●		
6- Pablo Marti	●	●	●			●	●		●				●		
7- project of Public Spaces (Danial maft 2005)						●	●								
8- capital Space/Cosumption Space 2012	●					●				●				●	●
9- Hossein Safari 2017			●	●		●	●					●			
10- Mohammad raza 2013			●						●	●					
11- juliana 2017		●				●							●		
12- helth Space &Space	●	●	●						●	●			●		
13- Garrette 2004															
14- harvard Gild lines 2000	●			●	●	●				●		●			
15-Plaza design Guidelines 1992		●				●		●							
16- Town Center Design Guild line 2011	●	●													
17-Report on consultant study on public open Space in private development	●	●													
18-open Space Combo		●	●			●							●		
19- P.o.s in new housing development															
20- public open Space guidelines		●			●	●									
21- guide 10															
22- Mayor Strategies in open spaces															●

(Table 2: - The most important Social Factors for Successful squares)

space usable one most time, as well as the number of this activities which effects on attracting more users to add a high level of vitality to squares. (barbara G.,Darby W.,2009.P.66).

4.5.2.10. liveliness*;* when the design square influence people usages patterns in a good

relation with the local climatic factors, for example; offering shaded areas, being close to the shops, cafes, buildings, and street will reveal as one of the significant items that brings livability to square. (Khalilah Z., Nor Z., H., Mazlina, M., 2014. P.10), to make the square an interesting space.

4.5.2.11. Visible; squares should have been seen as we arrived to the site, easy to realize, in order to be used, easy to increase visibility though applying more leveling in space and more transparence materials instead of using solid ones. (Report On [Pospd].2009, P.8).

4.5.2.12. Interaction with environment*;* integration within human interaction and physical features within the ecosystem in will gives meaning for using these squares daily and occasionally to improving local community which can be amplified by increasing commercial development and the whole integration lead to approach the local for protecting and managing their spaces. (Nor Zalina Harun & others.,2014, P.9), connecting these squares with nature considers as one of the important factors of inspiring users to use square and spend some time. (Juliana F., B. and others, 2017., P.6).

4.5.2.13. Commercialization; different formal and informal activities or some other small selling booths such as; coffee stands, and newspaper will attract people to stay longer time, square will be active day and night letting social interaction to take place there. (Urban Squares,2009, P.7).

Factors summarized by researcher through depending on most important papers as shown in (table 2), to be the important guidelines standards that research would depends on and analyzed to reveal the square success in Erbil city.

5. THE PRACTICAL PART

This part discusses and analyze research methods that used to comprehend the research hypothesis and achieve its goal.

5.1. Neshtiman Park

Neshtiman Park has been opened on the 21st of March 2012, on Nawroz ceremony, to be a celebration space not only for Erbil people, but also for all Kurdistan population and Iraq's people as well as, and those who are visiting the city from aboard. Erbil city capital of Kurdistan becomes more popular by its main square in the city center, which was established by the ministry of Municipality-Director of parks. As shown in (figure.1).

(Figure 1: Erbil city square)

5.2. Study Process

5.2.1. Site Analysis

Researchers try to analyze the important factors of Erbil city square, some of them by site visit and, others by survey, then illustrate them by using (Auto-Cad) and photos. as followings: -

5.2.1.1. Context

Neshtiman Park covers an area of approximately (17000m^2), in the center of Erbil city adjacent to Erbil Citadel, in front of Neshtiman bazar, surrounded with a series of commercial shops which complements the whole image of the space. It is (1km) away from (30m ring road). As shown in (figure 2).

(Figure. 2: Neshtiman Park)

5.2.1.2. Design Characteristics

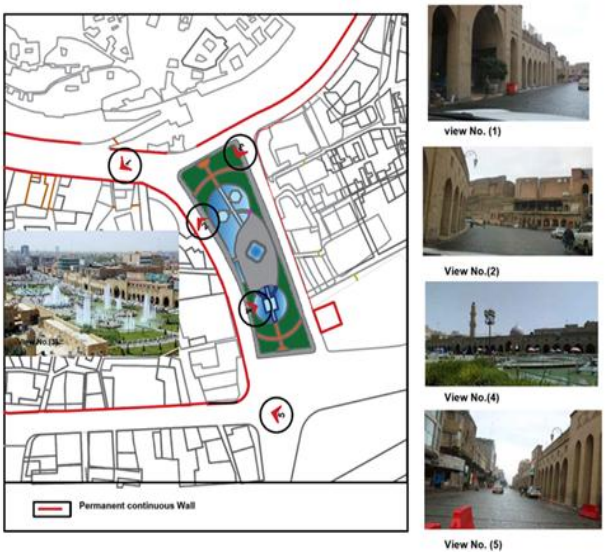
- **Land use;** Variety of land use such as, commercial, governorate, masjid, coffee shops, and restaurants have been added in the site. as shown in (figure 3)
- **Linkage**; Erbil City square contains; streets, sidewalks, leading way for adjacent buildings, this will be as the octopus's tentacles extending into surrounding spaces, as it is clear in (figure 4)
- **Permanent wall**; A permanent continues wall linked the citadel with the edges surrounding the park, as shown in (figure 5)
- **Accessibility;** Square was surrounded with three main roads, to the south the main intersection of the bazar, to the north surrounded with the (15m) road adjacent to citadel, to the west there is the (15m) road attached to Erbil grand bazar (Qaisary), the square attached with the pedestrian (12m) to the east side, which overlooks to series of coffee shops, restaurants, Masjid and (Baxi shar) garden, as in (figure 6).

(Figure 3: Existing land use)

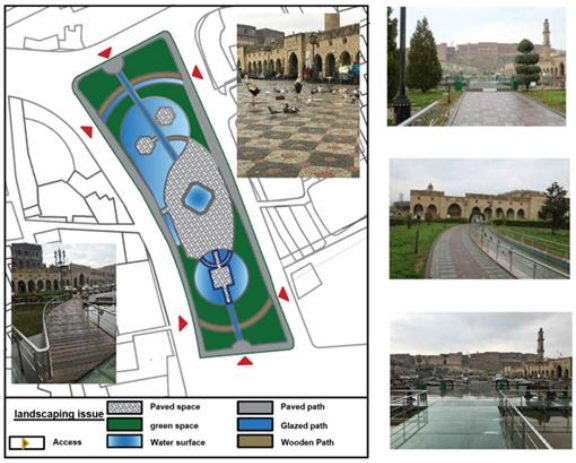
- **Landscaping issues;** Erbil square design classified in to main and secondary spaces which has too

many different hardscape and soft cape such as, green, paving, water surfaces, with sitting benches, sculpture, fountain, and colorful planting to strengthen the whole design and showed as one platform. Light have been distributed along pathways and around the green spaces as well as inside the water bodies and the fountain, giving a remarkable impression specially at night, as shown in (figure 7,8, and 9).

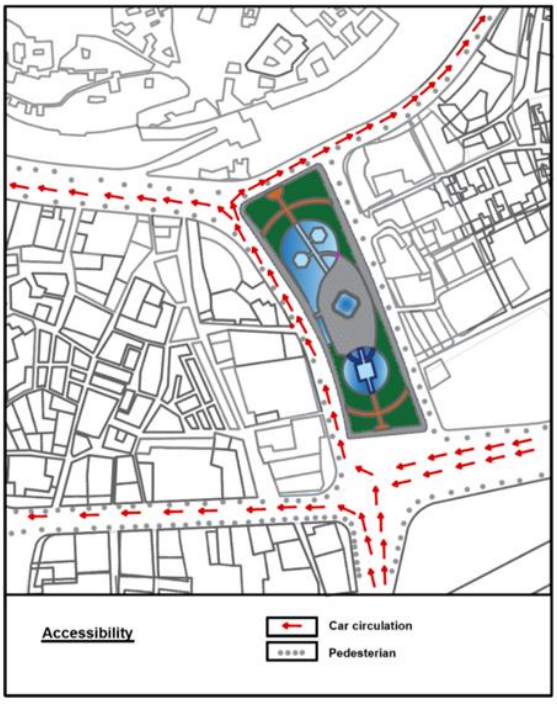
(Figure 4: Linkage with surrounding)



(Figure 5: permanent wall)

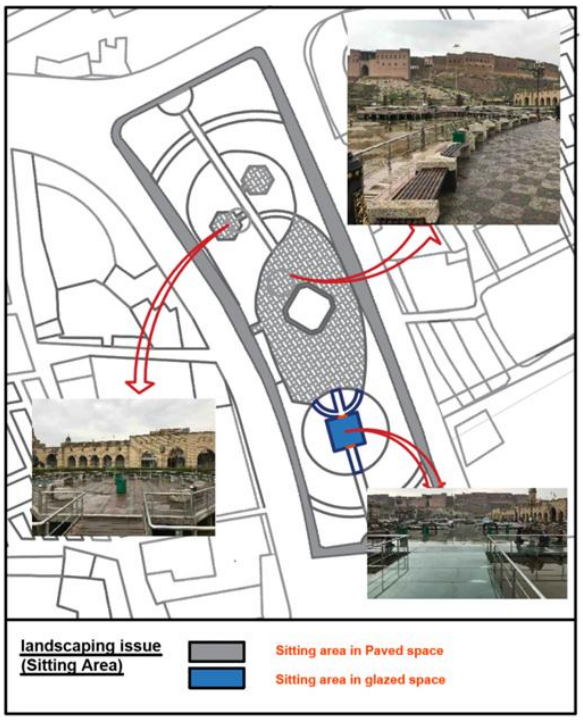


(Figure 7: landscaping issues)



(Figure 6: Accessibility)

(Figure 8: landscaping issues, green, fountain, and lighting)



(Figure 9: landscaping issues, sitting area)

5.3. Evaluation Method

The process of evaluation has been done in to two ways:

5.3.1. Data Collection

5.3.1.1. Architectural design data collection: - It refers to the questionnaire which have been submitted to a specialist people, (designers, town planners, city developers, and urban designers), for assessing Erbil city square in term of "Design guidelines" as shown in (appendix 1).

5.3.1.2. Social life data collection: - refers to the questionnaire which is directed towards ordinary people in order to indicate the quality of social life in Erbil city square from people point of view and their social need and desires in such spaces, as shown in (appendix 2).

5.3.2. Data Collection Analysis

This can be illustrated throw two simple ways;

- **Using table to show;** rate of design factors satisfaction in Erbil square as shown in (Table 3), rate of social life agreement as shown in (Table 4).
- **Using bar chart to show**; evaluation of design factors as shown in the figures (10,11,12,13,14, and 15). And the evaluation of social life, as shown in the figures, (16, 17, 18, 19, 20, and 21).

	Image & Idntity	Accessibility	Visibility & views	Functionality	Flexibility	Usability	Inclusivness	Linkages	Mjor and Minor space	enclouser	Lightings	amenites	Clarity	Attractivness	Availability	Transperency	contextual relationship of site	Human Scale	Location	Design Integration	Width &Length of Streets	Defensable Space	Area	Historical Landmark	materials	maintenance
very Satisfied	13	5	10	5	4	4	7	13	1	3	11	5	2	5	7	4	9	8	15	3	0	3	5	16	10	0
Some what Satisfied	10	15	15	10	7	10	8	9	17	7	12	16	17	14	17	8	12	13	10	2	10	7	13	12	10	6
Neutral	8	8	6	9	8	15	9	7	7	9	6	12	11	10	3	12	9	7	5	15	11	14	9	4	11	10
Some what Dissatisfied	3	5	4	9	8	5	9	5	10	10	5	1	5	3	6	9	3	5	4	7	11	9	8	2	2	12
very Dissatisfied	1	2	0	2	8	1	2	1	0	6	1	1	0	3	2	2	2	2	1	8	3	2	0	1	2	7
	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35

(Table .3: design factors Satisfaction of Erbil city square)

values	Safety	Comfort	Social factor	Event place	Historical significant	Diversity	Accessible	Noise	vitality	populer	interesting	Vissible	interaction with environment	Homogenity	Commercialization
totally agree	25	22	22	16	45	41	27	21	19	41	13	17	11	8	31
agree	42	35	47	40	26	25	34	45	41	36	36	47	38	24	49
Neutral	18	31	21	30	13	27	15	23	34	15	31	21	26	43	13
dis agree	9	10	7	7	11	7	17	8	4	7	16	13	22	20	4
Totally disagree	6	2	3	7	5	0	7	3	2	1	4	2	3	5	3
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

(Table 4: Social life agreement in Erbil city square)

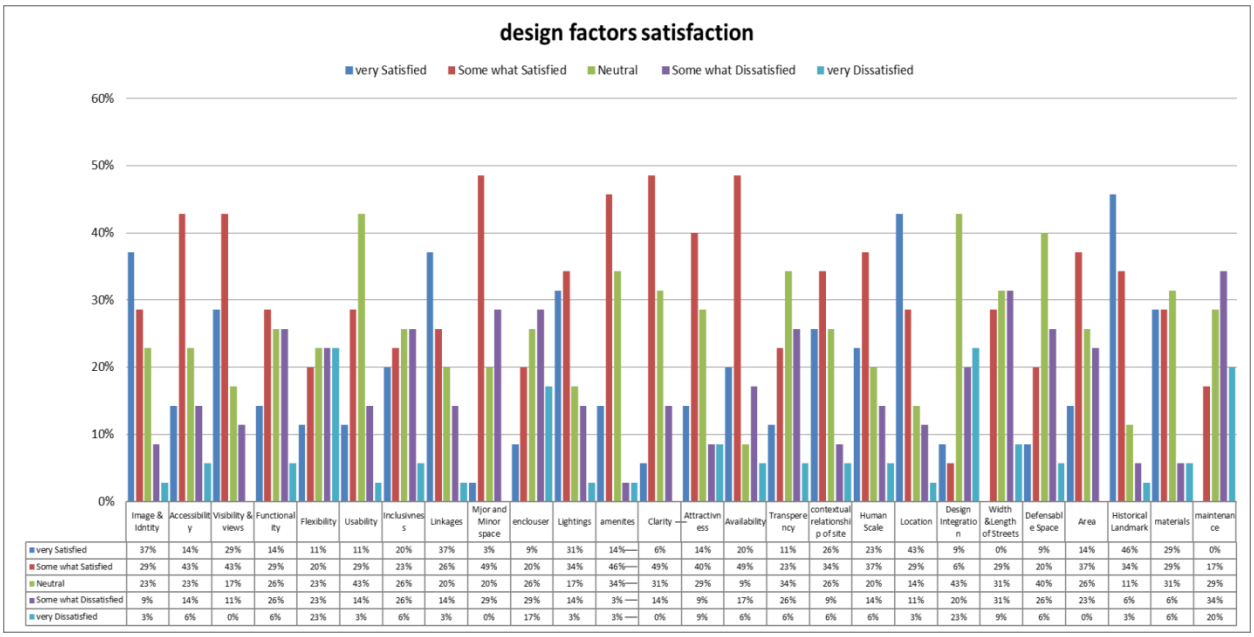
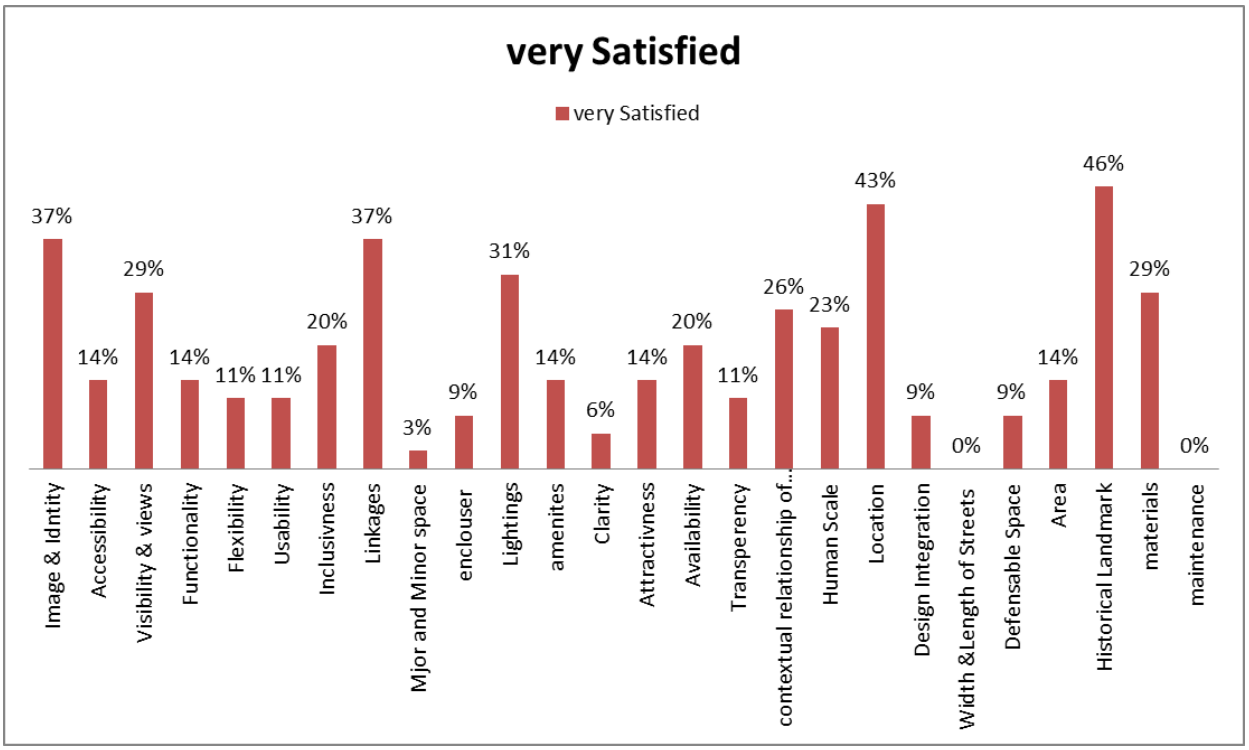
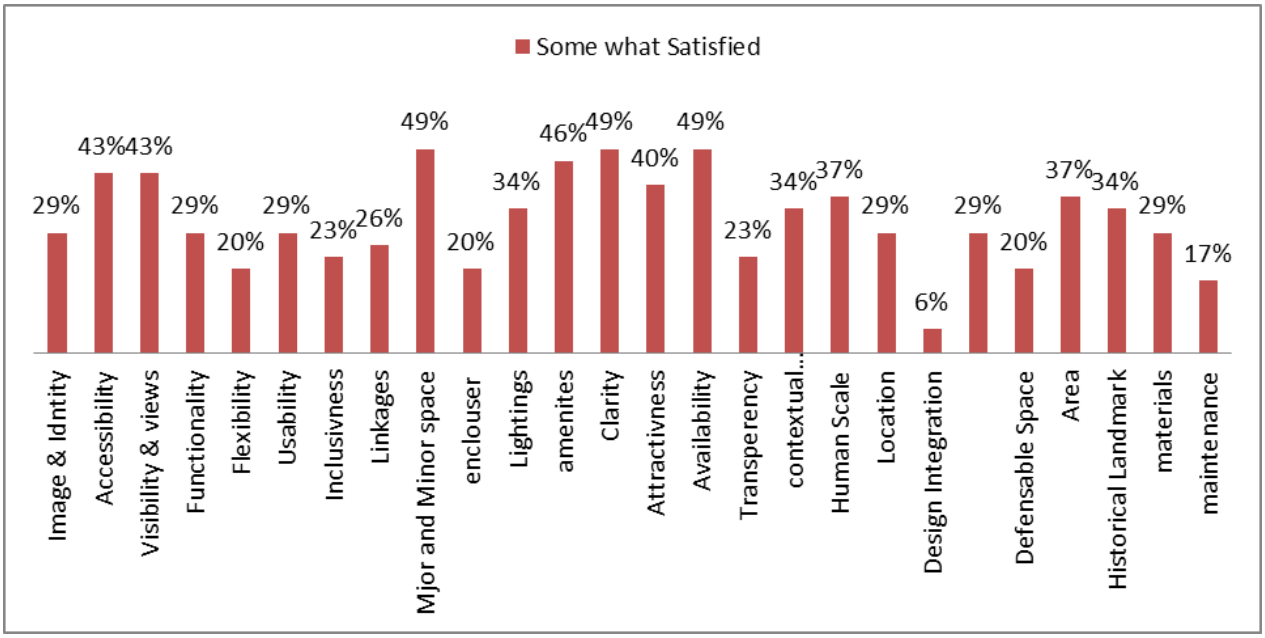


	Image & Idntity	Accessibility	Visibility & views	Functionality	Flexibility	Usability	Inclusivness	Linkages	Mjor and Minor space	enclouser	Lightings	amenites	Clarity	Attractivness	Availability	Transperency	contextual relationship of site	Human Scale	Location	Design Integration	Width &Length of Streets	Defensable Space	Area	Historical Landmark	materials	maintenance
very Satisfied	37%	14%	29%	14%	11%	11%	20%	37%	3%	9%	31%	14%	6%	14%	20%	11%	26%	23%	43%	9%	0%	9%	14%	46%	29%	0%
Some what Satisfied	29%	43%	43%	29%	20%	29%	23%	26%	49%	20%	34%	46%	49%	40%	49%	23%	34%	37%	29%	6%	29%	20%	37%	34%	29%	17%
Neutral	23%	23%	17%	26%	23%	43%	26%	20%	20%	26%	17%	34%	31%	29%	9%	34%	26%	20%	14%	43%	31%	40%	26%	11%	31%	29%
Some what Dissatisfied	9%	14%	11%	26%	23%	14%	26%	14%	29%	29%	14%	3%	14%	9%	17%	26%	9%	14%	11%	20%	31%	26%	23%	6%	6%	34%
very Dissatisfied	3%	6%	0%	6%	23%	3%	6%	3%	0%	17%	3%	3%	0%	9%	6%	6%	6%	6%	3%	23%	9%	6%	0%	3%	6%	20%

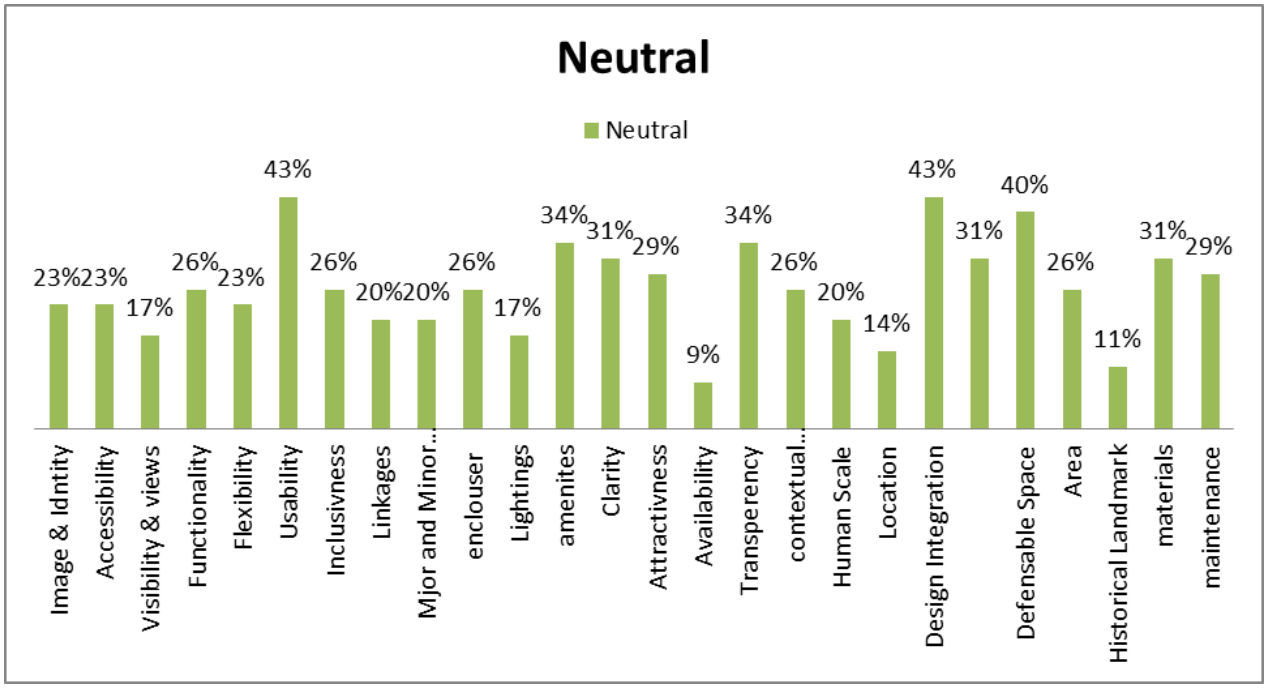
(Figure 10: design factor satisfaction)



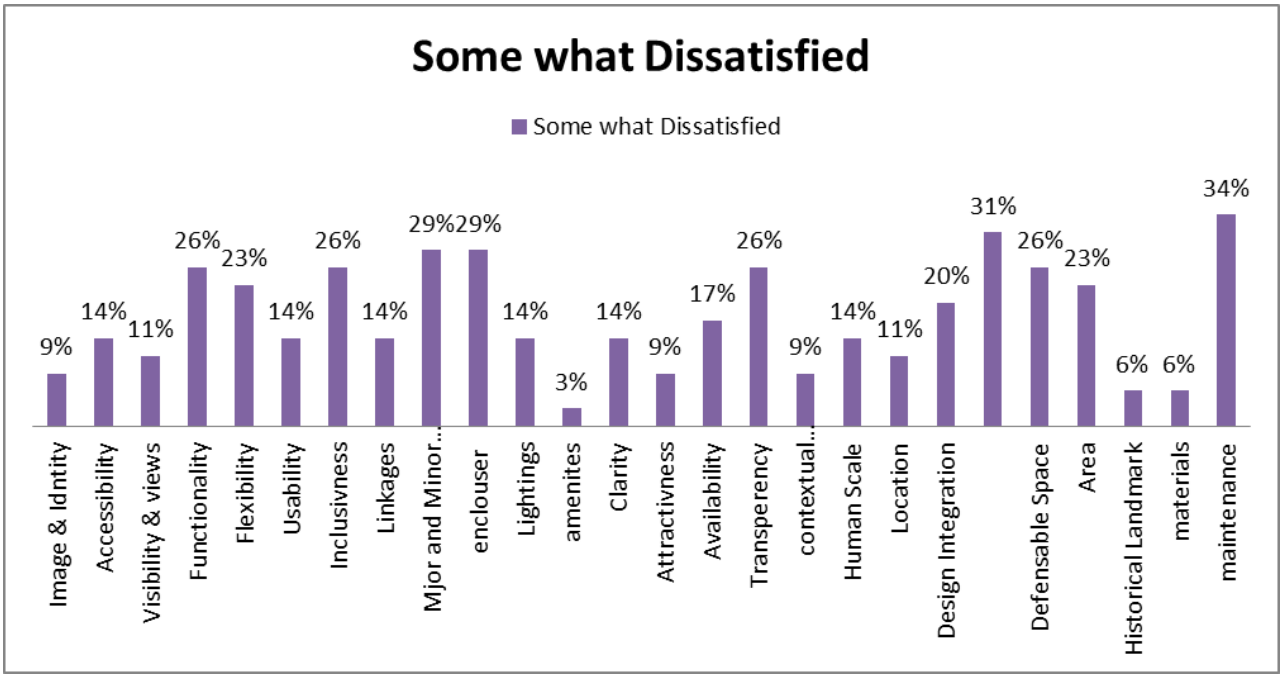
(Figure 11: very satisfied design factors)



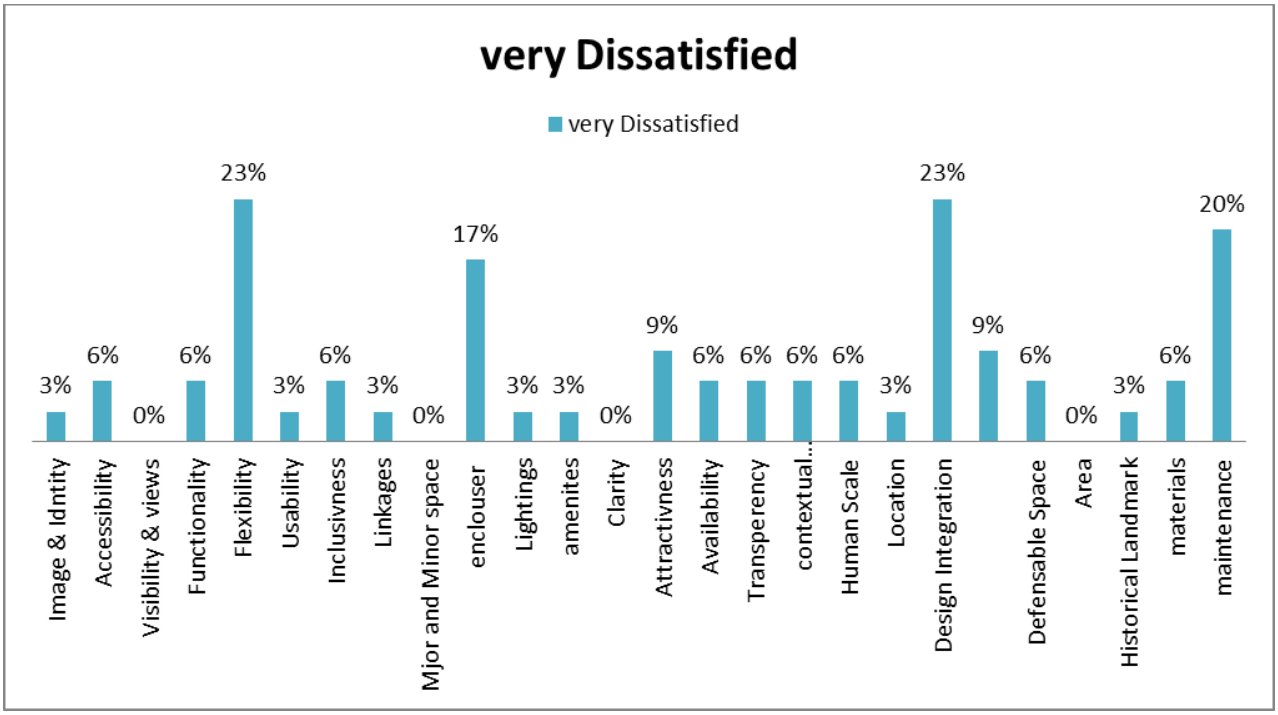
(Figure 12: somewhat satisfied design factors)



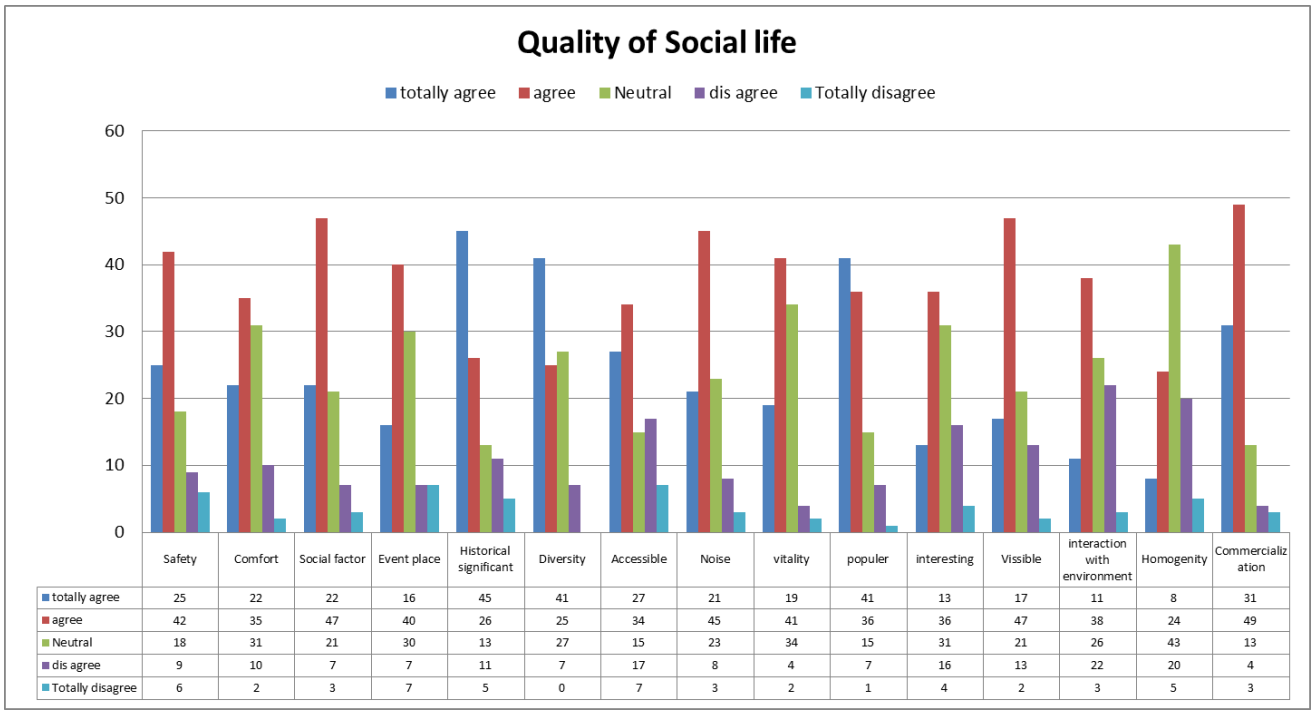
(Figure 13: Neutral satisfied design factors)



(Figure 14: somewhat dissatisfied design factors)

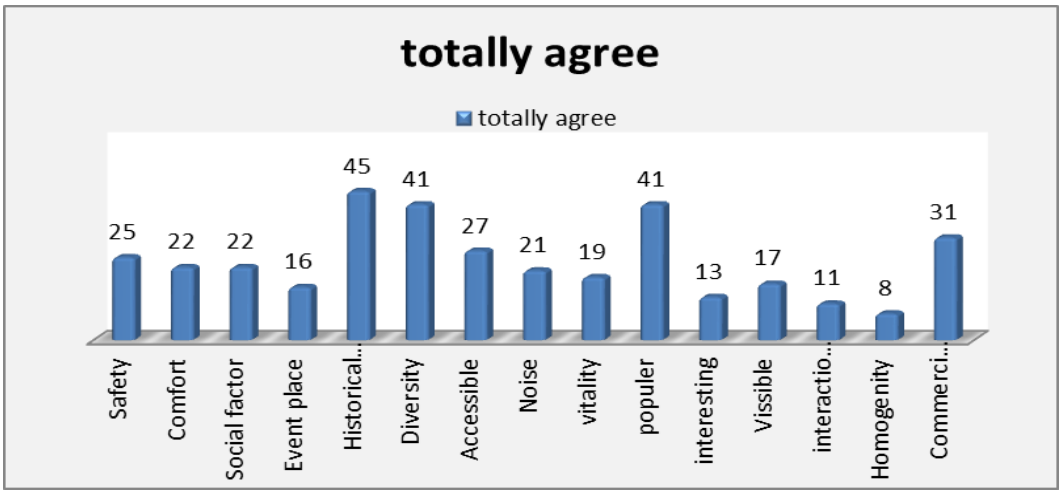


(Figure 15: very dissatisfied design factors)

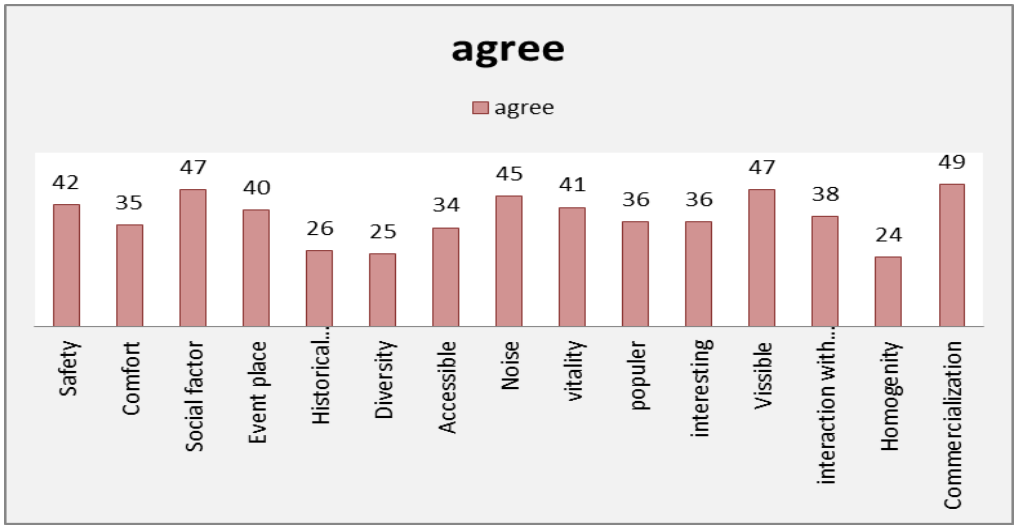


	Safety	Comfort	Social factor	Event place	Historical significant	Diversity	Accessible	Noise	vitality	populer	interesting	Vissible	interaction with environment	Homogenity	Commercialization
totally agree	25	22	22	16	45	41	27	21	19	41	13	17	11	8	31
agree	42	35	47	40	26	25	34	45	41	36	36	47	38	24	49
Neutral	18	31	21	30	13	27	15	23	34	15	31	21	26	43	13
dis agree	9	10	7	7	11	7	17	8	4	7	16	13	22	20	4
Totally disagree	6	2	3	7	5	0	7	3	2	1	4	2	3	5	3

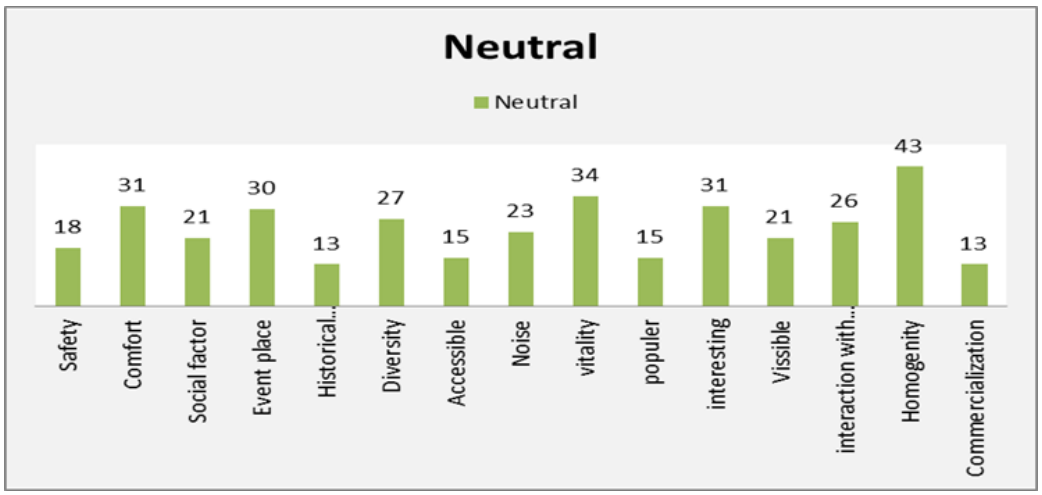
(Figure 16: quality of social life)



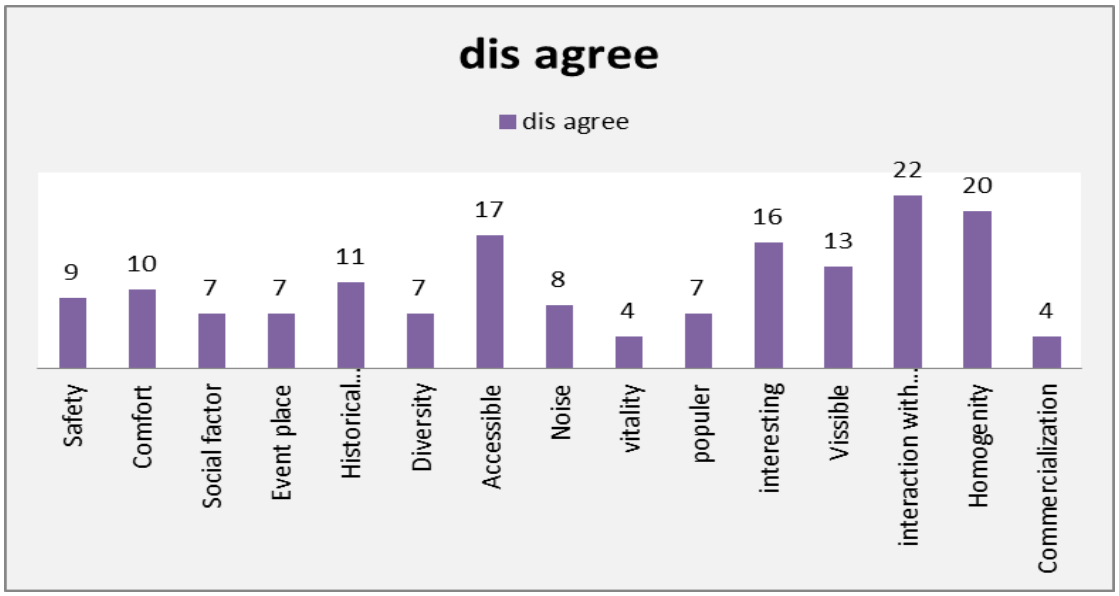
(Figure17: total agreement of people for social factors)



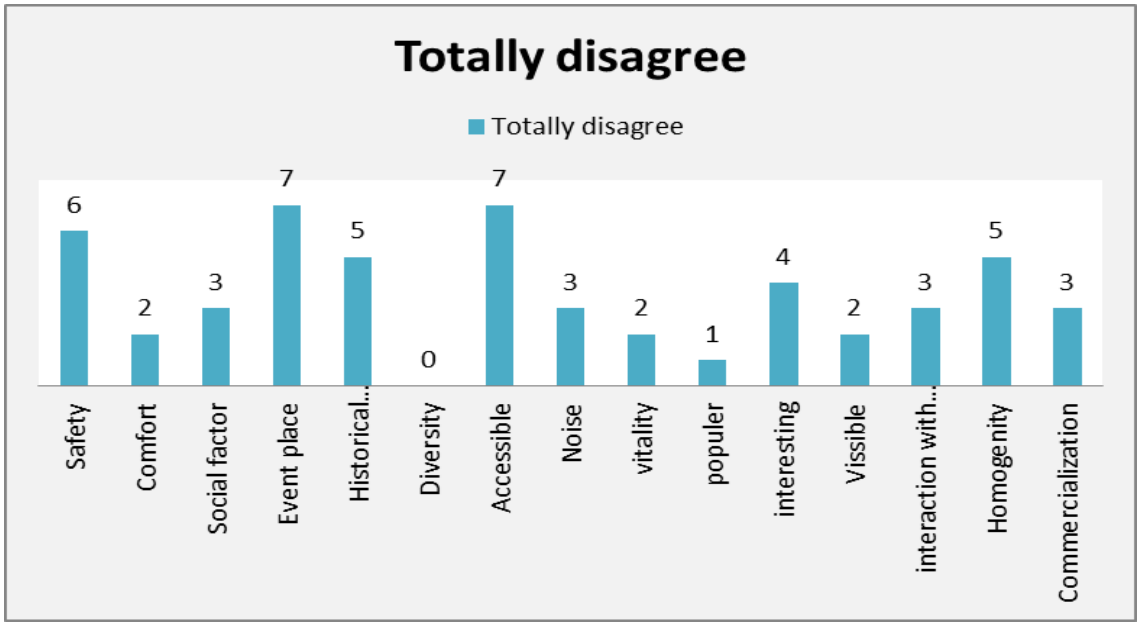
(Figure.18: agreement of people for social factors)



(Figure.19: Neutral agreement of people for social factors)



(Figure.20: disagreement of people for social factors)



(Figure.21: Evaluation of social life)

6. FINDINGS

6.1. Design factor's Survey: The very satisfaction design factors those were chosen by the Architects and Urban Designers in

Erbil city square in Descending sort were; [*historical land mark* that chosen by (%46), *Location* (%43), *image and identity* (%37), *Linkage* (%37)], while factors which takes the second place in satisfactory level were; [*Availability*, *Clarity*, *Major and minor space* chosen by (%49) of designers as somewhat satisfied, *Amenities* (%46), *Accessibility and visibility* factor (%43)], while the most very dissatisfied factors were ,[*Design integration and flexibility* (%23), *Maintenance* (%20), *enclosure* (%17)].

6.2. Social Survey: The totally agreement of Social factors has been chosen by people were, (*historical significant* (%45), *Popular and diversity* (%41), while the most social factors agreed by people, [*commercialize* (%49), *visible* (%47), *social place* (%47), *noise (*%45), safety (%42), *vitality* (%41), in addition to that there are few people totally disagreement with some factors, [*accessible and place for event* (%7)], both of two sides have been agreed to the importance of availability of the historical land mark as it connected to history and city culture where people usually seek about to understand the past, although beside that people preferred to get safety in such spaces within different amenities such as rest, shops and café.

7. CONCLUSION

7.1 Theoretical Part: - From the theoretical frame work the research achieves some important guidelines which totally effect Erbil square design in the future;

Square plays a significant role in gathering people and works as city lungs which attract users to have some time there, rest from shopping, socialize, or even get some food. **Successful city** square requires a series of uses and services with a strong design concept which have the ability to merge usages, services as well as landscape in one complementary platform, throw applying the most effective design principal functions and relationship with the adjacent public realm, as streets, pedestrian, edges, and land uses, it is not possible to judge squares the quality by viewing its plan only, however city square have to modify its sociability in a way that could serve its users efficiently. Good city square should provide maintenance, action and activity programming, which is generally effects on attracting users, seating place and surface areas, producing food service as café or restaurants, homogeneity, commercialize and diversity of uses will create a vital lively and attractive square.

7.1 Practical Part: from the practical part research achieves the following points;
Historical land mark available in Erbil city square insured by Architects as a very satisfaction (approval), as well as, image & identity, location and linkage were present as a very satisfactory factors, to be taken in account in designing squares in the future.
Several important design factors as; availability, clarity, major and minor spaces, amenities, accessibility, and visibility, have been satisfied (fulfilled) in Erbil city square, that means all these factors should be appear in different ways throw design, or providing different services.
From point (1 and 2), ten of twenty-six design factors have been chosen by architects and urban designers as very satisfied and somewhat satisfied in Erbil city square, and as shown in the tables above, these factors should be in consideration.
Availability of people belonging character to the site, as well as historical significant arises on the top of satisfactory factors that have been chosen by people as a very agreement which explains its important and availability and to rises the attention level of such factors. Popularity of the place to locals and foreign people and diversity of service and uses will give the spaces great spirit and its attraction level.

Commercialize, visible, social place, noisy, safety, vitality insured by people agreement, due to the daily need of such different services to be applied in squares.

Some of **design factors** dissatisfied by most of designers and architects as, design integration, flexibility, maintenance, and enclosure, as revealed in the site analysis, Neshtiman square lacking of enclose factor which is usually related with too many other factors such as safety, with a very bad maintenance, such factors effects totally on square using.

Few **social factors** have been chosen by people as some very disagreement ones as, accessible and place for events, the lack of public transportation and reaching this important space prevented from getting easy access to the square, all transportation were by taxies and private cars, the availability of public transportation in the historical city center would be more attractive to reach and get in by people.

- Due to that the Research will reveal some effective points could be followed and added to such effective space;

7.1.1. Pay more attention to the Architectural and design elements and details which added or may be added to such historical legacy like Erbil Citadel, to excrete and show this important element without effecting its great identity, as we appeal both guidelines to show the importance of such elements as well as the previous historical element.

7.1.2. design integration amongst the most effective factors which leads to other comprehensive ones to complete both sides; the design and social, if it is well applied, as well as square interaction with the surrounding environment.

7.1.3. the most effective factors that have been confirmed as a part of the research recommendation, design guidelines, space diversity, clarity in space, and availability of different services which related to be usable.

7.1.4. people asked for a diversity spaces also**,** with the availability of other overlapping factors, such as, safety, social space existence, control over the noise, space visibility, which all declares safety as well.

7.1.5. the other secondary factors which gain a medium range have been declared to be effective ones because it has been indicates or admitted to lead to such another one like, comfort, creating a space for events, vitality spaces to be, and interesting spaces, all to be put in minds in any adding or designing for such effective gathering space, the city square.

8. ACKNOWLEDGMENTS Researcher would like to offer a finest word to those who helped and offered their assist to complete the research and reach a very high level of reliability in its conclusions, and special thanks to our colleagues and friends for their efforts and support in answering the questionnaire honestly and sincerely which effect directly on the credibility of the research, as well as special thanks to the security of Erbil center for obtaining safety to researchers at the photography and survey time. As it was need more than three times visiting the site in a very accurate time.

Salahaddin University — Ansam Saleh Ali
College of Endeneering — Questionnair — Lana Abubakr Ali
Architectural Department

Dear Designers
A very Well come for you.....
This questionnair is for a research purpose,it will be part of Scientific upgrades, so Kindly be helpful in ansewring the questions below by clicking only one space which you prefferd the best choose among five choice.

Questios for the purpose of evaluating the architectural charecteristics of City Square and the success of it in your opinion as an Architect, Urban designer or a Town planner.(Park Neshtiman) has been taken for this purpose

Questions				I totally agree	I agree	Neutral	I disagre	I totally disagree
1-		Image & Idntity	Erbil City Square is a center of communities, traditionally helped to shape the identity of the entire city. Sometimes fountain used to give the square a strong image.					
2-	Connectivity	Accessibility	Accessibility and Connections of Park Nehtiman proximity to main axes of the City ,within (10-15) minutes walking distance radius from the public transport network ensures the urban connectivity to the site.					
		Visibility & views	locating at or as close as possible to streets level, The openness and Visibility of Park Neshtiman , take advantage of distant views to historical landmark often make it as the centre of city events and as a social nodes.					
3-	appropratenss	Functionality	The quality of park Neshtiman suited to serve a good purpose.					
		Flexibility	Park Neshtiman can be flourished with Multiple design management strategy, change with the seasons. outdoor cafés, markets, displays, help adapt our use for the space from one season to another.					
		Usability	park Neshtiman has an interior circulation pattern easy to access and realized, provides greater interest to site users which allows increasing choicese of movement patterns.					
		Inclusivness	Vareity of landuse ,Commercial ,Governarate , Resturant ....make a populer inclusive use in place.					
		Linkages	Just as important as the city edge, Park Neshtiman square contains ; streets, sidewalks and ground floors as a leading way for adjacent buildings . Like the tentacles of an octopus extending into the surrounding spaces.					
4-	Landscaping issue	Mjor and Minor space	Park Neshtiman classified in to main and secondery spaces ,which attributes as; Sittable Spaces, Food vendors, Sculpture, fountain and Vegetation to strengthen the design.					
		enclouser	Park Neshtiman Provid an open-air structure or canopy which helps to increase the comfort level and Sense of enclouser in the space for the public.					
		Lightings	The importance of lighting has a graet perception to human, after sunset, lighting affect peopl impression , Lighting design of Nehtiman Park have been done very well.					
		amenites	Park Neshtiman contains some site amenites such as benches, planters, sitting place...etc. to provide a comfortable space for people.					
5-		Clarity	park Neshtiman clarity gives the sense of a Difint space					
6-		Attractivness	Park Neshtiman lead the people to gather and inhance Community sense by; attractive façades,Natural elements,close to City center ,popularity of the place and to form a focal point in the public space network.					
7-		Availability	the avalibilty of different people needs such as; shops , resturants ,cafes,....etc. in the Squer center.					
8-	Spatial issues	Transperency	The street fronting uses, and their visual accessibility and Transperency, will give a positive experience for pedestrians in this square.					
		contextual relationship of site	Park Neshtiman has contexual relationship with the historical existing of site ,this will enhance the character sences and identity to the place.					
		Human Scale	Design , patterns,edges and buildings scale for Neshtiman park works as pedestrian-friendly and harmonize within human-scale.					
		Location	Erbil City Square is Location in the center of the City, Helpes to enhance Success and Populer.					
		Design Integration	park Neshtiman have an integrate Architectural design.					
		Width &Length of Streets	Streets around park Neshtiman create a central area ,length and width is propotional to park size, though it will be a safe way to reach the park.					
		Defensable Space	Erbil City Square afford good visual surveillance opportunities within both; space and edges. People will usually avoid dark hidden corners and vacant places					
		Area	The area of park Neshtiman is very suitable as it is not too small so it will nat fit to be an public open space and not too large to give a sence of loss.					
		Historical Landmark	Erbil Citadel is a historical land mark adjacent to Neshtiman Park give multiple meaning to be strong reason which encourages locals to utilize this place.					
		materials	The materials used in bulding facades surrounding the square can be represent as a good ,durable , traditional materials which gave the place a sense of Vernacular Architecture					
9-		maintenance	Park Neshtiman is easy to maintain by employers .					

(Appendix .1)

Salahaddin University										Ansam Saleh Ali	
College of Endeneering						Questionnair				Lana Abubakr Ali	
Architectural Department											
Dear Citizen											
Avery Well come											
This questionnair is for a research purpose,it will be part of Scientific upgrades, so Kindly be helpful in											
ansewring the questions below by slecting only one with the best choice among for you											
Questios for evaluating the architectural charecteristics of City Square and its success											
from your point of view as a user.(Park Neshtiman) has been selected for this purpose											

Questions			I totally agree	I agree	Neutral	I disagre	I totally disagree
1-	Safety	Park neshtiman is Safety, democratic and non discriminative place.					
2-	Comfort	placing tress along pedestrian path and sitting area and other natural elements in Park Neshtiman important to improve comfort, releaf and relaxing for people.					
3-	Social factor	Park Nshtiman becomes a conducive place for social interaction .					
4-	Event place	City square in Erbil City become a formal and informal space events like Nawroz Ceromory.					
5-	Historical significant	Erbil Citadel as a historical landmark adjacents to Neshtiman park gives a historical sense inspiration to the place .					
7-	Diversity	Variety of activites which surronds park Neshtiman make the space more Usable.					
8-	Accessible	Park Neshtiman accessible for different class or age of people, including disable people and informal sector.					
9-	Noise	too many people and multi function land use noisy that to be unpleasant place.					
10-	vitality	Natural Featurs ,fountains , sitting placese , make thid space more Vital.					
13-	populer	Due to human needs for markitngand its location in the center, became more populer among local and foreign people.					
14-	interesting	Park Neshtiman is enjoyable and intertainment place.					
15-	Vissible	due to the avilabilty of this park in Erbil center, its visible from all directions.					
16-	interaction with environment	People feel the interaction with the environment in Park Neshtiman.					
17-	Homogenization	People can hormonize with this zone,and spent hours in this place without feeling bored.					
18-	Commercialization	Erbil City center has a great commercialize activites due to the existing hundred of shops.					

(Appendix .2)

9.REFERENCES

Abdulkarim, D., & Nasar, J. L. (2014b). Do seats, food vendors, and sculptures improve plaza visitability? Environment and Behavior, 46, 805e825. http:// dx.doi.org/10.1177/0013916512475299

Achmad D., N., and W., Z., Public Open Space Privatization and Quality of Life, Case Study Merdeka Square Medan., ASEAN Conference on Environment-Behaviour Studies, Savoy Homann Bidakara Bandung Hotel, Bandung, Indonesia, 15-17 June, AcE-Bs Bandung., Procedia - Social and Behavioral Sciences 36., 466 – 475., www.sciencedirect.com, 2012.

Barbara G.,Darby W., gehl architects, public Spaces & Public Life., City of Seattle/Department of Planning and Development/Seattle Department of Transportation, Green Futures & Design University of Washington/College of Built,Environments.www.ISISeattle.org., Contact: Todd Vogel, info@ISISeattle.org, 2009.

Boris Johnson., Open Space Strategies, Best Practice Guidance, A Joint Consultation Draft by the Mayor of London and CABE Space, Published by Greater London Authority City Hall The Queen's Walk London SE1 2AA, www.london.gov.uk., September., 2008.

Boyce, P. R., Eklund, N. H., Hamilton, B. J., & Bruno, L. D. (2000). Perceptions of safety at night in different lighting conditions. Lighting Research and Technology, 32(2), 79e91. http://dx.doi.org/10.1177/096032710003200205.

Capital Spaces 2012., A Design Guide for London's Public Spaces., Capital Spaces – A Design Guide for London's Public Spaces, Bartlett School of Planning, UCL, Spring Term, Module coordinator: Prof. Matthew Carmona., Module tutors: Wendy Clarke, Sonia Freire Trigo., Collaborators: Urban Design Skills., 2012.

Carmona, M., Heath, T., Oc, T., & Tiesdell, S., *Public Places Urban Spaces: The Dimensions of Urban Design*. Oxford: Architectural Press, 2003

Hossein S., Fataneh F.,M., Syntactical analysis of the accessibility and sociability of a square in the Kuala Lumpur City Center, Department of Architecture, Rasht Branch, Islamic Azad University, Rasht, Iran School of Graduate Studies and Technical Complex, Islamic Azad University of Rasht Branch, Lahijan 44158-84715, Iran., Available online at www.sciencedirect.com., Frontiers of Architectural Research., www.keaipublishing.com/foar., Frontiers of Architectural Research (2017) 6, 456–468., 2017.

Jack L. Nasar, Saleheh Bokharae. Lighting modes and their effects on impressions of public squares, Journal of Environmental Psychology journal homepage: www.elsevier.com/locate/jep., Journal of Environmental Psychology 49., 2017 96e105).

Juliana Furlaneto Benchimol , Ana Paula do Nascimento Lamano-Ferreira, Mauricio Lamano Ferreira, Tatiana Tucunduva Philippi Cortese, Heidy Rodriguez Ramos., Decentralized management of public squares in the city of São Paulo, Brazil: Implications for urban green spaces., Contents lists available at Science Direct Land Use Policy.journalhomepage:www.elsevier.com/locate/landusepol.,UniversidadeNovedeJulhoUNINOVEAvenidaFranciscoMatarazzo,612,ÁguaBranca,SãoPaulo,SP,05001-100Brazil.,LandUsePolicy63, 2017.

Kallus, Rachel (2001) From Abstract to Concrete: Subjective Reading of Urban Space. *Journal of Urban Design*, Vol. 6, No. 2, 129.

Khalilah Zakariya, Nor Zalina Harun, Mazlina Mansor, Spatial Characteristics of Urban Square and Sociability:A review of the City Square, Melbourne *"Quality of Life in the Built & Natural Environment"* AicQoL2014Kota Kinabalu AMER International Conference on Quality of Life The Pacific Sutera Hotel, Sutera Harbour, Kota Kinabalu, Sabah, Malaysia 4-5 January 2014.

Larry D., Plaza Design Guidelines, City of Vancouver Land Use and Development Policies and Guidelines Community Services, 453 W. 12th Ave Vancouver, BC V5Y 1V4 F 604.873.7344 fax 873.7060 planning@city.vancouver.bc.ca., Adopted by City Council November 17, 1992.

M. Zawidzki, "Automated geometrical evaluation of a plaza (town square)", Department of Architecture, Massachusetts Institute of Technology,77 Massachusetts Ave.7 304G, Cambridge, MA02139, USA journalhomepage:www.elsevier.com/locate/advengsoft, 2016.

Madanipour, A. (1999) Why Are the Design and Development of Public Spaces Significant for Cities, Environment and Planning B; *Planning and Design*, 26 (6).

Mariani, L., Parisi, S.G., Cola, G., Lafortezzza, R., Colangelo, G., Sanesi, G., 2016.Climatological analysis of the mitigating effect of vegetation on the

urban heat island of Milan,Italy. Sci. TotalEnviron.569–570,762–773).

McColl, L., 2002. Making spaces places: creative activities can make idle public spacesengagingcentresofciviclife.Alternat.J.28(3).

Nor Zalina H., Khalilah Z., Mazlina M., Khairunnisa Z., "Determining Attributes of Urban Plaza for Social Sustainability"., AicQoL2014 Kota Kinabalu AMER International Conference on Quality of Life. The Pacific Sutera Hotel, Kota Kinabalu, Malaysia,4-5January 2014 *"Quality of Life in the Built and Natural Environment* Procedia - Social and Behavioral Sciences 153 (2014) 606 – 615,Available online at www.sciencedirect.com.

Project for Public Spaces, Squares. Retrieved December 14, 2014: http://www.pps.org/squares/.

Pugalis, L., Achieving place quality. Urban Des. 109, 14–15., C., 2004. Space syntax: some inconsistencies. Environ: Plan. Des., 2009.

Report On Consultancy Study On Public Open Space in Private Developments [Pospd]., Development Bureau, Rocco Design Architects Ltd. School of Architecture, Cuhk Land Elite Surveyors Ltd.

Town Centre Design Guidelines, City of White Rock, Urban Forum associates, Perry + Associates, Landscape Architecture, Site Planning, September, 2011.

Urban Design Principles and Development Guidelines., Garrett Square Revitalization Strategy Prepared for the Garrett Square Economic Development Corporation by Urban Design Center of Northeast Ohio, July 2004.

Urban Squares, Development Type-Urban Squares., Healthy spaces & places. www.healthyplaces.org.au., funded by the Australian Government Department of Health and Ageing., 29 May, 2009.

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Hepatotoxicity and Nephrotoxicity of Lead Nitrate in Toad *Bufo viridis*

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A R T I C L E I N F O

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A B S T R A C T

The present investigation dealt with the study of the effect of two doses of lead nitrate (40mg/kg and 80mg/kg) for three weeks on the liver and kidney of the male toad, *Bufo viridis*. Lead nitrate caused several histological alterations in the studied organs in a dose dependent pattern. The histological alterations included degeneration of hepatocytes, dilation of blood sinusoids, leucocytes infiltration in the liver and kidney, degeneration of kidney tubule epithelial lining cells. The most important finding was the significant dose dependent increase in number of melanomacrophage centers (MMGs) in the liver compared to control. The later results can be used as an important marker for water pollution by this heavy metal.

1. INTRODUCTION

Lead (Pb) is a ubiquitous environmental pollutant, widely distributed, representing a high toxicological and ecotoxicological risk. Lead is in frighteningly large array of consumer products, from art supplies and automobile components to specialty paints, some hair dyes, and even candy especially the local Kirkuk oil refineries (Al-Dabbas *et al.*, 2014, Al-Dabbas *et al.*, 2012). Lead (Pb) exposure is also considered to be a major public health problem; therefore(Chiesa *et al.*, 2006).

Lead has been found to induce a wide range of behavioral, biochemical and physiological effects. The liver, kidneys, and brain are considered to be the target organs for the toxic effects of lead (Jackie *et al.*, 2011) . Lead affects the metabolism of other minerals and has affinity for bone, where it acts by replacing calcium. Thus, the highest concentrations of lead are usually found in bone, kidney and liver (Gurer and Ercal, 2000, Al Zadjali *et al.*, 2015).

Oxidative stress with subsequent lipid peroxidation (LP) induced by production of reactive oxygen species (ROS) has been reported to be one of the important

mechanisms involved in toxic effects of lead (Yin *et al.*, 2008).

Amphibians are of interest, because during their development they move from aquatic to terrestrial habitats, which may be polluted by the metal since they are receptors of products generated by anthropogenic activities (Arrieta *et al.*, 2004). The most previous researches concerning the effect of lead ions on the liver or/and kidney included the species Rana (Vogiatzis and Loumbourdis, 2001, Loumbourdis, 2003, Fenoglio *et al.*, 2006, Jayawardena *et al.*, 2017)and few were included Bufo (Enuneku and Ezemonye, 2012).

Melanomacrophage centers (MMGs) of the spleen, liver, and kidney as part of the defense system of fish, amphibians, and reptile (Steinel and Bolnick, 2017, Vaissi *et al.*, 2017) are more and more often used as an object of micropathomorphological and toxicological studies(Pronina *et al.*, 2014) . A functional differences among MMGs of liver, kidney and spleen of fishes were determined (Ribeiro *et al.*, 2011). The main functions of MMGs are the iron capture and storage in haemolytic diseases storage, antigen trapping and presentation to lymphocytes, sequestration of products of cellular degradation and potentially toxic tissue materials, such as melanins, free radicals and catabolic breakdown products, destruction and detoxification of endogenous and exogenous materials (Agius and Roberts, 2003). They are thought to be the site of primary melanogenesis rather than mere storage (Agius and Roberts, 2003). There is evidence that liver MMGs represent a metabolically (melanin synthesis/degradation) and cytokinetically (proliferation/ death) active cell population during the annual cycle of the frog (Barni *et al.*, 2002) .

The aim of the present work was to investigate the hepatotoxicity and nephrotoxicity of lead giving as lead acetate in *Bufo viridis* (Amphibia: Anura: Bufonidae) with special attention to the effect on the MMGs.

2. MATERIALS AND METHODS

In this study, male toad, Bufo viridis, weighing 25-27 g collected from certain pond in Erbil city, were used. The experiments were conducted at College of science, Salahaddin University, Erbil-Iraq. The animals were kept in convenient plastic boxes in an environment mimic the natural pond. The fifteen toads were randomly and equally divided to three groups: control group (G1) given 1mL distilled water by gavage, lead nitrate (40mg/kg) as group 2 and lead nitrate (80mg/kg) as group 3. All the animals were treated for 3 weeks and they have been sacrificed 24 hours after the last oral dose. The liver and kidney of all animals were removed and processed for the histological study.

2.1. Histological studies

For histological study, fresh removed organs pieces were fixed in 10% buffered formalin, dehydrated in ethanol and embedded in paraffin. Serial sections with 5 μm thickness were obtained using miritome (Bright Co.) and stained according to hematoxylin and eosin procedure (Kiernan, 1981). Certain tissue samples (size ≤ 1mm3) were fixed in 3% glutaraldehyde in cacodylate buffer then postfixed in 1% OsO4, dehydration, clear and then embedded in araldite mixture for preparing plastic blocks. Semithin sections were stained by 1% toluidine blue in 1% borax (Burns, 1978). Counting cell number and photography were undertaken by special digital camera microscope (Olympus) per mm2.

3. RESULTS AND DISCUSSION

Amphibians living in ponds and rivers may exposed daily to environmental pollutants which may accumulate in their tissues and induce various histopathological alterations (Seixas Filho et al., 2017). In the present investigation, lead nitrate was used as pollutant to evaluate it's hepatotoxicity and nephrotoxicity in toad, Bufo viridis collected in autumn from local ponds in Erbil city.

3.1. Hepatotoxicity

Lead is a wide spread constituent of earth's crust (Needleman, 1999). It can cause hypertension, developmental defects, neurological problems, renal dysfunction, and anemia. The most important feature of lead hepatotoxicity in the toad was the dose dependent significant increase in the number of MMGs (Fig 1&2). As shown in Fig.3, this heavy metal was caused hepatocellular changes in the toad as compared with the normal histological structures in the control group. The low and high doses of the lead were found to induce several histological changes such as degeneration of the hepatocytes, dilation of blood sinusoids, congestion of blood vessels and the appearance of inflammatory infiltrated leukocytes.

Melanomacrophage centers are Melanophores exist mainly in the liver, kidney and spleen of fish (Agius and Roberts, 2003), frog and toad (Steinel and Bolnick, 2017). They are phagocytes that synthesize melanin (Gutierre *et al.*, 2018). These cells respond to catabolism processes (Kalashnikova, 2000, Steinel and Bolnick, 2017), immunological disorders (Pronina et al., 2014, Steinel and Bolnick, 2017) , Uv Uv exposure (Franco-Belussi *et al.*, 2016) and hibernation (Barni et al., 2002). Preliminary histological analyses suggested that MMGs are structurally similar to the mammalian germinal center (GC), leading to the hypothesis that the MMGs plays a role in the humeral adaptive immune response (Steinel and Bolnick, 2017). Different sizes of these cells were detected in the liver (Fig 2) and although such size different has been detected in normal environmental condition, such increase in size or frequency was detected in conditions of environmental stress and have been suggested as reliable biomarkers for water quality in terms of both deoxygenation and iatrogenic chemical pollution (Agius and Roberts, 2003). An increase in their number was detected in response of the frog *Rana (Pelophylax) ridibunda* to insecticide exposure (Paunescu *et al.*, 2010) and water polluted with fluoride (Bo *et al.*, 2018). An increase in the number of these MMGs was also recently detected in the liver of carp fish in response to mercury chloride exposure (Tjahjaningsih *et al.*, 2017) and liver, kidney and spleen of catfish, *Clarias gariepinus*, exposed to silver nanoparticles (Sayed and Younes, 2017) and this may rise the hypothesis of metal chelation by these cells and this should be confirmed by further investigations.

The degeneration of hepatocytes as a response to lead toxicity as revealed by the present investigation may be due to the oxidative stress which was considered as the main mechanism of lead induced toxicity in biological system (Flora *et al.*, 2012).

3.2 Nephrotoxicity

As with the liver, the lead has been found to cause nephrotoxic effect on toad kidney (Fig 4). The features of the nephrotoxicity were the degeneration of the epithelial cells lining the kidney tubules especially in the cortex region and infiltrated inflammatory leucocytes near

the glomerului and in the interstitial tissue between the renal tubules.

Similar to lead induced hepatotoxicity, it has been found that lead toxicity leads to kidney damage via two separate pathways: (1) the generation of reactive oxygen species (ROS) including hydroperoxides, singlet oxygen, and hydrogen peroxide and (2) the direct depletion of antioxidant reserves (Ercal et al., 2001).

The increase in MMGs noticed in liver couldn't be observed in kidney and this indicates that functional differences among MMGs of liver, kidney and spleen of toads are exist (Ribeiro et al., 2011).

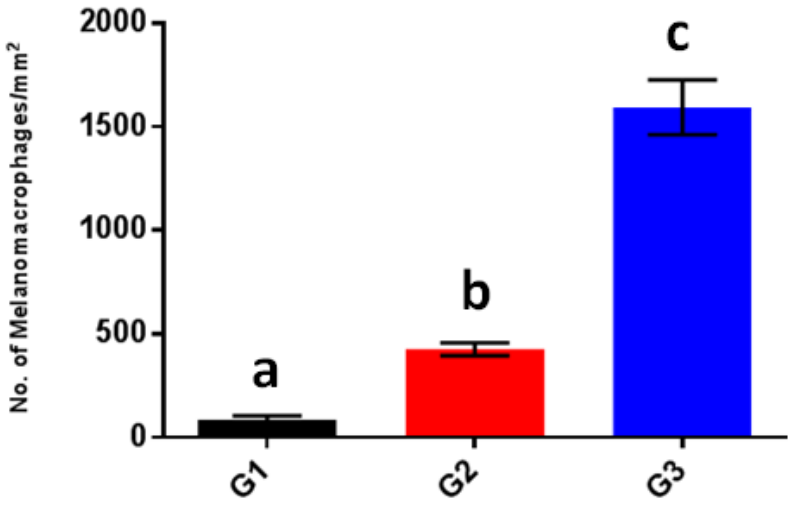

Fig (1): Number of MMGs in the liver of *Bufo viridis* exposed to lead nitrate Note: Columns superscript with different letters are significantly different at ($P \leq 0.05$)

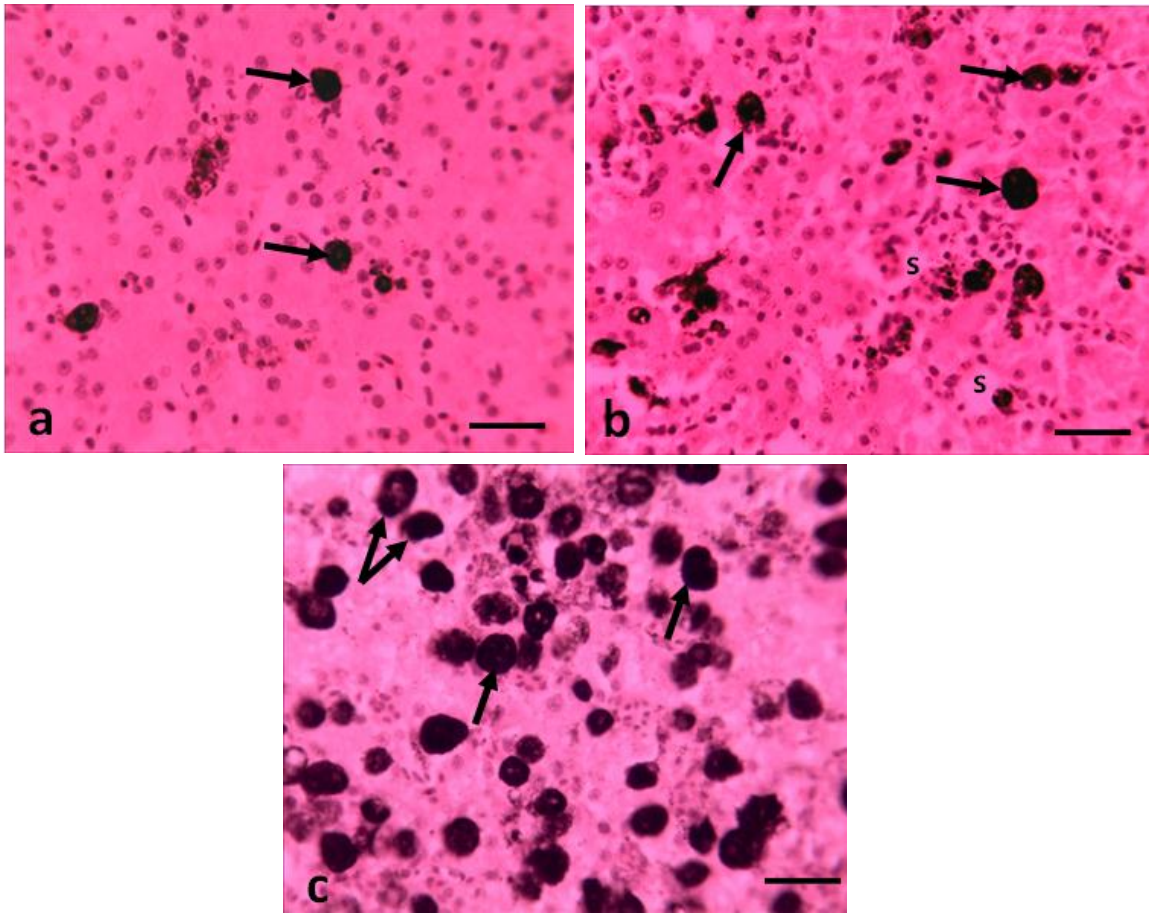

Fig. (2): Sections in liver of toad after exposure to lead nitrate showing MMGs (arrows). a) Control group, b) 40mg/kg lead nitrate treated group shown higher number of MMGs (arrows), c) 80mg/kg lead nitrate treated group showing higher number of MMGs compared to both groups, notice the different sizes of MMGs. H&E.

All scale bars=20µm.

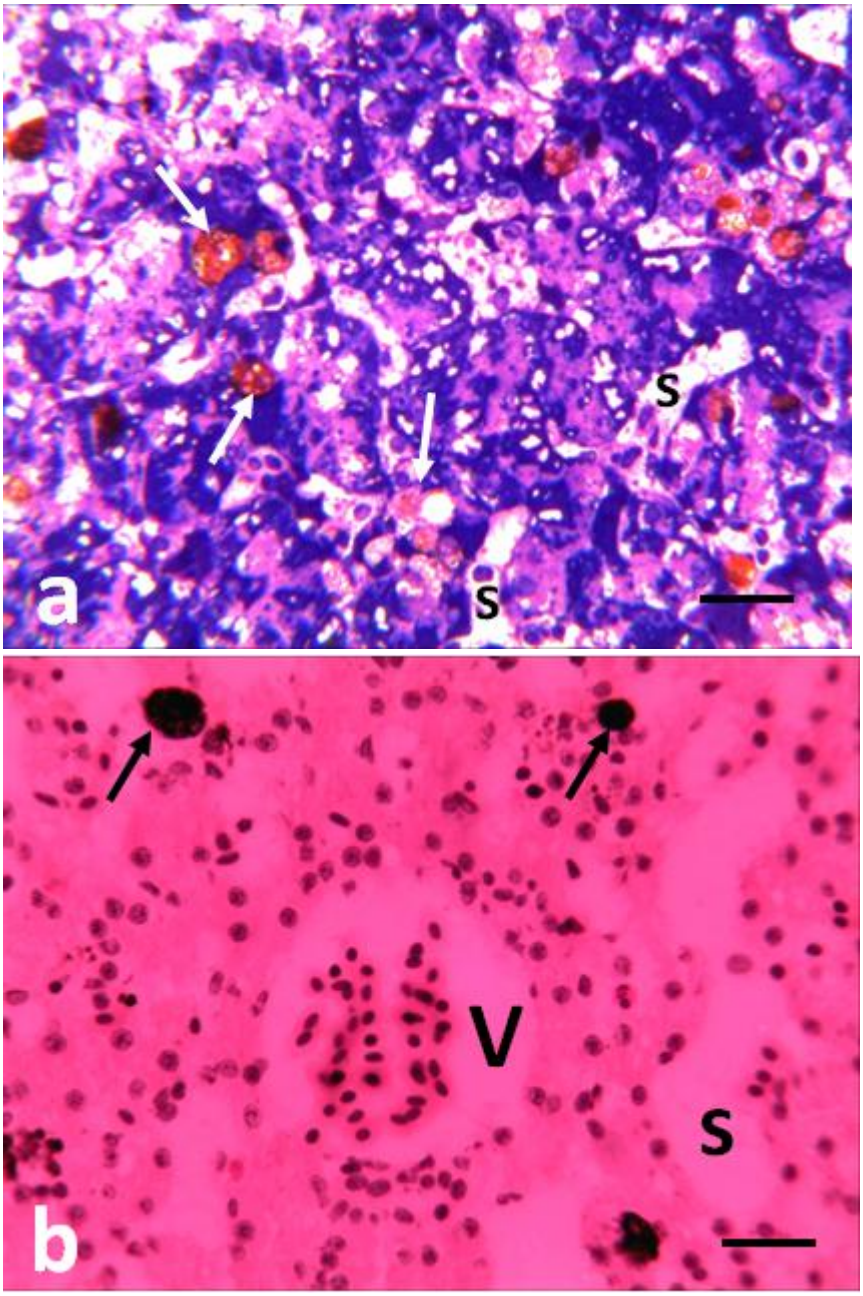

Fig. (3): Sections in the liver of toad after exposure to lead nitrate showing various histopathological alterations. a) a lot of MMGs (white arrows), dilated blood sinusoids(S), inverted section stained by toluidine blue, b) Some MMGs (arrows), portal vein (V) congested with blood cells and dilated blood sinusoids (S), paraffin section stained by H&E.

All scale bars=20µm.

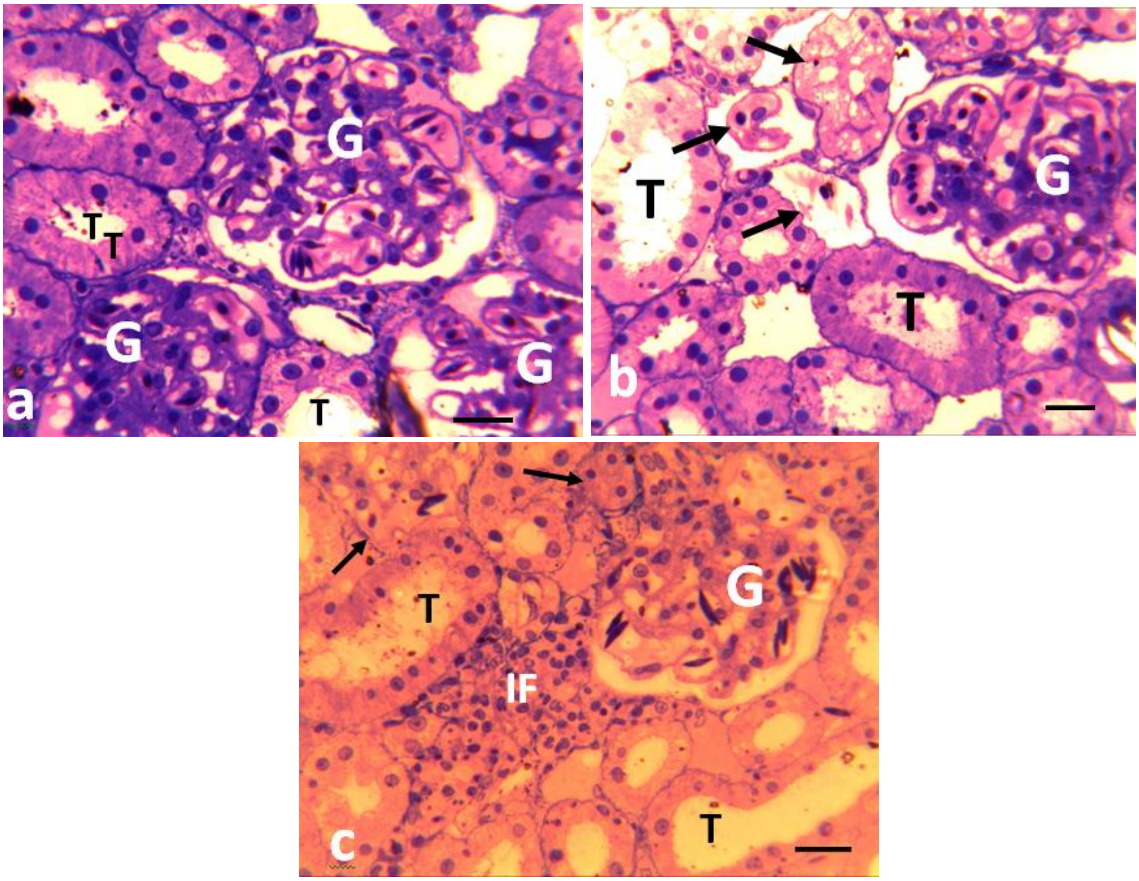

Fig. (4): Sections in the kidney of lead nitrate treated toads. a) Control group showing normal histological structure in the cortex region with glomerulus (G) and renal tubules (T). b) 40mg/kg lead nitrate treated group showing renal tubules (T) lined by degenerated epithelial cells (arrows), plastic sections stained by toluidine blue. c) 80mg/kg lead nitrate treated group showing inflammatory infiltrated leukocytes (IF), normal (T) and abnormal renal tubules which are lined by degenerated cells (arrows), paraffin section stained by H&E. All scale bars= 20µm.

REFERENCES

AGIUS, C. & ROBERTS, R. 2003. Melano-macrophage centres and their role in fish pathology. *Journal of fish diseases,* 26**,** 499-509.

AL-DABBAS, M. A., ALI, L. A. & AFAJ, A. H. 2012. The effect of Kirkuk Oil Refinery on Air pollution of Kirkuk City-Iraq. *Iraqi Journal of Science,* 53**,** 8-18.

AL-DABBAS, M. A., HUSSAIN, G. A. & AL-JUBORY, M. E. 2014. The Effect of North Refineries Company on Soil pollution of Baiji City-Iraq. *Iraqi Journal of Science,* 55**,** 1319-1329.

AL ZADJALI, S., NEMMAR, A., FAHIM, M. A. A., AZIMULLAH, S., SUBRAMANIAN, D., YASIN, J., AMIR, N., HASAN, M. Y. & ADEM, A. 2015. Lead exposure causes thyroid abnormalities in diabetic rats. *International journal of clinical and experimental medicine,* 8**,** 7160.

ARRIETA, M. A., BRUZZONE, L., APARTíN, C., ROSENBERG, C. E., FINK, N. E. & SALIBIáN, A. 2004. Biosensors of inorganic lead exposure and effect in an adult amphibian. *Archives of environmental contamination and toxicology,* 46**,** 224-230.

BARNI, S., VACCARONE, R., BERTONE, V., FRASCHINI, A., BERNINI, F. & FENOGLIO, C. 2002. Mechanisms of changes to the liver pigmentary component during the annual cycle (activity and hibernation) of Rana esculenta L. *Journal of anatomy,* 200**,** 185-194.

BO, X., MU, D., WU, M., XIAO, H. & WANG, H. 2018. The morphological changes and molecular biomarker responses in the liver of fluoride-exposed Bufo gargarizans larvae. *Ecotoxicology and environmental safety,* 151**,** 199-205.

BURNS, W. A. 1978. Thick Sections: Technique and Applications", Diagnostic Electron Microscopy, Ch. 4, B.F. Trump and R.J. Jones, eds., John Wiley & Sons, New York.

CHIESA, M. E., ROSENBERG, C. E., FINK, N. E. & SALIBIáN, A. 2006. Serum protein profile and blood cell counts in adult toads Bufo arenarum (Amphibia: Anura: Bufonidae): Effects of sublethal lead acetate. *Archives of environmental contamination and toxicology,* 50**,** 384-391.

ENUNEKU, A. & EZEMONYE, L. 2012. Acute toxicity of cadmium and lead to adult toad Bufo maculates. *Asian J Biol Life Sci,* 1**,** 238-41.

ERCAL, N., GURER-ORHAN, H. & AYKIN-BURNS, N. 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current topics in medicinal chemistry,* 1**,** 529-539.

FENOGLIO, C., GROSSO, A., BONCOMPAGNI, E., MILANESI, G., GANDINI, C. & BARNI, S. 2006. Morphofunctional evidence of changes in principal and mitochondria-rich cells in the epidermis of the frog Rana kl. esculenta living in a polluted habitat. *Archives of environmental contamination and toxicology,* 51**,** 690.

FLORA, G., GUPTA, D. & TIWARI, A. 2012. Toxicity of lead: a review with recent updates. *Interdisciplinary toxicology,* 5**,** 47-58.

FRANCO-BELUSSI, L., SKöLD, H. N. & DE OLIVEIRA, C. 2016. Internal pigment cells respond to external UV radiation in frogs. *Journal of Experimental Biology***,** jeb. 134973.

GURER, H. & ERCAL, N. 2000. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biology and Medicine,* 29**,** 927-945.

GUTIERRE, R. C., JARED, C., ANTONIAZZI, M. M., COPPI, A. A. & EGAMI, M. I. 2018. Melanomacrophage functions in the liver of the caecilian Siphonops annulatus. *Journal of anatomy,* 232**,** 497-508.

JACKIE, T., HALEAGRAHARA, N. & CHAKRAVARTHI, S. 2011. Antioxidant effects of Etlingera elatior flower extract against lead acetate-induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. *BMC research notes,* 4**,** 67.

JAYAWARDENA, U. A., ANGUNAWELA, P., WICKRAMASINGHE, D. D., RATNASOORIYA, W. D. & UDAGAMA, P. V. 2017. Heavy metal–induced toxicity in the Indian green frog: Biochemical and histopathological alterations. *Environmental toxicology and chemistry,* 36**,** 2855-2867.

KALASHNIKOVA, M. 2000. Ultrastructure of fish and amphibian liver during catabolism of degenerating erythrocytes. *Bulletin of Experimental Biology and Medicine,* 129**,** 101-104.

KIERNAN, J. A. 1981. Histological and Histochemistry Methods.: 1st ed. Pergomon press. Oxford.

.

LOUMBOURDIS, N. 2003. Nephrotoxic effects of lead nitrate in Rana ridibunda. *Archives of toxicology,* 77**,** 527-532.

NEEDLEMAN, H. L. History of lead poisoning in the world. International Conference on lead Poisoning Prevention and Treatment, Bangalore, 1999.

PAUNESCU, A., PONEPAL, C., GHICI, D. & GABRIEL, A. 2010. Liver histopathological alterations in the frog Rana (Pelephylax) ridibunda induce by the action of reldan 40EC. *Inset Marines, Tom. XVII/1:166-169*.

PRONINA, S., BATUEVA, M.-D. & PRONIN, N. 2014. Characteristics of melanomacrophage centers in the liver and spleen of the roach Rutilus rutilus (Cypriniformes: Cyprinidae) in Lake Kotokel during the Haff disease outbreak. *Journal of ichthyology*, 54**,** 104-110.

RIBEIRO, H., PROCóPIO, M., GOMES, J., VIEIRA, F., RUSSO, R., BALZUWEIT, K., CHIARINI-GARCIA, H., CASTRO, A. C. S., RIZZO, E. & CORREA, J. D. 2011. Functional dissimilarity of melanomacrophage centres in the liver and spleen from females of the teleost fish Prochilodus argenteus. *Cell and tissue research*, 346**,** 417-425.

SAYED, A. & YOUNES, H. A. 2017. Melanomacrophage centers in Clarias gariepinus as an immunological biomarker for toxicity of silver nanoparticles. *Journal of Microscopy and Ultrastructure*, 5**,** 97-104.

SEIXAS FILHO, J. T. D., CAMARGO FILHO, C. B., PEREIRA, M. M., MARTINS, A. M. C. R. P., RIBEIRO FILHO, O. P., MELLO, S. C. R. P., CASSIANO, L. L. & HIPOLITO, M. 2017. Histopathological aspects of the liver of free-living and farmed bullfrogs (Lithobates catesbeianus). *Revista Brasileira de Zootecnia*, 46**,** 275-279.

STEINEL, N. C. & BOLNICK, D. I. 2017. Melanomacrophage centers as a histological indicator of immune function in fish and other poikilotherms. *Frontiers in immunology*, 8**,** 827.

TJAHJANINGSIH, W., PURSETYO, K. T. & SULMARTIWI, L. Melanomacrophage centers in kidney, spleen and liver: A toxic response in carp fish (Cyprinus carpio) exposed to mercury chloride. AIP Conference Proceedings, 2017. AIP Publishing, 020012.

VAISSI, S., PARTO, P. & SHARIFI, M. 2017. Anatomical and histological study of the liver and pancreas of two closely related mountain newts Neurergus microspilotus and N. kaiseri (Amphibia: Caudata: Salamandridae). *Zoologia (Curitiba)*, 34.

VOGIATZIS, A. K. & LOUMBOURDIS, N. S. 2001. Exposure of Rana ridibunda to lead II. Impact of lead on various parameters of liver metabolism of the frog Rana ridibunda. *Journal of Applied Toxicology: An International Journal*, 21**,** 269-274.

YIN, S.-T., TANG, M.-L., SU, L., CHEN, L., HU, P., WANG, H.-L., WANG, M. & RUAN, D.-Y. 2008. Effects of Epigallocatechin-3-gallate on lead-induced oxidative damage. *Toxicology*, 249**,** 45-54.

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The Effect of Aqueous and Alcoholic Extracts of Galls of *Quercus infectoria* on the Growth of Some Pathogenic Fungi

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ABSTRACT

The present study was aimed to estimate the antifungal activity of galls of *Quercus infectoria* on the growth of eight species from six different genera of pathogenic fungi, which isolated from different sources, includes: Opportunistic (*Aspergillus flavus*, *A. fumigates*, *A. ochraceus*, *Cladosporium cladosporioides*, *Penicillium citrinum* and *Stachybotry schartarum*) and dermatophytes: *Microsporum gypseum* and *Trichophyton rubrum*. The Gall extracts were prepared by aqueous and ethanol extraction and two concentrations of each extract were used (2.5 and 5) mg/ml and tested for their activity as antifungal. The results of pour plate method showed that, ethanol extract at both concentrations affected more than aqueous extract on the growth of mycelia, that decreased the growth of mycelia of the pathogenic fungi when compared with control, while the antifungal activity of *Quercus* gall by filter paper disc diffusion method on the radial growth of pathogenic fungi showed that the aqueous extracts of *Quercus* gall have no any effect on the growth inhibition of all studied fungi, while the ethanol extracts of *Quercus* gall have low effect on the studied fungi.

1. INTRODUCTION

The kingdom fungi consist of a variety of species that are associated with a wide spectrum of diseases in animals and humans (Kavanagh, 2007). Pathogenic fungi cause a spectrum of infections from annoying to life-threatening with portals of entry that include skin contact, inhalation, and translocation across physical barriers as a result of host defects or accidental or iatrogenic abridgment of epithelial integrity (Calderone and Cihlar, 2002). The most common diseases of human that caused by fungi are the opportunistic fungal infections that take place in patients with immunity disorder (Kavanagh, 2007). *Aspergillus* sp. is an example of an opportunistic mold; it is responsible for a variety of infections referred to as aspergillosis (Dismukes *et al.*, 2003). The genus *Penicillium* is among the most common contaminant fungi in the environment. Around 15 species are known to cause opportunistic human mycoses in immunocompromised patients (Zanatta *et al.*, 2006). *Cladosporium* spp., which have a worldwide distribution and are among the most common air-borne fungi, they are the cause of opportunistic mycoses, some species are pathogenic and toxigenic to humans. They cause cerebral and cutaneous phaehyphomycoses. (Tasic and Tasic, 2007). *Stachybotrys* together with other molds such as *Penicillium*, *Aspergillis*, *Alternaria*, and *Cladosporium*, may play a role in the development of sick-building syndrome

(Johanning *et al.,* 1996). Humans may develop a disease that toxin-related by ingestion of food products contaminated with the *Stachybotry schartarum* or their toxins, exposure to mycotoxins in building and/or inhalation of conidia (Fung *et al.,* 1998). Dermatophytosis is one of the major public health problems that caused by dermatophytes, it includes three kinds of molds such as *Microsporum, Trichophyton* and *Epidermophyton,* which commonly causes humans and animals skin diseases. Dermatophytosis is an infectious disease of hair, skin and nails, which attack the keratinized tissue (Ameen, 2010 and Seebacher *et al.,* 2008).

To treat disease all over the world, there are various medicinal herbs have been used for years in daily life. They have a wide range of usage in folk medicine. The plant extracts and essential oils of many plants have been shown to exert biological activity, which leads to traditional medicine researchers focused on the characterization of antimicrobial activity of these plants (Essawi and Srour, 2000 and Iwu *et al.,* 1999).

Galls of *Q. infectoria* have been documented in pharmacology to possess astringent, antifungal (Digraki *et al.,* 1993) and anti-inflammatory (Kaur *et al.,* 2004) activities. The components of *Qurercus infectoria* galls comprise a large amount of syringic acid, tannins, gallic acid, ellagic acid, methylolenatebeta sitosterol, amentoflavone hexamethyl ether, isocryptomerin, methyl betulate and hexagalloyl glucose (Lodhi *et al.,* 2012).

The objectives of this research are to inquires the activity of gall as herbal medicine and to evaluate the capability of aqueous and ethanol gall extract have by two concentration and two methods to inhibit the growth of some pathogenic fungi such as opportunistic: *A. flavus, A. fumigates, A. ochraceous, C.cladosporioides P. citrinum*, and *S. chartarum* and dermatophytes: *M. gypseum* and *T. rubrum*.

2. Materials and Methods

2.1. Collection of Fungi

Different genera and species of fungi were isolated from different samples, such as soil, fruit, vegetables and human skin, which cultures on Potatoes dextrose agar (PDA) with chloramphenicol for opportunistic and Saboraud dextrose agar (SDA) with (chloramphenicol and cycloheximide) for dermatophytes, the grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures, covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics, then preserved in refrigerator in slant tubes until used for studying. Eight species from six different genera of fungi were chosen for this study. The chosen molds were transferred from the slant to Petri dishes for activation, by sub-culturing on PDA and SDA (Rajankar *et al.,* 2007). The spore suspension that used to test the plant aqueous and ethanol extracts were prepared by scraping the spore using sterilized glass rod and placed in a small-sterilized vial containing 10 ml of SDW (William *et al.,* 1976).

2.2. Gall samples collection and preparation

The galls of *Quercus infectoria* that used in this study were obtained from various locations in Erbil city. They cleaned then placed at room temperature, preserved in plastic sacs until used for extraction. After that they were crushed (by using pestle and mortar) to small pieces and powdered in an electric grinder, finally, these powders were stored in plastic containers until use (Basri and Fan 2005).

2.3. Plant extracts preparation

2.3.1. Preparation of aqueous extracts

Forty gram of Gall was weighted, and 160 ml of sterile distilled water (SDW) were added to it then mixed well in a shaker (shaker incubator-4045/gallenkamp-9B/ England) for one hour and kept at 4°C for 24 hours, filtered through 4-5 gauze and supernatant were placed in Petri dish to dried out then the powder collected and preserved in vials in refrigerator (Rashan *et al.,* 1992).

2.3.2. Preparation of alcoholic extracts

Twenty gram of gall samples were weighted, then 200 ml of ethanol (95%) were added to mixed well in shaker for one hour and kept at 4°C for 24 hours, filtered through 4-5 gauze and supernatant were placed in petri dish to dried out at room temperature, then the powder collected and preserved in vials in refrigerator (Grand *et al.,* 1988).

2.4. Aqueous and ethanol plant extracts sterilization and dilution preparation

The stock solution of plant extract was prepared by adding one gram of aqueous plant extracts to 5 ml of SDW, and adding one gram of ethanol plant extract to 5 ml of Dimethyl sulfoxide (DMSO) (Riedel-DeHaen AG-Germany), then this stock solution was sterilized by using (Millipore filters 0.2 µm). The MIC was determined using the two-fold serial dilution technique of concentrations, which were 5mg/ml and 2.5 mg/ml prepared from the stock solution (Rios *et al.,* 1987).

2.5. Determination of antimicrobial activity

2.5.1. Pour plate method:

The prepared concentration of 2.5 and 5 mg/ml were added to 200ml for each of PDA and SDA and poured into sterilized petri dishes then inoculated by one drop of spore suspension of Opportunistic (*Aspergillus flavus, A. fumigates, A. ochraceus, Cladosporium cladosporioides, Penicillium citrinum* and *Stachybotry schartarum*) and dermatophytes: *Microsporum gypseum* and *Trichophyton rubrum*. in to the center of the medium, a sterilized petri dishes with no addition of plant extract (PDA and SDA) was used as control and inoculated by fungi (two replication for each concentration), finally incubated at 25°C for 7 days for opportunistic fungi, and at 37°C for 10-15 days for dermatophytes. The diameter of growth (width and length) measured after incubation period (Rios *et al.,* 1987).

2.5.2. Antimycotic sensitivity test:

Fungal sensitivity for aqueous and ethanol plant extract was tested by using filter paper disc diffusion method, the filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vial, sterilized by oven and allowed to cool. The concentration 2.5 and 5 mg/ml of aqueous and ethanol plant extracts were prepared from the stock solution, blank discs were soaked in a known concentration of plant extract; another filter paper disc was soaked with SDW and DMSO used as negative control. Fungal spore suspension prepared from 5-7 day, were inoculated on PDA and SDA then spread by using sterilized glass rod, the discs were soaked with known concentration placed in petri dish. Finally, incubated at 25°C for 7 days for opportunistic fungi, 37°C for 10-15 days for dermatophytes. Zone of inhibition was obtained by measurement the radius growth of fungal colony from the center of the disc to the edge of the inhibition then measured both sides of the slope and their average accepted (Al-Refai, 2006).

2.6. Experimental microorganisms

Different genera and species of opportunistic fungi (*Aspergillus flavus, A. fumigates, A. ochraceous, Cladosporium cladosporioides, Penicillium citrinum* and *Stachybotrys chartarum*) and dermatophyte (*Microsporum gypseum* and *Trichophyton rubrum*) were isolated from different sources and diagnosed under microscope on the basis of their colony morphology and spore characteristics and used in this research to show the activity of plant extracts as an antifungal (Rajankar *et al.*, 2007).

2.7. Statistical analysis

Data entry and statistical analysis were performed using IBM Corp. Released for Windows: IBM SPSS software version 20. All values have been expressed as mean ± standard error (Mean±SE), that used for statistical comparison and the Results were considered to indicate statistically significant difference at P value ≤ 0.05.

3. Results

The results of aqueous and ethanol extract of *Quercus infectoria* gall were studied and evaluated for their antifungal activities against some of the human pathogenic fungi such as Opportunistic (*A. flavus*, *A. fumigates*, *A. ochraceous, C. cladosporioides, P. citrinum* and *S. chartarum*) and Dermatophytes (*M. gypseum* and *T. rubrum*). In general, the research indicated that the ethanolic extract of gall has more effects as antifungal on the growth of dermatophytes than opportunistic fungi.

Antifungal activities of both aqueous and ethanol extracts of galls on the inhibition of mycelium growth of pathogenic fungi showed in (Table 1: a and b) in the concentration 2.5 and 5mg/ml by pour plate method. It has found that ethanol extract of oak galls at both concentrations affected more than aqueous extract on the growth of mycelia of fungi and reduced the mycelia growth compared with the control.

In aquatic extract the diameter of colony growth of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: *A. flavus* (2 and 1) cm, *A. fumigates* (2.25 and 1) cm, *A. ochraceous* (5.75 and 3) cm, *C. cladosporioides* (3.5 and2) cm, *P. citrinum,* (2 and 1) cm and *S. chartarum* (2.25 and 0.7) cm and Dermatophytes: *M. gypseum* (1.75 and 0.1) cm and *T. rubrum* (2 and 0) cm, respectively at both concentration. While in ethanol extract the growth of all fungi in the concentration 2.5 and 5mg/ml were as shown, opportunistic: *A. flavus* **(**1.25 and 0.5) cm, *A. fumigates* (1.75 and 0.8) *A. ochraceous* (3.75 and 2.25) cm, *C. cladosporioides* (2.75 and 1.5) cm, *P. citrinum* **(**1.25 and 0) cm and *S. chartarum* (2 and 0) cm, respectively, while in both dermatophytes: *M. gypseum* and *T. rubrum*, there were no any growth at both concentration.

The higher effect of ethanol plant extract as shown in figure 1 (a and b), at the concentration 2.5 and 5 mg/ml was on inhibition the growth of both of *Microsporum gypseum* and *Trichophyton rubrum,* followed by *P. citrinum* and *S. chartarum,* while the lower effect of ethanol extract was found on the growth of *A. ochraceous.*

Antifungal activities of galls aqueous and ethanol extracts on the inhibition of mycelium growth of pathogenic fungi as shown in (Table 2: a and b) in the concentration (2.5 and 5mg/ml), by using filter paper disc diffusion method. It has revealed that ethanol extract of oak galls at both concentrations had more inhibition zone than aqueous extract and reduced the mycelia growth when compared with control.

In aquatic extract the diameter of inhibition zone of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: *Aspergillus flavus* ranging from (0.2 and 0.5) mm, while there had no inhibition zone in each of *A. fumigates, A. ochraceous, C. cladosporioides* and *P. citrinum* and the diameter of inhibition of *S. chartarum* was (0.25 and 1) mm and dermatophytes: *M. gypseum* (0 and 1.25) and *T. rubrum* had no inhibition zone. While in ethanol extract the diameter of inhibition zone of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: *A. flavus* ranging from (0.15 and 0.45) mm, *Aspergillus fumigates* (1.25 and 1.5) mm, *A. ochraceous* (1 and 1.5) mm, *Cladosporium cladosporioides* (0.5 and 1) mm, *Penicillium citrinum* (1 and 1.5) mm *Stachybotrys chartarum* (0.45 and 0.85) mm and dermatophytes: *Microsporum gypseum* (0.75 and 1.25) mm and *Trichophyton rubrum* (1 and1.5) mm, at both concentration respectively.

Aqueous extracts of *Quercus* gall have no significant effect on the growth of all studied fungi as shown in figure 2 (a and b), while the effect of the ethanol extracts of *Quercus* gall have significant effect on the studied fungi.

4. Discussions

In the present study the ethanol extract of oak galls were more effective on controlling all studied fungi than aqueous extract and caused significant reduction in mycelial growth for the fungi, this may be due to the differences between the chemical components of the plant extract and most of the biologically active chemicals are soluble in ethanol more than water such as volatile oils, tannin and glycosides and these components are physiologically active against fungi (Paaverurve and Raal, 2010). Oak galls

containing tannins, which have inhibitory effects on fungi (Vonshak *et al.,* 2003).

The results of the current study are in agreement with those found by Al-Refai, (2006), who demonstrated that the aqueous extract of galls of *Quercus* had no effect on *C. albicans*, *C. krusi*, *C. pseudotropicalis* and *Rhodotorula* sp., and also coincided with the results of Gulluce *et al.,* (2004), who have found that galls of *Quercus* have inhibitory effects on the growth of all *Candida albicans* isolates and also another study suggested that *Quercus* possesses compounds with antibacterial and antifungal properties (Nair *et al.,* 2007). These results are in agreement with Farahmand *et al.,* (2016), who have evaluated the antifungal activities of methanolic extract of plants against pathogens (*Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*) with pour plate method; they have determined that plant extracts inhibited the growth of mentioned fungi at all studied doses. Suleiman and Omafe, (2013), have demonstrated that anti-fungal activities of three medicinal plants such as: Lemon grass (*Cymbopogon citrates*), Morinda (*Morinda lucida*) and castor oil (*Ricinus communis*) extracts were more on *Penicillium digitata* than on *Aspergillus niger*, especially at higher concentrations by serial dilution method. These extracts on *Aspergillus niger* showed progressive retardations on the vegetative growth of *Zea Mays*, the inhibitory action of the extracts on the growth of mycelium increased with increase in concentrations. Ahmed and Kadhim, (2007), who evaluated two kinds of plant extracts for their antimicrobial activity against two molds (*Aspergillus flavus* and *Penecillium* sp.). The plant ethanol extracts were prepared from leaves and concentrated after that the two concentrations of each extract were prepared (1, 0.5) mg/ml and tested their activity as an antifungal. The results revealed that both extracts at both concentrations inhibited the growth of tested fungi as compared with control. Sarmamy *etal.,* (2011), who determine the effects of aqueous and ethanol extracts of pomegranate and oak gall to control *Penicillium* spp. and *Aspergillus niger*, the results revealed that all the plant extract concentrations used were effective against the two fungi. Ethanol extracts were more efficient against the growth of mycelia of the two fungi than aqueous extracts. *Penicillium* spp. was more sensitive to the plant extracts of pomegranate and oak gall more than *Aspergillus niger.*

5. Conclusion

In this study it found that *Q. infectoria* is an important medicinal plant to estimate there antifungal activity on the growth of some pathogenic fungi and it revealed that ethanol extract of affected more than aqueous extract on the growth of mycelia and reduced the mycelia growth of the pathogenic fungi as compared with control.

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Table (1a): Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of opportunistic fungi

Opportunistic Fungi	Plant extract	Concentration	The average diameter of mycelial growth (cm)		Mean ± SE
Aspergillus flavus	Aqueous	Control (D.W)	8	8.5	8.25±0.0121
		2.5	2	2	2.00± 0.013
		5	1	1	1.00±0.0899
	Ethanol	Control (DMSO)	8	8	8.00±0.0097
		2.5	1	1.5	1.25±0.0772
		5	0.5	0.5	0.50±0.0985
A. fumigatus	Aqueous	Control (D.W)	8.5	8.5	8.50±0.1114
		2.5	2	2.5	2.25±0.1383
		5	1	1	1.00±0.0989
	Ethanol	Control (DMSO)	8	9	8.50±0.1567
		2.5	2	1.5	1.75±0.0745
		5	1	0.6	0.80±0.0563
A. ochraceous	Aqueous	Control (D.W)	9	9	9.00±0.0344
		2.5	5.5	6	5.75±0.0448
		5	4	4.5	3.00±0.1455
	Ethanol	Control (DMSO)	9	9	9.00±0.1844
		2.5	3.5	4	3.75±0.0994
		5	2	2.5	2.25±0.0712
Cladosporium cladosporioides	Aqueous	Control (D.W)	7	6	6.50±0.1156
		2.5	3	4	3.50±0.0557
		5	2	2	2.00±0.0332
	Ethanol	Control (DMSO)	7	6	6.50±0.1777
		2.5	3	2.5	2.75±0.0666
		5	1	2	1.50±0.0224
Penicillium citrinum	Aqueous	Control (D.W)	9	8	8.50±0.0334
		2.5	2	2	2.00±0.0339
		5	1	1	1.00±0.0098
	Ethanol	Control (DMSO)	9	8	8.50±0.0332
		2.5	1	1.5	1.25±0.656
		5	-	-	-
Stachybotrys chartarum	Aqueous	Control (D.W)	3.5	4	3.75±0.0444
		2.5	2	2.5	2.25±0.0378
		5	0.6	0.8	0.70±0.0007
	Ethanol	Control (DMSO)	4	3	3.50±0.0346
		2.5	2	2	2.00±0.0556
		5	-	-	-

Table (1b): Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of Dermatophytes

Dermatophytes	Plant extract	Concentration	Average diameter (cm)		Mean±SE
Microsporum gypseum	Aqueous	Control (D.W)	6.5	6.5	6.50±0.1678
		2.5	1.5	2	1.75±0.0676
		5	-	0.2	0.10±0.0007
	Ethanol	Control (DMSO)	6.5	6	6.25±0.0675
		2.5	-	-	-
		5	-	-	-
Trichophyton rubrum	Aqueous	Control (D.W)	7	7	7.00±0.1222
		2.5	2	2	2.00±0.0444
		5	-	-	-
	Ethanol	Control (DMSO)	7	7	7.00±0.1547
		2.5	-	-	-
		5	-	-	-

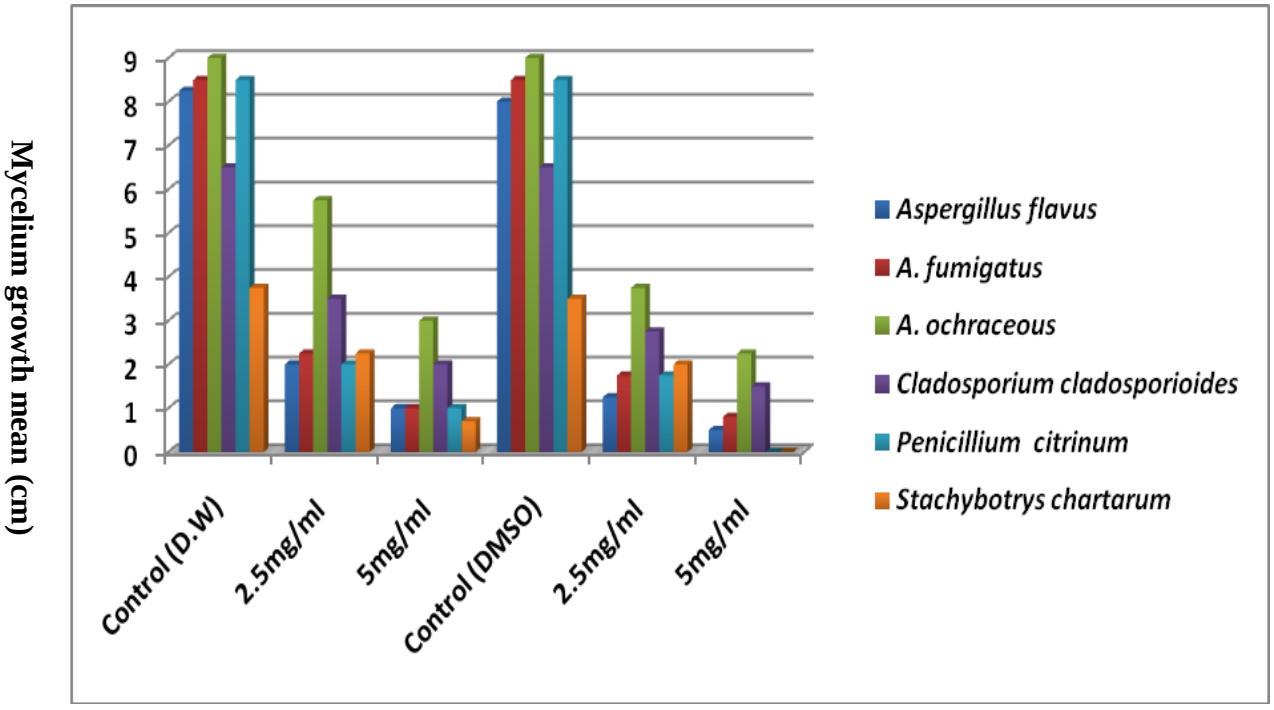

Figure (1a): Effect of aqueous and alcoholic extracts of oak galls by pour plate method on the growth of opportunistic fungi

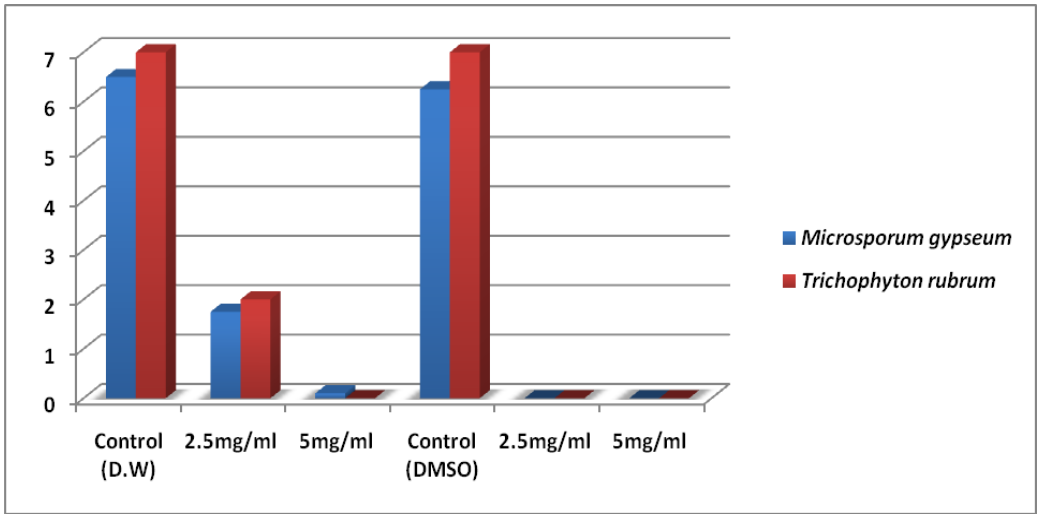

Figure (1b): Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of dermatophytes

Table (2a): Inhibitory activity of gall extracts against growth of opportunistic fungi by using disc diffusion method

Opportunistic Fungi	Plant extract	Concentration	Inhibition diameter (mm)		Mean±SE
Aspergillus flavus	Aqueous	Control (D.W)	0	0	0
		2.5	0.2	0.2	0.20±0.0004
		5	0.5	0.5	0.50±0.0011
	Ethanol	Control (DMSO)	0	0	0
		2.5	0.1	0.2	0.15±0.0032
		5	0.4	0.5	0.45±0.0034
A. fumigatus	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	0	0	0
	Ethanol	Control (DMSO)	0	0	0
		2.5	1.5	1	1.25±0.0364
		5	2	1	1.5±0.0221
A. ochraceous	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	0	0	0
	Ethanol	Control (DMSO)	0	0	0
		2.5	1	1	1.00±0.0065
		5	2	1	1.50±0.0088
Cladosporium cladosporioides	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	0	0	0

	Ethanol	DMSO	0	0	0
		2.5	0	1	0.50±0.0223
		5	1	1	1.00±0.0564
Penicillium citrinum	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	0	0	0
	Ethanol	Control (DMSO)	0	0	0
		2.5	1	1	1.00±0.0254
		5	1.5	1.5	1.50±0.0879
Stachybotrys chartarum	Aqueous	Control (D.W)	0	0	0
		2.5	0.2	0.3	0.25±0.0032
		5	1	1	1.00±0.0033
	Ethanol	Control (DMSO)	0	0	0
		2.5	0.4	0.5	0.45±0.0065
		5	1	0.7	0.85±0.0044

Table (2b): Inhibitory activity of gall extracts against growth of dermatophytes by using disc diffusion method

Dermatophytes	Plant extract	Concentration	Inhibition diameter (mm)		Mean±SE
Microsporum gypseum	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	1	1.5	1.25±0.0055
	Ethanol	Control (DMSO)	0	0	0
		2.5	1	0.5	0.75±0.0065
		5	1.5	1	1.25±0.0143
Trichophyton rubrum	Aqueous	Control (D.W)	0	0	0
		2.5	0	0	0
		5	0	0	0
	Ethanol	Control (DMSO)	0	0	0
		2.5	1	1	1.00±0.0017
		5	2	1	1.50±0.0073

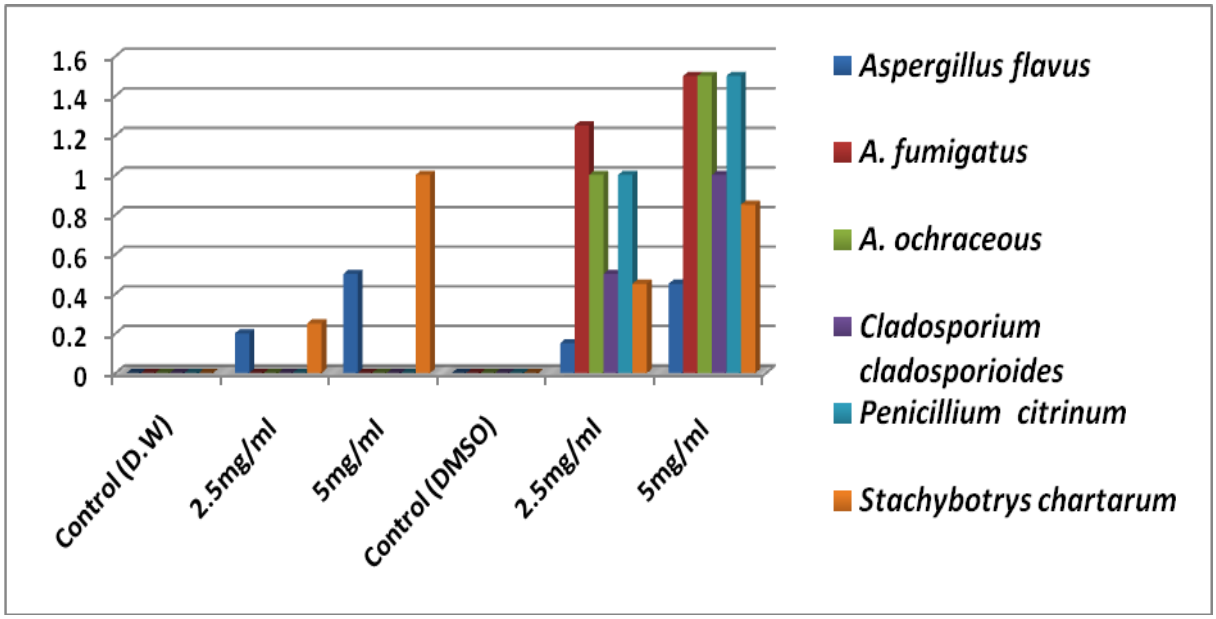

Figure (2a): Inhibitory activity of gall extracts against growth of opportunistic fungi by using disc diffusion method

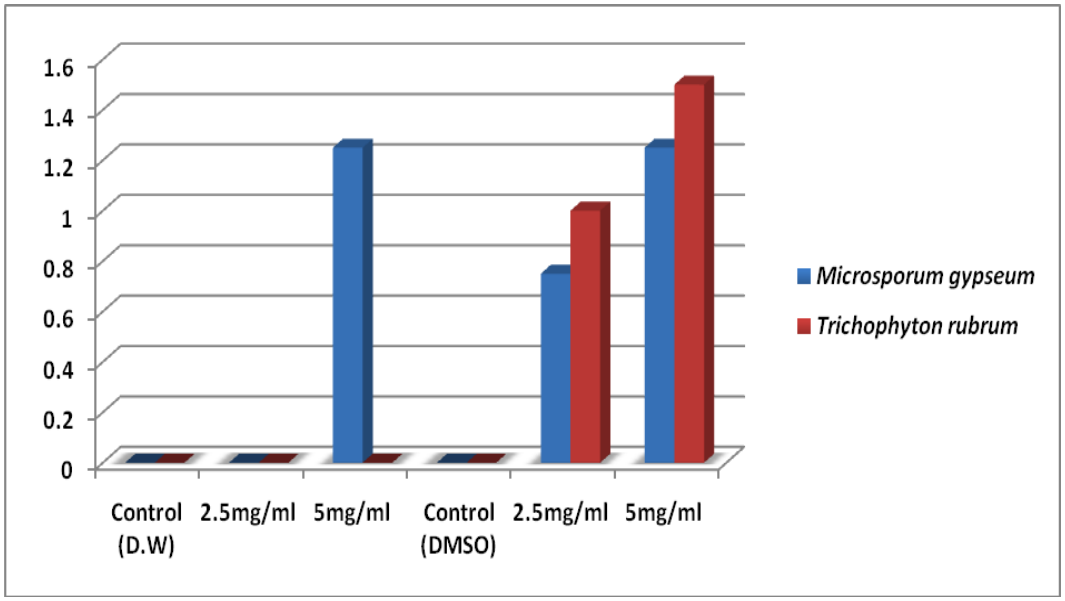

Figure (2b): Inhibitory activity of gall extracts against growth of Dermatophytes by using disc diffusion method

References

Ahmed, L.T. and Kadhim, H.M. 2007. The effect of some plant extracts on pathogenic fungi that cause human and animal infections; *Diala Jour.* 27:82-91.

Al-Refai, F.N. 2006. Isolation and identification of fungi from cosmetic using some plant extracts as a preservative agent, Ph.D. Thesis, College of science. Univ. of Mosul. Iraq.

Ameen, M. 2010. Epidemiology of superficial fungal infections. *Clinics in Dermatology*. 28: 197-201.

Basri, D.F. and Fan, S.H. 2005.The potential of aqueous and acetone extracts of galls of

Quercus infectoria as antibacterial agents. Indian J. Pharmacol. Vol 37, Issue 1: 26-29.

Calderone, R.A. and Cihlar, R.L. 2002. Fungal pathogenesis principles and clinical applications. Printed in the United States of America. 762 pp.

Digraki, M.; Alma, M.H.; Ilcim, A. and Sen, S. 1993. Antibacterial and antifungal effects of various commercial plant extracts. *Pharm. Biol.* 37: 216-220.

Dismukes, W.E.; Pappas, P.G. and Sobel, J.D. 2003. Clinical Mycology. Published by Oxford University Press, Inc. 198 Madison Avenue, New York. Printed in the United States of America.; New York.

Essawi, T. and Srour, M. 2000. Screening of some palestinian medicinal plants for antibacterial activity. *J.Ethnopharmacology* 70: 343-349.

Farahmand, S.; Rasooli, A. and Saffarpour, M. 2016. Antifungal activities of methanolic extract of plants. S1: 42-44.

Fung, F.; Clark, R. and Williams, S. 1998.

Stachybotrys, a mycotoxin-producing fungus of increasing toxicologic importance. *J. Toxicol.Clin. Toxicol*. 36: 629–631.

Grand, A.; Verpoort, R.; Wondergem, P.A. and Ponsset, J.L. 1988. Anti infection phytotherapies of the tree-savannah sengal (west-Africa), 11-antimicrobial activity of 33 species. *J. Ethnoph.* 22: 25-31.

Gulluce, M.; Adiguzel, A.; Ogutcu, H.; Sengul, M.; Karaman, I. and Sahin, F. 2004. Antimicrobial effects of *Quercus ilex* L. extract. *Phytother. Res*. 18 (3): 208-211.

Iwu, M.W.; Duncan, A.R. and Okunji, C.O. 1999. New antimicrobials of plant origin. In: Janick, J. (Ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria: 457-462.

Johanning, E.; Biagini, R.; Hull, D.; Morey, P.; Jarvis, B. and Landsbergis, P. 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in water damaged office environment. *Int. Arch. Occup. Environ. Health*. 68:207–218.

Kaur, G.; Hamid, H.; Ali, A.; Alam, M.S. and Athar, M. 2004. Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria. J. Ethnopharmacol*. 90: 285-292.

Kavanagh, K. 2007. New Insights in Medical Mycology. Published by Springer, Dordrecht. The Netherlands; page1.

Lodhi, G.; Singh, H.K.; Pant, K.K.; Rao, C.V. and Hussain, Z. 2012. Hepatoprotective effects of *Quercus infectoria* gall extract against carbon tetrachloride treated liver injury in rats. *Int. J. Applied Res. Nat. Product*. 5(3): 17-22.

Nair, R.; Kalariya, T. and Chanada, S. 2007. Antibacterial activity of some plant extracts used in folk medicine. *J. Herb Pharmacother.* 7: 191-201.

Paaverurve, V.M. and Raal, A. 2010. Total tannin content in distinct *Quercus robur L. galls. J. Med. plant Res*. 4 (8): 702-705.

Rajankar, P.N.; Tambekar, D.H. and Wate, S.R. 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of the Purna river basin. Research Journal of Agriculture and Biological Science. 3(6):701-703.

Rashan, I.J.; Abed, A.A. and Aziz, A.A. 1992. Further observations on the pharmacological activities of the aqueous extract of *Aristolochiabottae* stems. Fitotoerapia. 4: 350-352.

Rios, J.L.; Recio, M.C. and Villar, A. 1987. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *J. Ethnopharmacology*. 21:135-144.

Sarmamy, A.O.I. ; Taha, M.I. and Ismaeil, A. S. 2011. Antifungal Activity of *Pomegranate* and Oak Galls Extracts against *Penicillium* spp. and *Aspergillus niger*. Rafidain journal of science, vol.22, issue 2: 1-10.

Seebacher, C.; Bouchara, J.P. and Mignon, B. 2008. Updates on the epidemiology of dermatophyte infections. *Mycopathologia* 166: 335-352.

Suleiman, M.N. and Omafe, O.M. 2013. Activity of three medicinal plants on fungi isolated from stored maize seeds *Zea Mays* (L.). *Glob. J. Med. Plant Res.* 1(1): 77-81.

Tasic, S. and Tasic, N.M. 2007. *Cladosporium* spp. Cause of opportunistic Mycoses; *Acta. Fac. Med. Naiss*. 24 (1): 15-19.

Vonshak, A.; Barazani, O.; Sathiyamoothy, P.; Shalev, R.; Vardy, D. and Golan-Goldhirsh, A. 2003. Screening of south Indian medical plants for antifungal activity against cutaneous pathogens. *Phytother. Res.* 17:1123-1125.

William, S.; Wold, M. and Suzuk, I. 1976. The citric acid fermentation by *Aspergillus niger* regulation by zinc of growth and acidogenesis. *Can. J. Microbial*. 22: 1083-1092.

Zanatta, R.; Miniscalco, B.; Guarro, J.; Gene, J.; Capucchio, M.T.; Gallo, M.G.; Mikulicich, B. and Peano, A. 2006. A case of disseminated mycosis in a German Shepherd dog due to *Penicillium purpurogenum. Medical Mycology.* 44: 93-97.

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Erysimum amasianum Hausskn. & Bornm. (Brassicaceae) as a New Record for Flora of Iraq

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ABSTRACT

Erysimum amasianum Hausskn. & Bornm. regards as a new plant species for the Flora of Iraq within Brassicaceae family, found in Safin mountain (north-east of Erbil) in Rowanduz district (MRO). The characteristics of *E. amasianum* are the followings: Biennial plants; 2-fid hairy; leaves entire (or dentate, pinnatifid in some basal leaves), linear-oblanceolate (cultrate in lower cauline leaves, linear in upper cauline leaves); pedicel thickened, fruit a siliqua, terete, rigid, erect-spreading. The identification of the species was proved by applying the keys in the available references, morphological description was fixed. Pollen grains characters have been studied like shape, color, size, surface ornamentation and number. In addition, the features of the stem anatomy such as epidermis, cortex, vascular bundles and the pith have been examined.

1. INTRODUCTION

Brassicaceae (Cruciferae) is one of the families mentioned in the Flora of Iraq which have 3250 species around the world and distributed on 365 genera (Simpson, 2006). In Iraq, Al-Rawi (1964) stated that the family comprise 199 species distributed on 74 genera. In the Flora of U.S.S.R., Shishkin (1949) indicated 52 species of the genus *Erysimum* (Tourn.) L., in Saudi Arabia, Migahid (1978) did not remark any species of the genus, while Post (1932) declared 11 species in Syria, Palestine and Sinai. Chamberlain and Raven (1972) in Turkey stated 33 species of the genus implicating *E. amasianum*. In Iran, Raven (19--) indicated that 40 species of the genus present, also in Iran, Ghahreman and Attar (1999) mentioned 26 species. In the Flora of low land Iraq, Rechinger (1964) declared 3 species, while Guest (1933) did not mention any species of the genus in Iraq, Zohary (1946) stated 10 species of the genus in Iraq and Blakelock (1948) pointed to 3 species of the genus in Iraq, but Al-Rawi (1964) mentioned 13 species and Ridda and Daood (1982) with Townsend and Guest (1980) indicated 12 species. Faris (1983) mentioned 3 species in Piramagrun mountain, each of Fatah (2003), Darwesh (2017) and Khalaf (1980) mentioned 2 species in Haibat Sultan, Choman and Sinjar respectively, Ahmed (2010) did not state any species of the genus *Erysimum* in Gomaspan, whilst Hameed (2016) mentioned 1 species in Hujran Basin. Ahmad (2013) indicated 6 species in Hawraman mountains. Chakravarty (1976) stated that the seeds of the species *E. repandum* L. which present in Iraq when

soaked in water, become coated with a transparent mucilage matter. They are given in fever. They are also applied in the form of a poultice to relieve pain in the stomach. It is reported that the plant is employed in Spain as an antiscorbutic. It is also reported useful for sheep grazing.

The current study confirmed the presence of *E. amasianum* in Iraq depending on the recent collections, as well as morphological characters, pollen grains and stem anatomy, to add more information for aiding the identity of the species covered by the present study.

2. MATERIALS AND METHODS

Plant specimens have been collected within field trips in the different regions of northern districts of Iraq in 2017. By helping the keys of Flora of Iraq, Flora of Turkey and Flora Iranica, the specimens have been identified, then kept in herbarium of Education College, University of Salahaddin-Erbil, (ESUH). For the pollen grains, anthers fixed in FAA, a single anther removed and positioned in a drop of water or 50% glycerol (the latter to prevent the material from drying out). The anther dissected with a scalpel to extrude the grains. The anther wall material was removed, a drop of safranin was added on the grains, then a cover-slip was slide on top of the pollens. (Simpson, 2006). A Sony camera used for photographing the parts of plants and the scientific terms have been taken from Harris and Harris (2001), Hesse et al. (2009) and Agashe and Caulton (2009). For the stem anatomy, the procedure in Al-Mashhadani (1992) has been used and the information in Metcalfe and Chalk (1950) was applied.

3. RESULTS AND DISCUSSION

3.1. Morphological Study

E. amasianum Hausskn. & Bornm. In Mitt. Thur. Bot. Ver. 20: 2 (1904-5); Fl. Turkey, Cullen, 1: 474 (1965).

Biennial, herb, height (18-41) cm, stem erect, branched, costate, winged, white, 2-fid hairs, green, (7.5-34)x(1.0-1.5) cm. Leaves simple, sessile, exstipulate, alternate-spiral, margin entire (or dentate, pinnatifid in some basal leaves), apex acute, base truncate, densely 2-fid hairs; basal leaves narrowly oblanceolate, linear, green, (5.5-42.0)x(0.3-0.8) mm; lower cauline leaves cultrate, green, (45-60)x(0.7-1.4) mm; upper cauline leaves linear, green, (40-56)x(0.6-1.3) mm. Inflorescence simple raceme, ebracteate, 2-fid hairs, flowers actinomorphic, 4-merous, pedicel thickened, 2-fid hairs, green, (2.2-3.0)x(0.4-0.7) mm, Calyx of 4 sepals, saccate, free, in two decussate pairs, lateral ones lanceolate-narrowly lanceolate, upper and lower ones cultrate, margin entire, apex obtuse or acuminate, base obtuse, 2-fid hairs on the lower surface, green, (6.7-7.7)x(1.1-1.8) mm. Corolla of 4 petals, alternating with the sepals, obovate, margin entire, apex undulate, base truncate, 2-fid hairs on the lower surface (on the midrib of the petal limb), yellow, petal limb (3.5-4.5)x(2.8-3.2) mm, petal claw (7.0-7.6)x(0.3-0.4) mm, Stamens 6, tetradynamous, in two whorls, the outer of 2 shorter and the inner of 4 longer stamens; filaments filiform, yellow, longest (5.7-6.8)x(0.25-0.30) mm, shortest (4.0-5.2)x(0.15-0.20) mm; anthers narrowly oblong-cultrate, yellow, basifixed attachment with the filaments, (2-3)x(0.3-0.45) mm, two nectar glands at the base of each stamen, triangular, yellow, (0.20-0.25)x(0.15-0.20) mm. Pistil 1, ovary syncarpous, of 2 carpels, 2-locular, divided by a replum, superior, terete, , 2-fid hairs, yellow, (6.5-9.0)x(0.4-0.5) mm; style single, globoid or oblong, 2-fid hairs, yellow, (0.5-1.0)x(0.4-0.8) mm; stigma bilobed, rough, yellow, (0.2-0.5)x(0.8-1.1) mm. Fruit stalk terete, thickened, 2-fid hairs, (3-5)x(0.55-0.65) mm, fruit a siliqua, dehiscing upwardly by 2 valves, terete, rigid, erect-spreading, 2-fid hairs, yellow or green-yellow, (9-30)x(0.50-0.65) mm. Seeds numerous, broadly obovoid-very broadly obovoid or narrowly oblong-cultrate, yellow-brown, (0.35-0.80)x(0.2-0.3) mm. (Plates 1-3).

Type: [Turkey A5 Amasya] Amasia, 400-600 m, Bornmuller 404 (K!). Galatia. A5 Amasya: Amasya, Mt. Kirklar, 500 m, Bornm. 2686.

Studied specimens

MRO: ESUH/ Safin mountain (north-east of Erbil), 800 m, 25.4.2017, A. Sardar & S. Al-Dabagh, 7593.

Environment & Presence: Present as individuals on the rocky-clay soils; altitude: 800 m; flowering: April. (Figure 1).

3.2. Palynological Study

Pollens yellow, single, tri-colporate, prolate or spheroidal in equatorial view, spheroidal in polar view, small according to Erdtman (1971), equatorial axis (13.75-17.50) µm, polar axis (18.75-25.00) µm, reticulate surface ornamentation, few in number. (Plate 4).

3.3. Anatomical Study

The stem has been studied by a cross section of the middle of a flowering stem. The epidermis was of a single continuous layer of elongate or semi-circular cells having different sizes; each cell with two projections interact with the two adjacent cells. The thickness of the epidermis depending on the differences in the cell sizes. The external and internal walls of the epidermal cells were convex (the internal walls thickened), the radial walls were sinuous. The epidermis layer (4-13) µm, the cuticle layer is thin, (1.5-2.5) µm.

The cortex consists of parenchymal tissue (the first part collenchymal), have little intercellular spaces, the cells oblong or irregular of different sizes. The number of the cortical layers is (2-3) layers, (6-21) µm. The vascular tissue is lignified and parenchymatous, the phloem outwardly and the xylem inwardly, vessels small, sclerenchymal cells present among the vascular bundles, (70-90) µm. The pith consists of parenchymal cells, circular, semi-circular or oblong, with many intercellular spaces, (5.5-6.0) µm. The pericycle with the endodermis were unclear. (Plate 5).

The research treated with a new record of the genus *Erysimum* which was *E. amasianum* from Brassicaceae in Iraq, the study contained some aspects like the morphological characters with the environment. By reviewing the literature about the genus *Erysimum* in Iraq, including the 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 researcher did not find any plant belongs to *E. amasianum*, for this reason it regarded as a new record for the Flora of Iraq.

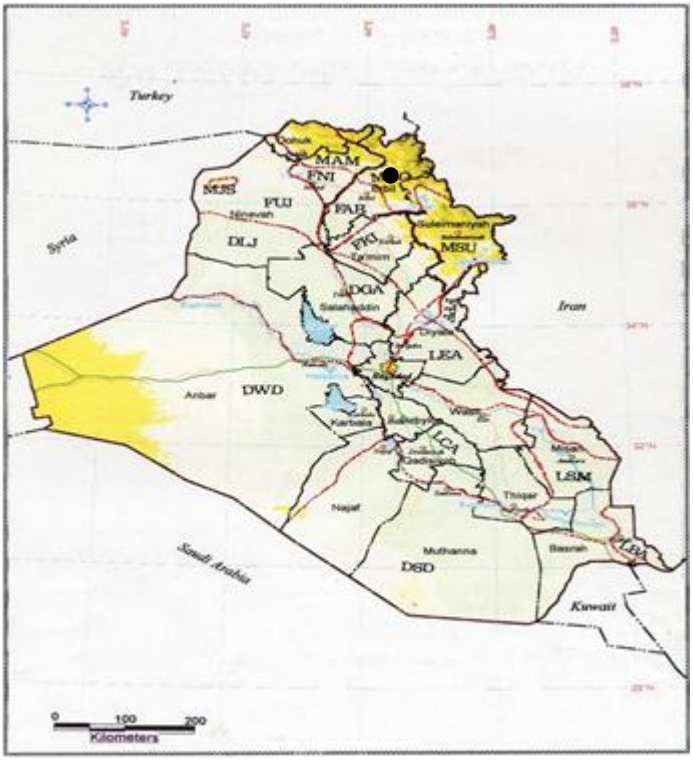


Fig (1): Iraq's map showing the regions and districts depending on [Guest (1966) and FAO. (2002) ● *E. amasianum*

Plate (1): Photograph of *E. amasianum*

A

10 mm

B

C

D

Plate (2): *E. amasianum*: A- Leaves; B- Basal leaves; C- Inflorescence D- Pedicel with sepal bases

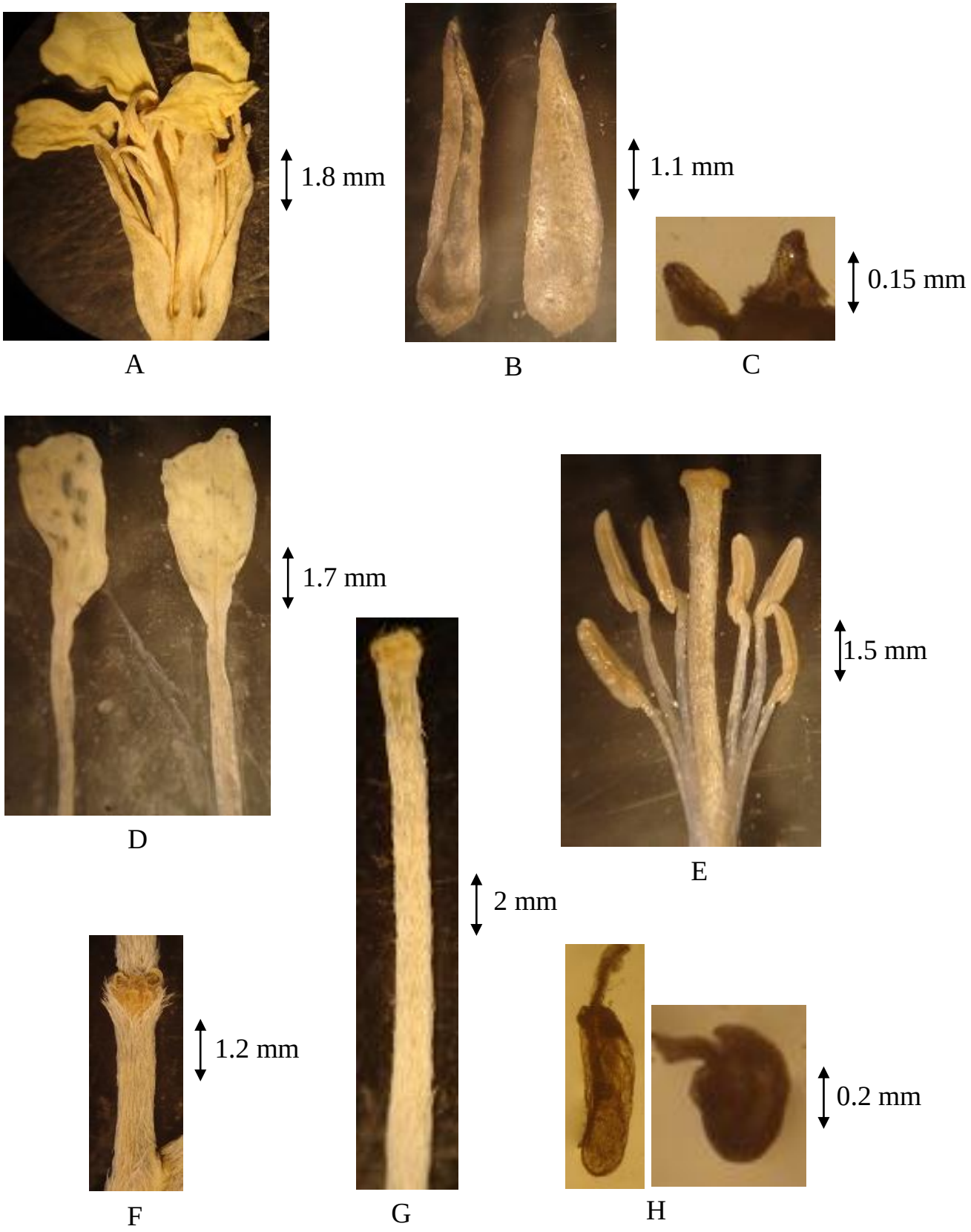
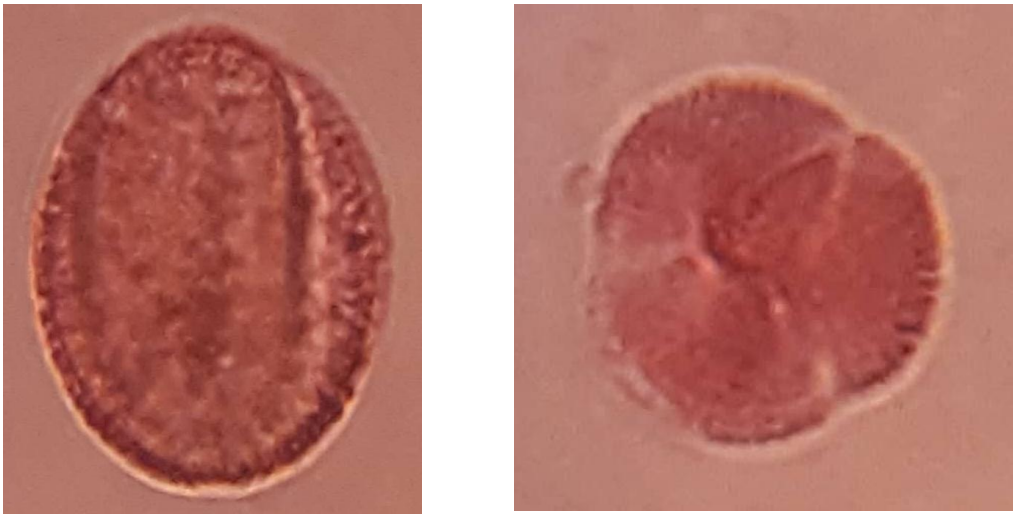
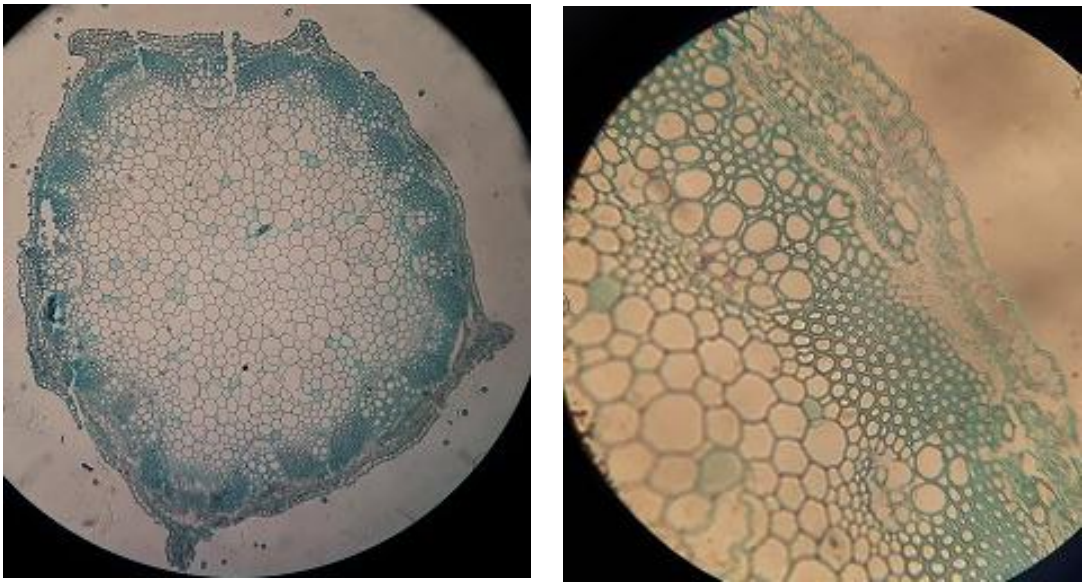


Plate (3): *E. amasianum*: A- Flower; B- Sepals; C- Nectar glands; D- Petals; E- Stamens & Pistil; F- Fruit stalk; G- Fruit (siliqua); H- Seeds



A B

Plate (4): Pollen grains of *E. amasianum*: A- Equatorial view; B- Polar view; A, B=100X



A B

Plate (5): A- C.S. of Stem of *E. amasianum*; B- Magnification view of A; A=10X, B=40X

4. CONCLUSIONS

The current study confirmed the presence of the plant *E. amasianum* from Brassicaceae family as a new record for the Flora of Iraq which collected from Safin mountain (north-east of Erbil), as well as, morphological, palynological and anatomical studies have been conducted for the plant under study.

REFERENCES

AGASHE, S. N. & CAULTON, E. 2009. Pollen and Spores, Applications with Special Emphasis on Aerobiology and Allergy, Science Publishers, Enfield, NH, USA: 400 pp.

AHMAD, S. A. 2013. Vascular Plants of Hawraman Region in Kurdistan Iraq. Ph.D. Dessartation, University of Sulaimani, Sulaimaniya, Iraq: 72.

AHMED, K. H. 2010. The Vascular Plants of Darband Gomaspan and the Adjacent Areas in Erbil Province. High Deploma Thesis, Salahaddin University, Erbil, Iraq: 38 pp.

AL-MASHHADANI, A. N. 1992. A Comparative Systematic Study of the genus Onosma L. (Boraginaceae) in Iraq. Ph.D. Dessartation, Baghdad University: 137-138.

AL-RAWI, A. 1964. Wild plants of Iraq with their distribution. Ministry of Agriculture & Irrigation, State board for agricultural & water resources research, National Herbarium of Iraq, Baghdad: 85.

BLAKELOCK, R. A. 1948. The Rustum Herbarium, Iraq. Systematic List. Part 1., kew Bull, 3: 430.

CHAKRAVARTY, H. L. 1976. Plant Wealth of Iraq, A Dictionary of Economic Plants. Vol.1, Botany Directorate, Ministry of Agriculture & Agrarian Reform, Baghdad-Iraq: 225-226.

CHAMBERLAIN, D. F. & RAVEN, P. H. 1972. In: Flora of Turkey. Vol. 4, Edinburgh at the University press, 183-196.

DARWESH, D. T. D. 2017. Plant Biodiversity and Ethnobotanical Properties of Various Plants in Choman (Erbil-Iraq). M. Sc. Thesis, Kahramanmaraş Sütçü İmam University, Graduate School of Natural and Applied Sciences University, Kahramanmaraş, Turkey: 130 pp.

ERDTMAN, G. 1971. Pollen Morphology and Plant Taxonomy, Angiosperms, Hafner publishing company, New York: 301-302.

FAO. 2002. Yearly report of Food and Agriculture Organization of the United Nations, Agricultural production department, Erbil-Iraq.

FARIS, Y. S. 1983. The Vascular Plants of Pira Magrun mountain. M. Sc. Thesis, Salahaddin University, Erbil, Iraq: 90.

FATAH, H. U. 2003. The Vascular Plants of Haibat Sultan mountain and the Adjacent Areas. M. Sc. Thesis, University of Sulaimani, Sulaimaniya, Iraq: 80.

GHAHREMAN, A. & ATTAR, F. 1999. Biodiversity of Plant Species in Iran, Central Herbarium, Tehran Univ., Tehran, Iran: 83.

GUEST, E. 1933. Notes on plants and plant products with their colloquial names in Iraq, Government Press: 31.

GUEST, E. 1966. Flora of Iraq. Vol. 1, Ministry of Agriculture of Iraq: 213 pp.

HAMEED, M. A. H. 2016. Vascular Plant Taxa of Hujran Basin-Erbil/ Iraq. M. Sc. Thesis, Kahramanmaraş Sütçü İmam University, Graduate School of Natural and Applied Sciences University, Kahramanmaraş, Turkey: 69-71.

HARRIS, J. G. & HARRIS, M. W. 2001. Plant Identification Terminology, An Illustrated Glossary. 2nd edition, Spring Lake Publishing, Spring Lake, Utah, United States: 206 pp.

HESSE, M., HALBRITTER, H., ZETTER, R., WEBER, M., BUCHNER, R., FROSCH - RADIVO, A. & ULRICH, S. 2009. Pollen Terminology, An illustrated handbook, Springer-Verlag/Wien, Austria: 219.

KHALAF, M. K. 1980. The Vascular Plants of Jabal Sinjar. M. Sc. Thesis, Baghdad University, Baghdad, Iraq: 125.

METCALFE, C. R. & CHALK, L. 1950. Anatomy of the Dicotyledons, Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses. Vol. 1, Oxford, at the Clarendon press: 664.

MIGAHID, A. M. 1978. Flora of Saudi Arabia.Vol.1, Riyadh Univ. publ.: 374.

POST, G. E. 1932. Flora of Syria, Palestine and Sinai. Vol. 1, American press, Beirut: 472-474.

RAVEN, P. H. 19--. In: Flora Iranica. No.7, Akademische Druck-u. Verlagsanstalt, Graz-Austria:, 3-18.

RECHINGER, K. H. 1964. Flora of low land Iraq, Weinheim verlag von. J. Cramer, wein: 552-554.

RIDDA, T. J. & DAOOD, W. H. 1982. Geographical distribution of wild vascular plants of Iraq, National Herbarium of Iraq: 56-57.

SHISHKIN, B. K. 1949. Flora of the U.S.S.R., Vol.15, Izdatelstro Akademii Nauk SSSR, Moskva-Leningrad: 429-465.

SIMPSON, M. G. 2006. Plant Systematics, Elsevier Academic Press, USA: 280,463.

TOWNSEND, C. C. & GUEST, E. 1980. Flora of Iraq. Vol.4, P.2, Ministry of Agriculture and Agrarian Reform, Baghdad-Iraq:1051-1063.

ZOHARY, M. 1946. The Flora of Iraq and its Phytogeographical Subdivisions, Iraq,Bull. No. 3, Dep. Agri.: 108.

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Water quality assessment for Duhok reservoir, Duhok-Iraq.

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A B S T R A C T

The physicochemical water quality and trophic status assessment for Duhok reservoir were carried out during four seasons in 2011. Water samples collected from three sites at three depths (surface, 2m, and 6m). Duhok reservoir constructed in 1988 for different purposes of water supply, irrigation, and recreations. The results showed that water quality is in an alkaline side of neutrality, conductivity value ranged from 750 to 1240 $\mu S.cm^{-1}$. Dissolved oxygen concentration decline under 5 $mg.l^{-1}$ during spring and summer seasons, with a trend of decreasing DO concentration from the surface toward depth (6m) in all studied sites. Based on TN: TP ratio nitrogen is a limiting factor for phytoplankton growth. Phosphate concentration of lake water falls within the oligotrophic status, while for chlorophyll *a* and Secchi depth values the lake categorised as eutrophic.

1. INTRODUCTION

Water quality is a composite of physical, chemical and biological characteristics of the given water (Straskraba and Tundisi, 1999). Water quality deterioration in reservoirs usually comes from excessive nutrient inputs, eutrophication, acidification, heavy metal contamination, organic pollution and obnoxious fishing practices. The effects of these "imports" into the reservoir do not only affect the socio-economic functions of the reservoir negatively but also bring loss of structural biodiversity of it (Djukic *et al*., 1994). The use of the physicochemical properties of water to assess water quality gives a good impression of the status, productivity, and sustainability of such water body (Tessema *et al*., 2014). The most important variables which directly and indirectly influencing the phytoplankton composition variability regarding abundance and composition are physical structure of the reservoir and the nutrient availability **(**Naselli, 2000; Padisák *et al*., 2006).

Phytoplankton is the main primary producer of water ecosystems and plays a major role in food chains (Reynolds, 1984). Phytoplankton communities are sensitive to changes in their environment, and therefore total phytoplankton biomass and many phytoplankton species are used as indicators of water quality (Reynolds *et al.*, 2002).

Phytoplankton is the focal point for biological studies of trophic status of the lake through the growth of primary producers is tied directly to nutrients, making phytoplankton the most likely group of organisms to respond to increased nutrient availability, as well as, phytoplankton community composition can have strong influences on water quality (Cottingham *et al*., 1998). Phytoplankton chlorophyll *a* concentrations may vary with

discharge, catchment area, water depth, or other physical factors (Kilkus *et al*., 1975; Søballe and Kimmel, 1987). The values of chlorophyll *a* concentration, a parameter which indicates the biomass of phytoplankton, are the highest after formation of the reservoir, which complies with the theory of its ageing (Gecheva *et al*., 2013). It is confirmed that the biomass of phytoplankton in freshwater lakes on certain occasions can limited by light (May *et al*., 2010; Xu *et al*., 2010).

Temperature and light have direct effects on phytoplankton production. Algal species exhibit photosynthetic optimum under different irradiance and temperature conditions (Wehr and Sheath, 2002). Photosynthetic response to light intensity is temperature dependent and species-specific (Wetzel, 2001).

This study aims to assess water quality and trophic status of Duhok Lake based on physicochemical parameters, chlorophyll *a* and Secchi depth.

2. MATERIALS AND METHODS

2.1. Study area

The Dohuk Lake dam is an earth-fill embankment dam (latitude: 36º 52′ 33″ N; longitude: 43º 00′ 13″ E). It was built on the Dohuk River just north of Dohuk city, Iraq. The dam completed in 1988 with the primary purpose of providing water for irrigation. It is 60 m tall and can withhold 52 million m^3 of water. The dam has a bell-mouth spillway with a maximum discharge of 81 m^3/s. The maximum length of the reservoir is 4km, and the maximum width is about 1.7km, with a maximum depth of 60m (Shekha *et al*., 2013) (Figure 1).

2.2. Sample collection and analysis

During four seasons in 2011 water samples were collected from three sites at three depths (surface, 2m and 6m). Different physicochemical water quality variables were analyses based on standard methods of water analysis. pH, electrical conductivity measured in field by using (pH meter Philips 4014 and EC meter Philips 4025 respectively). Dissolved Oxygen and BOD_5 determine by using azide modification of Winkler method as described by (APHA, 1998). Magnesium ion by (EDTA titrimetric method); total dissolved nitrogen (TDN) by wet mineralized method using potassium persulphate ($K_2S_2O_8$) as described by MacKareth *et al*. (1978); while persulphate digestion method was used for total dissolved phosphate (TDP) determination by treating water samples with (5% $K_2S_2O_8$) as described by Lind (1979). On the other hand, Indophenol's blue method and diazotized sulfanilamide procedure were applied for determination ammonia and nitrite respectively according to (APHA, 1998).

The chlorophyll-*a* concentration of prepared samples was determined spectrophotometrically using the cold acetone extraction method; while water transparency was measured by using Secchi disk (Bartram and Balance, 1996). Meanwhile, phytoplankton enumeration was determined by using filtration method as described by (Mcnabb, 1960).

3. RESULTS AND DISCUSSION

Water temperature is an important variable influenced other parameters related to water quality monitoring, such as the availability of many aquatic nutrients solubility of gasses, and the organism's activities (Ruttner, 1973). Results showed variability in water temperature during study seasons (9.0 to 28 °C at the surface water) (Table 1). Also decreases in water temperature recorded with the depth. While no variation observed between study locations (Table 2). Moyal and Hussain (2015) reported that the variation in water temperature might result from weather condition at the time of the study. Akindele *et al*. (2013) demonstrated that water temperature is not only influenced by seasonality but also by time of samples collection. Spatially and temporally surface water temperature decreased with the depth. The same pattern of variation was observed by (Medupin, 2011).

The pH values recorded during the study period shifted to be in the alkaline side of neutrality it ranged from (7.65 to 8.5) and (7.9

to 8.1) respectively for temporally and spatially variations. It falls within the acceptable range of natural water. In natural water, the pH value is usually 6.5-8.5 (Tebbutt, 1983). Most previous studies on inland water referred to the similar foundation, they returned reasons to the geological formation of the area and dominated of bicarbonate ions (Goran, 2014; Toma, 2011 and Al-Nimma, 1982).

Electrical conductivity (EC) estimate the amount of total dissolved ions in water (Goldman and Horne, 1983). Generally, variation in EC values between neither studied sites nor between depths observed, the lowest value recorded during the winter season (750 $\mu S.cm^{-1}$) and highest during summer season (1240 $\mu S.cm^{-1}$). It may be due to the dilution effect of rainfall in cold season, and to other factors on EC value such as temperature, geological origin and ionic salts of the water body. Carr and Neary (2006) commented that major ions were lower during the rainy season than in dry season as a result of dilution by rainfall.

Dissolved oxygen (DO) play a vital role in aquatic organisms (O'Sullivan and Reynolds, 2004). The results showed a decrease in DO concentration from the surface toward depth (6m) at all sites. It may be related to light transparency and intensity which decreased with depth (Dodds and Whiles, 2010). Highest DO concentration recorded during the winter season, coincided by low water temperature and dilution effect of rainfall. On the other hand, DO concentration decline below 5 $mg.l^{-1}$ during spring and summer seasons (4 $mg.l^{-1}$, table 1), which indicated bad aeration condition and non-acceptable level for aquatic organisms. It may be related to many factors such as high water temperature and activities organisms for decomposing organic matter (Welch and Jacoby, 2004). Oppositely, highest DO concentrations recorded during winter season at all depths, it coincided with lowest water temperature and chlorophyll *a* concentrations. Generally, the BOD_5 value was low and fall under 1.6 $mg.l^{-1}$ in all studied sites. Highest value noted during the summer season (2.2 $mg.l^{-1}$), which coincided by higher water temperature and low DO concentration. Increase in water temperature led to the decrease in solubility of gasses, also positively affect metabolic rate of organisms that required more DO for organic matter decomposition (Lampert and Sommer, 2007).

All inorganic form of nutrients (NO_2, NH_4, and PO_4) was within a permissible level of local and international drinking water standard. The main sources of nutrients input into the lake from human activities, fertilisers, animal manure and tourists. Higher nutrient concentration recorded during summer and spring seasons, attributed to decreased water volume. Similar results obtained by (Moyal and Hussain, 2015). Related to phosphate concentration of Duhok Lake, highest value noted during the summer season, with an increase in PO_4 toward the depth. The mean and range of PO_4 concentration in the lake (1.49 $\mu g.l^{-1}$, and 0.08 to 3.2 $\mu g.l^{-1}$, respectively) were lower than mean and range of oligotrophic lakes, then the lake could be categorised as oligotrophic lake according to (Wetzel, 1983).

The most two limiting nutrients to algal production in lakes are nitrogen and phosphorus. The normal total nitrogen to total phosphorus (TN: TP) ratio in a lake is 10:1. A higher ratio indicates a deficiency in phosphorus and a lower ratio indicates a deficiency in nitrogen (Horne and Goldman, 1994). Average TN: TP ratios calculated for Duhok Lake is (8.50), then nitrogen regarded as a limiting nutrient for primary production. The low nutrient inputs or high phosphorus level may be the reason for nitrogen shortage. Alemayehu and Hackett (2016) observed that when nitrogen effects on algal growth, it is usually because either the phosphorus level is high or nutrient input is very low. These results disagreement with that recorded by Shekha *et al*. (2017) in the same lakes, they commented that phosphorus was the limiting factors for algal growth.

Measurements of the algal pigment chlorophyll *a* can be used to estimate the water quality, trophic level and phytoplankton standing crop (Akbulut, 2003; Koenings *et al*., 1987). During this study chlorophyll *a* was above 10 µg.l^{-1} in all sites from the surface to depth (6m). Winter season characterised by lowest chlorophyll *a* concentration (below 10 µg.l^{-1}), with a trend to increase gradually to the spring season and highest concentration recorded during the summer season. It could be related to the water level. Ndungu *et al*. (2013) found an inverse relationship between chlorophyll a and water level. In addition to, decrease in sunlight and photosynthesis during winter season compared to other seasons. As showed in Figure (2) an opposite variation between chlorophyll a and magnesium concentration in water was noted. Magnesium is the major component of chlorophyll *a* structure (Lee, 2008).

According to Wetzel (1983), the level of chlorophylla in oligotrophic lakes is 0.33-3 µg/L, in a mesotrophic lakes 2-15 µg/L, and in eutrophic lakes 10-500 µg/L. Comparing the data of Duhok lake with Wetzel (1983) range suggests that the concentration of chlorophyll *a* (mean and range value was (22 µg.l^{-1} and 5.1-46.5 µg.l^{-1}, respectively during seasons) are within the range of eutrophic lakes. On the other hand, the Secchi depth in this study was range between 2m - 3.65m with mean value 2.8m, which also confirmed the eutrophic state of the Dukan Lake. These results were compared with (OECD, 1982) for the trophic status of the reservoir (Table 3), and Duhok Lake also can be categorised as eutrophic based on chlorophyll *a* and Secchi depth. It come accordance with that found by Shekha *et al*. (2017), they stated that water quality of Duhok lake was classified as eutrophic in all seasons except in Autumn found as a mesotrophic lake.

Phytoplankton numbers were high in site 1, average count (2277 cells.l^{-1}), and while for season variation, the highest mean number was recorded during the spring season (2948 cells.l^{-1}). Zheng and Jan Stevenson (2006) found that phytoplankton increased with an increase of light, which suggested increases in radiant energy are conducive to the growth of phytoplankton. As noted in Figure (3) similar variations were obtained between phytoplankton and chlorophyll a concentration during studied seasons. Same results were documented by Cao *et al*. (2016) in Lake Poyang, China. On the other hand, in this study during phytoplankton counting very low density of zooplankton was noted which indicated less predation stress on it. Vidovic *et al*. (2015) commented that the availability of nutrients with an absence of zooplankton predation causes the massive development of phytoplankton.

4. CONCLUSIONS AND RECOMMENDATION

Finally, it can be concluded that nitrogen is a limiting factor for producers and water quality of Duhok reservoir classified as eutrophic. It well be recommended for future studies to investigate faunal structural composition and diversity.

REFERENCES

AKBULUT, A., 2003. The relationship between phytoplanktonic organisms and chlorophyll a in Sultan Sazliği, Turk J. of Botany. 27: 421-425.

AKINDLE, E.O.; ADENIYI, I.F. and INDABAWA, I.I. 2013. Spatio- temporal assessment and water quality characteristics of Lake Tiga, Kano, Nigeria. Research J. of Environmental and Earth Science. 5(2): 67- 77.

ALEMAYEHU, D. and HACKETT, F. 2016. Water quality and trophic state of Kawa Lake, J. of Environmental Studies. 2(1): 1-7.

Al-NIMMA, B.A.B. 1982. A study on the limnology of the Tigris and Euphrates rivers, M.Sc. Thesis, University of Salahaddin - Erbil. Iraq.

AMERICAN PUBLIC HEALTH ASSOCIATION (A.P.H.A.). 1998. Standard methods for the examination of water and wastewater. 20th Ed. A.P.H.A., 1015 Fifteenth Street, NW, Washington, DC. 20005-2605.

BARTRAM, J. and BALANCE, R. 1996. Water quality monitoring (a practical guide to the design and implementation of freshwater quality studies and

monitoring programs). United Nation Environmental Programme- UNEP- and WHO. E & FN Spon, Chapman and Hall. UK. 383pp.

CAO, J.; CHU, ZHAOSHENG; DU, Y.; HOU, Z. and WANG, S. 2016. Phytoplankton dynamics and their relationship with environmental variables of Lake Poyang. Hydrology Research.

CARR, G.M. and NEARY, J.P. 2006. Water quality for ecosystem and human health. United Nations Environment Programme Global Environment Monitoring System.

COTTINGHAM, K. L.; CARPENTER, S.R., and AMAND, A.L. 1998. Responses of epilimnetic phytoplankton to experimental nutrient enrichment in three small seepage lakes. J. of Plankton Research. 20(10): 1889-1914.

DJUKIC, N.; MALETIN, S.; PUJIN, V.; IVANC, A. and MILAJOVIC, B. 1994. Ecological assessment of water quality of Tisze by physico-chemical and biological parameters. Tisca Szeged. 28: 37-40.

DODDS, W.K. and WHILES, M.R. 2010. Freshwater ecology: Concepts and environmental applications of limnology. 2nd Ed. Elsevier Inc., USA.

GECHEVA, G.; YURUKOVA, L.; CHESHMEDJIEV, S.; VARADINOVA, E. and BELKINOVA, D. 2013. Integrated Assessment of the Ecological Status of Bulgarian Lowland and Semi-Mountain Natural Lakes. Journal of Environmental Protection. 4:29-37. http://dx.doi.org/10.4236/jep. 2013.46A004.

GOLDMAN, C.R. and HORNE, A.J. 1983. Limnology. McGraw- Hill international Book Company. Japan.

GORAN, S. M. 2014. Ecological study on Dukan Lake with particular reference to bioaccumulation of some heavy metals and PAHs in fish and Gull tissues- Sulaimani, PhD Dissertation, Salahaddin University-Erbil, Iraq.

HORNE, A.J. and GOLDMAN, C.R. 1994. Limnology. 2nd Edition. McGraw-Hill, Inc., U.S.A.

KILKUS, S. P.; LAPERRIERE, J. D. and BACHMANN, R.W., 1975. Nutrients and algae in some central Iowa streams, Water Pollution Control Federation. 47(7): 1870–1879.

KOENINGS, J. P.; EDMUNDSON, J. A.; KYLE, G. B. and EDMUNDSON, J.M. 1987. Limnology field and laboratory manual: Methods for assessing aquatic production. Alaska Department of Fish and Game, FRED Division Report Series 71, Juneau.

LAMPERT, W. and SOMMER, U. 2007. Limnoecology: The ecology of lakes and streams. 2nd Ed. Oxford University Press.

LEE, R.E. 2008. Phycology. 4th Ed. Cambridge University Press.

LIND, O.T. 1979. Handbook of common methods in limnology. C.V. company. USA. 197pp.

MACKERETH, F.J.H.; HERON, J. and TALLING, J.F. 1978. Water analysis: Some revised methods for limnologists science publication. No.36. Freshwater Biol. Assoc., Titus Wilson and Sons Ltd. UK. 121pp.

MAY, L.; SPEARS, B.M.; DUDLEY, B.J. and HATTON-ELLIS, T.W. 2010. The Importance of Nitrogen Limitation in the Restoration of Langorse Lake, Wales, UK. J. of Environmental Monitoring. 12: 338-346. http://dx.doi.org/10.1039/ b912827a.

MCNABB, C. 1960. Enumeration of freshwater phytoplankton concentration on the membrane filter. Limnological and Oceanography. 5:57-61.

MEDUPIN, C. 2011. Phytoplankton community and their impact on water quality: An analysis of Hollingsworth Lake, UK. J. Applied Science Environmental Management. 15(2): 347- 350.

MOYAL, M.S. and HUSSAIN, N.A. 2015. Water quality assessment of the Shatt al Arab River, southern Iraq. Journal of Coastal Life Medicine. 3(5):459- 465.

NASELLI, F. L. 2000. Phytoplankton assemblage in twenty-one Sicilian reservoirs: relationships between species composition and environmental factors. Hydrobiologia. 424: 1-11.

NDUNGU, J.; AUGUSTIJN, D.C.; HULSCHER, S.J.; KITAKA, N. and Mathooko, J. 2013. Spatio-temporal variations in the trophic status of lake Naivasha, Kenya. Lakes Reservoir: Research Manage. 18:317- 328.

OECD. 1982. Eutrophication of Waters. Monitoring, Assessment and Control. Organization for Economic Co-Operation and Development. Paris.

O'SULLIVAN, P.E. and REYNOLDS, C.S. 2004. Limnology and limnetic ecology. Blackwell Publishing Company.

PADISAK, J.; BORICS, G.; GRIGORSKZKY, I. and SOROCZKI- PINTÉR, É. 2006. Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. Hydrobiologia. 502: 157-168.

REYNOLDS, C. S. 1984. Phytoplankton periodicity-the interactions of form, function and environmental variability. Freshwater Biology. 14: 111–142.

REYNOLDS, C.; HUSZAR, V.; KRUK, C.; NASELLI-FLORES, L. and MELO, S. 2002. Towards a functional classification of the freshwater phytoplankton. J. Plankton Research. 24:417-428.

RUTTNER, R. 1973. The fundamental of limnology. Univ. of Toronto Press.

SHEKHA, Y. A.; ALI, L.A. and TOMA, J.J. 2017. Assessment of Water Quality and Trophic Status of Duhok Lake Dam. Baghdad Science Journal. Vol.14(2): 335-342.

SHEKHA, Y. A.; ISMAEL, H. M. and AHMED, A. A. 2013. Bacteriological and mycological assessment for water quality of Duhok Reservoir. Jordan J. of Biological Sciences (JJBS). 6(4):1-10.

SØBALLE, D. M. and KIMMEL, B. L. 1987. Large scale comparison of factors influencing phytoplankton abundance in rivers, lakes, and impoundments. Ecology. 68 (6):1943–1954.

STRASKRABA, M. and TUNDISI, J.G. 1999. Reservoir Water Quality Management. Guidelines of Lake Management. Volume 9. International Lake Environment Committee (ILEC) Shiga, Japan.

TEBBUTT, T.H.Y. 1983. Principles of water quality control. 3rd.Ed. Pergamon Press. UK.

TESSEMA, A.; MOHAMMED, A.; BIRHANU, T. and NEGU, T. 2014. Assessment of Physico-chemical Water Quality of Bira Dam, Bati Wereda, Amhara Region, Ethiopia. J. Aquac. Res. Development. 5(6): http://dx.doi.org/10.4172/2155-9546.1000267.

TOMA, J.J. 2011. Limnological study in Dokan Lake, Kurdistan Region of Iraq. J. of Environmental Studies. 6:1-12.

VIDOVIC, M.M.; RODIC, M.N.; VIDOVIC, M.U.; TRAJKOVIC, I.S. and JOVANIC, S.Z. 2015. Assessment of the trophic status by monitoring of reservoirs water quality. J. of Water Resources and Protection. 7:1-13.

WEHR, J.D. SHEATH, and R.G. 2002. Freshwater Algae of North American: Ecology and Classification. Academic Press.

WELCH, E.B. and JACOBY, J.M. 2004. Pollutant effects in freshwater: Applied limnology. 3rd Ed. Spon Press, UK. 504.

WETZEL, R. G. 2001. Limnology: Lake and River Ecosystems, 3rd Ed. Academic Press, San Diego, CA, USA.

WETZEL, R.G. 1983. Limnology. 2nd Ed. Saunders College Publishing, U.S.A.

XU, Y.; CAI, Q.; HAN, X.; SHAO, M. and LIU, R. 2010. Factors regulating trophic status in a large subtropical reservoir, China, Environmental Monitoring and Assessment, 169: 237-248. http://dx.doi.org/10.1007/s10661-009-1165-5.

ZHENG, L. and JAN STEVENSON, R. 2006. Algal assemblages in multiple habitats of restored and extant wetlands. Hydrobiologia. 561:221–238.

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$\tilde{S}p_c$-OPEN SETS and $\tilde{S}p_c$-CONTINUITY in SOFT TOPLOGICAL SPACES

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A B S T R A C T

The main objective of this paper is to introduce and define a new class of sets called soft p_c-open sets in soft topological spaces, which is subclass of soft pre-open sets. Several properties of this kind of sets are obtained. By using this soft set we present and study the concept of soft p_c-continuous functions.

1. INTRODUCTION

The soft set theory was first introduced and studied by the Russian researcher Molodtsov in 1999, as a generalization or analogue to so many theories such as the theory of rough set, the theory of fuzzy set, the theory of intuitionistic fuzzy set, the theory of vague set. Each theory has its difficulties which are remarked in [Molodtsov 1999]. Molodtsov pointed out several directions for the applications of soft sets, such as Riemann integration, game theory, theory of measurement, probability theory etc. Throughout this paper X is an initial universe, A is a set of parameters and ∇ is an index set. A pair (F,A) is called a soft set over X if F is a mapping $F: A \to P(X)$. The family of all soft sets over the universal set X with the set of parameters A is denoted by $SS(X)_A$. We say that (F,A) is a soft subset of (G,A) or (G,A) is a soft super set of (F,A). and we write $(F, A) \subseteq (G, A)$ if for all $\alpha \in A$, $F(\alpha) \subseteq G(\alpha)$ .Two soft sets are equal if (G,A) is a soft subset of (F,A) and (F,A) is a soft subset of (G,A) . The complement of (F,A) is denoted by $(F, A)^c$ and is defined by $(F, A)^c = (F^c, A)$ where $F^c: A \to P(X)$ is a mapping given by $F^c(\alpha) = X - F(\alpha)$ for all $\alpha \in A$.

In particular, (X,A) will be denoted by $\tilde{X}$. Let (F,A) be the soft set over X and Y be a non-empty subset of X. Then the soft subset of

(F,A) over Y denoted by (^{Y}F,A) is defined $^{Y}F(\alpha) = Y \cap F(\alpha)$, for all $\alpha \in A$.

2- PRELIMINARIES

In this section, we present a brief introduction to some of the background in soft topological spaces. In section two, we define $\tilde{s}p_c$- open set and prove several properties of $\tilde{s}p_c$- open sets, and the relation of this set with other types of soft-open sets are stated , in section three we define a new kind of continuity by using $\tilde{s}p_c$-open sets.

Definition 2.1: [Zorlutuna 2014] Let I be an arbitrary index set and $\{(F_i, A): i \in I\}$ be a sub family of $SS(X)_A$.

1-The union of soft sets is a soft set. That is $G(\alpha) = \cup \{F_i(\alpha): i \in I\}$ for each $\alpha \in A$. Symbolically we write $(G, A) = \cup \{(F_i . A): i \in I\}$.

2- The intersection of soft sets is a soft set. That is $H(\alpha) = \cap \{F_i(\alpha): i \in I\}$ for each $\alpha \in A$. Symbolically we write $(H, A) = \cap \{(F_i . A): i \in I\}$.

Definition 2.2: [Shaber2011] Let τ be a collection of soft sets over X, then τ is said to be a soft topology on X if:
(1) $\tilde{\emptyset}$, $\tilde{X}$ belong to τ .
(2) The union of any number of soft sets in τ belongs to τ .
(3) The intersection of any two soft sets in τ belongs to τ .

The triple (X, τ, A) is called a soft topological space over X .

Let (X, τ, A) be a soft space over X , then the members of τ are said to be soft open sets in X denoted by SO(X) and their complement are said to be soft closed sets in X. If Y is a non-empty subset of X, then τ_Y = {(F, A)$\cap Y$: (F, A) $\in \tau$ } is said to be the soft relative topology on Y and (Y, τ_Y, A) is called soft subspace of (X, τ, A).

Proposition 2.3: [Shaber2011] Let (Y, τ_Y , A) be a soft subspace of a soft topological space (X, τ, A) and (F, A) $\in$ SS(X)$_A$. Then:
(1) If (F, A) is a soft open set in Y and $\tilde{Y} \in \tau$, then (F, A) $\in \tau$.
(2) (F, A) is a soft open set in Y if and only if (F, A) = $\tilde{Y} \cap$ (G, A) for some (G, A) $\in \tau$.
(3) (F, A) is a soft closed set in Y if and only if (F, A) = $\tilde{Y} \cap$ (H, A) for some soft closed (H, A) in X .

A soft subset (F,A) of a soft space X is said to be soft pre-open [IIango 2013] (resp., soft semi-open[Hosny 2014], soft α-open set [Akdag and Alkan 2014], soft β-open set[Yunak2015] and soft regular-open [IIango 2013]) if A$\subseteq \tilde{s}$int$\tilde{s}$clA (resp., A$\subseteq \tilde{s}$cl$\tilde{s}$intA, A$\subseteq \tilde{s}$int$\tilde{s}$cl$\tilde{s}$intA, A$\subseteq \tilde{s}$cl$\tilde{s}$int$\tilde{s}$clA and A=$\tilde{s}$int$\tilde{s}$clA). The complement of soft pre-open (resp., soft semi-open, soft α -open, soft β -open, soft regular open) set is said to be soft pre-closed (resp., soft semi-closed, soft α-closed, soft β -closed, soft regular closed).The family of soft pre-open (resp., soft semi-open, soft α- open, soft β --open, and soft regular open) set is denoted by $\tilde{s}$PO(X) (resp., $\tilde{s}$SO(X), $\tilde{s}\alpha$ O(X), $\tilde{s}\beta$ -O(X), $\tilde{s}$RO(X)).

The soft pre interior of (F,A) is denoted by $int^p(F, A)$ [IIango 2013], defined as the union of all soft pre-open sets contained in (F,A) and the pre closure of (F,A) is , denoted by $cl^p(F, A)$ defined as the intersection of all soft pre- closed sets containing (F,A). A soft set (F,A) in a soft topological space (X, τ, A) is said to be a soft pre neighbourhood of the soft point x_α[Ravindran 2014] if there is a soft pre open set (G,A) such that $x_\alpha \in (G, A) \subseteq (F, A)$. If (F,A) is a soft subset of a soft space X , then the soft pre-boundary of (F,A) [Ravindran 2014] is defined as $\tilde{s}$pcl(F, A) \ $\tilde{s}$pint(F, A).

Let (X, τ, A) be a soft topological space. A soft point $x_\alpha \in$ SP(X)$_A$ is said to be pre limit

soft point of a soft set (F,A) if for every soft pre-open set (G,A) containing x_α, then $(F,A) \cap [(G,A) \setminus \{x_\alpha\}] \neq \tilde{\emptyset}$. The set of all pre limit soft point (F,A) is called pre derived set of (F,A). A soft topological space is called soft locally indiscrete [Zorlutuna2014] , if every soft open set over X is soft closed and is said to be extremely soft disconnected [Akdag and Alkan2014] if the soft closure of every soft open set is soft open.

Dentition 2.4: [khalaf 2011] Let $SS(X)_E$ and $SS(Y)_K$ be soft classes. Let $u : X \to Y$ and $p : E \to K$ be mappings. Then a soft mapping $f_{pu} : SS(X)_E \to SS(Y)_K$ is defined as:

(1) For a soft set (F, A) in $SS(X)_E$. $(f_{pu}(F, A), B)$, $B = p(A) \subseteq K$ is a soft set in $SS(Y)_K$ given by

$$f_{pu}(F,A)(\beta) = \begin{cases} u\left(\cup_{\alpha \in p^{-1}(\beta) \cap A} \left(F(\alpha)\right)\right) & p^{-1}(\beta) \cap A \neq \emptyset \\ \emptyset & otherwise \end{cases}$$

for $\beta \in B \subseteq K$. $(f_{pu}(F, A), B)$ is called a soft image of a soft set (F, A). If B = K, then we shall write $(f_{pu}(F, A), K)$ as $f_{pu}(F, A)$.

(2) For a soft set (G, C) in $SS(Y)_K$. $(f_{pu}^{-1}(G, C), D)$, $D = p^{-1}(C)$ is a soft set in $SS(X)_E$ given by

$$f_{pu}^{-1}(G,C)(\alpha) = \begin{cases} u^{-1}\left(G(p(\alpha))\right) & p(\alpha) \in C \\ \emptyset & otherwise \end{cases}$$

for $\alpha \in D \subseteq E$. $(f_{pu}^{-1}(G, C), E)$ is called a soft inverse image of a soft set (G, C).

We shall write $(f_{pu}^{-1}(G, C), E)$ as $f_{pu}^{-1}(G, C)$.

Theorem 2.5: [Yuksel 2014] A soft topological space (X, τ, A) is soft regular if and only if for every $x \in X$ and every soft open set (F,A) containing x, there is a soft open set (G,A) containing x such that $x \in (G, A) \subseteq \tilde{s}cl(G, A) \subseteq (F, A)$

Corollary 2.6: [Yuksel 2014] A soft topological space (X, τ, A) is soft regular if for every $x_\alpha \in SP(X)_A$ and every soft open set (F, A) containingx_α, there is a soft open set (G, A) containing x_α such that $x_\alpha \in (G, A) \subseteq \tilde{s}cl(G, A) \subseteq (F, A)$.

Theorem 2.7: [Akdag and Ozkan 2014] A subset (F,A) of a soft topological spaces (X,τ, A) is soft pre-open set if and only if there exists a soft open set (G,A) such that $(F, A) \subseteq (G, A) \subseteq \tilde{s}cl(F, A)$.

Theorem 2.8: [IIango 2013] Arbitrary union of soft pre-open sets is a soft pre-open set.

Proposition 2.9: [Akdag and Ozkan 2014] Let (X, τ, A) be a soft topological space. If (F, A) ∈s̃αO(X) and (G, A) ∈ s̃PO(X), then (F, A) ∩ (G, A) ∈ s̃PO(X).

Proposition 2.10: [Khalf 2015] A soft space X is extremely soft disconnected if and only if every soft semi-open set is soft pre-open.

Theorem 2.11: [Akdag and Ozkan 2014] Let $(F, A) \subseteq \tilde{Y} \subseteq \tilde{X}$, where (X, τ, A) be a soft topological space and $\tilde{Y}$ is a soft pre-open subspace of $\tilde{X}$. then (F, A) ∈ s̃pO(X), if and only if (F, A) ∈ s̃pO(Y).

Theorem 2.12: [Ravindran 2014] If U is soft open and (F, A) is soft pre-open, then U ∩ (F, A) is soft pre-open.

Theorem 2.13: [Ravindran 2014] Soft topological spaces (X, A, τ) and (X, A, τ_α) have the same class of soft pre-open sets.

Theorem 2.14: Let $(F, A) \subseteq \tilde{Y} \subseteq \tilde{X}$, where (X,τ, A) be a soft topological space and $\tilde{Y}$ is a soft subspace of $\tilde{X}$. If (F, A) ∈ SpO(X), then (F, A) ∈ SpO(Y).

Proof: Since (F, A) ∈ SpO(X), then from Theorem 2.7 there exists a soft open set (G,A)

such that $(F, A) \subseteq (G, A) \subseteq Scl(F, A)$, which implies that $(F, A) \subseteq (G, A) \cap \widetilde{Y} \subseteq \tilde{s}cl(F, A) \cap \widetilde{Y}$. Putting $(G, A)^* = (G, A) \cap \tilde{Y}$, then there exist a soft open set $(G, A)^*$ in $\widetilde{Y}$ such that $(F, A) \subseteq (G, A)^* \subseteq \tilde{s}cl_Y(F, A)$. Consequently $(F, A) \in SpO(Y)$.

Proposition 2.15: [Khalf 2015]Let (Y, τ_Y, A) be a soft subspace of a soft space (X, τ, A). If (F, A) is soft closed subset in $\widetilde{X}$ and $(F, A) \subset \widetilde{Y}$, then (F, A) is soft closed in $\widetilde{Y}$.

Theorem 2.16: [Ravindran 2014] For any soft spaces (X, τ, A) and (Y, τ_Y, A). If soft sets $(F, A) \subseteq \widetilde{X}$ and $(G, B) \subseteq \widetilde{Y}$ we have

1- $\tilde{s}pcl_{X\times Y}\big((F, A) \times (G, B)\big) = \tilde{s}Pcl_X(F, A) \times \tilde{s}Pcl_Y(G, B)$
2- $\tilde{s}pint_{X\times Y}\big((F, A) \times (G, B)\big) = \tilde{s}pint_X(F, A) \times \tilde{s}pint_Y(G, B)$

Proposition 2.17: If $f_{pu}: (X, \tau, A) \to (Y, \tau_Y, B)$ be a soft continuous and soft open function. If (F, A) is a soft pre-open set of $\widetilde{Y}$, then $f_{pu}^{-1}(F, A)$ is a soft pre-open set of $\widetilde{X}$.

Proof: Let (F, A) be a soft pre-open set of $\widetilde{Y}$, then there exists a soft open set (G,A) in $\widetilde{Y}$ such that $(F, A) \subseteq (G, A) \subseteq \tilde{s}cl(F, A)$. Then $f_{pu}^{-1}(F, A) \subseteq f_{pu}^{-1}(G, A) \subseteq f_{pu}^{-1}(\tilde{s}cl(F, A))$. Since f_{pu} is soft continuous, then $f_{pu}^{-1}(G, A)$ is a soft open set in $\widetilde{X}$ and since f_{pu} is soft open , then $f_{pu}^{-1}(\tilde{s}cl(F, A)) \subseteq \tilde{s}clf_{pu}^{-1}(F, A)$. Hence we obtain that $f_{pu}^{-1}(F, A) \subseteq f_{pu}^{-1}(G, A) \subseteq \tilde{s}clf_{pu}^{-1}(F, A)$, therefore $f_{pu}^{-1}(F, A)$ is soft pre-open in $\widetilde{X}$.

Definition.2.18: Let (X, τ, A) be a soft topological space. The soft θ-interior of a soft subset $(F, A). \in SS(X, A)$ is the soft union of all soft open sets over X whose soft closures are soft contained in (F, A), and is denoted by $\tilde{s}\theta int(F, A)$. The soft subset (F, A) is called soft θ -open if $\tilde{s}\theta int(F, A). = (F, A)$. The complement of a soft θ -open set is called soft θ -closed.

3. $\tilde{s}P_c$-open sets :

In this section we introduce and study the concept of $\tilde{s}p_c$-open sets in soft topological spaces.

Definition 3.1: A soft pre-open set (F, A) in a soft topological space (X, τ, A) is called soft p_c-open if for each $x_\alpha \in (F, A)$, there exists a soft closed set (K, A) such that $x_\alpha \in (K, A) \subseteq (F, A)$. The family of all $\tilde{s}p_c$-open sets in a soft topological space (X, τ, A) is denoted by $\tilde{s}p_cO(X, \tau, A)$ or $\tilde{s}p_cO(X)$. This definition is utilized in the proof of Proposition 3.2.

Proposition 3.2: A soft set (F, A) in a soft topological space (X, τ, A) is $\tilde{s}p_c$-open if and only if (F, A) is soft pre-open and it is a union of soft closed sets. That is, a soft set (F, A) is $\tilde{s}p_c$-open if and only if $(F, A) = \cup(K_\lambda, A)$, where (F, A) is soft pre-open and (K_λ, A) is soft closed for each $\lambda \in \Lambda$.

Remark 3.3: The following examples show that every soft pre-open set need not be $\tilde{s}p_c$-open set and $\tilde{s}p_c$-open set need not be soft closed.

Example 3.4: Consider $U = \{u_1, u_2, u_3\}$, $E = \{e_1, e_2, e_3\}$, $A = \{e_1, e_2\}$, and $F_A = \{(e_1, \{u_1\}), (e_2, \{u_1, u_2)\}$. The class of all soft subsets over U is denoted by $S(F_A)$. Then $F_{A_1} = \{(e_1, \{u_1\})\}$, $F_{A_2} = \{(e_1, \{u_1\}), (e_2, \{u_1\})\}$, $F_{A_3} = \{(e_1, \{u_1\}), (e_2, \{u_2\})\}$, $F_{A_4} = \{(e_2, \{u_1, u_2\})\}$, $F_{A_5} = \{(e_2, \{u_1\})\}$, $F_{A_6} = \{(e_2, \{u_2\})\}$

$F_{A_7} = F_A$ $F_{A_8} = F_\emptyset$. Define the soft topology $\tau = \{F_A, F_\emptyset, F_{A_1}, F_{A_3}, F_{A_4}, F_{A_6}\}$, $\tilde{s}PO(X) = \{F_A, F_\emptyset, F_{A_1}, F_{A_3}, F_{A_4}, F_{A_6}\}$, $\tilde{s}P_cO(X) = \{F_A, F_\emptyset, F_{A_1}, F_{A_4}\}$. Then $F_{A_3} \in \tilde{s}PO(X)$ but $F_{A_3} \notin \tilde{s}P_cO(X)$.

Example 3.5: Let $\mathbf{R}$ be the set of all real numbers, $A = \{u, s\}$, and

$\beta = \{(F_a^b, A); a < b\}$ where the map $F_a^b: A \to P(R)$ defined as follows:

$$F_a^b(\alpha) = \begin{cases} (a,b) & if\ \alpha = u \\ R\ or\ \widetilde{\emptyset} & if\ \alpha = s \end{cases}$$

Let τ be the topology on R with the base β. Then the set $G_a^b(\alpha) = \begin{cases} Q & if\ \alpha = u \\ \widetilde{\emptyset} & if\ \alpha = s \end{cases}$ where Q is the set of all rational numbers.

Then $G_a^b(\alpha)$ is $\tilde{s}p_c$-open set since for each $x_\alpha \in \{x_\alpha\} \subseteq G_a^b(\alpha)$ which is not a soft closed set .

The following result shows that in a soft T_1-space, $\tilde{s}pO(X)$ coincide with $\tilde{s}p_cO(X)$.

Proposition 3.6: If a space $\tilde{X}$ is a soft T_1-space, then $\tilde{s}p_cO(X) = \tilde{s}pO(X)$.

Proof. Let $\tilde{X}$ be a soft T_1-space and $(F,A) \in \tilde{s}pO(X)$. If $(F,A) = \widetilde{\emptyset}$, then $(F,A) \in \tilde{s}p_cO(X)$. If $(F,A) \neq \tilde{\varphi}$, then for each $x_\alpha \in (F,A)$, $\{x_\alpha\}$ is soft closed. Hence, $x_\alpha \in \{x_\alpha\} \subseteq (F,A)$. Therefore, by Definition 3.1, $(F,A) \in \tilde{s}p_cO(X)$. Hence, $\tilde{s}pO(X) \subseteq \tilde{s}p_cO(X)$, but $\tilde{s}p_cO(X) \subseteq \tilde{s}pO(X)$, therefore $\tilde{s}p_cO(X) = \tilde{s}pO(X)$.

The following result shows that any union of $\tilde{s}p_c$-open sets of a soft space (X, τ, A) is $\tilde{s}p_c$ -open.

Proposition 3.7: Let $\{(F_\lambda, A) : \lambda \in \Lambda\}$ be a collection of $\tilde{s}p_c$ –open sets in a soft topological space X , then $\cup\{(F_\lambda, A) : \lambda \in \Lambda\}$ is $\tilde{s}p_c$ -open.

Proof: From (F_λ, A) is soft pre-open for each λ and hence by Theorem 2.9 , $\cup\{(F_\lambda, A): \lambda \in \Lambda\}$ is soft pre-open. Let $x_\alpha \in \cup\{(F_\lambda, A): \lambda \in \Lambda\}$, hence $x_\alpha \in (F_\lambda, A)$ for some $\lambda \in \Lambda$. Since (F_λ, A) is $\tilde{s}p_c$ -open for each λ, so there exists a soft closed set (K, A) such that $x_\alpha \in (K, A) \subseteq (F_\lambda, A) \subseteq \cup\{(F_\lambda, A) : \lambda \in \Lambda\}$, so $x_\alpha \in (K, A) \subseteq \cup\{(F_\lambda, A): \lambda \in \Lambda\}$. Therefore, $\cup\{(F_\lambda, A) : \lambda \in \Lambda\}$ is an $\tilde{s}p_c$ -open set

We will see by the following example that the intersection of two $\tilde{s}p_c$ -open sets need not be $\tilde{s}p_c$ -open set.

Example 3.8: Consider the co-finite soft topological space $\tilde{X}$, with the same set of parameters A, where X=A=N the set of all natural numbers, $\tau = \{X, \varphi\} \cup \{G_1(n), G_2(n)\}$, where $G: A \to P(X)$ such that $G_1(n) = \{2n; n \in N\}$ and $G_2(n) = \{2n+1; n \in N\} \cup \{0\}$, easily can be checked that both $G_1(n)$, $G_2(n)$ are soft pre-open sets and since co-finite soft topology is soft T_1-spaces then by Proposition $G_1(n)$, $G_2(n)$ are $\tilde{s}p_c$ -open sets, but $G_1(n), \cap G_2(n) = \{0\}$ which is not an $\tilde{s}p_c$ -open set.

Remark 3.9: The result of Proposition 3.10 Improve Example 3.8, where the family of all soft pre-open sets of a soft space $\tilde{X}$ is a soft topology on $\tilde{X}$.

Proposition 3.10: If the family of all soft pre-open sets of a soft space $\tilde{X}$ is a soft topology on $\tilde{X}$, then the family of $\tilde{s}p_c$ -open sets is also a soft topology on $\tilde{X}$.

Proof: It is enough to show that the intersection of two $\tilde{s}p_c$ -open sets is $\tilde{s}p_c$ -open. Let (F, A) and (G, A) be two $\tilde{s}p_c$ -open sets, then (F, A) and (G, A) are soft pre-open sets. Since $\tilde{s}pO(X)$ is a soft topology on X , so (F, A) $\cap$(G, A) is soft pre-open. Let $x_\alpha \in$ (F, A) $\cap$

(G, A), then $x_\alpha \in$ (F, A) and $x_\alpha \in$ (G, A), so there exist soft closed sets (H, A) and (K, A) such that $x_\alpha \in$ (H, A) $\subseteq$ (F, A) and $x_\alpha \in$(K, A) $\subseteq$ (G, A) which implies that $x_\alpha \in$ (H, A) $\cap$ (K, A) $\subseteq$(F, A) $\cap$ (G, A), since any finite intersection of soft closed sets is soft closed, then (H, A)$\cap$(K, A) is soft closed set. Thus, (F, A) $\cap$ (G, A) is $\tilde{s}p_c$ -open set. This completes the proof.

Proposition 3.11: A subset (F,A) of a space (X, τ, A) is $\tilde{s}p_c$-open if and only if for each $x_\alpha \in (F,A)$, there exists a $\tilde{s}p_C$-open set (G,A)such that$x_\alpha \in (G,A) \subseteq (F,A)$.

Proof. Assume that (F,A) is $\tilde{s}p_c$-open in the space (X, τ). Then for each $x_\alpha \in (F,A)$, take (F,A) = (G,A) is $\tilde{s}p_c$-open containing x_α such that $x_\alpha \in (G,A) \subseteq (F,A)$. Conversely, suppose that for each $x_\alpha \in (F,A)$, there exists a $\tilde{s}p_c$ -open set (G,A) such that $x_\alpha \in (G,A) \subseteq (F,A)$. Thus $(F,A) = \cup \{x_\alpha\} \subseteq \cup (G,A) \subseteq (F,A)$ for each $x_\alpha \in (F,A)$, this implies that $(F,A) = \cup (G,A)$, therefore (F,A) is $\tilde{s}p_c$ -open set.

The next corollary follows immediately from Definition 3.1

Corollary 3.12: For any soft subset (F,A) of a soft space (X, τ, A), the following statements are equivalent:

1- (F,A) is $\tilde{s}p_c$-open
2- (F,A) is soft pre-open and soft closed .

Proposition 3.13: Every soft θ-open set of a space X is $\tilde{s}p_c$ -open.

Proof: Let (F,A) be a soft θ-open set in $\tilde{X}$, then for $x_\alpha \in (F,A)$, there exists a soft open set (G,A) such that $x_\alpha \in (G,A) \subseteq \tilde{s}cl(G,A) \subseteq (F,A)$, so $\cup_{x_\alpha \in (F,A)} \{x_\alpha\} \subseteq \cup (G,A) \subseteq \cup \tilde{s}cl(G,A) \subseteq \cup (F,A)$.
Now by Proposition 3.2 (F,A) is $\tilde{s}p_c$ -open.

The following example shows that the converse of Proposition 3.13 is not true. **Example 3.14:** Soft co-finite space is $\tilde{s}p_c$ –open set which is not soft θ-open set.

Remark 3.15: The following diagram shows the relations between $\tilde{s}\theta O(X)$, soft open set, $\tilde{s}\alpha O(X)$, $\tilde{s}pO(X)$, and $\tilde{s}p_c O(X)$.

$$\begin{array}{ccccc} \tilde{s}p_c O(X) & \longrightarrow & & & \tilde{s}pO(X) \\ \nearrow & & & & \nearrow \\ \tilde{s}\theta O(X) & \longrightarrow & \tilde{s}O(X) & \longrightarrow & \tilde{s}\alpha O(X) \end{array}$$

Proposition 3.16: Suppose $\tilde{X}$ be a soft topological space, if $\tilde{X}$ is soft regular space, then every soft open set is a $\tilde{s}p_c$-open set.

Proof: Let (F,A) be a soft open, then (F,A) is a soft pre-open. Since $\tilde{X}$is regular, then by Corollary 2.6, for each $x_\alpha \in$ (F,A), there exists a soft open set (G,A) such that $x_\alpha \in$ (G,A) $\subseteq$ $\tilde{s}$cl(G,A) $\subseteq$ (F,A). So that, $x_\alpha \in \tilde{s}$cl(G,A) $\subseteq$ (F,A). Therefore, by Definition 3.1, (F,A) is $\tilde{s}p_c$-open set.

The next corollary follows immediately from Proposition 3.16 and Proposition 2.15.

Corollary 3.17: If a soft topological space (X, τ, A) is soft regular, then $\tau = \tilde{s}int\, \tilde{s}p_c(X))$.

Proposition 3.18: Let $\tilde{X}$ be an extremely soft disconnected topological space. If (F,A) is a $\tilde{s}p_c$ -open set and (G,A) a soft regular open. Then (F,A) $\cap$ (G,A) is a $\tilde{s}p_c$ -open set.

Proof: Suppose $\tilde{X}$ be an extremely soft disconnected topological space and let (F,A)

and (G,A) be soft subsets of $\tilde{X}$. If (F,A) is $\tilde{s}p_c$ -open and (G,A) is soft regular open then, (G,A) is soft open and (F,A) ∩ (G,A) ⊆$\tilde{s}$cl($\tilde{s}$int($\tilde{s}$cl((F,A)))) ∩ (G,A) ⊆$\tilde{s}$cl($\tilde{s}$int($\tilde{s}$cl((F,A))) ∩ (G,A)) = $\tilde{s}$cl($\tilde{s}$int($\tilde{s}$cl((F,A)) ∩ (G,A))) ⊆ $\tilde{s}$cl($\tilde{s}$int($\tilde{s}$cl((F,A)∩ (G,A)))). So that, (F,A) ∩ (G,A) is soft pre-open. If x_α ∈ (F,A) ∩ (G,A) then, since (F,A) is $\tilde{s}p_c$ -open, there exists a soft closed set (K,A) such that x_α∈ (K,A) ⊆ (F,A) and so, x_α ∈ (K,A) ∩ (G,A) ⊆ (F,A) ∩ (G,A). Since (G,A) is soft regular open in an extremely soft disconnected space so, (G,A) is soft closed and hence (K,A) ∩ (G,A) is soft closed set. Therefore, (F,A)∩ (G,A) is a $\tilde{s}p_c$ -open set.

Proposition 3.19: Suppose $\tilde{X}$ be an extremely soft disconnected topological space and let (F,A) be a soft δ-open subset of $\tilde{X}$, then (F,A) is a $\tilde{s}p_c$ -open set.

Proof: Let $\tilde{X}$ be an extremely soft disconnected topological space and let (F,A) be soft δ-open subset of $\tilde{X}$. Then for any x_α ∈ (F,A) there exists a soft open set (G,A) such that x_α∈ (G,A) ⊆ $\tilde{s}$int($\tilde{s}$cl(G,A)) ⊆(F,A). Which implies that $\cup_{x_\alpha \in (F,A)} (G,A) = (F,A)$ is soft open sets and so its soft pre-open sets. But $\tilde{X}$ is extremely soft disconnected so that, $\tilde{s}$int($\tilde{s}$cl(G,A))= $\tilde{s}$cl(G,A) and x_α∈ $\tilde{s}$cl(G,A) ⊆(F,A). Therefore, (F,A) is $\tilde{s}p_c$ –open set.

Theorem 3.20: If a soft topological space (X, τ, A) is finite, then every $\tilde{s}p_c$ -open is soft clopen.

Proof: Let (F,A) ∈ $\tilde{s}p_c$ O(X), then (F,A) is soft pre-open set, and by Proposition 3.2, $(F,A) = \cup (K_\lambda, A)$, where (K_λ, A) is soft closed for each λ. Since $\tilde{X}$ is finite, then there exists a soft closed set (K,A) in $\tilde{X}$ Such that$(K,A) = \cup (K_\lambda, A) = (F,A)$, so $(K,A) = (F,A)$, this implies that (F,A) is both soft pre-open and soft closed, thus $(F,A) \subseteq \tilde{s}int(\tilde{s}cl(F,A)) = \tilde{s}int(F,A)$. Hence $(F,A)$is soft clopen.

Proposition 3.21: Soft topological spaces (X, τ, A) and (X, τ_α ,A) have the same class of $\tilde{s}p_c$-open sets.

Proof: Let (F,A) be any subset of a space $SS(X)_A$ and (F,A) ∈ $\tilde{s}p_CO(X, \tau)$. If (F,A) =$\tilde{\emptyset}$, then (F,A)∈ $\tilde{s}p_CO(X, \tau_\alpha)$. In case (F,A)≠ $\tilde{\emptyset}$, since (F,A) ∈ $\tilde{s}p_CO(X, \tau)$, then (F,A) ∈ $\tilde{s}pO(X,\tau)$ and (F,A) = ∪ (K_λ, A) where (K_λ, A) is soft closed for each λ. Since (F,A)∈ $\tilde{s}pO(X,\tau)$, then by Theorem 2.14 (F,A) ∈ $\tilde{s}pO(X,\tau_\alpha)$. Again since (K_λ, A) is soft closed in (X, τ) for each λ, then by Theorem 2.14, (K_λ, A) is soft closed in (X, τ_α) for each λ. Therefore, by Proposition 3.2, (F,A) ∈$\tilde{s}p_CO(X, \tau_\alpha)$. Hence $\tilde{s}p_CO(X, \tau) \subseteq \tilde{s}p_CO(X, \tau_\alpha)$. On the other hand, by the same way we can prove $\tilde{s}p_CO(X,\tau_\alpha) \subseteq \tilde{s}p_CO(X, \tau)$. Therefore, we get $\tilde{s}p_CO(X,\tau_\alpha) = \tilde{s}p_CO(X, \tau)$.

Theorem 3.22: Let (X, τ, A) be a soft topological space, and (F, A),(G, A) ∈ $SS(X)_A$. If (F, A) ∈ $\tilde{s}p_c$ O(X) and (G, A) is soft clopen, then (F, A) ∩ (G, A) ∈ $\tilde{s}p_c$ O(X).

Proof: Let (F, A) ∈ $\tilde{s}p_c$ O(X) and (G, A) be a soft clopen set, then (G, A) is soft open and (F, A) is soft pre-open. Then by Theorem 2.12, (F, A)∩(G, A) ∈ $\tilde{s}pO(X)$. Now let x_α ∈ (F, A) ∩ (G, A), then x_α∈ (F, A) and x_α ∈ (G, A), so there exists a soft closed set (K, A) such that x_α ∈ (K, A) ⊆ (F, A). Again since (G, A) is soft clopen, so it is soft closed set implying that (K, A) ∩ (G, A) is soft closed set. Therefore, x_α ∈ (K, A) ∩ (G, A) ⊆ (F, A) ∩ (G, A). Thus, (F, A) ∩ (G, A) is $\tilde{s}p_c$ O(X).

Proposition 3.23: Suppose Y be a soft subspace of a soft space X. If (F, A) $\in \tilde{s}p_cO(X)$ and (F, A) $\subseteq$Y , then (F, A) $\in \tilde{s}p_cO(Y)$.

Proof: Let (F, A) $\in \hat{s}p_cO(X, \tau)$, then (F, A) $\in \hat{s}pO(X, \tau)$ and for each $x_\alpha \in$(F, A), there exists a soft closed set (K, A) such that $x_\alpha \in$ (K, A) $\subseteq$ (F, A). Since (F, A) $\in \hat{s}pO(X, \tau)$ and (F, A) $\subseteq \tilde{Y}$, then by Proposition 2.14, (F, A) $\in \hat{s}pO(Y, \tau_Y)$. Since (K, A) is soft closed in X and (K, A) $\subseteq \tilde{Y}$, then by Proposition 2.15, (K, A) is soft closed in $\tilde{Y}$. Hence (F, A) $\in \hat{s}p_cO(Y, \tau_Y)$.

We will see by the following example that the converse of Proposition 3.23 Is not true.

Example 3.24: Consider X = {a, b, c}, Y = {a, b}, and A = {0, 1}. And let

$\tau = \{(F_i, A): i = 1,2,\dots\} \cup \{\tilde{X}, \tilde{\emptyset}\}$ where $(F_i, A), i = 1,2,\dots 6$ over X defined as follows:

$$F_1(x) = \begin{cases} \{a\} & if\ x = 0 \\ \{c\} & if\ x = 1 \end{cases} \quad F_2(x) = \begin{cases} \{b\} & if\ x = 0 \\ \{a\} & if\ x = 1 \end{cases}$$

$$F_3(x) = \begin{cases} \{c\} & if\ x = 0 \\ \{b\} & if\ x = 1 \end{cases} \quad F_4(x) = \begin{cases} \{a,b\} & if\ x = 0 \\ \{a,c\} & if\ x = 1 \end{cases}$$

$$F_5(x) = \begin{cases} \{a,c\} & if\ x = 0 \\ \{b,c\} & if\ x = 1 \end{cases}$$

$$F_6(x) = \begin{cases} \{b,c\} & if\ x = 0 \\ \{a,b\} & if\ x = 1 \end{cases}$$

Then $\hat{s}p_cO(X) = \tilde{\tau}$, and $\tilde{s}P_cO(Y) = \{\tilde{\emptyset}, \tilde{Y}, (H_i, A): i = 1,2,\dots,6\}$ where

$$H_1(x) = \begin{cases} \{a\} & if\ x = 0 \\ \emptyset & if\ x = 1 \end{cases} \quad H_2(x) = \begin{cases} \{b\} & if\ x = 0 \\ \{a\} & if\ x = 1 \end{cases}$$

$$H_3(x) = \begin{cases} \emptyset & if\ x = 0 \\ \{a\} & if\ x = 1 \end{cases} \quad H_4(x) = \begin{cases} \{a,b\} & if\ x = 0 \\ \{a\} & if\ x = 1 \end{cases}$$

$$H_5(x) = \begin{cases} \{a\} & if\ x = 0 \\ \{b\} & if\ x = 1 \end{cases} \quad H_6(x) = \begin{cases} \{b\} & if\ x = 0 \\ \{a,b\} & if\ x = 1 \end{cases}$$

So $(H_1, x) \in \tilde{s}P_cO(Y)$ but $(H_1, x) \notin \tilde{s}P_cO(X)$.

Theorem 3.25: Let (Y, τ_Y , A) be a subspace of a space (X, τ, A) and (F, A) $\subseteq \tilde{Y}$. If (F, A) is $\hat{s}p_c$ -open in a subspace (Y, τ_Y , A) and Y is soft clopen, then (F, A) is $\hat{s}p_c$ -open set in $\tilde{X}$.

Proof: Let (F, A) be a $\hat{s}p_c$ -open in a subspace (Y, τ_Y , A), then (F, A) is soft pre-open in a subspace (Y, τ_Y , A) and for $x_\alpha \in$ (F, A), there exists a soft closed set (K,A) in $\tilde{Y}$ such that $x_\alpha \in$ (K,A) $\subseteq$ (F, A). Since Y is soft clopen, Y is soft pre-open in $\tilde{X}$ and (F, A) is soft pre-open in $\tilde{Y}$, then by Theorem 2.11, (F, A) is soft pre-open in $\tilde{X}$. Also, Y is soft closed in $\tilde{X}$ and (K,A) is soft closed in $\tilde{Y}$, then by Proposition 2.15, (K,A) is soft closed in $\tilde{X}$ .Hence, (F, A) is $\hat{s}p_c$ -open in $\tilde{X}$.

The next corollary follows immediately from Proposition 3.23 and Theorem 3.25.

Corollary 3.26: Let $\tilde{X}$ be a soft topological space , and (F, A), $\tilde{Y}$ be soft subsets of $\tilde{X}$ such that (F, A) $\subseteq \tilde{Y} \subseteq \tilde{X}$ and $\tilde{Y}$ is soft clopen . Then (F, A) $\in \tilde{s}p_cO(Y)$ if and only if (F, A) $\hat{s}p_cO(X)$

Corollary 3.27: Let (F, A) , Y be soft sub-sets of a soft topological space X. Such that (F, A) $\subseteq \tilde{Y} \subseteq \tilde{X}$. If (F, A) $\in \hat{s}p_cO(X)$ and $\tilde{Y}$ is soft clopen subset of $\tilde{X}$, then (F, A) $\cap \tilde{Y} \in \tilde{s}p_cO(Y)$.

Proof: Let (F, A) be a $\hat{s}p_c$-open, then (F, A) is soft pre-open. Since $\tilde{Y}$ is soft clopen. By Theorem 2.12, (F, A) $\cap$ Y is soft pre-open. Since (F, A) is $\hat{s}p_c$-open, then for each $x_\alpha \in$ (F, A), there exists soft closed set (K,A) in X such that $x_\alpha \in$ (K,A) $\subseteq$ (F, A). Hence, $x_\alpha \in$ (K,A)$\cap \tilde{Y} \subseteq$ (F, A) $\cap \tilde{Y}$ and therefore, (F, A) $\cap \tilde{Y}$ is $\hat{s}p_c$-open set such that (F, A) $\cap \tilde{Y} \subseteq \tilde{Y}$. Thus, by Proposition 322, (F, A) $\cap \tilde{Y}$ is $\hat{s}p_c$-open in$\tilde{Y}$.

Proposition 3.28: Let $(X, \tau, A \times B)$ be the soft product topological space of the soft topological spaces (X, τ, A) and (X, τ, B) .If (F, A) is a soft p_c-open of (X, τ, A) and (G, B) is a soft p_c-open subset of (X, τ, B) , then $(F, A) \times (G, B) = (H, A \times B)$ where $H(a, b) = F(a) \times G(b)$ is a $\tilde{s}p_c$-open in $(X, \tau, A \times B)$.

Proof: Let $F(a) \times G(b) \in (F,A) \times (G,B)$, then $F(a) \in (F,A)$, and $G(b) \in (G,B)$. Since $(F,A) \in \tilde{s}p_c O(X,A)$ and $(G,B) \in \tilde{s}p_c O(X,B)$, then $(F,A) \in \tilde{s}pO(X,A)$ and $(G,B) \in \tilde{s}pO(X,B)$. Also there exists a soft closed subset (K,A) of (X,τ,A) and a soft closed subset (L,B) of (X,τ,B) such that $F(a) \in (K,A) \subseteq (F,A)$, and $G(b) \in (L,B) \subseteq (G,B)$. Therefore $(a) \times G(b) \in (K,A) \times \in (L,B) \subseteq (F,A) \times (G,B)$, and since $(F,A) \in \tilde{s}pO(X,A)$ and $(G,B) \in \tilde{s}pO(X,B)$, then by Theorem 2.16 part 2 $(F,A) \times (G,B) = \tilde{s}Pint(F,A) \times \tilde{s}pint(G,B) = \tilde{s}pint((F,A) \times (G,B))$, so $(F,A) \times (G,B) \in \tilde{s}PO(X,A \times B)$. Since (K,A), (L,B) are soft closed subset$(X,\tau,A),(X,\tau,B)$ respectively, then by Theorem 2.16 part 1 we get

$(K,A) \times (L,B) = \tilde{s}cl(K,A) \times \tilde{s}cl(L,B) = \tilde{s}cl((K,A) \times (L,B))$

so $(K,A) \times (L,B)$ is soft closed subset of $(X,\tau,A \times B)$.Therefore $(F,A) \times (G,B)$ is soft a $\tilde{s}p_c$-open in $(X,\tau,A \times B)$..

4. $\tilde{s}p_c$-continuous mapping:

In this section we study soft p_c-continuous and discuss their relations with soft continuous mappings.

Definition 4.1: A soft mapping $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ is called $\tilde{s}p_c$-continuous at a soft point $x_\alpha \in SP(X)_A$, if for each soft open set (G, B) of $\tilde{Y}$ containing $f_{pu}(x)_\alpha$, there exists an $\tilde{s}p_c$ open set (F, A) of $\tilde{X}$ containing x_α such that f_{pu}(F, A) ⊆ (G, B). If f_{pu} is $\tilde{s}p_c$-continuous at every soft point of $\tilde{X}$, then it is called $\tilde{s}p_c$-continuous mapping.

The following corollary follows immediately from Definition (4.1).

Corollary 4.2: Every $\tilde{s}p_c$-continuous mapping is an $\tilde{s}p$-continuous mapping.

We will see by the following example that the converse of Corollary 4.2 is not true.

Example 4.3: Consider X = {a, b, c} and A = {u, s} with the soft topology

τ = {$\tilde{\varphi}$, $\tilde{X}$, (F_1, A), (F_2, A), (F_3, A)} where F_1(u) = {a} , F_1(s) = {b}

F_2(u) = {b} , F_2(s) ={a}, F_3(u) = {a,b} , F_3(s) ={a,b}, so $\tilde{s}$pO(X) ={$\tilde{\varphi}$, $\tilde{X}$, (F_1, A), (F_2,A), (F_3, A)}, and $\tilde{s}p_c$O(X)= {$\tilde{\varphi}$, $\tilde{X}$} . Then the identity mapping $I: (X,\tau,A) \to (X,\tau,A)$ is an $\tilde{s}p$-continuous but not $\tilde{s}p_c$-continuous.

Proposition 4.4: A soft mapping $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ is $\tilde{s}p_c$-continuous if and only if the inverse image of every soft open set in $\tilde{Y}$ is an $\tilde{s}p_c$-open in $\tilde{X}$.

Proof: Suppose f_{pu} be $\tilde{s}p_c$-continuous and let (G, B) be any soft open set in $\tilde{Y}$. To show that $f_{pu}^{-1}(G,B)$ is an $\tilde{s}p_c$-open in $\tilde{X}$. If $f_{pu}^{-1}(G,B) = \emptyset$, then $f_{pu}^{-1}(G,B)$ is $\tilde{s}p_c$-open set in $\tilde{X}$. If $f_{pu}^{-1}(G,A) \neq \emptyset$, then there exists $x_\alpha \in f_{pu}^{-1}(G,B)$ which implies that $f_{pu}(x)_\alpha \in (G,B)$. Since f_{pu} is $\tilde{s}p_c$continuous, there exists an $\tilde{s}p_c$-open set (F,A) in $\tilde{X}$ containing x_α such that $f_{pu}(F,A) \subseteq (G,B)$. This implies that $x_\alpha \in (F,A) \subseteq f_{pu}^{-1}(G,B)$. This shows that $f_{pu}^{-1}(G,B)$ is an $\tilde{s}p_c$-open.

Conversely, suppose (G,B) be any soft open set in $\tilde{Y}$, so its inverse image is an $\tilde{s}p_c$-open set in $\tilde{X}$. Since $f_{pu}(x_\alpha) \in (G,B)$ we can take $(F,A) = f_{pu}^{-1}(G,B)$ as an $\tilde{s}p_c$-open set in $\tilde{X}$ containing x_α such that $f_{pu}(F,A) = f_{pu}(f_{pu}^{-1}(G,B)) \subseteq (G,B)$. Hence f_{pu} is $\tilde{s}p_c$-continuous.

Proposition 4.5: A soft mapping

$f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$ is $\tilde{s}p_c$continuous if and only if f is a soft pre-continuous and for each $x_\alpha\in X$ and each soft open set (F,B) of $\tilde{Y}$containing$f_{pu}(x)_\alpha$, there exist a soft closed (K,A) such that $f_{pu}(K,A)\subseteq(F,B)$.

Proof: Necessity: Suppose $f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$ be an $\tilde{s}p_c$-continuous and also let $x_\alpha\in X$ and (F,B) be any soft open set of $\tilde{Y}$ containing $f_{pu}(x)_\alpha$. By hypothesis, there exist a $\tilde{s}p_c$-open set (G,A) of $\tilde{X}$ containing x_α such that $f_{pu}(G,A)\subseteq(F,B)$. Since (G,A) is $\tilde{s}p_c$-open there exists a soft closed set (K,A) of $\tilde{X}$ such that $x_\alpha\in(K,A)\subseteq(G,A)$. Therefore, we have $f_{pu}(K,A)\subseteq(F,B)$. And also f_{pu} is $\tilde{s}p_c$continuous. Then f_{pu} is soft pre-continuous.

Sufficiency: Let (F,B) be any soft set of $\tilde{Y}$. we have to show that $f_{pu}^{-1}(F,B)$ is $\tilde{s}p_c$open set in $\tilde{X}$. Since f_{pu} is soft pre-continuous, then $f_{pu}^{-1}(F,B)$ is soft pre open in $\tilde{X}$. Let $x_\alpha\in f_{pu}^{-1}(F,B)$. Then $f_{pu}(x)_\alpha\in(F,B)$. By hypothesis there exists a soft closed set (K,A) of $\tilde{X}$ containing x_α such that $f_{pu}(K,A)\subseteq(F,B)$, which implies that $x_\alpha\in(K,A)\subseteq f_{pu}^{-1}(F,B)$, therefore $f_{pu}^{-1}(F,B)$ is $\tilde{s}p_c$-open set in $\tilde{X}$. Hence by Proposition 3.4 f_{pu} is $\tilde{s}p_c$-continuous.

Theorem 4.6: Let a soft mapping $f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$ be a soft continuous and soft open. If (F,B) is $\tilde{s}p_c$open set of $\tilde{Y}$, then $f_{pu}^{-1}(F,B)$ is $\tilde{s}p_c$open set of $\tilde{X}$.

Proof: Suppose (F,B) be an $\tilde{s}p_c$-open set of $\tilde{Y}$, then (F,B) is soft pre-open set of $\tilde{Y}$ and $(F,B)=\cup(K_\alpha,B)$ where(K_α,B) is soft closed set of $\tilde{Y}$ for each α. Then $f_{pu}^{-1}(F,B)=f_{pu}^{-1}(\cup(K_\alpha,B)=\cup f_{pu}^{-1}(K_\alpha,B)$. Since f_{pu} is soft continuous and soft open mapping. Then by Proposition 2.17 $f_{pu}^{-1}(F,B)$ is soft pre-open set of $\tilde{X}$ and. Also since f_{pu} is soft continuous, then $f_{pu}^{-1}(K_\alpha,B)$ is soft closed in $\tilde{X}$ for each α. Hence by Proposition 3.2 $f_{pu}^{-1}(F,B)$ is $\tilde{s}p_c$-open set in $\tilde{X}$.

Corollary 4.7: Let $f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$ be a soft continuous and soft open mapping. If (K,B) is $\tilde{s}p_c$-closed set of $\tilde{Y}$, then $f_{pu}^{-1}(K,B)$ is $\tilde{s}p_c$-closed set of $\tilde{X}$.

Proof: Obviously by Theorem 4.6

Proposition 4.8: The following properties are equivalent for a soft mapping $f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$, whenever $\tilde{X}$ is soft T_1 -space.

1- f is $\tilde{s}p_c$-continuous. 2- f is $\tilde{s}p$-continuous

Proof: Follows immediately from Proposition 3. 6.

Theorem 4.9: For a mapping$f_{pu}:(X,\tau,A)\to(Y,\tau_Y,B)$, the following statements are equivalent:

1- f_{pu} is $\tilde{s}p_c$-continuous.

2-$f_{pu}^{-1}(G,B)$is an $\tilde{s}p_c$-open set in $\tilde{X}$, for each soft open set (G,B) in $\tilde{Y}$.

3- $f_{pu}^{-1}(H,B)$is an $\tilde{s}p_c$-closed set in $\tilde{X}$, for each soft closed set (H,B) in $\tilde{Y}$.

4- f_{pu} ($\tilde{s}p_c cl((F,A))\subseteq\tilde{s}cl(f_{pu}$ (F, A)), for each

soft subset (F, A) over $\tilde{X}$.

5- $\tilde{s}p_c\,cl(f_{pu}^{-1}(G,B)) \subseteq f_{pu}^{-1}(\tilde{s}cl(G,B)$, for each soft open set (G,B) over $\tilde{Y}$.

6- $f_{pu}^{-1}(\tilde{s}int(G,B) \subseteq \tilde{s}p_c\,int(f_{pu}^{-1}(G,B))$, for each soft open set (G,B) over $\tilde{Y}$.

7- $\tilde{s}int(f_{pu}$ (F, A)) $\subseteq f_{pu}$ $(\tilde{s}p_c\,int((F,A))$, for each soft subset (F, A) over $\tilde{X}$.

Proof: (1)→(2) Follows from Proposition 4.4.

(2)→ (3)Let (H,B) be any soft closed sub set in $\tilde{Y}$. Then $\tilde{Y} \setminus (H,B)$ is a soft open subset in $\tilde{Y}$. By (2) $f_{pu}^{-1}(\tilde{Y} \setminus (H,B)) = \tilde{X} \setminus f_{pu}^{-1}(H,B)$ is $\tilde{s}p_c$-open set in $\tilde{X}$ and hence $f_{pu}^{-1}(H,B)$ is an $\tilde{s}p_c$-closed set in $\tilde{X}$.

(3)→ (4) Let (F, A) be any soft subset over $\tilde{X}$, then f_{pu} (F, A) $\subseteq \tilde{s}cl(f_{pu}$ (F, A)) and $\tilde{s}cl(f_{pu}$ (F, A)) is soft closed set in $\tilde{Y}$. Hence (F, A) $\subseteq f_{pu}^{-1}(\tilde{s}cl(f_{pu}$ (F, A))). By (3) we have $f_{pu}^{-1}(\tilde{s}cl(f_{pu}$ (F, A))) is an $\tilde{s}p_c$-closed set in X. Hence $\tilde{s}p_c\,cl$(F, A) $\subseteq f_{pu}^{-1}(\tilde{s}cl(f_{pu}$ (F, A))). Hence, $f_{pu}(\tilde{s}p_c\,cl$(F, A) $) \subseteq \tilde{s}cl(f_{pu}$ (F, A)).

(4)→(5) Let (G,B) soft open subset over $\tilde{Y}$. Then $f_{pu}^{-1}(G,B)$ is a soft subset over $\tilde{X}$. By (4) we have

$f_{pu}(\tilde{s}p_c\,cl\left(f_{pu}^{-1}(G,B)\right)) \subseteq \tilde{s}cl($

$f_{pu}(f_{pu}^{-1}(G,B)) \subseteq \tilde{s}cl(G,B)$. Hence

$\tilde{s}p_c\,cl\left(f_{pu}^{-1}(G,B)\right) \subseteq f_{pu}^{-1}(\tilde{s}cl(G,B))$.

(5) → (6) Let (G,B) soft open subset over $\tilde{Y}$. Then, apply (5) to $\tilde{Y} \setminus (G,B)$, we obtain $\tilde{s}p_c\,cl(f_{pu}^{-1}(\tilde{Y} \setminus (G,B))) \subseteq f_{pu}^{-1}\left(\tilde{s}cl\left(\tilde{Y} \setminus (G,B)\right)\right)$, iff

$\tilde{s}p_c\,cl(\tilde{X} \setminus f_{pu}^{-1}(G,B)) \subseteq f_{pu}^{-1}(\tilde{Y} \setminus \tilde{s}int(G,B))$, iff

$\tilde{X} \setminus \tilde{s}p_c\,int f_{pu}^{-1}(G,B) \subseteq \tilde{X} \setminus f_{pu}^{-1}(\tilde{s}int(G,B))$, iff

$f_{pu}^{-1}(\tilde{s}int(G,B)) \subseteq \tilde{s}p_c\,int f_{pu}^{-1}(G,B)$.

(6) → (7) Let (F, A) be any soft subset over $\tilde{X}$. Then f_{pu} (F, A) is a soft subset over $\tilde{Y}$. By (6), we have

$f_{pu}^{-1}(\tilde{s}int\,f_{pu}$ (F,A) $) \subseteq \tilde{s}p_c\,int f_{pu}^{-1}(f_{pu}$ (F,A) $)$

$\subseteq \tilde{s}p_c\,int$(F,A) .

Therefore $\tilde{s}int\,f_{pu}$ (F,A) $\subseteq f_{pu}(\tilde{s}p_c\,int$(F,A)).

(7) → (1) Let $x_\alpha \in SP(X)_A$ and let (G,B) be any soft open set in $\tilde{Y}$ containing $f_{pu}(x_\alpha)$. Then $x_\alpha \in f_{pu}^{-1}(G,B)$ and $f_{pu}^{-1}(G,B)$ is a soft subset over $\tilde{X}$. By (7), we have $\tilde{s}int\,f_{pu}$ $(f_{pu}^{-1}(G,B)) \subseteq f_{pu}(\tilde{s}p_c\,int\,f_{pu}^{-1}(G,B))$.

$\tilde{s}int(G,B)) \subseteq f_{pu}(\tilde{s}p_c\,int\,f_{pu}^{-1}(G,B)$). Since (G,B) is a soft open set, then $(G,B)) \subseteq f_{pu}(\tilde{s}p_c\,int\,f_{pu}^{-1}(G,B))$ which implies that $f_{pu}^{-1}(G,B) \subseteq \tilde{s}p_c\,int\,f_{pu}^{-1}(G,B)$. So $f_{pu}^{-1}(G,B)$ is an $\tilde{s}p_c$-open set in $\tilde{X}$ which contains x_α. Hence f_{pu} is $\tilde{s}p_c$-continuous.

Theorem 4.10: Let $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ be a mapping. and $\boldsymbol{\beta}$ be any basis for τ in Y. Then $f_{pu}$is $\tilde{s}p_c$-continuous if and only if for each $(F,B) \in \boldsymbol{\beta}$, $f_{pu}^{-1}(F,B)$ is $\tilde{s}p_c$-open subset of $\tilde{X}$

Proof: Necessity. Suppose f_{pu} is $\tilde{s}p_c$-continuous. Then since each $(F,B) \in \boldsymbol{\beta}$ is an soft open subset of $\tilde{Y}$. Therefore, by Proposition 4.4 $f_{pu}^{-1}(F,B)$ is a $\tilde{s}p_c$-open subset of $\tilde{X}$.

Sufficiency. Let (G,B) be any soft subset of $\tilde{Y}$. Then $(G,B) = \cup\{(F_i,B), i \in I\}$, where every (F_i,B) is a member of $\boldsymbol{\beta}$ and I is suitable index set. It follows that

$f_{pu}^{-1}(G,B) = f_{pu}^{-1}(\cup\{(F_i,B), i \in I\})=$

$f_{pu}^{-1}(G,B) = \cup f_{pu}^{-1}(\{(F_i,B), i \in I\})$, since $f_{pu}^{-1}((F_i,B))$ is a $\tilde{s}p_c$-open subset of $\tilde{X}$ for each $i \in I$. Hence $f_{pu}^{-1}(G,B)$ is the union of a family of $\tilde{s}p_c$-open sets of $\tilde{X}$ hence is a $\tilde{s}p_c$-open set of $\tilde{X}$. Therefore by Proposition 4.4 f_{pu} is $\tilde{s}p_c$-continuous.

Proposition 4.11: Let $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ be $\tilde{s}p_c$-continuous. If $\tilde{Y}$ is a soft clopen subset of a soft topological space Z, then $f_{pu}: (X,\tau,A) \to (Z,\tau_Z,B)$ is $\tilde{s}p_c$-continuous.

Proof: Suppose $x_\alpha \in SP(X)_A$ and (G,B) be any soft open set of $\tilde{Z}$ containing $f_{pu}(x)_\alpha$, then $(G,B) \cap \tilde{Y}$ is a soft open set in $\tilde{Y}$. But $f_{pu}(x)_\alpha \in SP(Y)_B$ for each $x_\alpha \in SP(X)_A$, then $f_{pu}(x)_\alpha \in (G,B) \cap \tilde{Y}$. Since $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ is $\tilde{s}p_c$-continuous, then there exists an $\tilde{s}p_c$-open set (F,A) containing x_α such that $f_{pu}((F,A)) \subseteq (G,B) \cap \tilde{Y} \subseteq (G,B)$. Therefore $f_{pu}: (X,\tau,A) \to (Z,\tau_Z,B)$ is $\tilde{s}p_c$-continuous.

Proposition 4.12: A mapping $f_{pu}: (X,\tau,A) \to (Y,\tau_Y,B)$ is $\tilde{s}p_c$-continuous, if for each $x_\alpha \in SP(X)_A$, there exists a soft clopen set (F,A) of $\tilde{X}$ containing x_α such that $f_{pu}|(F,A): (F,A) \to \tilde{Y}$ is $\tilde{s}p_c$-continuous .

Proof: Let $x_\alpha \in SP(X)_A$, then by hypothesis, there exists a soft clopen set (F,A) of $\tilde{X}$ containing x_α such that $f_{pu}|(F,A): (F,A) \to \tilde{Y}$ is $\tilde{s}p_c$-continuous. Let (G,B) be any soft sub set of $\tilde{Y}$ containing $f(x)_\alpha$, there exists a $\tilde{s}p_c$-open subset (H,A) of (F,A) containing x_α such that $\left(f_{pu}|(F,A)\right)(H,A) \subseteq (G,B)$. Since (F,A) is soft clopen set. By Theorem 3.25 (H,A) is $\tilde{s}p_c$-open subset in $\tilde{X}$ and hence $f_{pu}(H,A) \subseteq (G,B)$, This implies that f_{pu} is $\tilde{s}p_c$-continuous.

References

Akdag, M. & Alkan, A., (2014), Soft α-Open Sets and Soft α-Continuous Functions, Abstract and Applied Analysis, ID 891341, 1-7.

Akdag., M. & Ozkan, A.. (2014), On Soft Pre-open Sets and Soft Pre Separation Axioms, Gazi University Journal of Science, GU J Sci, 27(4):1077-1083.

Hosny, R. A. & Deena, A. (2014), Soft Semi Open Sets with Respect to Soft Ideals, Applied Mathematics sciences, Vol. 8, No. 150, 7487-7501.

Ilango, G. & Ravindran, M.. (2013), On Soft Preopen Sets in Soft Topological Spaces, Int. Journal Math. Research, Vol. 5, No. 4, 399-409.

Khalaf, A. & Ahmad, B. (2011), Mappings of Soft Classes, New Math. Nat. Comput. , 7, 471-481.

Khalaf, A.. & Musa, S. (2015), SS_c-Open Sets in Soft Topological Spaces, Journal of Garmian University. Vol.1, (1-22).

Molotov, D. 1999, S0ft set theory- first results, Computer & Mathematics with Applications, 37, 19-31

Ravindran, M. & Francis, F. (2014), Some More Results on Soft Preopen Sets In Soft Topology, Int. Journal of Recent Scientific Research, Vol12, 2230-2235.

Ravindran, M. & Ilango, G. (2016), A Note

On Soft Pre-open Sets, Int. Journal of Pure and Applied Math. Vol. 106, No. 5, 63-78.

Shabir, M. & Naz, M. (2011), On Soft Topological Spaces, Computers and Mathematics with Applications, 61, 1786-1799.

Yuksel, S. , Guzel, Z. & Tozlu, N. (2014), Soft Regular Generalized Closed Sets in Soft Topological Spaces, Int. Journal of Math. Analysis, Vol. 8, No. 8, 35 – 367.

Yumak Y. & Kkaymakci A., (2015), Soft β-open sets and their applications, J. of New Theory, 4, 80-89.

Zorlutuna, M. Akdag, W. & Atmaca, S. (2014), Remarks on soft topological spaces, Annals of Fuzzy Mathematics and Informatics, Vol. 3, No. 2, 171- 185.

كورته

ئامانج له م تويزينه وه ناساندنی جوريكی نوييه له به شه كومه له ی نه رمی كراوه ی له جوری p_c له بوشايی نه رمی توبولوجی دا . كه به شه كومه له يه له كومه له ی نه رمی pre وه به شيك له سيفه ته كانی ئه م كومه له يه مان خستوته روو. به به كارهينانی ئه م كومه له يه بيناسه ی نه خشه ی به رده وام له جوری نه رمی p_c مان كرد وه و خويندومانه ته وه..

الملخص

ألهدف من هذا البحث ادخال صنف جديد من المجموعات سميت بالمجموعات المفتوحه المرنه من نوع p_c

في الفضاءات التبولوجيه المرنة , هذا الصنف مجموعة جزئية من المجموعات المرنة المفتوحة من نوع pre- وقد حصلنا على بعض خواص هذه المجوعة . باستخدام هذه المجموعة عرفنا و درسنا مفهوم الدوال المستمرة المرنة من النمط p_c .

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Statistical Analysis of Municipal Solid Waste Landfill Leachate Characteristics in Different Countries

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A B S T R A C T

This research was aimed to study municipal solid waste landfills leachate parameters for different sanitary landfill designs such as open dumping, anaerobic, and semi-aerobic with various ages in different countries. Landfill leachate characteristics such as pH, electrical conductivity (EC), turbidity, color, ammonia-nitrogen (NH_3-N), biochemical oxygen demand (BOD), chemical oxygen demand (COD), biodegradability ratio, total solids (TS), total suspended solids (TSS), iron (Fe) and phenols and the age of landfills were used for examining in the statistical analysis. Application of statistical package for social sciences (SPSS) for statistical analysis for the mentioned parameters was another goal for this work. Results showed that leachates parameters such as turbidity, color, NH_3-N, BOD, COD, TSS, Fe, and phenols were surpassed the standard limits for disposal. SPSS results presented that strong correlation ($r > 0.5$ at $P < 0.005$) discovered amongst NH_3-N with BOD/COD, TS, and TSS; between BOD/COD with TS and TSS; among TS with TSS, Fe, and EC, and amid Fe and EC. The age of landfills generally hadn't important statistical correlation with the aforementioned landfill leachate parameters; merely color approached to have a correlation with age ($r = - 0.986$ at $P = 0.054$).

1. INTRODUCTION

Sanitary landfill is the greatest public municipal solid waste (MSW) disposal technique due to such benefits as easy disposal process, low cost, and landscape-restoring result on holes from mineral workings. Though, the production of highly polluted leachate is a chief disadvantage of MSW landfilling system. Landfill leachate is liquid produced mainly by the infiltration of precipitation from an open landfill or through the cap of a completed landfill site. MSW landfill leachates may comprise large quantities of organic matters, ammonia-nitrogen (NH_3-N), phenols, heavy metals, inorganic salts etc. (Renou et al., 2008; Bashir et al., 2010; Maulood and Aziz, 2016). Landfill leachate may be a threaten source for the soil,

groundwater and surface water sources, if not treated and carefully disposed to the natural environment (Aziz et. al., 2015; Aziz and Maulood, 2015). Normally, the risks of the landfill leachate on the environment are found by comparing leachate parameters characteristics with the standards.

Matsufuji et al. (1993) and Yamamoto (2002) reported that in an anaerobic landfill, MSWs are dumped in an excavated site, which is filled with water in an anaerobic condition. Usually, anaerobic sanitary MSW landfills are known by its sandwich-shaped cover. In contrast, semi-aerobic (Fukuoka method) landfills have a landfill leachate collection pipes. The opening of the pipe is surrounded by air, and the pipe is covered with appropriate crushed stones. Moisture content in disposed MSW is low, and oxygen is provided to the MSW from the leachate collection conduit (JICA, 2005; Aziz et al., 2010). Age of landfill has a significant impact on the formed fresh landfill leachate. Age of landfill arranges the landfill leachate to young (< 5 years), intermediate (5 to 10 years) and matured/stabilized leachate (> 10 years) (Aziz at al., 2011). MSW landfill leachate characteristics such as pH, organic matters, nitrogen compounds, electrical conductivity (EC), color, solids etc. are changes with the age and types of landfills (Renou et al., 2008; Aziz et al., 2010; Mojiri et al., 2015; Aziz and Ali, 2018). Huge surface and cross-sectional areas of MSW landfill sites causes more production of landfill leachate to the surrounded areas.

Amount of disposed MSW in Erbil landfill site (ELS) - Iarq, Pulau Burung landfill site (PBLS) - Malaysia and Palestine were 2000 tons/ day, 1800 tons/day, and 3605 tons/day, respectively (ARIJ, 2015; Kamaruddin et a., 2016; Aziz. and Mustafa, 2018). High quantity of disposed MSW leads to increase in leachate production. Quantity of produced leachate at ELS-Iraq and PBLS-Malaysia were 158.68 m^3/d and 69.201 m^3/d, respectively (Maulood and Aziz, 2016; Aziz et al., 2002).

The current research was focused on the MSW landfill parameters, such as age of landfill site, type of landfills (open dumping, anaerobic landfill, semi-aerobic landfill, and fully aerated landfill) and characteristics of landfill leachate. pH, electrical conductivity (EC), turbidity, color, NH_3-N, biochemical oxygen demand (BOD), chemical oxygen demand (COD), biodegradability ratio (BOD/COD), total solids (TS), total suspended solids (TSS), iron (Fe) and phenols were used for illustrating quality of leachate for different MSW landfills. Statistical Package for the Social Sciences (SPSS) was applied to find statistical relations between mentioned parameters. In literature, a number of factors influences on the landfill leachate quality (Renou, 2008; Aziz et al., 2010). So far, determining descriptive statistics and correlations among landfill site and aforementioned landfill leachate characteristics using IBM SPSS was not conducted yet.

2. MATERIALS AND METHODS

2.1. Landfills

To show the effect of age and type of landfill on the quality of formed leachate, different MSWs were studied. The landfills were located in Malaysia, Iraq, and Palestine. In Malaysia, MSW landfills of Pulau Burung, Kulim, Kuala Sepatang, Sungai Patani, and Alor Pongsu were selected. While Erbil and Deir El-balah MSW landfills were chosen in Iraq and Palestine, respectively. The landfills had various ages and designs. Studying MSW landfill leachate in Malaysia, Iraq and Palestine landfills are related to: 1) generation rate and characteristics of MSW are close to each others and all countries are located in Asia, 2) availability of information on the mentioned

landfills, and 3) commonly life style, climate and topography for the countries and especially for Erbil-Iraq and Palestine are close to each other. Details of landfills are shown in Table 1. Data from 15 various landfills were collected.

2.2. Landfill leachate

Characteristics of produced MSW landfill leachate at mentioned landfills were reported. The parameters of pH, EC, turbidity, color, NH_3-N, BOD, COD, BOD/COD, TS, TSS, Fe, and phenols were collected at different MSW landfills. Table 1 illustrates the quality parameters for landfill leachates. In literature, data collection, transportation, and analyses were carried out according to (APHA, 2005).

2.3. SPSS program

SPSS is a complete system for analyzing collected data. It can obtain data from any type of file and use them to produce tabulated reports, plots of distributions and trends, charts, descriptive statistics, and complex statistical analysis. It is one of the best widespread statistical packages which can implement extremely complex data manipulation and analysis with easy instructions. SPSS is expert of handling huge quantities of data and can execute all of the analyses covered in the text and much more long produced by SPSS Inc., it was developed by IBM in 2009. The present version (23) is formally called IBM SPSS Statistics. Confidant products in the similar family are used for survey authoring and placement (IBM SPSS Data Collection), data mining (IBM SPSS Modeler), collaboration and deployment, and text analytics (Field, 2009). Statistical analysis for the quality parameters for the collected samples was carried out using IBM SPSS software. Descriptive statistics and correlations between wastewater samples were prepared.

3. RESULTS AND DISCUSSIONS

3.1. MSW landfills

Data on MSW characteristics is significant for the design of new waste management policy (Idris et al., 2004). Active MSW management and minimization strategy is practically difficult without dependable MSW data. Acceptable information on MSW characteristics is necessary to evaluate the influences of various MSW kinds and to appraisal the age of landfills. However, obtaining responsible and accurate data on MSW characteristics is not easy (Idris et al., 2004). A MSW landfill is a separated land that receives domestics, commercial, non hazardous … etc. wastes, Fig. 1. (https://www.epa.gov/landfills/municipal-solid-waste-landfills).

MSW landfills are simple, economical, and widely investigated disposal systems adopted all over the world. In a traditional MSW landfills, efforts are made to minimize moisture infiltration, which, in turn, leads to a longer decomposition time for the disposed MSW. Biodegradation of the MSW could be enhanced in different methods. Among some techniques, recirculation of landfill leachate and associated fluids within the landfill system (i.e., bioreactor landfills) was found to be effective method (Rajesh et al., 2016).

MSW disposal techniques contain open dumping, incineration, sanitary landfill, grinding and discharge to sewer, composting, hog feeding, milling, dumping, compaction, reduction, and anaerobic digestion. Sanitary landfill is the greatest common urban MSW treatment method (Aziz et al., 2010). The landfill must be designed and operated so as to separate the MSW from the environment until it may be extracted safe over physical, chemical, and biological decay processes in the landfill. Generally, a sanitary landfill will be determined by the following: site selection, planned capacity, extensive site preparation,

Table 1: Characteristics of different landfill leachates

No.	MSWL			Characteristics												References
	Site	Age (Years)	Type	pH	EC (µS/cm)	Turb. (NTU)	Color (Pt.Co.)	NH_3-N (mg/L)	BOD (mg/L)	COD (mg/L)	BOD/COD	TS (mg/L)	TSS (mg/L)	Fe (mg/L)	Phenols (mg/L)	
1	Pulau Burung-Malaysia	18	Semi-aerobic	8.28	22100	180	3347	1568	243	2345	0.124	9925	837	3.4	6.7	(Aziz et al., 2010)
2	Kulim-Malaysia	14	Anaerobic	7.76	8550	1936	4041	538	326	1892	0.205	6336	707	5.3	2.6	(Aziz et al., 2010)
3	Pulau Burung-Malaysia	18	Semi-aerobic	8.7	23655	145	5545	2010	100	2615	0.038	-	123	-	-	(Bashir et al., 2010)
4	Pulau Burung-Malaysia	19	Semi-aerobic	8.36	22360	-	3615	1627	181	1819	0.1	9507	815	4.9	6.95	(Aziz et al., 2015)
5	Kulim-Malaysia	15	Anaerobic	8.02	7660	-	3029	562	71	1580	0.04	4832	553	3.82	1.54	(Aziz et al., 2015)
6	Kuala Sepetang-Malaysia	17	Anaerobic	8.66	9680	-	6398	564	257	1456	0.19	6615	693	3.43	1.56	(Aziz et al., 2015)
7	Sungai Petani-Malaysia	23	Open dumping	8.45	3945	451	1690	532	269	1310	0.205	5723	710	6.03	169	(Mojiri et al., 2015)
8	Erbil-Iraq	12	Anaerobic	8.05	10170	120	77000	930	32	-	-	18000	2000	-	-	(Aziz and Maulood, 2015)
9	Pulau Burung-Malaysia	20	Semi-aerobic	8.5	17880	-	4530	1170	75	2170	0.034	-	197	-	-	(Abu amr et al., 2012)
10	Alor Pongsu Landfill Site (APLS)	16	anaerobic stabilized	8.29	10788	-	12475	1674	274	3125	0.088	-	6483			(Abu Amr et al., 2016)
11	*Deir El-balah Landfill Site (DBLS), Gaza, Palestine*		anaerobic stabilized	8.24	40800	537	-	3400	1821	20448	0.9	-	-	-	-	(Hilles et al., 2016)
12	*Concentrated Landfill Leachate in Shanghai Laogang*	10	Covered and anaerobic	7.34	-	-	2113	49	285	3018	0.09	-	-	15.7	5.11	(Not Published)
13	*Sahom Landfill, Kampar, Malaysia*	>15	anaerobic stabilized	8.42	10980	40.8	1450	3330	37	550	0.08		207			(Shehzad et al., 2016)
14	*Papan Landfill, Ipoh, Malaysia*	>5	Anaerobic Intermidiate	7.99	21720	421	4200	700	1260	6650	0.24		410			(Bashir et al., 2016)
15	*Batu Gajah leachate*	>10	Dump Site	9		100	1069	330	131	561	0.23		19			(Not Published)

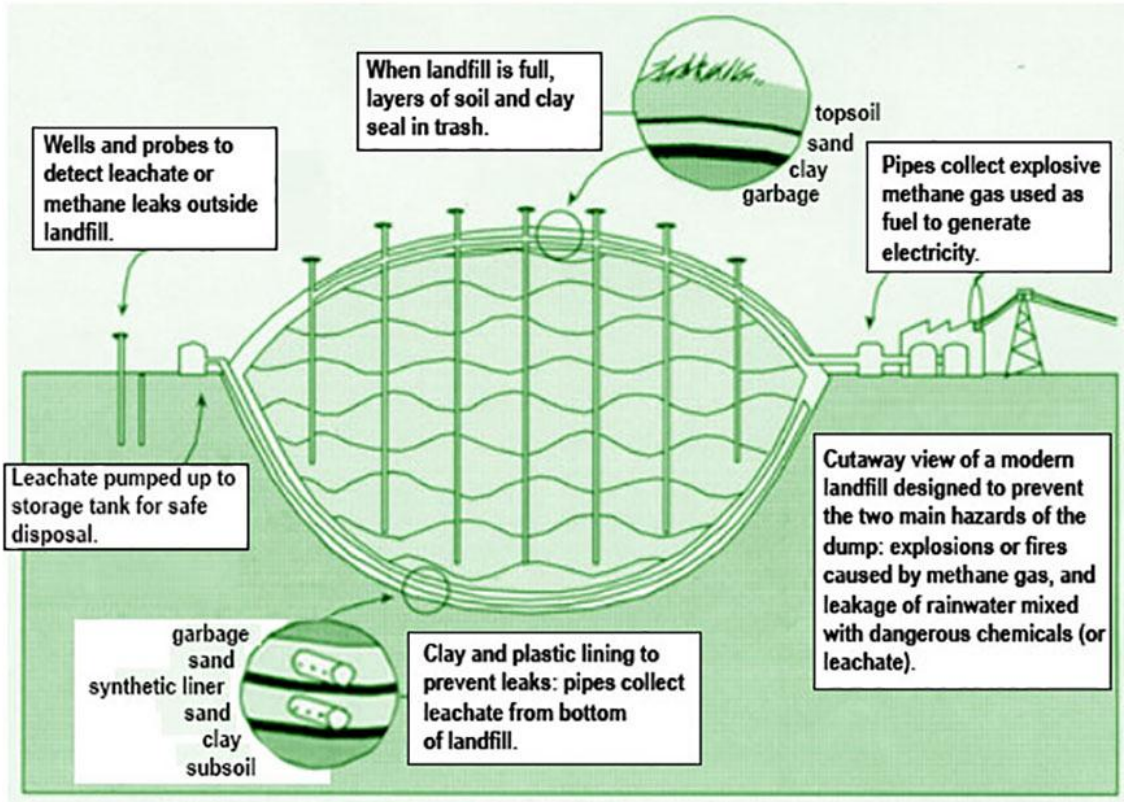

Figure (1): A cross-section of a municipal solid waste landfill (Source: https://www.epa.gov/landfills/municipal-solid-waste-landfills).

designed cell advance, full gas management, full leachate management, compaction, daily and final cover, fence and gate etc. A key component of separation is in the management and treatment of leachate. A number of processes can be applied to achieve isolation of leachate from the surrounding environment, depending on available resources. The systems range from prevention of leachate generation, to sophisticated leachate treatment methods, to controlled release of leachate into the environment (Liermann, 2009). In order to be designated a sanitary landfill, a disposal site should the following three common but basic situations: 1) compaction of the wastes, 2) daily covering of the wastes using soil or other material, and 3) control and prevention of negative impacts on the community health and on environment (e.g. odors, polluted water supplies, etc.) (UNEP, 2005). Advantages of sanitary landfills are: 1) Simple disposal method, 2) Low cost, and 3) Landscape-restoring effect on holes from mineral working. On the other hand, the shortcomings are the production of highly polluted leachate and emission of methane gas. Landfills are classified according to their structures (Table 2).

Table 2: Classification of landfill structures (Aziz, 2011)

No.	Type	Details
1	Anaerobic landfill	Disposed solid wastes are filled in dug areas of plane field or valley. The wastes are filled with water under anaerobic conditions.
2	Anaerobic sanitary landfill	Anaerobic landfill with sandwich-shaped cover. Conditions of disposed wastes are similar to the conditions of Type 1.
3	Improved anaerobic sanitary landfill	This type has a leachate collection system at the bottom of the landfill site. Other parameters are the same as those of the anaerobic sanitary landfill. The conditions are anaerobic and moisture content is significantly less than that in the anaerobic sanitary landfill.
4	Semi-aerobic landfill	The leachate collection pipe is larger than that of the improved sanitary landfill. The openings of the pipes are surrounded by air and the pipe is covered with small crushed stones. Moisture content in disposed solid waste is low. Oxygen is supplied to solid waste from the leachate collection pipe.
5	Aerobic landfill	Aside from the leachate collection pipe, air supply pipes are attached and air is able to mix with the solid waste. Thus, the landfill becomes more aerobic than semi-aerobic.

3.2 Characteristics of landfill leachate

Landfill leachates are produced when moisture mixes with refuse in the landfill; pollutants are dissolved into the liquid phase, after which they accumulate and then percolate. Leachates fluctuate from one landfill to another in the short- and long-terms periods because of differences in waste composition, hydrogeology, and climate. Enhancements in landfill engineering are intended to decrease leachate production and collection, as well as recover treatment prior to discharge (Visvanathan et al., 2000). Landfill leachates are regarded as wastewater that has the strongest environmental effect. The greatest important feature of leachates is the high concentrations of pollutants.

Leachate is the liquid percolation that drains through the waste in the landfill varies usually depend on MSW form and the MSW age (Christensen et al., 1994). Normally, the leachate can be categorized into three chief groups as shown in Table 3.

The three main clusters are mostly inorganic matters, organic matters, and xenobiotic organic compounds. Leachate quality is meaningfully impact by the landfill age (Table 4).

Clearly, as landfill age increases, the biodegradable portion of organic contaminants in leachate decrease as an outcome of the anaerobic decomposition occurring in landfill site. Consequently, mature or stabilized leachate contains much more refractory organics than young leachate. In this respect, young landfill leachate (age < 5 years) is normally categorized by high BOD and COD concentrations, quite high amount of NH_3-N, high ratio of BOD/COD, and a pH value < 6.5. In contrast, stabilized landfill leachate (age>10 years) usually comprises high amount of NH_3–N, moderately high strength of COD, and a low BOD/COD ratio of less than 0.1. Regarding the parameters such as COD, BOD_5, NH_3-N, phenols, heavy metals etc. that surpassed the standards, it is recommended to treat the landfill leachate using physical-chemical and/or biological processes (Bashir et al., 2010; Aziz et al., 2011; Bashir et al., 2016). Commonly, leachate characterization varies with the climatic regions in addition to the landfill operational practices. The key factors that affect the leachate characteristics are: 1) Design and operation of the landfill and its age,

2) MSW composition, soluble or insoluble biodegradable or non-biodegradable, liquid or solid, organic or inorganic, and toxic or nontoxic, 3) Site hydrology, and 4) Availability of moisture and oxygen (Bagche, 2004). Aziz and Maulood (2015) recognized that produced leachate from Erbil landfill site-Iraq affected on groundwater and the surrounding soil; Improving/upgrading and design of sanitary MSW landfills are solutions for minimizing environmental pollution caused by landfill leachate.

Table 3: Pollutants in Leachate (Lee et al., 2010)

Group of Pollutants In Leachate	Components
Organic matters	Acids, alcohols, aldehydes and others usually quantified as COD (Chemical Oxygen Demand), BOD (Biochemical Oxygen Demand), DOC (Dissolved Organic Carbon), Other Volatile fatty acid and refractory compound include fulvic-like and humic like compounds
Inorganic matters	Sulfate, chloride, ammonium, calcium, magnesium, sodium, potassium , hydrogen carbonate, iron and manganese and heavy metal like lead, nickel, copper, cadmium, chromium and zinc
Xenobiotic organic compounds	Aromatic hydrocarbon, phenols, chlorinated aliphatics, pesticides and plastizers include PCB, Dioxin, PAH, etc.

Table 4: Landfill leachate classification versus age (Ngo et al., 2008)

Parameter	Young	Intermediate	Old
Age (years)	<5	5 to 10	>10
pH	<6.5	6.5 to 7.5	>7.5
COD (mg/L)	>10,000	4,000 to 10,000	<4,000
BOD_5/COD	>0.3	0.1 to 0.3	<0.1
Organic compound	80% volatile fatty acids (VFA)	5% to 30% VFA + humic and fulvic acids	Humic and fulvic acids
Heavy metals	Low to medium	Low	Low
Biodegradability	Important	Medium	Low

3.3 Statistical analysis by IBM SPSS

IBM SPSS was used for analyzing landfill leachate data shown in Table 1. Effect of age of landfill site, and characteristics of landfill leachate (such as pH, EC, turbidity, color, NH_3-N, BOD, COD, BOD/COD, TS, TSS, Fe and phenols) were studied. Descriptive statistics for landfill parameters are illustrated in Table 5. Results revealed that mean static

values for age of landfill, pH, EC, turbidity, color, NH_3-N, BOD, COD, BOD/COD, TS, TSS, Fe, and phenols were 16.54545 years, 8.27067, 16176 µS/cm, 436.75556 NTU, 3418.91667 Pt .Co, 1265.60000 mg/L, 357.46667 mg/L, 2237.76923 mg/L, 0.18314, 8705.42857, 1058.00000, 6.08286, and 27.63714, respectively. The mean static value for age of 16.54545 years indicated that landfills are in methane formation phase and mature/stabilized leachate, Table 4 (Tchobanoglous and Kreith, 2002**;** Ngo et al., 2008; Aziz, 2011; Aziz and Maulood, 2015). pH and COD mean static values for landfill leachate confirm that the leachates are old, Table 4. According to Malaysian standards for disposal of wastewater, turbidity, color, NH_3-N, BOD, COD, TSS, Fe, and phenols mean static values were exceeded the permissible limits (Environmental Quality Regulation, 2009). Based on Iraqi standards for disposal of wastewater to water sources, color, NH_3-N, BOD, COD, TSS, Fe, and phenols were surpassed the standards (IES, 1993). Therefore, the produced landfill leachates require treatment processes prior disposal to the natural environment. Based on the BOD/COD value of 0.18314, physical-chemical treatment methods are suitable for treatment of produced landfill leachates (Bashir et al.,2010; Aziz et al., 2011; Mojiri et al., 2015; Aziz and Ali, 2018). Range, standard deviations and variance of landfill leachate parameters are given in Table 5. Standard deviation can be calculated by taking square root of variance. Wide variety for standard deviations and range are reflects of MSW characteristics, age and structure of landfill, location and weather. BOD/COD and pH have smallest figures for standard deviations and range. This is due to closing of BOD/COD and pH values from the mean.

Table 6 shows the correlations among landfill leachate parameters. A Pearson product-moment correlation coefficient was carried out to examine the relationship between age of landfill, pH, EC, turbidity, color, NH_3-N, BOD, COD, BOD/COD, TS, TSS, Fe, and phenols. Pearson's correlation coefficient requires only that data are interval for it to be an accurate measure of the linear relationship between two variables (Utts and Heckared, 2007; Field, 2009). It can be noticed from Table 6 that probability (P) values for relation of NH_3-N with BOD/COD, TS, and TSS were 0.002, 0.042, and 0.008, respectively. Pearson correlation coefficient for NH_3-N with BOD/COD, TS, and TSS were -1, 0.992, and 1, respectively. Positive relationship means higher scores on variable 1 are associated with higher scores on variable 2. While, negative relationship means higher scores on variable 1 are associated with lower scores on variable 2 and vice versa. In some cases, no predictable relationships occur between variable 1 and variable 2. Correlation coefficient (r) from 0.5 to 1 and from -1 to -0.5 regard as strong relation (DeCoursy, 2003; Soong, 2004). Consequently, a strong r found between NH_3-N with BOD/COD, TS, and TSS. Additionally, BOD/COD had a strong r with TS and TSS as well at P values of 0.043 and 0.006. The P values for correlation amongst TS with TSS, Fe, and EC were less than 0.05. The r figures between TS with TSS, Fe, and EC were 0.988, -0.991, and 0.994, respectively. A strong relation between TS with TSS, Fe, and EC was detected. In addition, a strong relation between Fe and EC was obtained; r between Fe and EC was -1.0.

4. CONCLUSIONS

MSW Landfills leachate parameters in different deigned landfills with various ages were studied. Some landfill leachate such as turbidity, color, NH_3-N, BOD, COD, TSS, Fe, and phenols were exceeded the standard limits for disposal. SPSS outcome revealed that strong correlation ($r > 0.5$ at $P < 0.005$) found

Table 5: Descriptive statistics for landfills using SPSS

Parameters	N Statistic	Range Statistic	Minimum Statistic	Maximum Statistic	Sum Statistic	Mean Statistic	Mean Std. Error	Std. Deviation Statistic	Variance Statistic
Age (year)	11	13.000	10.000	23.000	182.000	16.54545	1.114888	3.697665	13.673
pH	15	1.660	7.340	9.000	124.060	8.27067	0.104688	0.405453	0.164
EC (µS/cm)	13	36855.000	3945.000	40800.000	210288.000	16176.00000	2754.747075	9932.381831	98652208.830
Turb. (NTU)	9	1895.200	40.800	1936.000	3930.800	436.75556	196.489960	589.469879	347474.738
Color (Pt. Co.)	12	5329.000	1069.000	6398.000	41027.000	3418.91667	476.061182	1649.124311	2719610.992
NH_3-N (mg/L)	15	3351.000	49.000	3400.000	18984.000	1265.60000	263.785515	1021.636908	1043741.971
BOD (mg/L)	15	1789.000	32.000	1821.000	5362.000	357.46667	129.466336	501.420962	251422.981
COD (mg/L)	13	6100.000	550.000	6650.000	29091.000	2237.76923	430.278207	1551.390138	2406811.359
BOD/COD	14	0.866	0.034	0.900	2.564	0.18314	0.058502	0.218894	0.048
TS (mg/L)	7	13168.000	4832.000	18000.000	60938.000	8705.42857	1707.137270	4516.660671	20400223.620
TSS (mg/L)	13	6464.000	19.000	6483.000	13754.000	1058.00000	472.972095	1705.325140	2908133.833
Fe (mg/L)	7	12.300	3.400	15.700	42.580	6.08286	1.646498	4.356224	18.977
Phenols (mg/L)	7	167.460	1.540	169.000	193.460	27.63714	23.576213	62.376796	3890.865

Table 6: Person correlations and significant values for data sets using SPSS

		BOD (mg/L)	pH	Color (Pt. Co.)	NH_3-N (mg/L)	COD (mg/L)	BOD/COD	TS (mg/L)	TSS (mg/L)	Fe (mg/L)	Age (year)	EC (µS/cm)	Phenols (mg/L)	Turb. (NTU)
Pearson Correlation	BOD (mg/L)	1.000	-.853	.483	-.738	-.237	.741	-.644	-.755	.533	-.622	-.555	-.232	.986
	pH	-.853	1.000	-.869	.276	-.306	-.281	.149	.300	-.013	.940	.038	.706	-.928
	Color (Pt .Co.)	.483	-.869	1.000	.235	.736	-.230	.359	.210	-.483	-.986	.461	-.964	.622
	NH_3-N (mg/L)	-.738	.276	.235	1.000	.831	-1.000	.992	1.000	-.965	-.069	.971	-.486	-.615
	COD (mg/L)	-.237	-.306	.736	.831	1.000	-.828	.896	.816	-.948	-.613	.940	-.890	-.072
	BOD/COD	.741	-.281	-.230	-1.000	-.828	1.000	-.991	-1.000	.963	.064	-.970	.481	.619
	TS (mg/L)	-.644	.149	.359	.992	.896	-.991	1.000	.988	-.991	-.198	.994	-.595	-.507
	TSS (mg/L)	-.755	.300	.210	1.000	.816	-1.000	.988	1.000	-.958	-.044	.965	-.463	-.635
	Fe (mg/L)	.533	-.013	-.483	-.965	-.948	.963	-.991	-.958	1.000	.330	-1.000	.699	.385
	Age (year)	-.622	.940	-.986	-.069	-.613	.064	-.198	-.044	.330	1.000	-.306	.906	-.744
	EC (µS/cm)	-.555	.038	.461	.971	.940	-.970	.994	.965	-1.000	-.306	1.000	-.681	-.409
	Phenols (mg/L)	-.232	.706	-.964	-.486	-.890	.481	-.595	-.463	.699	.906	-.681	1.000	-.391
	Turb. (NTU)	.986	-.928	.622	-.615	-.072	.619	-.507	-.635	.385	-.744	-.409	-.391	1.000
Sig. (1-tailed)	BOD (mg/L)	.	.175	.340	.236	.424	.234	.277	.228	.321	.286	.313	.426	.053
	pH	.175	.	.165	.411	.401	.409	.452	.403	.496	.111	.488	.250	.122
	Color (Pt. Co.)	.340	.165	.	.424	.237	.426	.383	.433	.339	.054	.348	.086	.286
	NH_3-N (mg/L)	.236	.411	.424	.	.188	.002	.042	.008	.085	.478	.077	.339	.289
	COD (mg/L)	.424	.401	.237	.188	.	.190	.146	.196	.103	.290	.111	.151	.477
	BOD/COD	.234	.409	.426	.002	.190	.	.043	.006	.087	.480	.078	.340	.288
	TS (mg/L)	.277	.452	.383	.042	.146	.043	.	.050	.044	.436	.035	.297	.331
	TSS (mg/L)	.228	.403	.433	.008	.196	.006	.050	.	.093	.486	.085	.347	.281
	Fe (mg/L)	.321	.496	.339	.085	.103	.087	.044	.093	.	.393	.008	.254	.374
	Age Y	.286	.111	.054	.478	.290	.480	.436	.486	.393	.	.401	.139	.233
	EC (µS/cm)	.313	.488	.348	.077	.111	.078	.035	.085	.008	.401	.	.262	.366
	Phenols (mg/L)	.426	.250	.086	.339	.151	.340	.297	.347	.254	.139	.262	.	.372
	Turb. (NTU)	.053	.122	.286	.289	.477	.288	.331	.281	.374	.233	.366	.372	.

between NH3-N with BOD/COD, TS, and TSS, among BOD/COD with TS and TSS, amongst TS with TSS, Fe, and EC, and between Fe and EC. The age of landfills commonly hadn't significant statistical correlation with the mentioned landfill leachate parameters; only color neared to have a correlation with age (r = - 0.986 at P = 0.054).

REFERENCES

A practical Guide to Landfill Management in Pacific Island Countries. In: Inland- Based Waste Disposal, vol.1, (2005) Japan International Cooperation Agency (JICA).

Abu Amr S., Aziz H. A, Aziz S. Q (2012) Effect of Ozone and Ozone/Fenton in the Advanced Oxidation Process on Biodegradable characteristics of Semi-aerobic Stabilized Leachate. CLEAN - Soil, Air, Water, 41(2): 148 -152.

Abu Amr S., Zakaria SNF, Aziz H. A (2016) Performance of combined Ozone and zirconium tetrachloride in stabilized landfill leachate treatment, Journal of Material cycles and waste management, 19(4): 1384-1390.

American Public Health Association (APHA) (2005) Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington, DC.

Applied Research Institute – Jerusalem (ARIJ) (2015) Status of the Environment in the State of Palestine, Karam Muamar Street, P. O. Box 860, Bethlehem – Palestine.

Aziz, H.A., Adlan, M.N., Amilin, K., Yusoff, M.S, and Ramly, N. H. (2002) Quantification of generation rate of leachate from semi-aerobic landfill: Field data, Proceedings, The 5th Asian Symposium on Academic Activities for Waste Management (AAAWM), Kuala Lumpur, 9–12 September, 2002.

Aziz, S. Q., and Mustafa, J. S. (2018) Thermal and Financial Evaluations of Municipal Solid Waste from Erbil City-Iraq, 4th International Engineering Conference on Developments in Civil & Computer Engineering Applications, Erbil-Iraq, Feb 26-27, 2018, 86-97.

Aziz S. Q. (2011) Landfill Leachate Treatment Using Powdered Activated Carbon Augmented Sequencing Batch Reactor (SBR) Process, PhD thesis, School of Civil Engineering, Universiti Sains Malaysia (USM), Malaysia

Aziz S. Q., Aziz H. A, Bashir M. J. K, Mojiri A. (2015) Assessment of various tropical municipal landfill leachate characteristics and treatment opportunities, Global NEST Journal, 17: 1-3.

Aziz S. Q., Aziz H. A, Yusoff M. S., Bashir M. J. K (2011) Landfill leachate treatment using powdered activated carbon augmented sequencing batch reactor (SBR) process: Optimization by Response Surface Methodology. Journal of Hazardous Materials, 189: 404–413.

Aziz S. Q., Aziz H. A, Yusoff M. S., Bashir M. J. K, Umar M. (2010) Leachate characterization in semi-aerobic and anaerobic sanitary landfills: A comparative study. Journal of Environmental Management, 91: 2608-2614.

Aziz S. Q., Maulood Y. I (2015) Contamination Valuation of Soil and Groundwater Source at Anaerobic Municipal Solid Waste Landfill Site, Environmental Monitoring and Assessment, 187(12):155-165.

Aziz, S.Q., and Ali, S. M. (2018) Characteristics and potential treatment technologies for different kinds of wastewaters. ZANCO Journal of Pure and Applied Sciences, Salahaddin University-Erbil, ZJPAS (2018) 30 (s1), 122-134.

Bagchi A. (2004) Design, of landfills and integrated solid waste management. Third edition. John Wiley & Sons, Inc.

Bashir M. J. K., Aziz H. A,Yusoff M. S, Aziz S. Q, Mohajeri S. (2010) Stabilized Sanitary Landfill Leachate Treatment Using Anionic Resin: Treatment Optimization by Response Surface Methodology. Journal of Hazardous Materials, 182: 115-122.

Bashir M. J. K.,, Xian T. M, Shehzad A., Sethupahi S., Aun N. C., Abu Amr S. (2016) Sequential treatment for landfill leachate by applying coagulation-adsorption process. Geosystem Engineering, 20 (1): 9-20.

Christensen T. H, Kjeldsen P., Albrechtsen H. J, Heron G., Nielsen P. H, Bjerg P. L, Holm P. E. (1994) Attenuation of landfill leachate pollutants in aquifers. Crift. Rev. Environ. Sci. Technol., 24: 119.

DeCoursey, W.J. (2003)Statistics and Probability for Engineering Applications With Microsoft® Excel. Elsevier Science (USA). Library of Congress Cataloging-in-Publication Data, ISBN: 0-7506-7618-3.

Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009, Malaysian Environmental Quality Act 1974 [Act 127].

Field A. (2009) Discovering statistics using SPSS, 3rd Edition, SAGE Publications Ltd.

Hilles H. A, Abu Amr S. S, Hussein R. A, Arafa A. I, El-Sebaie M. D (2016) Performance of combined sodium persulfate/H2O2 based advanced oxidation process in stabilized landfill leachate treatment, Journal of Environmental Management, 166: 493 – 498.

https://www.epa.gov/landfills/municipal-solid-waste-landfills

Idris A., Inank B., Hassan M. N. (2004) Overview of waste disposal and landfills/dumps in Asian countries. Journal of Materials Cycles and Waste Management, 6: 104–110.

Iraqi Environmental Standards (IES) (1993) Ministry of Oil, Investigation, Planning and Development Division.

Kamaruddin, M. A., Yusoff, M. S., Aziz, H. A.and Alrozi, R. (2016) Current status of Pulau Burung Sanitary Landfill leachate treatment, Penang Malaysia, AIP Conference Proceedings 1774, 030014 (2016); doi: 10.1063/1.4965070.

Lee A. H, Nikraz H., Hung Y. T. (2010) Influence of Waste Age on Landfill Leachate Quality. International Journal of Environmental Science and Development, 1(4): 347-350.

Liermann T. A. (2009) Fiji Islands' Naboro Landfill Leachate Quality Analysis and the Applicability of Developed versus Small Island Developing State Discharge Standards. M.Sc Thesis of Environmental Engineering. MICHIGAN TECHNOLOGICAL UNIVERSITY.

Matsufuji Y., Hanashima M., Nagano S., Tanaka A. (1993) Generation of greenhouse effect gases from different landfill type. Eng. Geol. 34, 181e197.

Maulood, Y.I. and Aziz, S. Q. (2016) Soil and Municipal Solid Waste Leachate Characterization at Erbil Anaerobic Landfill Site. ZANCO Journal of Pure and Applied Sciences, Salahaddin University-Erbil, Vol. 28, No.3, 104-113.

Mojiri A., Aziz H. A, Ziyang L., Nanwen Z., Tajuddin R. M, Aziz S. Q, Dongdong G. (2015) Zeolite and activated carbon combined with biological treatment for metals removal from mixtures of landfill leachate and domestic wastewater, Global NEST Journal, 17(4): 727-737.

Ngo H. H, Guo W., Xing W. (2008) Applied technologies in municipal solid waste landfill leachate treatment. Encyclopedia of Life Support Systems (EOLSS), pp: 17.

Rajesh S., Babel K., Mishra S. K (2016). Reliability-Based Assessment of Municipal Solid Waste Landfill Slope. J. Hazard. Toxic Radioact. Waste, Article Number: 04016016-11

Renou S., Givaudan G. J, Poulain S., Dirassouyan F., Moulin P. (2008) Landfill leachate treatment: review and opportunity, J. Hazard. Mater. 150: 468–493.

Shehzad A, Mohammed J.K. Bashir, Sethupathi S., Lim J. (2016) Simultaneous removal of organic and inorganic pollutants from landfill leachate using sea mango derived activated carbon via microwave induced activation. International Journal of Chemical Reactor Engineering, 14, 991–1001.

Soong, T.T. (2004) Fundamentals of Probability and Statistics for Engineers. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England

Tchobanoglous, G., and Kreith., F. (2002) Handbook of solid waste management. Second edition. Mcgraw-Hill, Library of Congress Cataloging–in-Publication Data.

UNEP. (2005) Solid Waste Management, (Volume I). pp 524.

Utts, J.M., and Heckard (2007) Mind on Statistics, 3rd edition. Thomson Learning, Inc. All Rights Reserved. Thomson Learning WebTutor™ is a trademark of Thomson Learning, Inc.

Visvanathan C., Ben Aim R., Parameshwaran K. (2000) Membrane Separation Bioreactors for Wastewater Treatment. Critical Reviews in Environmental Science and Technology, 30(1): 1-48.

Yamamoto O. (2002) Solid waste treatment and disposal experiences in Japan. In: Proceedings of International Symposium on Environmental Pollution Control and Waste Management (EPCOWM'2002), Tunis, 417-424.

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Numerical Treatment of Mixed Volterra-Fredholm Integral Equations Using Trigonometric Functions and Laguerre Polynomials

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A B S T R A C T

In this paper, numerical solution of linear mixed Volterra-Fredholm integral equations of the second kind by using trigonometric functions and Laguerre polynomials approximation accompanied with the least square technique is presented. For the explanation of the idea and more illustration, an algorithm is introduced, and several examples are solved. Also, comparison between the exact and the approximate solutions are given to show the efficiency of the methods and accuracy of the results.

1. Introduction

Many problems in mathematical physics, mechanics, many related fields of engineering, mixed boundary value problems, and contact problems in the theory of elasticity lead to integral equations, (Abdou, M., 2002), (Mkhitarian, S. and Abdou, M., 1990) and (Aleksandrow, V., 1968). These integral equations have received considerable interest in a different area of sciences in mathematics Applications, (Wazwaz, A., 2011) and (Constanda, C., 1995). The solution of Volterra-Fredholm integral equation can be obtained theoretically in (Abdou, M., 2003), (Aghajani, A., et al., 2012) and (Abdou, M., 2002) . At the same time, in (Maleknejad, K., Hadizadeh, M., 1999) and (Shehab, S. et al., 2010) the sensing of numerical methods takes an important place in solving this type of equations. Double integral equations had been

treated numerically by (Abdou, M. 2005), (Kauthen, P., 1989), (Wang, Q. and Wang, K.), (Ezzati, R. and Najafalizadeh, S., 2012), and (Ahmed, S., 2011) where different methods are used.

Throughout this paper, we consider the integral equation of the form

$$u(x) = f(x) + \int_0^x \int_a^b k(r,t)u(t)dtdr; \ x \in [a,b] \quad (1)$$

where $f(x)$ and $k(x,t)$ are known on the interval [a, b], and $u(x)$ is the continuous function to be determined.

Orthogonal functions and polynomials receive attention in dealing with various problems. One of those is integral equations. The main property of using orthogonal basis is that it reduces these problems to a system of linear algebraic equations by seeking a solution of the form:

$$u(x) \cong \tilde{u}_n(x) = \sum_{i=0}^{n} \alpha_i \emptyset_i(x) \quad (2)$$

where $\emptyset_0(x), \emptyset_1(x), \dots, \emptyset_n(x)$ are the orthogonal functions defined on a certain interval [a, b]. Here we would like to choose $\emptyset_i(x)$ as trigonometric functions on [-π, π] or Laguerre polynomials on [0, ∞) with the least square approximation for solving this type of integral equation.

2. Principal Concepts

This section deals with some definitions and concepts (Atkinson, K., 1997) and (Delves, L. and Walsh, J., 1974) which are used in this work.

2.1. Orthogonal Polynomials

Two functions $p(x)$ and $q(x)$ are orthogonal over the interval [a, b] with weight function $w(x)$ if

$$\langle p(x), q(x) \rangle = \int_a^b p(x)q(x)w(x)dx = 0$$

2.2. Trigonometric Functions (T-F)

A trigonometric polynomial of order n is a function of x of the form

$$p(x) = a_0\tau_0(x) + \sum_{i=1}^{n} \left(a_i\tau_{2i-1}(x) + b_i\tau_{2i}(x)\right); \quad (3)$$

where $\tau_0(x) = 1$, $\tau_{2i-1}(x) = \cos ix$, and $\tau_{2i}(x) = \sin ix$ for $i = 1, \dots, n$, while the coefficients $a_0, a_1, \dots, a_n, b_1, \dots, b_n$ are real numbers such that $a_n \neq 0$, or $b_n \neq 0$.

The set $\{\tau_0(x), \tau_1(x), \dots, \tau_{2n}(x)\}$ is orthogonal set on $[-\pi, \pi]$ with respect to the weight function $w(x) = 1$. This orthogonally follows from the fact that for every integer i the integrals of $\sin ix$ and $\cos ix$ over $[-\pi, \pi]$ are zero. They are periodic functions with period (2π). Hence we can regard them as element of the space [-π, π], then the space of trigonometric polynomials of order n will be denoted by $\mathcal{T}_n$ that is

$\mathcal{T}_n = \{p \in C[-\pi, \pi], p(x) = a_0\tau_0(x) + \sum_{k=1}^{n}\left(a_k\tau_{2k-1}(x) + b_k\tau_{2k}(x)\right)\}$.

2.3. Laguerre Polynomials (L-P)

The general form of the Laguerre polynomials of n*th* degree is defined by

$$L_n(x) = \sum_{k=0}^{n} \frac{(-1)^k}{k!} \binom{n}{k} x^k \quad (4)$$

The Rodrigues representation for them is

$$L_n(x) = \frac{e^x}{n!}\frac{d^n}{dx^n}(x^n e^{-x});$$

they are orthogonal on [0, ∞) with the weigh function $w(x)=e^{-x}$, and satisfies recurrence relation

$$L_{n+1}(x) = \frac{2n+1-x}{n+1}L_n(x) - \frac{n}{n+1}L_{n-1}(x); \quad n \geq 2$$

where $L_0(x) = 1$ and $L_1(x) = 1 - x$.

3. Function Approximation

It is sometimes more comfortable to seek an approximate solution $\tilde{u}_n(x)$ in terms of delineative functions $\emptyset_0(x), \emptyset_1(x), \dots, \emptyset_n(x)$, which depends on *n* and independent on the kernel $k(x,t)$.

In this section, we find an approximate solution of equation (1) in the form that defined in equation (2). The substitution of $\tilde{u}_n(x)$ in the presented integral equation will gives

$$\tilde{u}_n(x) = f(x) + \int_0^x \int_a^b k(r,t)\tilde{u}_n(t)dtdr + e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n) \qquad (5)$$

where e_n is the error term which depends on *x* and on the way that $\alpha_i's$ are chosen, then

$$\sum_{i=0}^{n} \alpha_i \emptyset_i(x) = f(x) + \int_0^x \int_a^b k(r,t) \sum_{i=0}^{n} \alpha_i \emptyset_i(t)\, dtdr + e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n) \qquad (6)$$

Thus

$$e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n) = \sum_{i=0}^{n} \alpha_i \left(\emptyset_i(x) - \int_0^x \int_a^b k(r,t)\emptyset_i(t)dtdr \right) - f(x) \qquad (7)$$

Put

$$y_i(x) = \emptyset_i(x) - \int_0^x \int_a^b k(r,t)\emptyset_i(t)dtdr \;;$$

$$i = 0,1, \dots, n$$

So equation (7) becomes:

$$e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n) = \sum_{i=0}^{n} \alpha_i y_i(x) - f(x) \qquad (8)$$

Let

$$E(\alpha_0, \alpha_1, \dots, \alpha_n) = \int_a^b [e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n)]^2 . w(x)dx$$

where $w(x)$ is any positive function defined on $[a, b]$ which is called the weight function [15], then

$$E(\alpha_0, \alpha_1, \dots, \alpha_n) = \int_a^b \left(\sum_{i=0}^{n} \alpha_i \{ \emptyset_i(x) - \int_0^x \int_a^b k(r,t)\emptyset_i(t)dtdr \} f(x) \right)^2 w(x)dx$$

The main point here is how to find the coefficients $\alpha_i's, i = 0,1, \dots, n$ such that the error is minimized, and this equivalent to finding best approximation of the solution of the presented integral equation. Here the necessary condition for obtaining the minimum value of *E* is

$$\frac{\partial E}{\partial \alpha_i} = 0, \qquad i = 0,1, \dots, n$$

That is for each $i = 0,1, \dots, n$, we can get

$$\frac{\partial E}{\partial \alpha_i} = \frac{\partial \int_a^b [e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n)]^2 w(x)dx}{\partial \alpha_i}$$

$$= 2\int_a^b w(x)e_n(x, \alpha_0, \alpha_1, \dots, \alpha_n)\frac{\partial [e_n(x, \alpha_0, \dots, \alpha_n)}{\partial \alpha_i}dx$$

$$= 2\int_a^b w(x)\left(\sum_{j=0}^{n}\alpha_j\left\{\emptyset_j(x) - \int_0^x\int_a^b k(r,t)\emptyset_j(t)dtdr\right\} - f(x)\right).\left(\emptyset_i(x) - \int_0^x\int_a^b k(r,t)\emptyset_i(t)dtdr\right)dx$$

$$= 2\left(\int_a^b\sum_{j=0}^{n}\alpha_j y_j(x)y_i(x)w(x)dx - \int_a^b f(x)y_i(x)w(x)dx\right) = 0$$

Thus, we have

$$\sum_{j=0}^{n}\alpha_j.\int_a^b y_j(x)y_i(x)w(x)dx = \int_a^b f(x)y_i(x)w(x)dx$$

$$i = 0,1,\dots,n.$$

Finally by putting the last equation as a system of linear algebraic equations the following results will be concluded

$$\sum_{j=0}^{n}\alpha_j y_{ij} = \beta_i \quad i = 0,1,\dots,n. \qquad (9)$$

for $i = 0,1,\dots,n$ and $j = 0,1,\dots,n$

$$\left.\begin{aligned} y_{ij} &= \int_a^b y_j(x)y_i(x)w(x)dx \\ \beta_i &= \int_a^b y_i(x)f(x)w(x)dx \end{aligned}\right\} \qquad (9a)$$

while

$$y_i(x) = \emptyset_i(x) - \int_0^x\int_a^b k(r,t)\emptyset_i(t)dtdr \qquad (9b)$$

Rewriting equation (9) in the matrix form yields:

$$Y_n A = B \qquad (10)$$

where,

$$Y_n = \begin{bmatrix} y_{00} & y_{01} & \cdots & y_{0n} \\ y_{10} & y_{11} & \cdots & y_{1n} \\ \vdots & \vdots & \cdots & \vdots \\ y_{n0} & y_{n1} & \cdots & y_{nn} \end{bmatrix},$$

$$A = [\alpha_0, \alpha_1, \dots, \alpha_n]^T ,$$

and

$$B = [\beta_0, \beta_1, \dots, \beta_n]^T .$$

By applying the collocation of points in the form $x_i = a + ih$; for $i = 0,1,\dots,n$ and $h = (b-a)/n$ and solving the system of linear equations, the unknown points $\alpha_0, \alpha_1, \dots, \alpha_n$ will be obtained, then they will be substituted in equation (2) to get the approximate solution of integral equation (1).

4. The Solution of LMV-FIE2nd with (T-F) and (L-P)

In this section, we solve equation (1) by using the trigonometric functions and Laguerre polynomials based on the above technique.

4.1. Using Trigonometric Functions (T-F)

Here the unknown function $u(x)$ in Equation (1) can be expanded in terms of trigonometric functions as follows:

$$\tilde{u}_n(x) = \sum_{i=0}^{n}\alpha_i\,\tau_i(x) \qquad (11)$$

where $\tau_i(x)$ are as defined in section 2.2., by applying the steps which described in section 3 we will get the system (9) that could be easily solved to get the values of $\alpha_i's$. In this case, $w(x) = 1$ and equation (9a-9b) will be replaced by

$$\left.\begin{aligned} y_{ij} &= \int_a^b y_i(x)y_j(x)dx \\ \beta_j &= \int_a^b y_i(x)f(x)dx \end{aligned}\right\} \qquad (11a)$$

and

$$y_i(x) = \tau_i(x) - \int_0^x \int_a^b k(r,t)\,\tau_i\,(t)dtdr \qquad (11b)$$

At last, by substituting these values of $\alpha_i's$ in equation (10) we obtain the approximate solution $\tilde{u}_n(x)$ of $u(x)$.

4.2. Using Laguerre Polynomials (L-P)

By substituting an approximate solution of the form

$$\tilde{u}_n(\mathrm{x}) = \sum_{\mathrm{i}=0}^{\mathrm{n}} \alpha_{\mathrm{i}}\, L_i(x) \qquad (12)$$

in equation (1), where $L_i(x),\ i = 0,1,\dots,n$ are Laguerre polynomials defined in equation (4), then by following the same description in section (3), we get the system of equations (9), while here

$$\left.\begin{aligned} y_{ij} &= \int_0^b y_i(x)y_j(x)e^{-x}dx \\ \beta_j &= \int_0^b y_i(x)f(x)e^{-x}dx \end{aligned}\right\} \qquad (12a)$$

and

$$y_i(x) = L_i(x) - \int_0^x \int_a^b k(r,t)L_i(t)dtdr \qquad (12b)$$

then the values of $\alpha_0, \alpha_1, \dots, \alpha_n$ will be founded and then substituted in equation (11) to get the solution of equation (1).

5. The Algorithm

In this section, we will consider each step of the method which is solved by MATLAB and we distinguish the algorithm in six steps:

Step 1: Input the value of *n* (the number of participant functions $\emptyset_i(x),\ i = 0,1,\dots,n$).

Step 2: Let

$$\tilde{u}_n(x) = \sum_{i=0}^{n} \alpha_i \emptyset_i(x)$$

be an approximate solution of equation (1), where $\emptyset_i(x)$ are Trigonometric functions (T-F) or Laguerre polynomials (L-P).

Step 3: Input $\tau_i(x)$ (the trigonometric functions), or $L_i(x)$ (the Laguerre polynomials), where $i = 0,1,\dots,n$.

Step 4: For all $i = 0,1,\dots,n$ and $j = 0,1,\dots,n$

In the first case (T-F), evaluate

- $y_i(x)$ from equation (11b).
- y_{ij} and β_i in equation (11a).

while in the second case (L-P), compute

- $y_i(x)$ in equation (12b).
- y_{ij} and β_i using equation (12a).

Step 5: In both cases, solve the system of equations (10) to get the values of $\alpha_0, \alpha_1, \dots, \alpha_n$.

Step 6: substitute these values of $\alpha_i's$ in the assumption that we have in the first step to get the approximate solution $\tilde{u}_n(x)$ of equation (1).

6. Numerical examples

In this section, we study some examples and demonstrate that, in spite of the above mentioned method in the previous section, the computations, associated with the examples are performed by MATLAB.

Example 1: Consider the LMV-FIE2nd

$$u(x) = 2 + 4x - \frac{9}{8}x^2 - 5x^3 + \int_0^x \int_0^1 (r-t)u(t)dtdr;\ 0 \le x \le 1$$

the exact solution is

$$u(x) = 2 + 3x - 5x^3$$

Using the formula derived in the previous section and solving the system of equations

for *n*=4, we get the following approximate solutions:

1- By using Trigonometric Functions:

Here we take equation (11) as an approximation of *u*(*x*), by applying the algorithm with its program and taking the value of *n* =4, we have the formula

$$\begin{aligned}\tilde{u}_5(x) = (&-16.0447931435)\\ &+ (23.3670600343)\cos(x)\\ &- (0.5810322888)\sin(x)\\ &- (5.3274104457)\cos(2x)\\ &+ (1.8660347551)\sin(2x)\end{aligned}$$

and we get the results of the solution and consequently the least square error (L.S.E.) that shown in the Table 1, where $L.S.E. = \sum_i \ (u(x_i) - u_n(x_i))^2$ for some *i*.

1- By Laguerre Polynomials:

In equation (11), put *n* =4 and use the algorithm described in section 5 with its program to get the value of $\alpha_i's$, then we get the related approximate solution

$$\begin{aligned}\tilde{u}_4(x) = &-25 + 87(-x + 1)\\ &- 90\left(\frac{1}{2}(x^2 - 4x + 2)\right)\\ &+30\left(\frac{1}{6}(-x^3 + 9x^2 - 18x + 6)\right)\\ &+ \ 0\left(\frac{1}{24}(x^4 - 16x^3 + 72x^2\right.\\ &\left. - 96x + 24)\right).\end{aligned}$$

Table 1 gives a comparison between the numerical results using (T-F) and (L-p) methods with *n*=4, while Figure 1 gives a comparison between the exact and the approximate solution using (T-F) and (L-p) solution for different values of *n.*

Example 2: Consider the LMV-FIE

$$u(x) = \sin(x) - \tfrac{1}{2}(x^2 + 2x)$$

$$+ \int_0^x \int_0^{\frac{\pi}{2}} (1 + rt)u(t)dtdr\,; \ \ 0 \le x \le \frac{\pi}{2}$$

the exact solution is

$$u(x) = \sin(x).$$

In the first case, we use Trigonometric functions and as defined in equation (11) with *n*=2, then the values $\alpha_0 = 0, \alpha_1 = 0, and\ \alpha_2 = 1$ will be obtained, which acquires the exact solution of the problem, thus the *L.S.E*= 0.

While in the second case, the Laguerre Polynomials will be taken in the approximate solution as in equation (12), different values of *n* have been chosen in some distinct points and the results are listed in Table 2. Exact solutions and numerical results using (L-P) are given in Figure 2 for *n*=1, 2, 3, and 4.

In both cases, our results are compared with the exact solutions by computing the absolute error and the L.S.E. of them.

Example 3: Consider the LMV-FIE2nd

$$u(x) = xe^x - \tfrac{x^2}{2}$$

$$+ \int_0^x \int_0^1 ru(t)dt; \ \ 0 \le x \le 1$$

the exact solution $u(x) = xe^x$.

Perform the prescribed steps in the above algorithm with n=4, we get the values of $\alpha_0, \ldots, \alpha_4$ in both cases as shown in Table 3, while Table 4 and Figure 3 present the comparison between the exact and the numerical solutions.

Table 1. The (T-F) & (L-P) results with n=4 compared with exact solution of example 1.

x	Exact solution $u(x)$	Approximate solutions with n=4	
		using(T-F)	using(L-P)
0	2	1.9948564450	2
0.1	2.295	2.2970294341	2.295
0.2	2.560	2.5698463229	2.560
0.3	2.765	2.7636460722	2.765
0.4	2.880	2.8783985642	2.880
0.5	2.875	2.8749715021	2.875
0.6	2.720	2.7215870510	2.720
0.7	2.385	2.3864075992	2.385
0.8	1.840	1.8391842323	1.840
0.9	1.055	1.0528984137	1.055
1.0	0	0.0053270672	0
L.S.E.		7.364991e-05	0

Table 2. Absolute error**s** and L.S.E of example 2 using (L-P) with distinct values of n .

Table 3. The value of $\alpha_i's$ in (T-F) and (L-P) methods for n=4 for example 3.

Method / α_i	(T-F)	(L-P)
α_0	8.014712541	12.705876238
α_1	-9.819635818	-1.454772743
α_2	0.839835858	53.211914728
α_3	1.806476364	-31.88929412
α_4	0.057682302	07.426502596

0	1.6645e-04	9.9301e-06	7.0077e-07
0.1π	3.7018e-06	2.8661e-06	2.4906e-07
0.2π	4.4708e-05	2.4446e-06	2.3466e-07
0.3π	8.2437e-05	3.8178e-07	2.8371e-07
0.4π	7.3433e-05	1.6831e-06	3.4237e-07
0.5π	2.9626e-04	2.3266e-05	1.3179e-06
L.S.E	1.2967e-07	6.5706e-10	2.5428e-12

Table 4. The comparison between the exact and the approximate (T-F) & (L-P) solutions of example 3 for n=4.

x	Exact values $u(x)$	Approximate values $u_n(x)$	
		using(T-F)	using(L-P)
0	0	0.00155309	0.00022670
0.1	0.11051709	0.10990450	0.11041829
0.2	0.24428055	0.24400276	0.24425685
0.3	0.40495764	0.40536376	0.40503966
0.4	0.59672988	0.59723946	0.59680660
0.5	0.82436064	0.82439147	0.82434025
0.6	1.09327128	1.09277725	1.09316582
0.7	1.40962690	1.40916255	1.40955115
0.8	1.78043274	1.78067721	1.78050678
0.9	2.21364280	2.21433362	2.21378584
1	2.71828183	2.71652912	2.71788417
L. S. E		7.35874e-06	2.75680e-07

Figure 1. comparison between the numerical results of example 1 using (T-F) and (L-P) for n=2, 3, and 4.

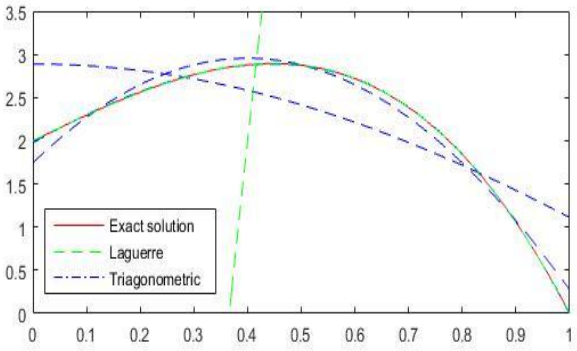


Figure 2. Exact solutions and numerical results of example 2 using (L-P) for n=1, 2, 3, and 4.

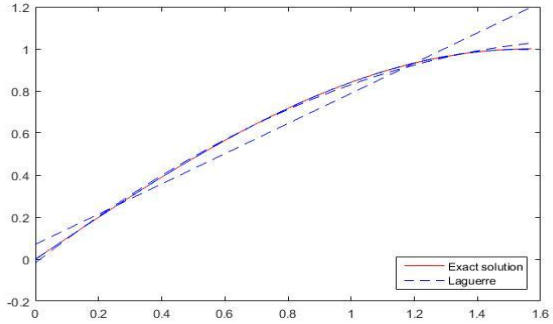
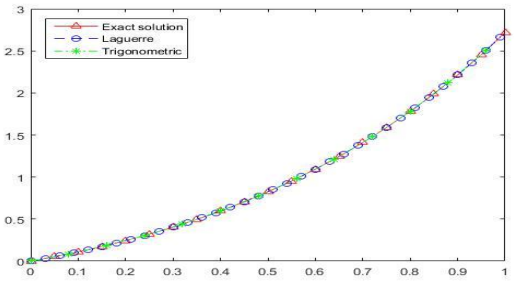


Figure 3. Exact solutions and Numerical solutions of example 3 using (T-F) and (L-P) for n= 4.



7. Conclusion

In this paper, the trigonometric functions and Laguerre polynomials depending on the principle of the least square technique are introduced to solve the second kind linear mixed Volterra–Fredholm integral equations. Several examples are applied for illustration and good approximate (sometimes exact) results are found. Moreover, the results of (T-F) and (L-P) are compared to each other and with the exact solutions to demonstrate the propriety and implementation of the method. Also, better results have been obtained by increasing the value of (n) which represents the number of basis functions. The given numerical examples and the outcomes in Tables 1-4 and Figures 1-3 are supported these claims.

8. Appendix A

```
disp('Trigonometric Polynomials ')
```

```
for n=2:4;
LSE=0;
bb=1;%(.5)*pi;
syms r x
for i=1:n
q(1)=1;
q((2*i)-1)=cos(i*x);
q(2*i)=sin(i*x);
end
q
q=[1  cos(x) sin(x) cos(2*x) sin(2*x)
cos(3*x) sin(3*x),cos(4*x) ,sin(4*x),
cos(5*x) sin(5*x)];
%Ex -1-
f=2+4*x-(9/8)*(x)^2-5*(x)^3;
k=r-x;
E=2+3*x-5*(x)^3;
%Ex -3-%k=r; E=x*(exp(x));
%f=x*(exp(x))-(1/2)*(x^2);
%Ex -2-%k=1+(r*x);E=sin(x);
%f=sin(x)-(x*(x + 2))/2;
for i=1:n
y(i)=q(i)-int(int(k*q(i),x,0,bb),0,x);
end

for i=1:n
  for j=1:n
      Y(i,j)=int(y(i)*y(j),0,bb);
  end
end
for i=1:n
    b(i)=int(y(i)*f,0,bb);
end
format long
for p=0:.1:bb;
    for i=1:n
      b(i)=subs(b(i),x,p);
        for j=1:n
         a(i,j)=subs(Y(i,j),x,p);

        end
    end
    A=inv(a);
    B=(b');
    c=mtimes(A,B);
    %  c=inv(a)*b'
    double(c)
ss=c(1)*q(1)+c(2)*q(2);%+c(3)*q(3)
  +c(4)*q(4)+c(5)*q(5);%+c(6)*q(6);
   s=subs(ss,x,p);
  Ex=subs(E,x,p);
  er=Ex-s;
  e=(er)^2;
%disp(double([Ex  s er]))
%disp(double([s Ex]))

%disp(er)
%disp(e)
LSE=LSE+e;
end
double(c)
ss=0;
end
disp('L.S.E=')
 disp(double(LSE))
 disp('c=')
disp(double(c))
```

Appendix B

```
disp('Laguerre Polynomials ')
LSE=0;
n=5;
bb=1;%pi/2;
syms r x
q=[ 1 -x+1,((3-x)/2)*q(2)-(1/2)*q(1),
((5-x)/3)*q(3)-(2/3)*q(2),((7-x)/4 )*
q(4)-(3/4)*q(3)]
%,q(6),q(7),q(8),((15-x)/8)*q(8)-
(7/8)*q(7)  ]
%Ex -1-
f=2+4*x-(9/8)*(x)^2-5*(x)^3;k=r-
x;E=2+3*x-5*(x)^3;
for i=1:n
y(i)=q(i)-int(int(k*q(i),x,0,bb),0,x);
end
for i=1:n
  for j=1:n
   Y(i,j)=int(y(i)*y(j)*exp(-x),0,bb);
  end
end
for i=1:n
    b(i)=int(y(i)*f*exp(-x),0,bb);
end
')
format long
  for i=1:n
    for j=1:n
     a(i,j)=Y(i,j);
    end
  end
    A=inv(a);
    B=(b');
    c=mtimes(A,B);
   ss=0;
 for i=1:n
     ss=ss+c(i)*q(i);
 end
for p=.5
  s=subs(ss,x,p);
   Ex=subs(E,x,p);
    er=Ex-s;
    e=(er)^2;
    disp(double([ Ex  s er    ]))
    LSE=LSE+e;
end
disp('L.S.E=')
disp(double(LSE))
```

References

Abdou, M.A. 2002. Integral Equation of Mixed Type and Integrals of Orthogonal Polynomials. *Journal of Computational and Applied Mathematics.* 138(2): 273-285.

Abdou, M.A. 2002. Fredholm-Volterra Integral Equation of the First Kind and contact Problem, *J. Applied Mathematics and Computation*. 125(2-3): 177-193.

Abdou, M.A.2003. On Asymptotic Method for Fredholm Volterra Integral Equation of the Second Kind in Contact problem, *Journal of Computational and Applied Mathematics.* 154: 431-446.

Abdou, M.A. and Abd Al-Kader G.M., 2005. Mixed Type of Fredholm-Volterra Integral Equation. *Le Matematiche*. LX: 41-58.

Aghajani, A. , Jalilian, Y. and Sadarangani, K., 2012. Existence of Solution for Mixed Volterra- Fredholm Integral Equations. *Elecronic Journal of Differential Equations*.137: pp. 1-12.

Ahmed, S.S. 2011. Numerical Solution for Volterra-Fredholm Integral Equation of the Second Kind by Using Least squares technique. *Iraqi Journal of Science*. 52(4): 504-512.

Aleksandrow, V.M. and E.V. Kovalenko. 1968. *Problems in Mechanic Media with Mixed Boundary Conditions*. Nauka Moscow.

Atkinson, K.E. 1997. *Numerical Solution of Integral Equation of the Second Kind*. Cambridge.

Constanda, C., 1995. Integral Equation of the First Kind in Plane Elasticity. *Quartly of Aplied Mathematics.* v. LIII (4): 783-793.

Delves, L. and Walsh, J., 1974. *Numerical Solution of Integral Equations*. Oxford.

Ezzati, R. and Najafalizadeh, S., 2012. Numerical Methods for Solving Linear and Nonlinear Volterra-Fred holm Integral Equations by Using Cas Wavelets. *World Applied Sciences Journal*. 18 (12): 1847-1854.

Kauthen, P.G., 1989. Continuous Time Collocation Methods for Volterra-Fredholm Integral Equation. *Numer Math*. 56: 409-424.

Maleknejad, K. and Hadizadeh, M. A. 1999. New Computational Method for Volterra-Fredholm Integral Equation, *J. Comput. Math. Appl.* 37: 37-48.

Mkhitarian, S.M. and M.A. Abdou. 1990. On Different Methods of Solution of the Integral Equation for the Planer Contact Problem of Elasticity. *Dokl. Acad. Nauk.Arm. SSR*. 89(2): 59-74.

Shehab, S.N., Ali, H.A. and H.M. Yaseen. 2010. Least Square Method for Solving Integral Equations with Multiple Time Lags. *Eng & Tech Journal*. 28(10): 1893-1899.

Wang, Q., Wang K., and Chen, S., 2014. Least Square Approximation Method for the Solution of Volterra-Fredholm Integral Equations. *Journal of Computational and Applied Mathematics*.272:141-147.

Wazwaz, A.M. 2011. *Linear and Non-linear Integral Equations*. Springer. Saint Xavier University of Chicago, USA.

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COMPRESSIVE STRENGTH OF THREE TYPES OF PIT AND FISSURE SEALANTS (AN IN-VITRO COMPARATIVE STUDY)

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A B S T R A C T

This research presents an experimental and theoretical investigation of compressive strength and stress distribution of three different types of pit and fissure sealants (PFS). The PFS had been divided into three major categories, i.e. resin-modified glass-ionomer (RMGI), resin-based PFS, and compomer Sealant, for each given type, commercial brands photac fil quick aplicap (3M ESPE), ultraseal XT hydro (Ultradent), and compoglass F (Ivoclar Vivadent) have been selected, respectively. Thirty samples, which were divided into three groups (ten specimens for each PFS type) were loaded in compression until failure. The SPSS (Statistical Package for the Social Science) version 23 program was used to perform the statistical analysis and assess the difference between the compressive strength of each study group.

The results of the experiment showed that the compressive strength of the resin-based PFS overcomes the RMGI and compomer sealant by 116% and 30% respectively. By using ABAQUS program performed the 3D finite element model (consisting 13050 elements and 14508 nodes) to evaluate the effect of chemical composition on the compressive strength of the PFS and compared stresses with the experimental results. The results of the analysis of these two methods showed that the vertical stress values differ even by 100% at stress concentration zones. This research showed that the filler fraction and particle size and uniformity of the filler distribution are the main determining factor affecting the compressive strength of PFS.

1. INTRODUCTION

Selection of the type of pit and fissure sealants for a specific clinical case becomes a priority task against preventive dentistry because it may effect on the long-term treatment result and the patient satisfaction with the result of the work. The mechanical properties of PFS play the decisive role in achieving this goal, especially the strength of the material under compression. The primary goal of using these materials is to coat and seal the pits and fissures which were used for preventing the teeth from the harmful bacteria and subsequent caries. In recent decades, many attempts have been made to prevent the

development of caries, in particular, occlusal caries, since it was once generally recognized that the pits and fissure in the teeth would be infected with bacteria within ten years of the eruption in the mouth. (Babu et al., 2014; Cohen and Sheiham, 1988; Subramaniam et al., 2008).

Since the first PFS was produced in 1922 (History and Evolution of Pit and Fissure Sealants), the composition of these materials have changed to improve them, and several modified types have been introduced with the purpose of enhancing their mechanical properties and expanding their clinical applications (Beauchamp et al., 2008). The mechanical properties of dental materials, in general, and PFS, in particular, have been investigated since their development, and the problems have remained up to now (Cattani-Lorente et al., 1999; Xie et al., 2000; Xu and Burgess, 2003). Durability and retention of PFS, apparently, are associated with their mechanical strength (Aratani et al., 2005; Lerech et al., 2017).

It is important to ensure that the mechanical properties of the material are strong enough to withstand the mastication load. It is believed that the chemical composition of PFS materials played a significant role in their mechanical properties and their subsequent success of the application in dentistry (Cildir and Sandalli, 2007a; Feigal, 2002; Geiger et al., 2000; Nunn et al., 2000). Moreover, when comparing materials that are brittle and generally weak in tension, compressive strength is a useful benchmark (Cattani-Lorente et al., 1999; Xu and Burgess, 2003).

Given the fact that over the last decades a lot of scientific works have been devoted to the mechanical properties of these materials (Bayne et al., 1998; Beun et al., 2012, 2008; Crisp et al., 1976), but yet, the correlation between chemical composition and compressive strength of various PFS has not been widely studied. Based on this, in this study, the uniaxial compressive strength of three categories of these materials was compared.

This research focused on the compressive strength, as the most important mechanical property of materials for dentistry, depending on their type and chemical composition. Simultaneously with the experimental test, the modelling of the tested samples was simulated in the environment of the ABAQUS computer complex, then investigated the stress field at specified sections of the samples on the base of the finite element method (FEM). Which in turn, allows having a complete image of the distribution of the stresses in the body of the materials and their direction.

Accordingly, the present study aimed to evaluate and compare the compressive strength of three different types of the PFS (photac fill, ultraseal XT hydro, and compoglass F) after 24 hours of storage in distilled water at 37^{o}C according to ISO and ANSI/ADA specifications, then performed the statically axially loading test and analysis done by equilibrium equations, as well as, used FEM, so as to find the material of choice for stress-bearing areas.

2. MATERIAL AND METHODS

2.1.Materials

The selected materials used in this study have been divided into three major categories, i.e. resin-modified glass-ionomer (RMGI), resin-based PFS, and compomer sealant, for each given type, commercial brands photac fil (3M ESPE), ultraseal XT hydro (Ultradent), and compoglass F (Ivoclar Vivadent) have been selected, respectively (Table 1).

Table 1 Characteristics of the used pit and fissure sealants.

PFS Type	Brand Name	Manufacturer	Color	Filler Wt. %	Mean Filler Size µm	Composition
RMGI	Photac Fil Quick Aplicap	3M ESPE, Deutschland	A3	76-77	5-7	**Powder:** (Na, Ca, Al, La Fluorosilicate Glass), activator (amin). **Liquid:** monomer, oligomer, copolymer acid (acrylic and maleic acid), water.
Resin-Based	Ultraseal XT hydro	Ultradent, Douth Jordan, UT, USA	Opaque White	53	<0.001- >1	**Filler:** Al_2O_3<0.4%wt, TiO_2<0.3%wt, Na_2PO_3F<0.2% wt. **Matrix:** Triethylene Glycol Dimetharrylate (TEGDMA)<20%wt Diurethane Dimethacrylate (DUDMA)<8%wt, Methacrylic acid (MAA)<1%wt
Compomer	Compoglass F	Ivoclar Vivadent Schaan, Liechtenstein	A2	80.5	0.2-3	**Filler:** Ytterbium Trifluoride, Ba-Al-Fluorosilicate Glass, and Spheroid mixed oxide 80%wt, initiators, stabilizers and pigments <0.20%wt. **Matrix:** Dimethacrylate 19.30%wt.

2.2. Preparation of Specimens

In this research, a cylindrical mold with double open ends (height=6mm and diameter=4mm) according to "ISO 6874 for the testing of the polymer-based pit and fissure sealants" using stainless-steel two-parts mold were used to make samples with standardized size h/d≤10 to avoid buckling, unwanted deformations, and failure (Figure 1-a). Ten samples from each material were used for the compressive strength using the universal testing machine Gunt-Hamburg WP 300, with measuring ranges force: 0…20kN, graduation: 0.5kN, and travel: 0…20mm, graduation: 0.01mm.

The form was filled, in three consecutive layers, by material, approximately with 2 mm thickness.

Polymerization of each layer was carried out with a visible light curing unit with 1mm distance for 20 seconds at 1200mW/cm^2 by

using Elipar S10 (3M Espe, USA) (Figure 1-b).

Samples were stored in their mold for one hour before having their thickness and diameter measured. Each sample was transferred to plastic test tubes containing 3ml of distilled water. All samples and containers were incubated at 37^{o}C for 24 hours, Figure 1-c, d (Cildir and Sandalli, 2007b).

The compressive strength test of different fissure sealants was measured using the universal mechanical testing machine Gunt-Hamburg WP 300. The specimens were placed between the bottom support block and top loading bar with 4 mm in diameter, and a compressive load was applied axially at cross-head speed of 1mm/min to the specimen at the age of 24 hours (ISO 6874:2015 - Dentistry - Polymer-based pit and fissure sealants) (Figure 1-e, f).

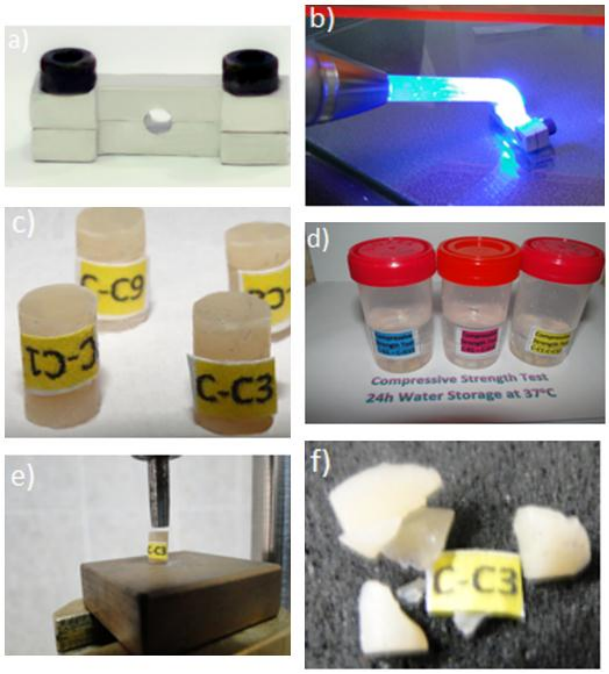

Figure 1 Compressive test procedure: mold preparation, filling and light curing, water curing, testing, and failure mode. a)mold, b)light curing, c)specimens, d)sample stored in distilled water at 37oC, e)compressive test, f)sample's failure mode.

2.3.Methods

Done comparative analysis of three different sealant materials by using the analytical calculation of the performed tests and FEM. Next statistical analysis was performed on the test results.

a) Experimental method

Maximum failure load was recorded for each specimen and divided by the net cross-sectional area to determine the compressive stress in MPa by using the following equation:

$$\sigma_c = \frac{P}{A} = \frac{P}{\pi d^2/4} = \frac{4P}{\pi d^2}$$

where P is the failure load, in Newton; d is the average measured the diameter of the specimen, in mm, and A is the net cross-sectional area (mm^2) of samples (Aratani et al., 2005; Cildir and Sandalli, 2007a; Walsh et al., 2014).

b) Finite element modelling and simulation of the PFS by ABAQUS software

The mechanical properties of the materials obtained during the experimental testing became the input data for numerical analysis. Performed the numerical modelling of the cylinder shape (h=6mm, d=4mm) in the ABAQUS computer complex environment. Then illustrated the comparison of these two different methods and outlined the findings and conclusions.

To achieve the more accurate results, after meshing, the samples are subdivided into 13050 3D small elements of simple shapes connected by 14508 nods. The mechanical properties of the PFS, input data, such as modulus of elasticity, density, poison's ratio, .. for the modelling were taken from the test results. Thus stress of all small elements was calculated, and there was a complete oblique of

the stress-strain state of the entire sample (Figure 2).

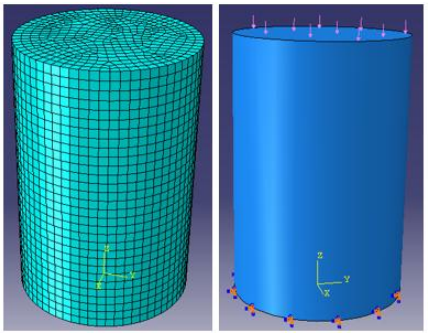

Figure 2 Meshed FE model and loading and supporting illustration of the tested sample

c) Statistical Calculation

The SPSS (Statistical Package for the Social Science) version 23 program was used to perform the statistical analysis and assess the difference between the compressive strength of each study group, one-way ANOVA was conducted to examine mean comparison, and then the posterior test was carried out through Duncan test.

3. RESULTS

The compressive strengths of all types of PFS materials are shown in Figure 3 and displayed in Table 2. The results of the statistical analysis demonstrated significant difference within a group ($p<0.05$), at the same time the significant difference between groups (Figure 3). In which resin-based PFS showed the highest compressive strength value (264 MPa).

Table 2 Compressive strength of the three types of PFS.

Material Group	Commercial Brand	N	Mean Compressive Strength±SD MPa	95% Confidence Interval for Mean		Min	Max	p
				Lower Bound	Upper Bound			
RMGI	Photac Fil	10	122±18	109.51	135.29	97	149	<0.05
Resin-Based	Ultraseal XT hydro	10	264±19	250.01	277.59	230	290	<0.05
Compomer Sealant	Compoglass F	10	202±27	182.42	221.78	157	237	<0.05

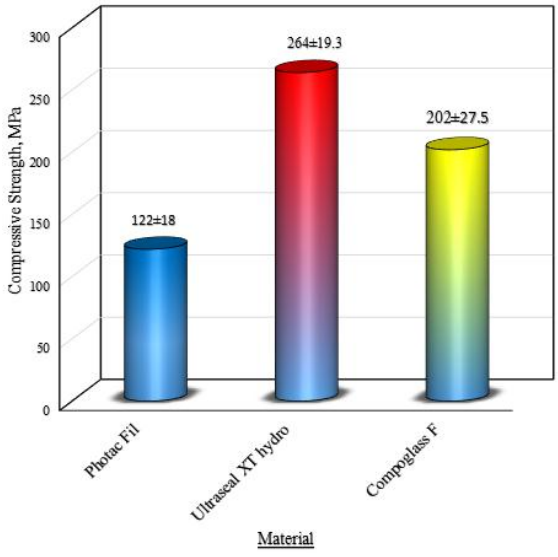

Figure 3 Mean Value and standard deviation of the Compressive Strength of three different types of PFS.

The results of the Tukey HSD and Duncan test, which show the highly significant difference between different types of PFS are presented in Table 3.

The complete illustrative image of the stress distribution could not be achieved without the help of a powerful computer program like ABAQUS. For this purpose, all of three kinds of PFS were modelled and simulated under surface loading applied to the top face of the specimens, such as applied during testing (Figure 2). The derived results in the form of stress field, as well as the distribution of stress in the body of the materials and stress concentration zones (bottom of the tested samples), are presented in Figures 4 and 5.

Table 3 Post Hoc tests- Tukey HSD and Duncan tests for compression strength.

Test	PFS	N	Subset for alpha = 0.05			Duncan grouping[a]
			1	2	3	
Tukey HSD	Photac Fil	10	122			
	Compoglass F	10		202		
	Ultraseal XT hydro	10			264	
	Sig.		1.000	1.000	1.000	
Duncan	Photac Fil	10	122			A
	Compoglass F	10		202		B
	Ultraseal XT hydro	10			264	C
	Sig.		1.000	1.000	1.000	

[a] there is no group with the same letter – there is a significant difference between groups

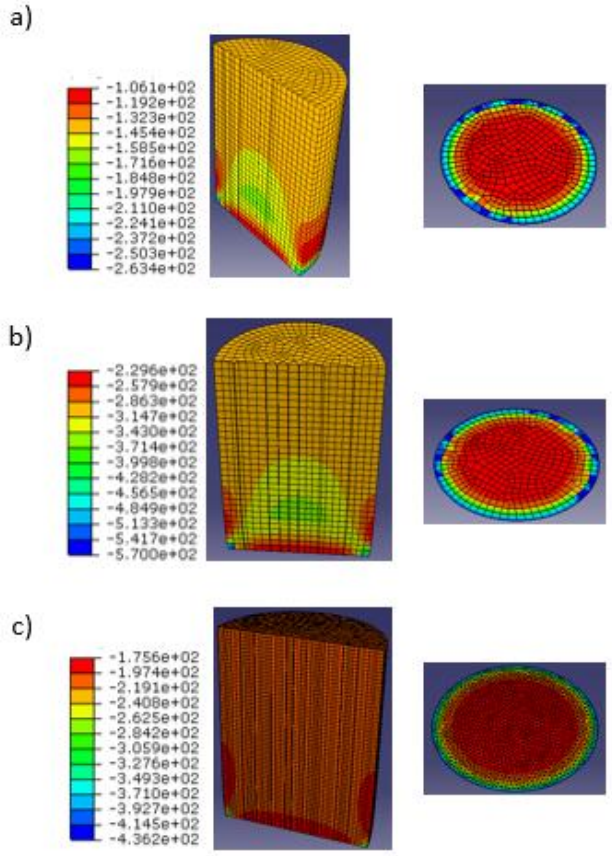

Figure 4 Vertical stress –Ϭz (MPa) distribution in the body of three categories of PFS and stress concentration at the bottom of the tested samples. a) Photac Fil, b) Ultraseal XT hydro, c) Compoglass F

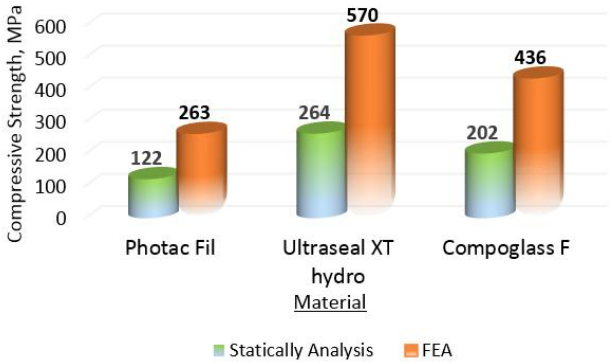

Figure 5 Comparison of the vertical stresses calculated by analytic and FE methods

4. DISCUSSION

It is well known that the mechanical characteristics of the PFS are strongly associated with the filler content, filler distribution, particle size, and type. As well as coupling between particles and matrix are also factors that influence mechanical properties such as strength and modulus of elasticity (Obici et al., 2005).

Two predominant types of PFS (materials are available: resin-based sealants and glass ionomer cement (Ripa, 1993; Singh et al., 2011).

Moezzyzadeh (2012) concludes: "Various filler particle sizes can result in increased number of fillers within the composite matrix which subsequently confers extra strength because there is a direct relationship between the increased compressive strength of composites and increased number of fillers". At the same time, other points of view do not agree with Moezzyzadeh. The filler content may be considered as a factor; however, the filler type and its fraction may have the stronger effect on the mechanical properties than the percentage of the filler because Compoglass F which had a high filler content 80.5% wt. (Table 1) was not as strong as Ultraseal XT hydro which had a filler content 53% (Taher, 2001).

The experimental results of this study presented in Table 2 showed that the Ultra seal XT hydro had significantly highest compressive mean value (264MPa), while the photac fil had significantly lowest compressive mean value (122MPa) and this agrees with the study of Xiaoming Xu and John O. Burgess (2003) that observed significantly higher strength of resin-based dental sealant in comparison to GIC dental sealants.

Based on previous work, it can be noted that tri-ethylene-glycol-dimethacrylate (TEGDMA) and bisphenol A-glycidyl methacrylate (BISGMA) are the backbone molecules of composite resin. TEGDMA is a linear co-monomer which terminates with two functional methacrylate groups identical to those terminating Bis-GMA. After polymerization, acrylic bonds are created with Bis-GMA as well as with other molecules of TEGDMA, and also with the mineral filler particles, by means of a coupling agent grafted on these filler particles and also having a terminal functional methacrylate group. A tridimensional network is thus formed, characterized by a high mechanical and chemical resistance (Meyer, et al. 1998).

The result of the present study showed that the compressive strength of the resin-based material overcomes the RMGI and compomer sealant by 116% and 30% respectively.

The findings of this study agree with Meyer et al. (1998) and El-Kalla and Franklin Garcia-Godoy (1999) that the compomer had higher compressive strength than RMGI, because the polyacid-modified composite resins absorbed less water than the resin-modified glass-ionomer cement, so the water diffuses more slowly through the polyacid-modified composite resin specimens.

Also, the higher mechanical strength of compomers can be interpreted by the fact that compomers have their glass particles silanated to bond covalently to the polymer matrix. It is easy to note, that compressive strength increase by moving from glass ionomers and resin-modified glass ionomers to compomers and composite resins. On the other hand, this work proves that the filler content cannot be the only and decisive factor affected on the compressive strength of the PFS. In general, the difference between the test results of all types of PFS depends on the some other factors, such as, water content, calcium amount, variability of monomers, dehydration of the specimen during the testing, presence of hydrophilic, and sometimes the complex of these factors plays the decisive role in determining the strength of

the material under compression (Meyer et al., 1998; Munhoz et al., 2016; Uno et al., 1996).

The multivariate results indicated that the chemical composition of material had a significant influence on their micro-mechanical properties. Certainly, the presence of calcium as an integrating part in the photac fill is a clear indication that its strength is lower than other tested materials (Xu and Burgess, 2003).

In this work, the specimens are tested by the applied centric-axially compressive load. Thus, it was imitated the chewing pressure on the tooth. During the test, the material is compressed into the barrel shape. As a result of the loading, the tensile stress occurs in horizontal directions, and as a result, the samples collapsed.

The biomechanics has more ambitious goals to investigate the stress state of the PFS using numerical methods and simulation of the samples to have a clearer idea about the behavior of materials in the chewing process. It is of fundamental importance in the field of dental biomechanics to know how the applied load is transferred to the stress and what is the pattern of the distribution of all kinds of the stress within the tested specimens (Natali, 2003).

For the complete presentation and illustration of the stress distribution, a three-dimensional model of the samples was simulated by using the ABAQUS software system, and as the result of the simulation, all types of stresses in the body of the material were investigated. Illustrated in Figure 4 specimen finite element model allowed to clearly identify the location of the stress concentration. As it can be seen from the Figure 4, the stress distribution is not completely uniform, even one cannot say that the samples were destroyed by the compressive stress, and as it is known, failure occurred because of the influence of the tensile stresses (Figure 4-b, c).

The FEM is one of the most powerful and effective approaches for analyzing and investigating the stress state of the materials under different types of loading. FEM is a theoretical technique which in its turn is the basis for computational analysis using software programs.

For a very long time, it has been proved that the numerical calculation is more accurate than the statically analysis. As is seen from Fig. 4-a, b, c, starting from the top surface, where the load is applied to the middle of the height of the sample, the value of the vertical stresses- σ is almost the same as in the static analysis. Then, in addition to approaching the bottom surface (supported plane), the value of the stress gradually grows and at lowest plane reaches its peak, which is more than 100% greater than stress on the upper plane (Figure 4). The first cracks and subsequent fractures of the samples were observed in these so-called, zones of stress concentration.

The results of this study revealed a strong correlation between individual specimens of the same type of PFS material, and commonly had a significant statistical correlation between different types of material groups.

5. CONCLUSION

In this work, theoretical and experimental approaches to investigating one of the most important mechanical properties of the PFS are used, and on the base of the in-vitro measurements and theoretical analysis it can be concluded that the PFS application have some physicochemical and mechanical characteristics that have to be taken into account by clinicians to provide adequate preventing sealing.

Ultraseal XT hydro and compoglass F showed higher compressive strength values, while lowest values were found for photac fil.

This research showed that the filler fraction and particle size and uniformity of the filler distribution are the main determining factor affecting on the compressive strength of PFS.

Finite element analysis by ABAQUS software makes it possible to reveal the place of stress concentration where the values of the vertical stresses significantly more than its values in the usual sections of the specimens.

REFERENCE

Aratani, M., Pereira, A.C., Correr-Sobrinho, L., Sinhoreti, M.A.C., Consani, S., 2005. Compressive strength of resin-modified glass ionomer restorative material: effect of P/L ratio and storage time. J. Appl. Oral Sci. 13, 356–359.

Babu, G., Mallikarjun, S., Wilson, B., Premkumar, C., 2014. Pit and fissure sealants in pediatric dentistry. SRM J. Res. Dent. Sci. 5, 253.

Bayne, S.C., Thompson, J.Y., Swift Jr, E.J., Stamatiades, P., Wilkerson, M., 1998. A characterization of first-generation flowable composites. J. Am. Dent. Assoc. 129, 567–577.

Beauchamp, J., Caufield, P.W., Crall, J.J., Donly, K., Feigal, R., Gooch, B., Ismail, A., Kohn, W., Siegal, M., Simonsen, R., 2008. Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. J. Am. Dent. Assoc. 139, 257–268.

Beun, S., Bailly, C., Devaux, J., Leloup, G., 2012. Physical, mechanical and rheological characterization of resin-based pit and fissure sealants compared to flowable resin composites. Dent. Mater. 28, 349–359.

Beun, S., Bailly, C., Devaux, J., Leloup, G., 2008. Rheological properties of flowable resin composites and pit and fissure sealants. Dent. Mater. 24, 548–555.

Cattani-Lorente, M.A., Dupuis, V., Moya, F., Payan, J., Meyer, J.-M., 1999. Comparative study of the physical properties of a polyacid-modified composite resin and a resin-modified glass ionomer cement. Dent. Mater. 15, 21–32.

Cildir, S.K., Sandalli, N., 2007a. Compressive strength, surface roughness, fluoride release and recharge of four new fluoride-releasing fissure sealants. Dent. Mater. J. 26, 335–341.

Cildir, S.K., Sandalli, N., 2007b. Compressive strength, surface roughness, fluoride release and recharge of four new fluoride-releasing fissure sealants. Dent. Mater. J. 26, 335–341.

Cohen, L., Sheiham, A., 1988. The Use Of Pit And Fissure Sealants In The General-Dental-Service In Great-Britain And Northern-Ireland. Brit Dent J 165, 50–53.

Crisp, S., Lewis, B.G., Wilson, A.D., 1976. Characterization of glass-ionomer cements 1. Long term hardness and compressive strength. J. Dent. 4, 162–166.

El-Kalla, I.H., Garcia-Godoy, F., 1999. Mechanical properties of compomer restorative materials. Oper. Dent. 24, 2–8.

Feigal, R.J., 2002. The use of pit and fissure sealants. Pediatr. Dent. 24, 415–422.

Geiger, S.B., Gulayev, S., Weiss, E.I., 2000. Improving fissure sealant quality: mechanical preparation and filling level. J. Dent. 28, 407–412.

History and Evolution of Pit and Fissure Sealents [WWW Document], n.d. URL http://www.juniordentist.com/history-and-evolution-of-pit-and-fissure-sealents.html (accessed 5.11.18).

ISO 6874:2015 - Dentistry -- Polymer-based pit and fissure sealants [WWW Document], n.d. URL https://www.iso.org/standard/67595.html (accessed 5.12.18).

Lerech, S.B., Tarón, S.F., Dunoyer, A.T., Arrieta, J.M.B., Caballero, A.D., 2017. Compressive strength of glass ionomer and composite resin. In vitro study. Rev. Odontológica Mex. 21, e107–e111.

Meyer, J.-M., n.d. TEGDMA and Bisphenol-A: the same level of risk in dental medicine?

Meyer, J.M., Cattani-Lorente, M.A., Dupuis, V., 1998. Compomers: between glass-ionomer cements and composites. Biomaterials 19, 529–539.

Moezzyzadeh, M., 2012. Evaluation of the compressive strength of hybrid and nanocomposites. Shahid Beheshti Univ. Dent. J. 30, 23–28.

Munhoz, T., Nunes, U.T., Seabra, L.M.-A., Monte-Alto, R., 2016. Characterization of Mechanical Properties, Fluoride Release and Colour Stability of Dental Sealants. Pesqui. Bras. Em Odontopediatria E Clin. Integrada 16, 149–158.

Natali, A.N. (Ed.), 2003. Dental biomechanics. Taylor & Francis, London ; New York.

Nunn, J.H., Murray, J.J., Smallridge, J., 2000. British Society of Paediatric Dentistry: a policy

document on fissure sealants in paediatric dentistry. Int. J. Paediatr. Dent. 10, 174–177.

Obici, A.C., Sinhoreti, M.A.C., Correr-Sobrinho, L., Góes, M.F. de, Consani, S., 2005. Evaluation of mechanical properties of Z250 composite resin light-cured by different methods. J. Appl. Oral Sci. 13, 393–398.

Ripa, L., 1993. Sealants revisted: an update of the effectiveness of pit-and-fissure sealants. Caries Res. 27, 77–82.

Singh, R.D., Chand, P., Jurel, S.K., Tripathi, S., 2011. Evaluation of Adhesive and Compressive Strength of Glass Ionomer Cements. J. Indian Prosthodont. Soc. 11, 210–214.

Subramaniam, P., Konde, S., Mandanna, D.K., 2008. Retention of a resin-based sealant and a glass ionomer used as a fissure sealant: a comparative clinical study. J. Indian Soc. Pedod. Prev. Dent. 26, 114.

Taher, N.M., 2001. Mechanical properties of flowable composites. situations 2, 6–7.

Uno, S., Finger, W.J., Fritz, U., 1996. Long-term mechanical characteristics of resin-modified glass ionomer restorative materials. Dent. Mater. 12, 64–69.

Walsh, R.M., Woodmansey, K.F., Glickman, G.N., He, J., 2014. Evaluation of compressive strength of hydraulic silicate-based root-end filling materials. J. Endod. 40, 969–972.

Xie, D., Brantley, W.A., Culbertson, B.M., Wang, G., 2000. Mechanical properties and microstructures of glass-ionomer cements. Dent. Mater. 16, 129–138.

Xu, X., Burgess, J.O., 2003. Compressive strength, fluoride release and recharge of fluoride-releasing materials. Biomaterials 24, 2451–2461.

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Molecular Identification and Genotyping of *Pseudomonas aeruginosa* Isolates Using Double-Locus Sequence Typing (DLST) Analysis

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ABSTRACT

The opportunistic pathogen *Pseudomonas aeruginosa* is responsible for many life-threatening nosocomial and environmental acquired infections. In this study, the antibiotic susceptibility, molecular identification and genotyping of *P. aeruginosa* were performed. A total of 100 isolates of *P. aeruginosa* out of 523 specimens (19.12%) from clinical and environmental sources were analyzed. The results of antibiotic sensitivity profile grouped these isolates into non-multidrug resistant (non-MDR), multidrug resistant (MDR), and extensively drug resistant (XDR) by the ratios of 23.7%, 40.5% and 35.6%, respectively. The double-locus sequence typing (DLST) scheme was employed for genotyping a collection of 36 isolates of *P. aeruginosa* recovered from different clinical and environmental sources. Isolates were successfully typed into 19 different DLST genotypes with high discriminatory power (0.9206). In addition, three new alleles were recognized for the locus ms172 namely; ms172-128, ms172-129 and ms172-130. Thus, three novel DLST genotypes of this pathogen have been identified, which were previously not reported, with the combinations of DLST128-60, DLST129-79 and DLST130-17, respectively. All new genotypes, which were exclusively belonged to the clinical sources, were exhibited XDR pattern. The phylogenetic analysis differentiated these genotypes into seven different genetic clusters supported by strong bootstrap values. However, there were indications of distinct evolutionary origins for some of the un-clustered genotypes (5/8). The DLST type 32-39 was the predominant cluster in this region with a majority of XDR pattern. Hereby, it can be concluded that DLST was capable of discriminating the phenotypically and genetically related isolates of *P. aeruginosa* and offered a reliable phylogenetic analysis.

1. INTRODUCTION

The bacterial pathogen *Pseudomonas aeruginosa* is well-known as a Gram-negative, extremely versatile and important opportunistic human pathogen that causes significant healthcare- associated infections, particularly in immunocompromised patients (Burstein *et al*., 2015). Also, it has been recognized as a ubiquitous microorganism due to its extraordinary survival capability that can adapt to a wide range of ecological niches including soil, water, sewage, hospitals etc (Hare *et al*., 2012). This pathogen is responsible for many serious and frequent hospital as well as environmental acquired illnesses including;

urinary tract infections (UTIs), folliculitis and skin rashes, pneumonia, bacteremia, osteomyelitis and ear infections (Burstein *et al*., 2015, Park *et al*., 2015 and Gba *et al*., 2018). The *P. aeruginosa* notorious fame is markedly contributes to its remarkable power to produce resistance against most commonly prescribed antibacterial drugs (Firouzi-Dalvand *et al*., 2016). This is either through innate resistance to many of the commonly used antibiotics by mutation in chromosomally encoded genes or through horizontally acquired resistance (Valot *et al*., 2015, Miranda *et al*., 2015 and Gba *et al*., 2018). In spite of many efficient infection control policies, outbreaks and infections in hospitals have been increasingly reported worldwide due to the prevalence of multi-drug resistant (MDR) strains of *P. aeruginosa* with significantly rising in patients' morbidity and mortality (Janam *et al*., 2011 and Khosravi *et al*., 2016). Therefore, in order to understand the epidemiology of *P. aeruginosa*, it might be important to address the antibiotic resistance pattern, genotyping and phylogenetic relationship of its isolates from different environmental and clinical sources.

To date, many genotyping techniques have been employed to study and characterize *P. aeruginosa* isolates. Although the electrophoresis-based typing methods of bacterial strain typing such as pulsed field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have many advantages and have been long used for genotyping of this pathogen, they are relatively time-consuming and require extensive inter-laboratory comparison of the results as well as coordination of protocols (Tang and Stratton, 2013). Therefore, the DNA sequencing-based genotyping approaches are becoming increasingly popular for bacterial typing. In contrast to current fragment based methods, DNA sequencing methods yield accurate, reproducible and highly-sensitive sequence results. Moreover, sequence data can be easily stored in online databases and shared between laboratories and/or compared to well-established libraries (Li *et al*., 2009 and Chen *et al*., 2015). Among these DNA sequence-based techniques is the recently developed scheme of double-locus sequence typing (DLST) based on the DNA sequencing and nucleotide analyses of two extremely variable loci (ms172 and ms217) of *P. aeruginosa* genome (Pappa *et al*., 2017). This technique allows obtaining unambiguous and uniformly systematized definition of types, since each isolate could be assigned with a definitive type and the data can be stored in databases for the purpose of surveillance investigations and/or comparing results with future studies (Cholley *et al*., 2015).

The main objectives of this study were molecular identification, investigation of the antibiotic resistance pattern, application of an efficient and recently developed DNA sequence-based genotyping technique, the DLST, to investigate the local epidemiology of *P. aeruginosa* and to detect the DLST genotypes of this pathogen in different districts in Kurdistan region-Iraq and eventually to assess the phylogenetic diversity among *P. aeruginosa* isolates obtained from different clinical and environmental sources.

2. MATERIALS AND METHODS

2.1. Bacterial isolates

The collection of *P. aeruginosa* isolates was comprised of 100 isolates out of 523 specimens investigated. Samples were obtained from both clinical (n=385) and environmental (n=138) sources, over a period from April to December 2014. This collection included: 86 clinical and 14 environmental isolates. Clinical specimens

were obtained from patients of both genders (various ages) at some of the major public hospitals in Kurdistan Region-Iraq including; the Emergency and Azadi (Teaching) Hospitals/Duhok, Rizgari (Teaching) Hospital/Erbil, Teaching, Emergency and General Medicine Hospitals/Sulaymaniyah, whereas the environmental samples were obtained from a number of animal farms (soils) at these three districts. The clinical specimens were taken from different wards at these hospitals and from various infection sources; such as infected burns and wounds, ear infections, urine samples and respiratory tract (bronchial-alveolar-lavage and sputum).

2.2. Bacterial identification

The classical identification of *P. aeruginosa* isolates was performed according to the methods described by Khan *et al*., (2008), with minor modifications, using both selective and non-selective culture media, catalase and oxidase tests. Samples were first cultured into brain heart infusion broth and incubated at 37°C for 24 hours. Each sample was then streaked onto the MacConkey agar (Difco) plate and was kept incubated for 24 hours at 37°C. An isolated suspected colony of the organism was selected and streaked onto Pseudomonas Isolation Agar (Sigma-Aldrich-UK) plate and incubated overnight at 37°C. Isolates that showed growth and colony characteristics of this bacterium at 37°C on this medium, and showed positive reaction to catalase and oxidase tests with gram negative feature, were primarily identified as *P. aeruginosa*. A single pure bacterial colony was then cultured into 5ml of nutrient broth and kept incubated for 24 hours at 37°C. Bacterial growth was eventually stored in 50% glycerol and kept at -20°C before use.

2.3. Antimicrobial susceptibility test

The Kirby Bauer disc diffusion method on Mueller-Hinton agar was used for screening the antibiotic resistance pattern according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014). All isolates were subjected to ten commonly used antibiotics (Oxoid, Basingstoke, UK); including imipenem 10µg, ciprofloxacine 5µg, amikacin 30µg, ceftazidime 30µg, erythromycin 15µg, gentamicin 10µg, cefotaxime 30µg, ticarcillin 75µg, amoxacillin/clavulanic acid 30/10µg, and cefixime 5µg discs.

Isolates that showed resistant to at least one agent in three or more antimicrobial categories were defined as multi-drug-resistant, while isolates showed susceptibility to only one or two in all antimicrobial categories were considered as extensively drug-resistant (Magiorakos *et al*., 2012).

2.4. DNA extraction and molecular confirmation of isolates

The High yield DNA Purification Kit was implemented to extract DNA from all 100 bacterial isolates as recommended by the supplier's manual (Cinnagen-Iran). Molecular confirmation was performed for all isolates of *P. aeruginosa* by PCR using a primer pair *PA-SS* (Table 1) as previously described by (Spilker *et al*., 20104) with minor PCR condition adjustments. The amplification reaction comprised of master mixture (12.5µl) (Promega/USA), primers (10pmol/µl) forward and reverse (1µl each), DNA sample (2µl) with a final concentration of 25-50ng/µl. The final reaction volume was completed to 25µl by adding 8.5µl of sterile nuclease free water. A Thermal Cycler (Applied Biosystems-2720) was used and the amplification conditions were; 1 cycle at 94^{o}C for 5min as initial denaturation, 30 cycles starting with denaturation at 94^{o}C for 1min, annealing at 58^{o}C for 1min and amplification at 72^{o}C for 1min with the final extension step at 72^{o}C for 5min (Spilker *et al*., 20104).

Table 1: Primers used for molecular identification and DLST typing of P. aeruginosa.

Primer	Sequences (5′-3′)	Product size	Target	Reference
PA-SS	F- GGGGGATCTTCGGACCTCA R- TCCTTAGAGTGCCCACCCG	956bp	16SrDNA	Spilker *et al*., 2004
ms172	F-GGATTCTCTCGCACGAGGT R-TACGTGACCTGACGTTGGTG	variable	ms172 locus	http://www.dlst.org/CSS/images/LTM_PR_029.pdf
ms217	F-TTCTGGCTGTCGCGACTGAT R-GAACAGCGTCTTTTCCTCGC	variable	ms217 locus	http://www.dlst.org/CSS/images/LTM_PR_029.pdf

2.5. PCR amplification and sequencing of DLST loci

PCR amplification was performed for each of the two loci ms172 and ms217 using primers reported in (Table 1) as previously described by Basset and Blanc (2014) using a full DLST protocol available on the DLST website (http://www.dlst.org/Paeruginosa/) for genotyping of *P. aeruginosa*. Briefly, the PCR amplification reactions for each locus was carried out in 50µl reaction mixture composed of 25µl of master mix, 2µl of each of forward and reverse primers (10pmol/µl), 0.4µl of *Taq* DNA polymerase (1U/µl), 2µl of (1:7) diluted *PWO* DNA Polymerase (1U/µl), 16.6µl of dH_2O and 2µl of genomic DNA (25-50ng/µl). The PCR amplification conditions were as follow: initial denaturation at 94°C for 5 min; 35 cycles consisting of 30s at 94°C, 30s at 60°C (ms172) or 64°C (ms217), 45s at 72°C; with a final extension at 72°C for 5min (14). Gel electrophoresis was performed using 1.2% agarose gel. Gels were stained with ethidium bromide and evaluated for success and specificity of one clear band visible per PCR. The PCR products for each locus were purified with MinElute PCR Purification Kit (50) (Qiagen), according to the supplier's protocol. Sequencing reactions were carried out with a reverse primer for each locus independently with a BigDye Terminator V3.1 (Applied Biosystems, USA). The purified PCR products were then sequenced using a 4 capillary ABI3130 Genetic Analyzer for Sanger sequencing at the DNA Sequencing Facility Unit of the Scientific Research Center, College of Science, University of Duhok.

2.6. Data analysis, Allele and DLST type assignment

The nucleotide sequences were imported, assembled, edited, trimmed and verified in Geneious, version R8.1 (Kearse *et al*., 2012) and then saved as Fasta format for sequence alignment and phylogenetic purposes. The *P. aeruginosa* DLST website at (http://www.dlst.org/Paeruginosa/) was used to assign numbers to each distinct allele within a locus after submitting good quality trace file sequences for each of ms172 and ms217 loci per each tested isolate. Thus, each individual isolate was given double numbers known as allelic profile that yielded to its DLST type. Any allele that did not match with the existing alleles in the database was designated and registered as "new" and a new allele number was given by the database.

The discriminatory power for DLST typing scheme, in the current study, was obtained through the index of diversity (ID) which was calculated by using an online tool at (http://www.comparingpartitions.info/).

3. RESULTS AND DISCUSSION

In the current study, out of 523 specimens investigated searching for the presence of *P. aeruginosa*, a total of 100 isolates of this pathogen have been identified from both hospitalized patients at Duhok, Erbil and Sulaymaniyah hospitals and from environmental samples, representing 19.12% of the total investigated specimens. This prevalence rate was found to be slightly lower than that previously reported in this region by Hassan *et al*., (2012) in which *P. aeruginosa* accounted for 26.1% of the total nosocomial infections in Kurdistan. However, remarkably higher prevalence rates have been reported by studies elsewhere in Iraq, such as 46.6% in Najaf (Al-Dahhan, 2015) and 67.7% in Basrah (AbdulQader *et al*., 2015). Probable variation and fluctuations in the prevalence rate of this bacterium over time and from study to another might be obvious due to the variation in study period, geographical location, sample type, hospital level, clinical department, economic condition, variation in the resistance pattern of this opportunistic pathogen in each hospital and/or province as well as type and quality of antibiotics used.

In this investigation, vast majorities (86%) of the isolates were from clinical sources with a prevalence rate of 22.3% of the total investigated clinical specimens; meanwhile, 14% were from the environmental source with a prevalence rate of 10.1% of the total investigated environmental specimens. Isolates that have been identified as *P. aeruginosa* at the initial stages through means of selective agar cultures, biochemical and microscopic tests, were further confirmed at the molecular level by 16S rDNA-based PCR assay using species-specific primer pair (PA-SS). This primer pair yielded a DNA band of 956bp (Figure 1) corresponding with the specific band of *P. aeruginosa* across all tested isolates. Thus, all were identified and confirmed at the molecular level as *P. aeruginosa*.

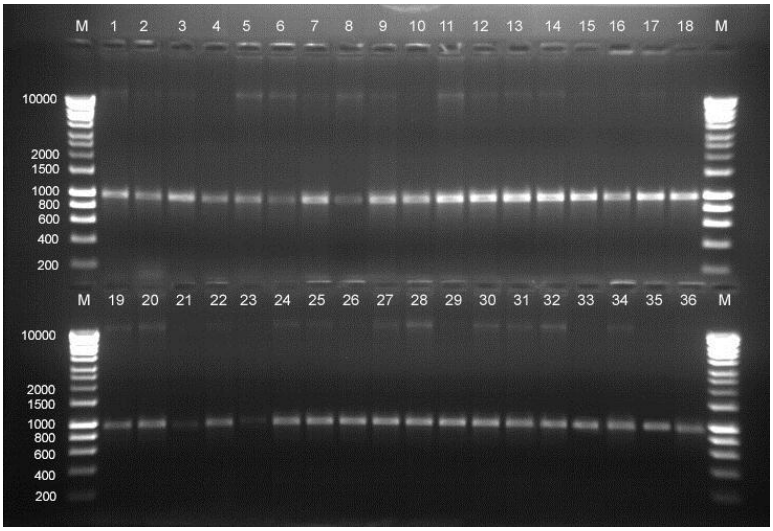

Figure 1: Example of molecular identification of *P. aeruginosa* by PCR based assay using PA-SS primer pair. Lanes M represent DNA ladder (10kb). Numbers from 1-36 represent the tested samples. Electrophoresis was carry out using 1.2% agarose gel at 85V for 1hour.

The results of antibiotic sensitivity test of these isolates across 10 commonly used antibiotics were as follow: imipenem (18.8%), ceftazidime (26.7%), amikacin (39.6%), ciprofloxacin (42.5%), cefotaxime (44.5%), gentamicin (58.4%), cefixime (64.3%), ticarcillin (71.2%), amoxacillin/clavulanic acid (73.2%), and erythromycin (82.1%). These results were compatible with those obtained in the investigation of the antibiotic susceptibility profile of this pathogen from both clinical and hospital environmental samples in Nasiriyah, Iraq (Al-Zaidi, 2016). Also, variable results have been reported elsewhere in the antibiotic resistant pattern of 88 strains of *P. aeruginosa* against twelve commonly used antibiotics from 10 different wards of the university hospital centre of Abidjan- Côte d'Ivoire (Gba *et al*., 2018).

It has been reported that a proportion of the resistant *P. aeruginosa* as high as 34% is considered alarming and worrying (Pappa *et al*., 2017), therefore, based on the antibiotic sensitivity results obtained in the current study, surveillance investigations of such resistant pathogen in this region are recommended. The results of high drug resistance rate in the current investigation indicated that patients might be in high antibiotic resistance risk due to the circulation of highly drug-resistant strains of this pathogen in our hospitals as well as in the environment, which might also serve as a potential source of bacterial pathogens with multidrug resistant.

The prevalence of such high resistance rate in this region could be explained by the acquisition of highly resistant strains of this pathogen among clinical and environmental sources. The frequent and widely prescription and administration of antibiotics, the abuse and uncontrolled use of antibiotics by self-medication as well as flooding of the markets by antibiotics which might not always WHO pre-qualified that casts doubt on their true quality.

The results of PCR amplifications of the ms172 and ms217 loci revealed clear single amplified DNA bands with variable size which indicated that they are highly polymorphic loci (Figure: 2).

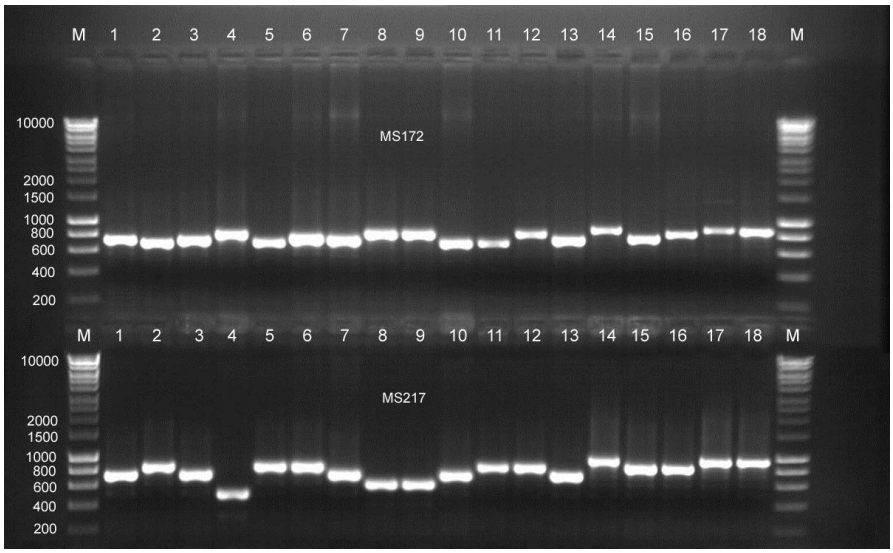

Figure 2: Example of PCR amplification of ms172 and ms217 loci. Lanes M represent DNA ladder (10kb). Numbers from 1-18 represent the tested samples. Electrophoresis was carry out using 1.2% agarose gel at 85V for 1hour.

The nucleotide sequences of both ms172 and ms217 loci have been determined for a collection of 36 isolates; represented different infection sources, environment and antibiotic resistant pattern. The nucleotide sequences obtained for each locus (allelic sequences) have been tested for their specificity implementing the BLAST algorithm of the GenBank database at the National Center for Biotechnology Information (NCBI) and then were submitted to the *P. aeruginosa* DLST database at (http://www.dlst.org/Paeruginosa/) to assign allelic numbers. The allelic numbers were assigned to each distinct allele within a locus. Data revealed that 13 different alleles for the locus ms172 and 15 different alleles for the locus ms217 have been determined. Furthermore, three new alleles for the locus ms172 study have been identified in the current namely; ms172-128, ms172-129 and ms172-130 which were previously not reported. These novel alleles were deposited at Allele definitions of ms172 section at the *P. aeruginosa* DLST database at (http://www.dlst.org/Paeruginosa/). Thus, three novel DLST genotypes have also been identified with the combinations of DLST128-60, DLST129-79, and DLST130-17 respectively, which were previously not described. The DLST technique has successfully typed all these isolates into 19 different DLST genotypes (Table 2).

Table 2: location, Site, DLST types and antibiotic pattern of *P. aeruginosa* isolates.

Isolate	Location	Site	DLST Type ms172-ms217	Antibiotic pattern
Pa-1	Duhok	Soil	16-9	non-MDR
Pa-2	Erbil	Burn	16-130	MDR
Pa-3	Erbil	Wound	16-9	non-MDR
Pa-4	Erbil	Middle Ear	4-101	MDR
Pa-5	Erbil	Urine	28-130	non-MDR
Pa-6	Erbil	Respiratory	8-78	XDR
Pa-7	Erbil	Soil	16-9	XDR
Pa-8	Duhok	Burn	16-11	MDR
Pa-9	Duhok	Wound	16-11	XDR
Pa-10	Duhok	Middle Ear	28-9	non-MDR
Pa-11	Duhok	Urine	32-39	non-MDR
Pa-12	Duhok	Respiratory	23-22	XDR
Pa-13	Sulaymaniyah	Soil	16-9	XDR
Pa-14	Sulaymaniyah	Burn	32-22	XDR
Pa-15	Sulaymaniyah	Wound	6-54	MDR
Pa-16	Sulaymaniyah	Middle Ear	4-101	XDR
Pa-17	Sulaymaniyah	Urine	32-39	MDR
Pa-18	Sulaymaniyah	Respiratory	32-39	XDR
Pa-19	Duhok	Wound	128*****-60	XDR
Pa-20	Sulaymaniyah	Burn	16-11	MDR

Pa-21	Sulaymaniyah	Burn	129*****-79	XDR
Pa-22	Sulaymaniyah	Wound	10-76	XDR
Pa-23	Sulaymaniyah	Burn	32-39	XDR
Pa-24	Sulaymaniyah	Burn	9-124	XDR
Pa-25	Sulaymaniyah	Burn	32-39	MDR
Pa-26	Duhok	Respiratory	28-130	XDR
Pa-27	Sulaymaniyah	Burn	32-39	non-MDR
Pa-28	Duhok	Middle Ear	23-22	XDR
Pa-29	Erbil	Respiratory	4-101	MDR
Pa-30	Sulaymaniyah	Burn	32-39	non-MDR
Pa-31	Sulaymaniyah	Wound	32-39	XDR
Pa-32	Duhok	Respiratory	23-39	XDR
Pa-33	Sulaymaniyah	Middle Ear	32-39	MDR
Pa-34	Duhok	Respiratory	10-25	XDR
Pa-35	Duhok	Urine	21-96	non-MDR
Pa-36	Sulaymaniyah	Urine	130*****-17	XDR

* New alleles identified in this study.

This sequence typing scheme showed a high discriminatory power in distinguishing each isolate from others which was 0.9206. This was evaluated according to the Simpson's index of diversity (ID), in which an index of more than 0.90 is considered as an ideal indication that the typing method has the ability to distinguish each isolate from all others. In contrast, an index of zero is the indication that all isolates are of an identical type (Cholley *et al*., 2015). Furthermore, employing this sequence typing technique not only allowed the discrimination of the studied isolates from each other, but also was capable of identifying new DLST genotypes which were previously not reported. This genotyping scheme is available at the DLST website (www.dlst.org), which allows researchers to submit novel types directly and coordinately (Basset and Blanc, 2014). To the best of our knowledge, this is the first report investigating *P. aeruginosa* DLST types and elucidating the predominant clones of this pathogen in this region. All novel DLST genotypes of *P. aeruginosa* were found to exhibit XDR pattern. Additionally, all new types were exclusively found to be from clinical sources; including wound, burn and urine. In contrast, none of the environmental isolates were presented new alleles. More so, the DLST scheme was successfully capable of discriminating the environmental isolates in different geographical locations (Duhok, Erbil and Sulaymaniyah), from clinical isolates by clustering them into a separate genetic cluster and all were typed as DLST16-9 genotype.

The allelic nucleotide sequences of the two loci (ms172 and ms217) were concatenated and used to construct phylogenetic tree among these strains, in order to allow better discrimination and greater tree robustness. Thus, the genotypes were differentiated into seven different clusters in the phylogenetic tree, supported by strong bootstrap values (Figure 3). However, eight genotypes were not grouped with any of these genetic clusters. This might indicate distinct evolutionary origins for some of these un-clustered genotypes (5/8; 62.5%) in Kurdistan, as they were found to be

unrelated and shared no alleles with other genetic clusters. Furthermore, one of these unclustered genotypes was assigned as new (DLST129-79).

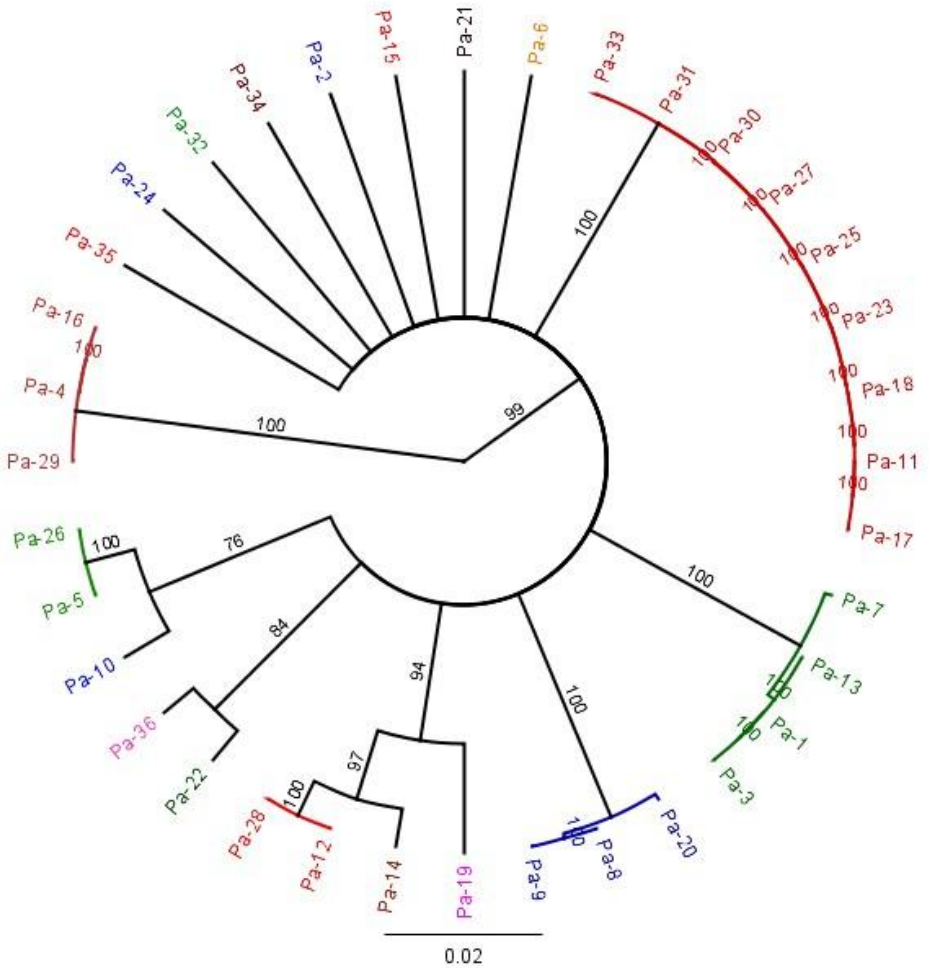

Figure 3: Phylogenetic relationships of *P. aeruginosa* isolates.

Also, the phylogenetic analysis revealed that the DLST-32-39 was the predominant cluster (9/36; 25%) in this region. In addition, the vast majority of the predominant cluster isolates (7 out of 9; 77.7%) were found to exhibit an XDR pattern and identified from different infection sources. This may be due to their adaptation and fitness to a variety of environmental niches that make them squeeze away from the immune as well as the antibiotic barriers. Identification of the new types as well as the predominant cluster of this pathogen with extensively drug-resistant patterns might further confirm that patients are in high antibiotic resistance risk. Therefore, applying efficient infection control policies and measures might be required in order to minimize hospital as well as environmental related infections due to particular persistent MDR and/or XDR clones of *P. aeruginosa*. In the present study, the results of DLST typing indicated that different DLST genetic profiles might serve as a driving force and a potential source for the distribution of the resistant strains in our hospitals and environments as well. However, further investigations might be

required to provide important information in terms of the possible correlations between a particular DLST clusters and the production as well as the transmission of the resistant mechanisms.

The major goal of investigating the local epidemiology is to identify the transmission of isolates. The markers used for this purpose are required to be stable through the period of investigations. The new, cost effective and promising DLST typing scheme has been proved to produce a stable results for several months or even years for comparison of isolates recovered throughout the duration of investigations (Pappa *et al*., 2017). Therefore, it can be implemented to study the epidemiology of isolates with low cost and in a short of time as well. In the present study, the DLST technique was implemented to assess the diversity of *P. aeruginosa* isolates through a period of one year and it was proved to be reliable and powerful molecular tool to distinguish and discriminate clusters of isolates belonging to the same infection sources and environments. Also, it does not require a special search algorithm as well as no assembly procedure is needed. Additionally, a web-based database (http://www.dlst.org), which has recently been developed, can be used to identify allele profiles and DLST types as well as to identify new alleles and types in unambiguous way. Also, in terms of the *P. aeruginosa* population, this database could be used as a reference and implemented to assess the local and/or international diversity of this pathogen.

5. CONCLUSIONS

Lastly, it can be concluded that *P. aeruginosa* strains, which have been isolated from different geographical and infection sources, have showed variations in their antibiotic resistant pattern, allelic profiles and DLST types. This may reflect their diversity as well as their adaptive ability to different environmental niches and diseases caused. The exact identifications and characterization of this pathogen in clinical settings and assessing the phylogenetic relationship among its strains are crucial for epidemiological investigations. Thus, this study may consequently have significant role in diagnosis, antibiotic treatment, and infection control policies as well. The DLST typing scheme was successfully used for genotyping and to investigate the local epidemiology of a collection of *P. aeruginosa* isolates in Kurdistan. This technique was also found to be highly reproducible, typable as well as to have high discriminatory power to distinguish between these isolates.

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Conflict of Interest

The author has no conflicts of interest to disclose.

REFERENCES

ABDULQADER N K, RAOOF W M, & AL-MUSSAWI A A. (2015). Biofilm Forming-*Pseudomonas aeruginosa* isolated from burn patients in Basra city, south of Iraq. *International Journal of Current Research*; 7(10):21750-21753.

AL-DAHHAN H A A. (2015). Molecular Characterization of *P. aeruginosa* isolated from Patients with URTI. *JCBPS; Section B*; 5 (No. 3): 2736-2747.

AL-ZAIDI J R. (2016). Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolated from clinical and hospital environmental samples in Nasiriyah, Iraq. *Afr J Microbiol Res*., 10(23):844-849. doi: 10.5897/AJMR2016.8042.

BASSET P & BLANC D S. (2014). Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method. *Eur J Clin Microbiol Infect Dis*, 33:927–932. doi: 10.1007/s10096-013-2028-0.

BURSTEIN D, SATANOWER S, SIMOVITCH M, BELNIK Y, ZEHAVI M, YERUSHALMI G, BEN-AROYA S, PUPKO T, & BANIN E. (2015). Novel type III effectors in *Pseudomonas aeruginosa*. *mBio.*, 6(2):e00161-15. doi:10.1128/mBio.00161-15.

CHEN Y, FRAZZITTA A E, LITVINTSEVA A P, FANG C, MITCHELL T G, SPRINGER D J, DING Y, YUAN G & PERFECT J R. (2015). Next generation multilocus sequence typing (NGMLST) and the analytical software program MLSTEZ enable efficient, cost-effective, high-throughput, multilocus sequencing typing. *Fungal Genet Biol.*, 75:64-71. doi: 10.1016/j.fgb.2015.01.005.

CHOLLEY P, STOJANOV M, HOCQUET D, THOUVEREZ M, BERTRAND X, & BLANC D S. (2015). Comparison of double-locus sequence typing (DLST) and multilocus sequence typing (MLST) for the investigation of *Pseudomonas aeruginosa* populations. *Diagnostic Microbiology and Infectious Disease* 82, 274–277.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). (2014). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. *CLSI document M100-S24. Wayne, PA*. 34(1).

FIROUZI-DALVAND L, POOLADI M, NOWROOZI J, AKHVAN-SEPAHI A, & HOOSHIYAR M. (2016). Presence of exoU and exoS genes in *Pseudomonas aeruginosa* isolated from urinary tract infections. *Infect Epidemiol Med.*, 2(2):8-11. doi: 10.7508/iem.2016.02.003.

GBA K M K, GUESSENND N K, MAKAYA N P N D, TAHOU E J., KONAN F, TOTY A A & N'GUETTA S P A. (2018). Detection of Metallo-beta-lactamase Producing *Pseudomonas aeruginosa* in an Abidjan Hospital, Côte d'Ivoire. *JAMB*, 8(1): 1-8. doi: 10.9734/JAMB/2018/38792.

HARE N J, SOLIS N, HARMER C, MARZOOK N B, ROSE B, HARBOUR C, CROSSETT B, MANOS J, & CORDWELL S J. (2012). Proteomic profiling of *Pseudomonas aeruginosa* AES-1R, PAO1 and PA14 reveals potential virulence determinants associated with a transmissible cystic fibrosis-associated strain. *BMC Microbiology*, 12:16. http://www.biomedcentral.com/1471-2180/12/16.

HASSAN K I, RAFIK S A & MUSSUM K. (2012). Molecular identification of *Pseudomonas aeruginosa* isolated from Hospitals in Kurdistan region. *J Adv Med Res*., 2(3): 90-98.

JANAM R, GULATI A K & NATH G. (2011). Antibiogram and genotyping of *Pseudomonas aeruginosa* isolated from human, animal, plant, water and soil sources in north India. *Southeast Asian J Trop Med Public Health*, Vol. 42 No. 6, p.1477-1488.

KEARSE M, MOIR R, WILSON A, STONES-HAVAS S, CHEUNG M, STURROCK S, BUXTON S, COOPER A, MARKOWITZ S, DURAN C, THIERER T, ASHTON B, MEINTJES P, & DRUMMOND A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* ; 28(12): 1647-1649.

KHAN N H, AHSAN M, YOSHIZAWA S, HOSOYA S, YOKOTA A, & KOGURE K. (2008). Multilocus sequence typing and phylogenetic analyses of *Pseudomonas aeruginosa* isolated from the ocean. *Appl Environ Microbiol*., 74:6194-6205. doi:10.1128/AEM.02322-07.

KHOSRAVI A D , HOVEIZAVI H, MOHAMMADIAN A, FARAHANI A, & JENABI A. (2016). Genotyping of multidrug-resistant strains of *Pseudomonas aeruginosa* isolated from burn and wound infections by ERIC-PCR. *Acta Cirúrgica Brasileira*, Vol. 31 (3).p.206-2011.

doi: http://dx.doi.org/10.1590/S0102-865020160030000009.

LI W, RAOULT D & FOURNIER P E. (2009). Bacterial strain typing in the genomic era. *FEMS Microbiol Rev.*, 33(5):892-916. doi: 10.1111/j.1574-6976.2009.00182.x.

MAATALLAH M, CHERIAA J, BACKHROUF A, IVERSEN A, GRUNDMANN H, DO T, LANOTTE P, MASTOURI M, ELGHMATI M S, ROJO F, MEJDI S, & GISKE C G. (2011). Population structure of *Pseudomonas aeruginosa* from five mediterranean countries: Evidence for frequent recombination and epidemic occurrence of CC235. *PLoS ONE*, 6(10): e25617. doi:10.1371/journal.pone.0025617.

MAGIORAKOS A P, SRINIVASAN A, CAREY R B, CARMELI Y, FALAGAS M E, GISKE C G, HARBARTH S, HINDLER J F, KAHLMETER G, OLSSON-LILJEQUIST B, PATERSON D L, RICE L B, STELLING J, STRUELENS M J, VATOPOULOS A, WEBER J T & MONNET D L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*., 18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x.

MIRANDA C C, DE FILIPPIS I, PINTO L H, COELHO-SOUZA T, KBIANCO, CACCI L C, PICÃO R C, CLEMENTINO M. (2015). Genotypic characteristics of multidrug-resistant *Pseudomonas aeruginosa* from hospital wastewater treatment plant in Rio de Janeiro, Brazil. J Appl Microbiol., 118(6):1276-86. doi: 10.1111/jam.12792.

PAPPA O, BELOUKAS A, VANTARAKIS A, MAVRIDOU A, KEFALA A M, GALANIS A. (2017). Molecular Characterization and Phylogenetic Analysis of *Pseudomonas aeruginosa* Isolates Recovered from Greek Aquatic Habitats Implementing the Double-Locus Sequence Typing Scheme. Microb Ecol, 74:78–88. doi: 10.1007/s00248-016-0920-8.

PARK J-W, SHIN I-S, HA U-H , OH S-R, KIM J-H, AHN K-S. (2015). Pathophysiological changes induced by Pseudomonas aeruginosa infection are involved in MMP-12 and MMP-13 upregulation in human carcinoma epithelial cells and a pneumonia mouse model. Infect Immun, 83:4791–4799. doi:10.1128/IAI.00619-15.

SPILKER T, COENYE T, VANDAMME P & LIPUMA J J. (2004). PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *J Clin Microbiol*., 42: 2074-2079. doi: 10.1128/JCM.42.5.2074-2079.2004.

STOVER C K, PHAM X Q, ERWIN A L, MIZOGUCHI S D, WARRENER P, HICKEY M J, BRINKMAN F S, HUFNAGLE W O, KOWALIK D J, LAGROU M, GARBER R L, GOLTRY L, TOLENTINO E, WESTBROCK-WADMAN S, YUAN Y, BRODY L L, COULTER S N, FOLGER K R, KAS A, LARBIG K, LIM R, SMITH K, SPENCER D, WONG G K, WU Z, PAULSEN I T, REIZER J, SAIER M H, HANCOCK R E, LORY S, & OLSON M V. (2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*, 406:959-964.

TANG Y W & STRATTON C W. (2013). Advanced techniques in diagnostic microbiology, Second Edition. *Springer Science*, *Springer New York Heidelberg Dordrecht London*. ISBN: 978-1-4614-3970-7(eBook). doi: 10.1007/978-1-4614-3970-7.

VALOT B, GUYEUX C, ROLLAND J Y, MAZOUZI K, BERTRAND X, & HOCQUET D. (2015). What it takes to be a *Pseudomonas aeruginosa*? The core genome of the opportunistic pathogen updated. *PLoS ONE*, 10(5): e0126468. doi:10.1371/journal.pone.0126468.

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Ichthyofauna of Darbandikhan Lake in Kurdistan Region, Iraq

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A B S T R A C T

In the present study, random samples of fishes were taken by gill net from Darbandikhan Lake, southeast of Sulaimani City, Kurdistan Region, Iraq, to find out the fishes that are naturally found in lake Darbandikhan. The study was carried out during the period from March to the end of October 2012. A total of 255 freshwater fishes, belong to 17 species, namely: *Arabibarbus grypus*, *Barbus barbulus*, *Capoeta trutta*, *Capoeta umbla*, *Carasobarbus luteus*, *Carassius auratus*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Cyprinus carpio*, *Garra rufa*, *Hemiculter leucisculus*, *Hypophthalmichthys molitrix*, *Luciobarbus esocinus*, *Squalius lepidus* (Family Cyprinidae), *Mystus pelusius* (Bagridae), *Silurus triostegus* (Siluridae) and *Mastacembelus mastacembelus* (Mastacembelidae), were collected from this lake. The study demonstrated that *Capoeta trutta*, *Cyprinion macrostomum* and *H. leucisculus* were the most abundant and wide spread in lake Darbandikhan, while *M. pelusius* and *H. molitrix* were scarce.

1. INTRODUCTION

Fishes have been ecological dominants in aquatic habitats they have occupied nearly all major aquatic habitats, from lakes and polar oceans that are ice-covered through much of the year, to tropical swamps, temporary ponds, intertidal pools, ocean depths, and all the more benign environments that lie within these various extremes (Gene *et al.*, 2009).

Fishes are excellent sources of protein, containing all the ten essential amino acids in desirable concentrations for human beings and available at cheaper rates (Agrawal, 1999). The white meat of fish contains 16-29% of protein and has a food value of 300-1600 calories per pound (Shaukat, 2008).

Kurdistan Region is very rich in freshwater bodies as there are many lakes, rivers, stream, karees and springs e.g. Dokan Lake, Darbandikhan Lake, Duhok Lake, Bawashaswar Lake, Greater Zab River, Lesser Zab River and Sirwan River. These water bodies are a good habit for many aquatic organisms especially Ichthyofauna. Unfortunately the study of this fauna in theses water bodies were very limited (Abdullah *et al.*, 2007).

In Darbandikhan Lake there are many species of fishes; some are native species and

others are exotic, some are large and others never grow to enough size for human consumption. The purpose of this paper is to identify the fish species in this lake and to know which species was more abundance in this lake.

2. MATERIALS AND METHODS

2.1. Description of Study Area

Darbandikhan Lake is 7500 hectare, located about 60 km southeast of Sulaimani city, north of Iraq. It is situated between 35° N and 45° E, with the altitude of 485 m above sea level. The surface area is about 121 km^2 and the lake capacity is 3,000,000,000 m^3 with the maximum depth of 75 m, while the Mean depth is 14.8-24.9 m, the main structure is a 128 m high dam with a central clay core and rock fill shoulders. The crest length of the dam is 445 m. Darbandikhan lake was constructed in 1962 at Sirwan river for the purposes of irrigation, control of flood, generating electricity power, fish raising and tourism (Al-Saudi, 1976).

The lake is fed by two rivers, Tanjero River in the north and Sirwan River in the east. It is surrounded by mountains, from north of the lake toward the east are Baranan, Kolkarash, Gulan, Jardsna, while to the south are Zmnako, Zawale, Psht- Qala, Dilf and Shameran, and to the south east is Bashi perk (Al-Saudi, 1976).

Water levels decline in summer due to end of the raining season, and dam releases until raining season resumes in late autumn. The lake and surrounding area is a biological diverse area for wildlife (Bachmann *et al.*, 2008).

2.2. Collection and Preservation of Fishes

A total of 255 fish specimens were collected from Darbandikhan Lake by local fishermen by using gill netting (2 x 100 m mesh sizes 1.5 x 1.5, 3 x 3, 4 x 4 and 6 x 6 cm), cast netting (2 x 50m mesh sizes 2 x 2 cm), electro fishing, and hook biweekly during the period from March to the end of October 2012.

The fishes were placed in containers with local lake water, transferred immediately to the laboratory and examined as soon as possible after their capture. The fishes were identified according to Beckman (1962) and Coad (2010) and the scientific names for fishes were according to Froese and Pauly (2016).

Specimens were preserved, without removal of the guts or gills so that no key characters are lost. Fishes were dropped into 1 part full-strength formalin to kill the fish quickly and then 9 parts of water were added to form a 10% preserving solution according to Coad (2010).

3. RESULTS AND DISCUSSION

A total of 255 different specimens of fishes were collected from Darbandikhan Lake during the period from March to the end of October 2012. Table (1) shows different species of fishes and their abundance in the lake of Darbandikhan. The fish fauna of Darbandikhan lake included 13 native species (plate 1) with the ratio of 76.47%; namely *Arabibarbus grypus*, *Barbus barbulus*, *Capoeta trutta*, *Capoeta umbla*, *Carasobarbus luteus*, *Chondrostoma regium*, *Cyprinion macrostomum*, *Garra rufa*, *Luciobarbus esocinus*, *Squalius lepidus* (Family Cyprinidae), *Mystus pelusius* (Bagridae), *Silurus triostegus* (Siluridae) and *Mastacembelus mastacembelus* (Mastacembelidae). The exotic species (plate 2) included four species with a total ratio of

23.52%; namely *Carassius auratus*, *Cyprinus carpio*, *Hemiculter leucisculus* and *Hypophthalmichthys molitrix*. Coad (2010) mentioned that there are 13 species of exotic fishes in the Tigris-Euphrates Basins including the four recorded species in the present study.

The most abundant species recorded in this investigation belonged to family Cyprinidae (14 species with a ratio 82.35%), followed by families Bagridae, Siluridae and Mastacembelidae (one species for each family with a total ratio of 5.88%). It was obvious that *Capoeta trutta* was the most abundant and wide spread, followed by *Cyprinion macrostomum*, then in the third rank *H. leucisculus* while *Mystus pelusius* and *H. molitrix* were scarce. Abdullah (2005) indicated that *A. grypus* and *C. carpio* were the most abundant species in Darbandikhan Lake. Abdullah *et al.* (2007) also, showed that *Capoeta damascinus* was the most abundant species followed by *C. carpio* and *A. grypus* in Darbandikhan Lake.

It seems from the present study that the distribution of fish populations in the Darbandikhan lake is changing, due to the period, place, and way of fishing, besides the nature of the lake itself which is characterized by changing its water level from year to year and season to season, thus affecting fish distribution (Abdullah *et al.*, 2007).

Moreover, the reason might belong to the introduction of some fish (*C. auratus*, *C. carpio* and *H. molitrix*) into this environment at the end of the seventies of the last century and culturing processes of some of such species are continuous which are leading to their quick spread that affects the density of the rest of species. The evidence supporting this idea is the increase of their fishing and marketing into the local markets nearby the lake. It is inevitable that the increase of these fishes is at the expense of the other species that are similar in their nutrition to the carp like *A. grypus* and *Luciobarbus xanthopterus* (Al-Saadi *et al*., 1986; Abdullah *et al*., 2007).

Table 1: Scientific names of collected fish from Darbandikhan Lake and their total lengths and numbers.

Family and Scientific Names	Total length (cm)	Number
Cyprinidae		
Arabibarbus grypus (Heckel, 1843)	33-88	10
Barbus barbulus Heckel, 1847	28-32.5	10
Capoeta trutta (Heckel, 1843)	19-35.5	69
Capoeta umbla (Heckel, 1843)	25-40	12
Carasobarbus luteus (Heckel, 1843)	23-27.5	11
Carassius auratus (Linnaeus, 1758)	20-28	13
Chondrostoma regium (Heckel, 1843)	18-25.5	14
Cyprinion macrostomum Heckel, 1843	21.5-23	22
Cyprinus carpio Linnaeus, 1758	23.5-41	13
Garra rufa (Heckel, 1843)	14-16	6
Hemiculter leucisculus (Basilewsky, 1855)	9.5-12.5	20
Hypophthalmichthys molitrix (Valenciennes, 1844)	40-67	4
Luciobarbus esocinus Heckel, 1843	24.5-51	11
Squalius lepidus Heckel, 1843	21.5-33	17
Bagridae		
Mystus pelusius (Solander, 1794)	20-25	2

Siluridae		
Silurus triostegus Heckel, 1843	54.5-80	7
Mastacembelidae		
Mastacembelus mastacembelus (Banks and Solander, 1794)	49-64	14
Total		**255**

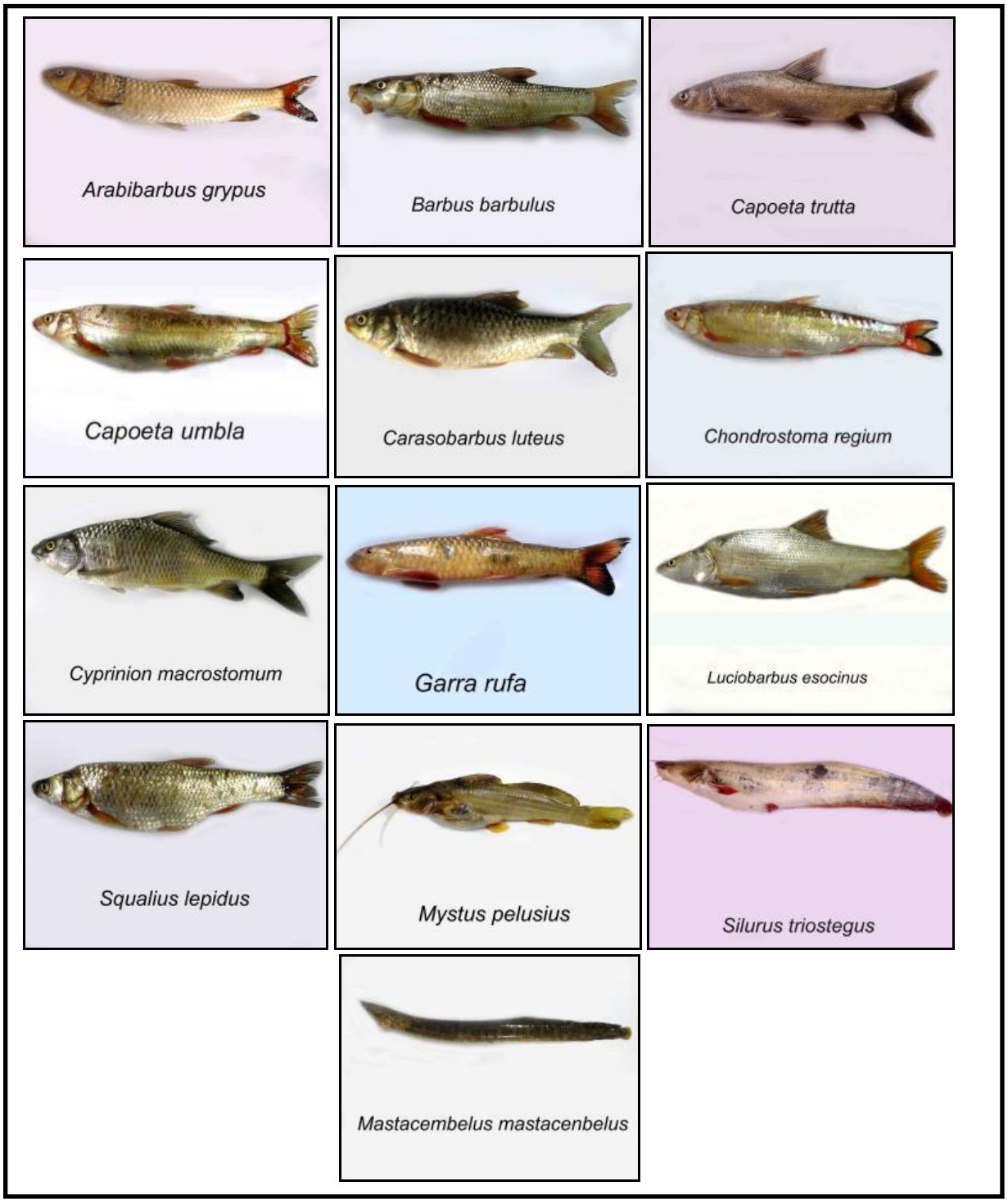

Figure 1: Native fish species in Darbandikhan Lake.

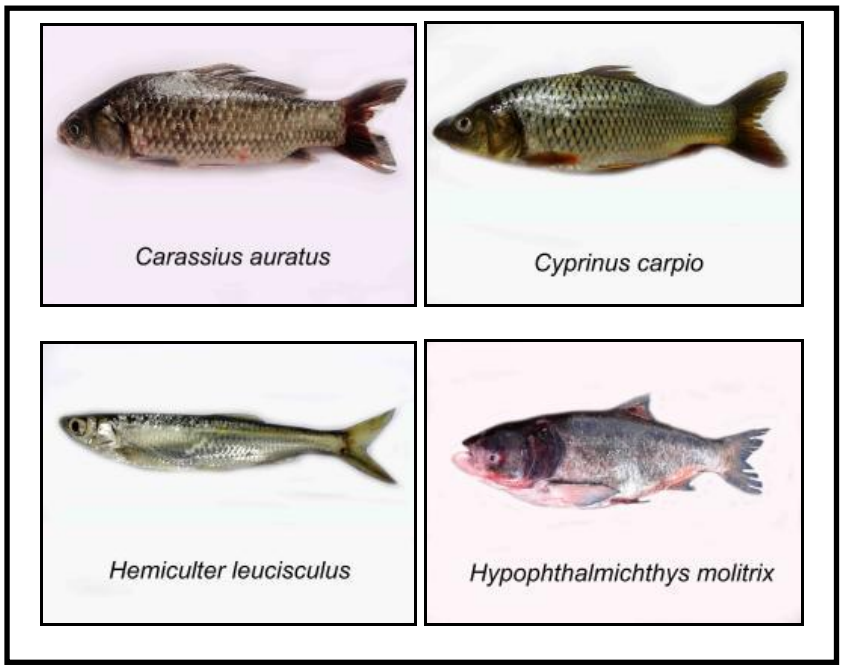

Figure 2: Exotic fish species in Darbandikhan Lake.

REFERENCES

ABDULLAH, S. M. A. 2005. Parasitic fauna of some freshwater fishes from Darbandikhan lake, north of Iraq. J. Dohuk Univ. 8(1). p. 29-35.

ABDULLAH, S. M. A.; Rahemo Z. I. F. and Shwani A. A. 2007. The inhabitant fishes in Darbandikhan lake in north of Iraq and methods for developing their culture. Egypt. J. Aquat. Biol. Fish. 11(3). P. 1-7.

ABDULLAH, S. M. A. 2005. Parasitic fauna of some freshwater fishes from Darbandikhan lake, north of Iraq. J. Dohuk Univ. 8(1). P. 29-35.

ABDULLAH, S. M. A.; RAHEMO Z. I. F. and SHWANI A. A. 2007. The inhabitant fishes in Darbandikhan lake in north of Iraq and methods for developing their culture. Egypt. J. Aquat. Biol. Fish. 11(3). P. 1-7.

AGRAWAL, V. P. 1999. Recent trends in Aquaculture. Meerut, India, Soc. Bio-sci. p. 1-3.

AL-SAADI, H. A.; AL-DAHAM N. K. and AL-HASSAN L. A. 1986. Aquatic Ecology. Mousul Univ. Press.

AL-SAUDI, A. F. 1976. Little Zab region in Iraq. Asad Press, Baghdad.

BACHMANN, A.; DELA CRUZ C. R.; DAVIES J. and MALONE L. R. 2008. Key biodiversity survey of Kurdistan, northern Iraq, Sulaimani, Kurdistan, Iraq.

BECKMAN, W.C. 1962. The freshwater fishes of Syria and their general biology and management. FAO Fish. Biol. Branch Tech. Pap. No.8. p. 179.

COAD, B. W. 2010. Freshwater fishes of Iraq. Pensoft Publisher, Sofia-Moscow.

FROESE, R. and PAULY D. 2016. FishBase. World web electronic publication. www.fishbase.org,version (3/3/2016).

GENE S. H.; BRUCE B. C.; DOUGLAS E. F. and BRIAN W. B. 2009. The diversity of fishes. 2nd edition, Printed in Malaysia.

SHAUKAT, N. 2008. Studies on digenetic trematodes of some fishes of Karachi Coast. Ph. D dissertation, Jinnah Univ. Nazimabad, Karachi, Pakistan.

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Cytogenetic analysis of human lymphocytes exposed to various concentrations of aqueous and methanolic extracts of *Brassica oleracea var. italic* (Broccoli)

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A B S T R A C T

The chromosomal aberration, Mitotic index (MI) were employed to investigate the *in vitro* effect of broccoli (*Brassica oleracea)* on human chromosomes. *In vitro* incubation for 48 hrs of normal human lymphocytes were studied with various concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of Aqueous and Methanolic extract of broccoli. Results indicated a positive relationship between Mitotic index (MI) values and the methanolic extract at concentrations (0.19±0.012, 0.29±0.021, 0.26±0.003, 0.3±0.006, 0.32±0.011 and 0.34±0.008) respectively, and (0.12±0.006, 0.15±0.002, 0.19±0.011, 0.21±0.015, 0.22±0.021, 0.30±0.003) respectively, for aqueous extract, a significant differences noted (P≥0.01) among effects of different concentrations. MI value of positive control treatment cyclophosphamide (CP) (0.13±0.003) as compared with a negative control group (0.401±0.018). In addition the value of the damaged cells in negative control group was (0.06±0.01), whereas for positive control group was (43.65±0.003). The damaged cells for the treated group with extract revealed increasing in value in a concentration dependent manner from (4.99±0.08) to (26.53±0.004) and (5.75±0.08) to (23.43±0.023) for both aqueous and methanolic crude extract respectively as compared with the negative control (0.06±0.01). This effect maybe attributed to anti oxidative activity induced by *Brassica oleracea.*

1. INTRODUCTION

In last recent years, researches on plants has been increased all over the world (Nsimba *et al.* 2008). Many secondary metabolites of plant are commercially important and used in a number of pharmaceutical compounds (Joy *et al.* 1998). The use of plants for treating diseases is as old as the human species (Silva and Fernandes, 2010).

Broccoli (*Brassica oleracea L. var. italica*) belongs to *Brassica oleracea* species together with other commonly grown Brassica vegetables. Broccoli has great potential to prevent several diseases, such as cancer (Keck and Finley, 2004; Hartikainen, 2005) and cardiovascular disease (Mukherje *et al.*, 2008), and the intake of this vegetable results in an improvement of the general health status, mainly due to its antioxidant (Borowski *et al.*, 2008) and anticarcinogenic properties (Jefery

and Araya, 2009). The beneficial effects of broccoli is by substantial quantities of bioactive compounds, such as vitamin C, β-carotene, phenolic compounds, and glucosinolates (Heimler *et al.,* 2006; Jagdish *et al.,* 2006), which are good free radical scavengers (Eberhardt *et al.*, 2005).

Polyphenols act as potent antioxidants as they protect the cells against oxidative damage. They exhibit free radical scavenging and metal chelating activities. Therefore, the intake of these compounds may result in reduction of the risk to develop various degenerative diseases triggered by oxidative stress (D'Archivio *et al*., 2003).

The cellular macromolecules of humans, such as DNA, proteins and lipids, are continuously at risk for endogenous and environmentally induced structural alterations. (Lee and Kohn, 2009). According to rather controversial information, about 5% of environmental chemicals are characterized by potential mutagenic activity. At the same time, data on mutagenic activity of overall pollutants of air, water, and foodstuffs are being accumulated (Bach *et al*., 1973; Knudsen *et al.,* 1999).

Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. In epidemiological studies, it has been shown that people with elevated frequencies of CA in their peripheral blood lymphocytes have a significantly elevated risk of developing cancer (Obe *et al.,* 2002)

The aim of this work was to study the Cytogenetic effect of aqueous and methanolic crude extracts of Brassica oleracea var. Italic on human blood lymphocyte by evaluation MI, damaged cells and chromosomal aberrations.

2. MATERIALS AND METHODS

2.1. Plant sampling:

Brassica oleracea was obtained from the wet market in Erbil, identified by herbarium in College of Agriculture, Salahaddin University, Erbil, Iraq. Then dried at room temperature according to Harbon (1984), and ground in to powder by electrical grinder (mesh No. 0.5mm), then extracted and isolated using aqueous and methanol as an extracting solvent in research center laboratories of Erbil Polytechnic University.

2.2. Broccoli extracts preparation:

Repeated extraction with water and methanol recommended by Harborne, was applied as described by (Al-Atby, 2001), fifty grams of the broccoli powder was placed in a flask separately, with 300 ml of deionized distilled water (D.D.W) and absolute methanol then each flask was tightly sealed. The mixture was left to stir on a magnetic stirrer for 3 days at room temperature. Then filtered through gauze and Whatman no.1 filters paper. Filtrate extract was dried in an electric oven at 37°C. Dried extract was scrubbed off the petri dishes, and stored in tightly sealed plastic test tube at -20°C (Harbon, 1984).

2.3. Cytogenetic study on human lymphocyte:

1. **Blood collection:** The blood samples were taken from apparently normal human adult by venous puncturing, using disposable syringe. Five ml of blood was transferred into heparinized tubes.
2. **Procedure**

   Blood samples (0.5 ml) was added to 4.5 mL RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.3ml Phytohaemagglutinin (PHA), and then incubated at 37 ºC in

CO2-incubator with shaking test tubes gently each 15 minute.

After 24 hrs, cultures were treated with 1ml of each effective concentration of both aqueous and methanolic extracts (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) (three replicates for each concentration) of *Brassica oleracea var. Italic* , 1ml of PBS as a negative control, and 1 ml of 50 µg/ml of cyclophosphamide (CP), as a positive control. Lymphocytes were harvested after 52 hrs incubation by adding 0.1 g/ml colchicine (to arrest the cells at metaphase in mitosis) and incubation at 37oC for 1hr, then centrifuging cell suspension to remove culture medium (1000 rpm), addition of hypotonic solution (KCl 0.075 M) at 37 ºC for 20 min to swell the cells, and treated twice with Carnoy's fixative (3:1 ratio of methanol: acetic acid) (Shubber and Juma, 1999).

3. Slide preparation:

The slide was prepared according to the procedure applied by Iraqi center for cancer and medical genetic research (ICCMGR) by which the cell suspension removed from freezer or used immediately. The suspension was mixed very well by Pasture pipette; 3-4 drops of cells suspension dropped evenly from appropriate distance (30-50 cm) onto wet, chilled, oil-free slides and allowed drying at room temperature.

4. Staining:

The slides were stained using freshly made Giemsa stain (stock solution) and rapidly washed with warmed Sorenson's buffer, after that left to dry at room temperature.

5. Microscopic examination

Microscopic examination was performed to determine the Chromosomal Aberration (CA) analysis, 100 well spread complete metaphase cells in first cell cycle were evaluated per subject under a microscope at 100× magnification to identify numerical and structural CA. Chromosome-type CAs: (break; gap; ring, dicentric) were observed. The MI was determined by scoring at least 1000 cells, and then MI was calculated by the following formula:

Mitotic Index (%) = Number of the dividing cells X 100 / The total number of cells (Shubber and Juma, 1999).

3. RESULTS AND DISCUSSION

The most sensitive tests for the effect of carcinogenic and mutagenic agent are the quantifying of cytogenetic parameters including MI (%MI) (Shubber *et al.*, 1998).

The cytogenetic effects for six concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of *B. oleracea* aqueous and methanolic extract were studied on lymphocytes of peripheral blood in vitro after 48hrs incubation. The cell division induced by mitogen (PHA in comparison with CP 50µg/ml as positive control. (Fig. 1).

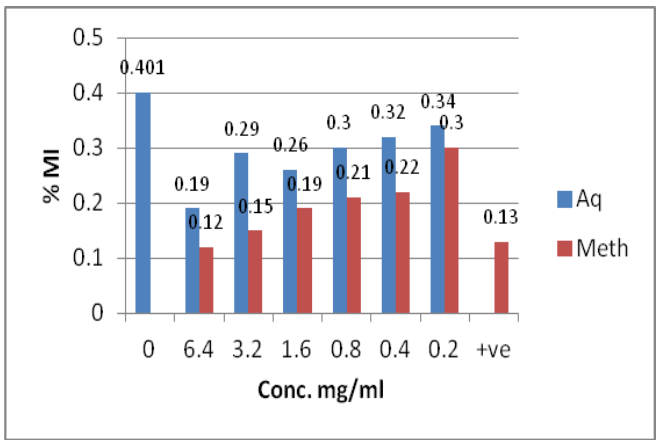

Figure (1): MI of human lymphocyte cells after 48hrs treatment with *B. oleracea* aqueous and methanolic extract.

The result of the present study revealed that there was significant difference in MI after 48hr treatment from (0.19, 0.12 to 0.34, 0.30) for both aqueous and methanolic extracts respectively. The exposure of lymphocytes to known and potent antimitotic agent (CP) resulted in sharp decrease in MI (0.13±0.003) as compared with a negative control group (0.401±0.018), because CP is an alkylating agent that adds an alkyl group (CnH2n+1) to DNA. It attaches the alkyl group to the guanine base of DNA, phosphoramide mustard forms DNA crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand cross linkages, respectively). This is irreversible and leads to cell death (Takimoto and Calvo, 2008). Krishna *et al.*, (1986), observed in their study that CP cause dose related chromosomal aberrations and sister chromatid exchange (SCE).

Figure (2), shows damaged cells (cell containing one or more chromosome aberration) among treatments. The value of the damaged cells in negative control group was (0.06±0.01), whereas for positive control group was (43.65±0.003). The damaged cells of the treated group with extract revealed increasing in value in a concentration dependent manner from (4.99±0.08) to (26.53±0.004) and (5.75±0.08) to (23.43±0.023) for both aqueous and methanolic crude extract respectively, and the percentage of damaged cells that induced after application the plant extract, was less than positive control group which received cyclophosphamide. This observation indicates that *B. oleracea* extract had an inhibitory effect on cellular proliferation but without increase of chromosomal aberration.

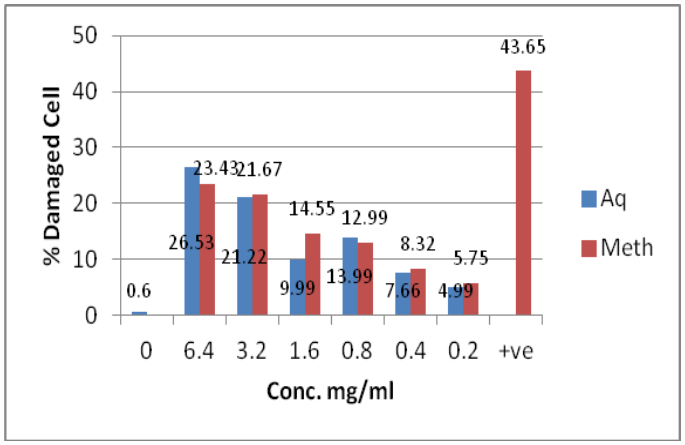

Figure (2): Damaged cell (%) of human lymphocyte cells after 48hrs treatment with *B. oleracea* aqueous and methanolic extract.

Also the M.I. of the treated groups decreased in a concentration dependent manner. All concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of *B. oleracea* of both extract have anti mitotic effects as compared with the positive control. (Fig. 3)

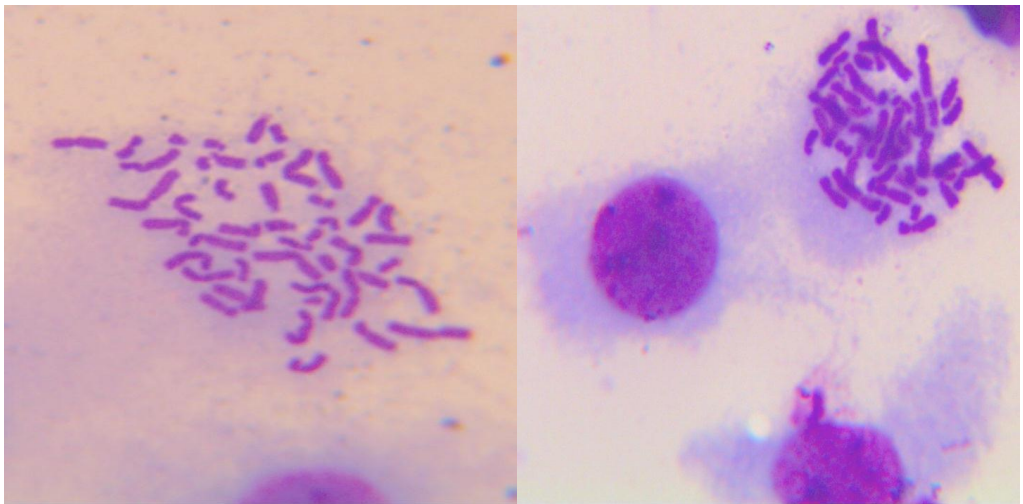

Figure (3): Karyotyping of human chromosome at different concentration of (A) Aqueous and (B) methanolic extract of *B. oleracea var. italica* (1000X, Giemsa stain).

The main isothiocyanate in broccoli is sulforaphane, which can up regulate the expression and activity of thioredoxin reductase (TR) in humans as well as thioredoxin thus playing a crucial role in the regulation of the redox state in cells (Bacon *et al.*, 2007).

Yanyan *et al.*, (2010) in their researches about sulforaphane, a dietary component of Broccoli, found that it inhibits Breast Cancer Stem Cells. Munters *et al*., (2010), was study the effects of broccoli sprouts intake on oxidative stress. Broccoli sprouts have been reported to inhibit skin and urinary bladder carcinogenesis *in vivo* (Dinkova- Kostova *et al.*, 2007), and also inhibit the proliferation of human bladder and prostate cancer cells *in vitro* (Tang *et al.*, 2006). The anticancer properties of broccoli sprouts occur through their primary active micronutrient, sulforaphane, by the induction of mitochondria-mediated apoptosis (Tang *et al*. 2007). Melchini *et al.*, (2013) showed in his study that erucin which is a dietary component of broccoli, is considered to be a major cancer chemopreventive phytochemical, and showed a lower potency in inhibiting the proliferation of prostate adenocarcinoma cells (PC3).

The chromosomal aberrations that include dicentric chromosome, chromosomal gap, chromosomal break and ring chromosome were found in all the positive control and in other different concentrations of extract (Fig. 3) (table 1 & 2).

The number of chromosomal aberrations (damaged cells) of treated groups with extracts decreased in comparison with the positive control. The chromosomal aberrations as chromosomal gap, break and ring chromosome where observed in all concentration but there values were lower in comparison with the positive control, table (1 & 2).

Table (1):Mean±SE for types of chromosomal aberrations among human lymphocyte cells after 48hrs treatment with *B. oleracea* aqueous extract:

Treatment	Conc. mg/ml	Metaphase examined	Types of chromosomal aberration			
			Di Centric	Ring	Gap	Break
-ve Control (PBS)	0	**100**	**1.33±0.41**	**-**	**1.23±0.33**	**6.66±0.33**
+ve conrol (CP)	50	**100**	**2±1.66**	**4.33±0.33**	**21.66±0.33**	**15.66±0.66**
Brassica oleracea var. italica (Aqueos)	6.4	**100**	**2.1±1.33**	**3±0.33**	**8±0.57**	**10.33±0.66**
	3.2	**100**	**3.33±0.33**	**-**	**8.11±0.66**	**10.23±0.33**
	1.6	**100**	**1.11±0.67**	**2.12±0.66**	**6.66±0.33**	**4.66±0.66**
	0.8	**100**	**2.33±0.6**	**-**	**5.33±0.88**	**5.33±1.33**
	0.4	**100**	**1.66±0.23**	**0.33±0.63**	**3±0.55**	**3.33±0.33**
	0.2	**100**	**0.66±0.67**	**-**	**1.33±0.33**	**3.76±0.33**
LSD		**LSD**	**2.57**	**0.79**	**1.8**	**2.93**

SE=standard error

Table (2): Mean±SE for types of chromosomal aberrations among human lymphocyte cells after 48hrs treatment with *B.oleracea* methanolic extract:

Antioxidant provide protection to living organism from damage caused by uncontrolled production of free radicals, reactive oxygen

Treatment	Conc. mg/ml	Metaphase examined	Types of chromosomal aberration			
			Di Centric	Ring	Gap	Break
-ve Control (PBS)	0	**100**	**1.33±0.41**	**-**	**1.23±0.33**	**6.66±0.33**
+ve conrol (CP)	50	**100**	**2±1.66**	**4.33±0.33**	**21.66±0.33**	**15.66±0.66**
Brassica oleracea var. italica (Aqueos)	6.4	**100**	**3.2±0.33**	**3±0.66**	**7±0.66**	**13.33±0.66**
	3.2	**100**	**4.66±0.33**	**1.12±0.66**	**6.11±0.66**	**9.33±0.66**
	1.6	**100**	**1±0.67**	**-**	**5.66±0.33**	**3.33±0.66**
	0.8	**100**	**3.33±0.6**	**0.33±0.63**	**4.33±0.66**	**6±0.33**
	0.4	**100**	**1.33±0.66**	**-**	**3±1.33**	**3.33±0.33**
	0.2	**100**	**-**	**-**	**2.66±0.33**	**2.33±0.33**
LSD		**LSD**	**2.57**	**0.79**	**1.8**	**2.93**

species (ROS) and concomitant lipid peroxidation, protein denaturation and DNA-strand breaking (Yadavet *et al.,* 2003). A major advantage of antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous (Kohlmeier *et al.,* 1995).

4. CONCLUSIONS

Phytochemicals that are present in broccoli have great cytogenetic effect to reduce the risk of chemotherapies on human lymphocytes.

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REFERENCES

Al-Atby S.M.H. (2001). Effect of crude alcoholic extract of Withania somnifera dun on growth of cancer cell line in Vitro and on some physiological parameters in mice. Ph. D. Thesis, Faculty of Veterinary Medicine, University of Baghdad. In Arabic.

Bach Q T, Do Mink P, Ho T, and Tran T. T. (1973). Aberrations chromosomiques provoquees par lesherbicides et defoliants a doses massives et repetees au Sud Vietnam. Etudesur 58personnes.Y. Viet-Nam,Nl, 11-18 (in French).

Bacon J.R; Plumb G.W; Howie A.F; Beckett G.J; Wang W, and Bao Y. (2007). Dual Action of Sulforaphane in the Regulation of Thioredoxin Reductase and Thioredoxin in Human HepG2 and Caco-2 Cells. J. Agric. Food Chem. 55, 1170–1176.

Borowski J; Szajdek A; Borowska EJ; Ciska E and Zielinski H. (2008). Content of selected bioactive components and antioxidant properties of broccoli (Brassica oleracea L.).European Food Research and Technology. 226: 459–465.

D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C and Masella R. (2007). Polyphenols, dietary sources and bioavailability. Annali dell'Istituto Superiore diSanita 43: 348–361.

Dinkova-Kostova AT; Fahey JW; Wade KL; Jenkins SN; Shapiro TA; Fuchs EJ, et al. (2007). Induction of the phase 2 response in mouse and human skin by sulforaphane-containing broccoli sprout extracts. Cancer Epidemiol Biomarkers Prev 16: 847–851.

Eberhardt MV, Kobira K, Keck AS, Juvik JA, Jefery EH. (2005). Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (Brassica oleracea L. var. italica). J Agric Food Chem, 53, 7421–7431.

Harborne J.B. (1984). Phytochemical methods. 2nd edition. Chapman and Hall Ltd. London. UK.

Hartikainen H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. Journal of Trace Elements in Medicine and Biology 18: 309–318

Heimler D; Vignolini P; Dini MG; Vincieri FF and Romani A. (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chem, 99, 464–469

Jagdish S, Rai M, Upadhyay AK, Bahadur A, Chaurasia SNS, and Singh KP. (2006). Antioxidant phytochemicals in broccoli

(Brassica oleracea L. var. italica Plenck) cultivars. J Food Sci Technol, 43, 391–393.

Jeffery EH, and Araya M. (2009). Physiological effects of broccoli. Phytochemical Reviews 8: 283–298

Joy, P. P; Mathew J. T, and Skaria BP. (1998). Aromatic and Medicinal Plants Research Station, Kerala Agricultural University, India.

Keck AS, and Finley JW. (2004). Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integrative Cancer Therapies 3:5–12.

Knudsen L. E.; Norppa H. ; Gamborg M. O. ; Nielsen Per S. ; Okkels H. ; Soll-Johanning H. ; Raffn E. ; Järventaus H. and Autrup H. (1999). Chromosomal Aberrations in Humans Induced by Urban Air Pollution: Influence of DNA Repair and Polymorphisms of GlutathioneS-Transferase M1 and N-Acetyltransferase 2. Cancer Epidemiology, Biomarkers & Prevention. Vol. 8, 303–310.

Kohlmeier L; Simonsen N, and Mottus K. (1995): Dietary modifiers of carcinogenesis .Environ .Persp, 130:177-178.

Krishnaa G; Nath J; Petersena M, and Ong T. (1986). In vivo and in vivo/in vitro kinetics of cyclophosphamide-induced sister-chromatid exchanges in mouse bone marrow and spleen cells. Mutation Research/Genetic Toxicology. Volume 204, Issue 2, P 297-305

Lee SH, and Kohn H, (2009). Nucleophilic Activation of a Tetra-Substituted Mitomycin Cyclic Bis-Disulfide. Chem. Pharm. Bull. 57(2): 149-157.

Melchini A; Traka MH; Catania S; Miceli N; Taviano MF, et al. (2013). Antiproliferative activity of the dietary isothiocyanate erucin, a bio active compound from cruciferous vegetables, on human prostate cancer cells. Nutr Cancer 63: 132-138.

Mukherjee S, Gangopadhyay H and Das DK. (2008). Broccoli: a unique vegetable that protects mammalian hearts through the redox cycling of the thioredoxin super-family. Journal of Agricultural and. Food Chemistry. 56:609–617

Munters E; Pieters N; Cuypers A, and Penders J. (2010). Effects of broccoli sprouts intake on oxidative stress, inflammation, microalbuminuria and platelet function in human volunteers: a cross-over study. Proceedings of the Nutrition Society. 69 (OCE8), E590.

Nsimba R; Kikuzaki H and Konishi Y. (2008). Antioxidant activity of various extracts and fractions of Chenopodiumquinoa and Amaranthus spp. seeds. Food Chem., 106, 760–766.

Obe G; Pfeiffer P.; . Savage J.R.K; Johannes C.; Goedecke W.; Jeppesen P.; Natarajan A.T.; Mart´ınez-López W.; Folle G.A. and Drets M.E. (2002). Chromosomal aberrations: formation, identification and distribution. Mutation Research. 504;17–36.

Shubber E.K; Amin B.H, and El-Adhami B.H. (1998). Cytogenetic effects of copper containing intrauterine contraceptive device (IUCD) on blood lymphocytes. Mutat. Res.; 2665: 1-7.

Shubber EK, and Juma AS. (1999). Cytogenetic effects of plants extract of Urtica dioca on mouse somatic cells. The Nucleus, 42 (3)182-187.

Silva NCC, and Fernandes JA. (2010). Biological properties of medicinal

plants: a review of their antimicrobial activity. The Journal of Venomous Animals and Toxins including Tropical Diseases. Vol.16, n.3, pp.402-413.

Takimoto C. H, and Calvo E. (2008). Principles of oncologic pharmacotherapy: in Cancer management, a multidisciplinary approach. Edited by R. Pazdur, L. D. Wagman, K. A. Camphausen and W. J. Hoskins, PRR, Melville, New York.

Tang D; Kari M; Warren G, and Yanzhuang W. (2007). Molecular mechanism of mitotic Golgi disassembly and reassembly revealed by a defined reconstitution assay. JBC. Doi; 10.1074.

Yadavet J.S; Thakur S, and Chadha P. (2003): Chyawanprah a genoprotective agent for bidi smoker.Int.J.Hum.Genet. 3:33-38.

Yanyan Li; Tao Z; Hasan K.; et al. (2010). Sulforaphane, a Dietary Component of Broccoli/Broccoli Sprouts, Inhibits Breast Cancer Stem Cells. Clin Cancer Res. 1; 16(9): 2580–2590.