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RESEARCH PAPER

Determination of diazinon residues in artificially polluted soils.

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ABSTRACT:

The combination effects of diazinon insecticide (O, O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidynyl]), three different soil orders (Entisols, Vertisols and Inceptisols) and two soil moisture contents (50% and 100% of field capacity) was studied in a pot experimental design. This experiment last for two months from with five sampling periods at biweekly intervals. The main obtained results of this study were the lowest pH values (6.53 ± 0.006) shown by the combined treatment DS3W2. The combinations DS3W1, DS3W2 and CS1W1 showed the highest organic matter levels (20.698 ± 0.001 g.kg⁻¹). The combined treatment DS3W2 showed the highest total nitrogen contents (2.811 ± 0.003 g.kg⁻¹). Diazinon residues were above the maximum residue limit (MRL) after 24h of application. Whereas, residues have not detected in the treated soils during the other sampling periods and this may indicate complete degradation of diazinon, which may refer to diazinon properties, environmental factors, microbial activities and soil characteristics.

KEY WORDS: Soil Pollution, Pesticides, Diazinon, Residue. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.1</u> ZJPAS (2019), 31(5);1-8 .

1.INTRODUCTION:

The continuous interaction among soil minerals, organic matter and organisms produce a dynamic system called soil. These three major components influences the physicochemical and properties of terrestrial biological system (Khudhur, 2018). The undesirable changes in soil's physical, chemical or biological characteristics known as pollution that harmfully affect the organism's life (Pandey et al., 2005). The environmental pollution by agricultural and industrial sources increase the amount of effluents in air, water and soil which affect the quality of food and human health (Bordjiba et al., 2001). Because of the relative lack of new areas suitable

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Nashmeel Saeed Khudhur E-mail: <u>Nashmeel.khudhur@su.edu.krd</u>, <u>nashmeel@gmail.com</u> **Article History:** Received: 13/03/2019 Accepted: 30/05/2019 Published:17 /10 /2019 for agriculture, the performance of the existing agricultural areas has substantially enhanced. Pesticides have extensively used in agrochemical practice to protect plant against different pests. Although these pesticides have intended for use at low concentrations, their application may cause pollution of soil, resulting in chemical and biological disturbance of this environment (Cserháti *et al.*, 2004). As pesticides are applied, they usually meet soil and undergo a variety of transformations that provide a complex pattern of metabolites (Sanchez *et al.*, 2011).

Diazinon [O, O-diethyl O-(2-isopropyl-4methyl 6-pyrimidinyl) phosphorothioate] is a non-selective and non-systemic contact, organophosphorus insecticide with a wide range of insecticidal activity and it is first registered in the United States in 1956 (Kendall et al., 1993 and NPIC, 2003). This compound is available as a dust, granules, seed dressings, wettable powder emulsifiable solution. After diazinon and

application, it is often found in the surrounding soil, surface waters and the surface of plants (Tang *et al.*, 2009). Diazinon has been widely used to control crawling insects, ticks, adult and juvenile forms of flying insects, mites and spiders at concentrations about 3-15 kg.ha⁻¹ (Kendall *et al.*, 1993 and NPIC, 2003).

Pesticide residue is the part of a pesticide that remains in the environment for a period after application. The residues of persistent pesticides stay in the environment without breaking down for long periods. These pesticides are sometimes desirable, because there is no need for repeated application and they provide long-term pest control (Milne, 1998). The derivatives of pesticide include degradation residues products, metabolites, reaction products, and impurities that may have toxicological significance (Stenersen, 2004). Soil properties such as organic matter content, soil texture and soil pH play an important role in the carryover potential of residual pesticides (Johnson, 2004). The characteristics of the pesticide, including its over-all stability either as parent compound or metabolites, its volatility, solubility, formulation and the method and its site of application are among the factors that influence the persistence of pesticides. Moreover, the environmental factors like temperature in particular, precipitation, humidity and wind are among the other factors affecting pesticides' persistence (Edwards, 1975). The rate of dispersal and the persistence time of pesticides in soil depend on the physico-chemical and biological properties of the soil (Medved, 1975). Kabir et al., (2008) observed that diazinon left residue up to 6 d after spray of 3 supervised fields at the field dose 1.5 ml.1⁻¹ of water, however the level of residue was above the maximum residue limit (MRL) which established by FAO 1970.

Unfortunately, there are few studies conducted on pesticides in Kurdistan Region. Khider (1981) studied the effect of insecticides and fungicides on 3 strains of Rhizobium leguminosarum. Darwesh (2004) studied the effect of some pesticides on denitrification in different textured soils. Other studies conducted on pesticide adsorption to soil media by some authors (Hamadamin 2006, Shariff, 2008, Abdul Al-Rahman, 2010). Alshaikh (2011) studied the effect of four fungicides on the growth rate of infected soybean with mycorrhiza. Khudhur (2011) studied the effect of some pesticides on soil and growth characteristics of faba bean.

Due to lack of studies on determination of pesticide residues in Kurdistan soils, we found an importance to conduct this study and we aim to determine the effect of diazinon pesticide in combination with three different soil orders and two levels of moisture content on some soil chemical properties.

2.MATERIALS AND METHODS

2.1. Soil sampling and analysis

Different soil orders, which had no previous history of pesticide use including Entisols, Vertisols and Inceptisols, were collected at a random pattern (0-30 cm depth) from three different agricultural fields of Erbil province in May 2011. The fields were Agholan, Debaga and Girdarasha (Figure 1).



Figure 1: Map showing: Iraq, Erbil and the studied aria.

Soil samples were brought to the greenhouse of Science College. Samples from each soil order were separately screened from gravels and stones, pulverized, air-dried and sieved by 2-mm sieve. Physical, chemical and microbiological properties (Table 1) were determined according to (Ryan *et* al., 2001, Jones, 2001, van Reeuwijk, 2002, Pansu and Gautheyrou, 2006 and Bashour and Sayegh,

2007).

Table 1: Physical, chemical and microbiological properties of the studied soils.

	Soil properties		Agholan	Debaga	Girdarasha
		Sand	23.79	20.51	19.23
	Particle size distribution (g.kg ⁻¹)	Silt	52.56	58.97	64.10
Dhydiaal		Clay	25.64	20.51	16.67
Physical	Texture		Silt loam	Silt loam	Silt loam
	Moisture content (%)		16.23	8	4.5
	Moisture content (%) Moisture content (ml.100g ⁻¹) pH Electrical conductivity (EC) (dS.m ⁻¹)		32.50	25.00	24.00
	7.22	7.20	7.16		
	Electrical conductivity (EC) (dS.m ⁻¹)		4.22	4.16	3.16
	Moisture content (%) Moisture content (ml.100g ⁻¹) pH Electrical conductivity (EC) (dS.m ⁻¹) Cation exchange capacity (CEC) (c.mol.kg ⁻¹) Organic Matter (OM) (g.kg ⁻¹) Total nitrogen (N) (g.kg ⁻¹) Total nitrogen (N) (g.kg ⁻¹)	33.168	26.959	28.895	
Chemical	Organic Matter (OM) (g.kg ⁻¹)		15.524	8.624	12.074
	Total nitrogen (N) (g.kg ⁻¹)		2.519	1.079	2.412
	Total phosphorus (P) (g.kg ⁻¹)		0.143	0.127	0.452
	Total sulfur (S) (g.kg ⁻¹)		0.007	0.004	0.008
Misushialasiaal	Total bacterial count (Bacterial cells.g ⁻¹	lry soil)	3.80×10^5	1.40×10^{5}	1.77×10^{6}
witcrobiological	Total fungal count (Fungal propagul.g ⁻¹	lry soil)	3.70×10^{5}	6.70×10^4	6.68×10^5

2.2. Analysis of soil physicochemical properties

In the laboratory, the collected soil samples from each pot in pre-labelled nylon bags were analysed for physical and chemical properties. Soil pH has measured from the saturation extract using pH-meter as mentioned by Ryan *et al.*, (2001). The instrument was standarized before use with buffers pH (4, 7 and 9). Walkley-Black procedure 1934 for soil organic matter and micro-Kjeldahl procedure for soil total nitrogen have followed according to van Reeuwijk (2002).

2.3. Diazinon treatment

Diazinon insecticide (10%) (Table 2 and Figure 2) was prepared at its commercial recommended dose according to its active ingredients (a.i.) as described by (Hill, 2008). It is then placed in a sprayer (the amount of spraying water was as 60 liters/donum) and ready for application. Each soil order was sprayed separately by diazinon and a portion from each soil order was left without pesticide treatment (control). Soil portions were mixed to obtain homogenous distributions. Then the pesticidetreated soils were sealed and left for an overnight at room temperature.



Figure 2: Diazinon structure (Xu, 2000).

Source: Xu, (2000); Plimmer *et al.*, (2003); Pretty, (2005); Moros *et al.*, 2007; Meister *et al.*, (2008); Tomlin, (2009) and WHO, (2010).

Table 2: Description of diazinon pesticide.

Common name	diazinon
Chemical name	[O,O-diethyl O-(2-isopropyl-4- methyl 6-pyrimidinyl)
Chemical hame	phosphorothioate]
Trada nama	Knox Out, Spectracide and
I rade name	Basudin
Chemical family	Organophosphate
Molecular formula	$C_{12}H_{21}N_2O_3PS$
Molecular weight	304.3 g.mol ⁻¹
Recommended dose	1-2.5kg.donum ⁻¹
Half-life in soil	9-11 days
Analysis method	HPLC-UV, GC/MS
LD50	1000 mg.kg ⁻¹
Toxicity class	II=Moderately hazardous

2.4. Pot experiment layout and statistical analysis

3

ZANCO Journal of Pure and Applied Sciences 2019

A factorial experiment $(2\times3\times2 \text{ i.e. pesticide} \times \text{soil order} \times \text{moisture content})$ was conducted using Completely Randomized Design (CRD) with three replications under twelve combined treatments (Table 3). The experiments last for two months with conducting five samplings at biweekly intervals and the climatic condition during the study has presented in (Table 4).

Combined treatments	Pesticide	Soil order	Moisture content	Combined treatments	Replications
DS1W1		Agholon	50%	Diazinon.Agholan.50%	3
DS1W2		Agnotan	100%	Diazinon.Agholan.100%	3
DS2W1	Diaginon	Dahaga	50%	Diazinon.Debaga.50%	3
DS2W2	Diazilioli	Debaga	100%	Diazinon.Debaga.100%	3
DS3W1	Cirdorasha		50%	Diazinon.Girdarsha.50%	3
DS3W2		Giruarasiia	100%	Diazinon.Girdarsha.100%	3
CS1W1		Agholon	50%	Control.Agholan.50%	3
CS1W2		Agiiolali	100%	Control.Agholan.100%	3
CS2W1	Control	Dahaga	50%	Control.Debaga.50%	3
CS2W2	(no pesticide)	Debaga	100%	Control.Debaga.100%	3
CS3W1		Cindonasha	50%	Control.Girdarasha.50%	3
CS3W2		Giruarasha	100%	Control.Girdarasha.100%	3

Table 3: Pot experimental design.

4

Table 4. Chimatic Condition during the study	Table 4:	Climatic	condition	during	the	study
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Parameters	1 st sampling	2 nd sampling	3 rd sampling	4 th sampling	5 th sampling	Mean
Maximum temperature (°C)	37.0	36.7	37.0	44.6	41.2	39.3
Minimum temperature (°C)	24.4	26.4	28.6	33.3	30.0	28.54
Dry temperature (°C)	31.1	31.5	33.3	38.9	35.1	33.98
Humidity (%)	29	31	23	20	29	26.4
Wind velocity (m.sec ⁻¹)	1.0	1.4	2.5	3.9	1.5	2.06
Wind direction	320	240	220	40	320	228
Maximum wind velocity (m.sec ⁻¹)	3	4	7	14	6	6.8

From: Directorate of Meteorology and Seismology in Erbil.

For this purpose, similar plastic pots (average diameter 15 cm and height 17 cm) have used and each filled with 4 kg pesticide-treated soil and control treatments remained without pesticide treatment. The pots have provided by a belowcontainer to collect the irrigation water and returnback to the pots. Then the pots have covered with filter papers and they have irrigated daily by tap water at 50% and 100% moisture content Sarmamy, 2018). Statistical (Khudhur and analysis was performed using SPSS version 11.5 and Microsoft Office Excel 2010 and the means were compared using Duncan's Multiple Range test at the level of significant of 0.05 (Khudhur and Sarmamy, 2016).

2.5. Determination of diazinon residues

Soil samples of 20 g have extracted for 10 minutes by ultrasonic agitation with 20 ml of

acetonitrile as recommended by Sáncheza et al. (2003). The suspensions have then centrifuged at 2000 rpm for 10 minutes and the supernatant removed. The centrifugation step has repeated twice to obtain a clear supernatant. The supernatants have then filtered through glass-fiber filters. The acetonitrile fractions were evaporated to dryness at room temperature and the residues were reconstituted in 0.4 ml mobile phase acetonitrile:water (65:35, v:v) by sonicating for 1 minute and then injected to chromatographic column. Diazinon has submitted to chromatographic analysis using high performance liquid chromatography in a reversed-phase C-18 column. The mobile phase has consisted of (65:35, acetonitrile:water v:v) at ambient temperature with injection volume of 100 µl and flow-rate of 1ml.min⁻¹. The detection wavelength of the UV-detector (Perkin Elmer) was 245 nm and the retention time was 2.77 minutes.

Stock diazinon solution (Analytical Standard 99.8% obtained from Sigma Aldrich) was prepared at 500 mg.l⁻¹ with acetonitrile as described by Sáncheza et al. (2003). This stock solution has further diluted to prepare additional working solutions with distilled-deionized water in order to obtain final concentrations of 0.01, 0.1, 1, 10 and 100 µg.ml⁻¹. From working solutions, a calibration standard curve was prepared for soil samples pesticide peak areas. Unknown concentrations of pesticide in the soil samples have calculated by interpolation of sample's peak areas on the calibration graph (Khudhur, 2013).

3.RESULTS AND DISCUSSION

The lowest pH values shown by the combination the combination DS3W2 during the 4^{th} sampling period (Table 5). This is probably because diazinon contains sulfur in its composition, which may use as a source of nutrient in the form of sulfate (SO₄⁻²) by microorganisms, and it must undergo a biological oxidation. This process produces large amounts of acid, and occasionally elemental sulfur has used to decrease soil pH (Hodges, 2012).

		L			
Treatments	1st sampling (24h after	2nd sampling (2 weeks after	3rd sampling (4 weeks after	4th sampling (6 weeks after	5th sampling (8 weeks after
	application)	application)	application)	application)	application)
DS1W1	7.89 ± 0.000^{b}	8.27±0.012 ^{ab}	7.01±0.006 °	$6.57\pm0.000^{\text{h}}$	$6.64 \pm 0.000^{\text{ f}}$
DS1W2	7.95 ± 0.000^{a}	7.95±0.000 ^g	7.27 ± 0.006^{a}	6.84 ± 0.006^{a}	6.73±0.006 ^d
DS2W1	7.82 ± 0.006^{d}	8.13±0.000 ^e	7.16±0.006 ^b	6.55 ± 0.006^{i}	6.75±0.000 °
DS2W2	7.82 ± 0.006^{d}	7.92±0.017 ^h	7.07 ± 0.006 ^c	6.63±0.006 ^g	6.48 ± 0.006^{i}
DS3W1	7.89±0.006 ^b	8.22±0.006 ^c	7.19±0.006 ^b	6.75±0.012 °	6.76±0.000 ^{bc}
DS3W2	7.88±0.006 ^b	7.91±0.012 ^h	7.06±0.012 °	6.46±0.006 ^j	6.53±0.006 ^h
CS1W1	7.50±0.000 ^e	8.29±0.017 ^a	7.09±0.028 °	$6.65 \pm 0.000^{\text{f}}$	6.57±0.000 ^g
CS1W2	7.89±0.006 ^b	8.26±0.006 ^b	7.28±0.012 ^a	6.79±0.003 ^b	6.78±0.012 ^a
CS2W1	$7.38\pm0.012^{\text{ f}}$	8.12±0.000 ^e	7.16±0.012 ^b	$6.58\pm0.000^{\text{h}}$	6.43±0.006 ^j
CS2W2	7.82 ± 0.338^{d}	8.12±0.006 e	7.06±0.006 °	6.71±0.006 ^d	6.77±0.006 ^{ab}
CS3W1	$7.84\pm0.006^{\circ}$	8.19±0.006 ^d	7.20±0.058 ^b	6.69±0.006 ^e	6.49±0.006 ⁱ
CS3W2	7.82 ± 0.000^{d}	$8.08 \pm 0.012^{\text{ f}}$	7.06±0.012 °	6.58±0.012 ^h	6.68±0.012 ^e

Table 5: Effects of different treatments on soil pH values during five sampling periods (Mean±S.E.).

Different significant effects on soil organic matter have shown by the combined treatments

between pesticides, soil orders and soil moisture content (Table 6).

Table 6: Effects of different treatments on soil organic matter (g.kg⁻¹) values during five sampling periods (Mean±S.E.).

Treatments	1st sampling (24h after application)	2nd sampling (2 weeks after application)	3rd sampling (4 weeks after application)	4th sampling (6 weeks after application)	5th sampling (8 weeks after application)
DS1W1	10.349±0.001 ^d	17.249±0.002 ^a	3.450 ± 0.001^{d}	6.899±0.001 ^c	13.799±0.001 °
DS1W2	13.799±0.002 °	6.899±0.003 ^d	6.899±0.002 ^c	6.899±0.002 ^c	10.349±0.001 ^d
DS2W1	13.799±0.002 °	10.349±0.001 °	3.450 ± 0.001^{d}	13.799±0.002 ^b	17.249±0.000 ^b
DS2W2	17.249±0.002 ^b	10.349±0.000 °	3.450 ± 0.001^{d}	13.799±0.001 ^b	17.249±0.001 ^b
DS3W1	10.349±0.000 ^d	17.249±0.000 ^a	13.799±0.001 ^a	17.249±0.000 ^a	20.698±0.001 a
DS3W2	20.698±0.001 ^a	10.349±0.001 °	3.450 ± 0.001^{d}	17.249±0.000 ^a	20.698±0.001 ^a
CS1W1	13.799±0.003 °	6.899±0.003 ^d	13.799±0.000 ^a	6.899±0.002 °	20.698±0.000 ^a
CS1W2	17.249±0.000 ^b	3.450±0.001 ^e	10.349 ± 0.000^{b}	13.799±0.000 ^b	17.249±0.000 ^b
CS2W1	10.349±0.001 ^d	10.349±0.001 °	3.450 ± 0.001^{d}	17.249±0.001 ^a	17.249±0.000 ^b
CS2W2	13.799±0.001 °	3.450±0.002 ^e	10.349±0.000 ^b	17.249±0.001 ^a	17.249±0.002 ^b
CS3W1	6.899±0.001 ^e	13.799±0.000 ^b	3.450±0.001 ^d	6.899±0.001 ^c	17.249±0.000 ^b
CS3W2	10.349±0.001 ^d	10.349±0.001 °	3.450 ± 0.000^{d}	6.899±0.001 ^c	17.249±0.001 ^b

At the beginning of the study, the combined treatment DS3W2 showed the highest level of soil organic matter. During the 2nd sampling the combined treatments DS1W1 and DS3W1 showed

the highest level of soil organic matter. The combination of DS3W1, DS3W2, CS2W1 and CS2W2 during the 4th sampling showed the highest organic matter amounts. At the end of the

5

study, the combinations of DS3W1, DS3W2 and CS1W1 showed the highest organic matter levels.

The combined treatments CS1W2 in the 1^{st} sampling, DS2W2 in the 2^{nd} sampling and DS1W2 in the 4^{th} sampling showed the highest total nitrogen contents (Table 7). This increase may be due to some organisms that were able to

adapt to some re-adjustment and adaptation to the strange environment created by diazinon and later participated in degradation of the organic compound whose cumulative effect translated to nitrogen content increase before the 4 weeks of the experiment as concluded by Shittu *et al.*, (2004).

Table 7: Effects of different treatments on soil total nitrogen (g.kg⁻¹) values during five sampling periods (Mean±S.E.).

6

Treatments	1st sampling (24h after application)	2nd sampling (2 weeks after application)	3rd sampling (4 weeks after application)	4th sampling (6 weeks after application)	5th sampling (8 weeks after application)
DS1W1	3.676 ± 0.000^{ab}	5.996±0.001 ^{bcd}	3.043±0.001 °	11.123±0.002 ^a	2.863±0.002 ^{ab}
DS1W2	2.899±0.002 ^{abc}	7.778 ± 0.000^{abcd}	$2.414\pm0.002^{\circ}$	12.579±0.002 a	1.414 ± 0.001 bc
DS2W1	3.731±0.001 ^{abcd}	7.353±0.001 ^{ab}	4.421±0.000 ^{bc}	2.869±0.002 ^{bc}	0.713±0.002 ^{bc}
DS2W2	1.545±0.001 ^{bcde}	18.421±0.001 a	5.518±0.001 ^{abc}	3.638±0.001 ^{bc}	2.113±0.002 ^{abc}
DS3W1	1.528±0.000 ^{bcde}	4.384±0.001 ^{cd}	16.279±0.000 ^a	2.887±0.000 ^{bc}	0.710±0.003 abc
DS3W2	2.298±0.001 abcde	3.030±0.001 ^{cd}	5.315±0.002 ^{bc}	2.947±0.001 ^{bc}	2.811±0.003 ^a
CS1W1	1.455±0.001 abcde	4.497±0.001 ^{cd}	3.024±0.002 °	2.917±0.001 ^{bc}	2.138±0.001 ab
CS1W2	4.970±0.002 ^a	4.839±0.001 ^{cd}	2.344±0.002 °	2.211±0.002 ^{bc}	2.134±0.001 abc
CS2W1	1.423±0.001 ^{de}	2.201±0.000 ^c	3.653±0.002 ^{abc}	5.072 ± 0.000^{b}	1.426±0.001 abc
CS2W2	0.735±0.001 ^e	2.966±0.002 ^{cd}	14.301±0.000 ^{ab}	2.917±0.000 ^{bc}	2.121±0.002 abc
CS3W1	1.461±0.002 ^{cde}	2.220±0.006 ^{cd}	3.024±0.001 °	0.000 ± 0.001 ^c	1.423±0.002 ^{bc}
CS3W2	0.783 ± 0.001^{e}	9.251±0.001 abc	$2.381 \pm 0.000^{\circ}$	1.471 ± 0.001 bc	0.707 ± 0.001 ^c

Diazinon residues were 1.654, 1.645, 1.654, 1.654, 1.654, 1.654 and 1.649 μ g.g⁻¹ air-dried soil in the combined treatments DS1W1, DS1W2, DS2W1,

DS2W2, DS3W1 and DS3W2 respectively (Table 8).

(1000 m 0								
Treatments	1st sampling (24h after application)	2nd sampling (2 weeks after application)	3rd sampling (4 weeks after application)	4th sampling (6 weeks after application)	5th sampling (8 weeks after application)			
DS1W1	1.654	0	0	0	0			
DS1W2	1.645	0	0	0	0			
DS2W1	1.654	0	0	0	0			
DS2W2	1.654	0	0	0	0			
DS3W1	1.654	0	0	0	0			
DS3W2	1.649	0	0	0	0			
CS1W1	0	0	0	0	0			
CS1W2	0	0	0	0	0			
CS2W1	0	0	0	0	0			
CS2W2	0	0	0	0	0			
CS3W1	0	0	0	0	0			
CS3W2	0	0	0	0	0			

Table 8: Diazinon residues ($\mu g. g^{-1}$ air-dried soil) during five sampling.

The levels were above the maximum residue limit (MRL) (0.5 ppm) set by FAQ-WHO 1970 after 24h of application, similar observations given by Kabir *et al.*, (2008) who revealed that diazinon residue could detected up to 6 days after spread (DAS). Whereas, residues were not detected in the treated soils during the other sampling periods and this may indicate complete degradation of diazinon which may refer to diazinon properties, environmental factors, microbial activities and soil characteristics. The presence of nutrient and microorganisms in the soil has been found to have greatest influence on the biodegradation of pesticides (Massiha *et al.*, 2011). Microorganisms have the ability to interact both chemically and physically with substances

ZANCO Journal of Pure and Applied Sciences 2019

leading to structural changes or complete degradation of the target pesticide molecule. *Pseudomonas* sp. utilizes diazinon as a source of carbon and energy and the enzymes responsible for organophosphorus pesticide degradation is carboxylesterase and phosphotriesterase which attack the P–O alkyl and P–O aryl bonds of organophosphates (Drufovka *et al.*, 2008).

4. Conclusions and recommendations

Diazinon does not show residue even two weeks after application and this indicate that it does not adversely affect soil microbial activity if applied at its recommended application rate, thus there is no need for soil inoculation for its biodegradation, but the problem is with repeated application, which requires biodegradation. Different significant effects on soil characteristics have shown by the interactions among pesticide, orders and soil moisture soil contents. Accordingly, we can recommend the application of pesticides at recommended doses and avoiding repeated application, and recommend the application of organic matter to the soil to improve soil characteristics and increase soil microbial activity, which may have beneficial effects on the persistence and rate of movement of pesticide residues through the soil profile.

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RESEARCHPAPER

Macroinvertebrates as bioindicators of Water Quality of Effluentreceiving Ossah River, Umuahia, Southeast Nigeria.

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ABSTRACT:

Bioindicators are biological organisms that can be used to monitor the quality of the environment. Macroinvertebrates are known to be extremely sensitive to organic pollutants coupled with their wide distribution, ease and minimal cost of sampling which makes them perfect bioindicators. Macroinvertebrate fauna of an effluent-receiving River Southeast Nigeria was studied in 3 stations between January and June 2018; to assess their community structure in relation to effluent discharge and other anthropogenic activities. The modified kick sampling technique and sweeping of aquatic macrophytes with hand net were used in the sampling of the macroinvertebrates. Five taxonomic groups and twenty (20) taxa were recorded; contributing 119 macroinvertebrate individuals. The composition of the taxa showed that non-biting midge, *Chironomus* sphad the highest number (39.5%). In terms of spatial distribution, the highest number of individuals (57) was recorded in station 2 while stations 3 and 2 had 37 and 25 individuals respectively. The macroinvertebrates fauna was dominated by tolerant species (75%). The diversity indices as reflected in Shannon-Wiener index (H) (1.717 - 1.923), Magalef Species Richness (2.769 - 2.968) and Evenness index (0.4285 - 0.6843) were low indicating lower number of species and environmental degradation due to anthropogenic impacts. The physicochemical parameters and macroinvertebrate assemblages showed that the river was adversely impacted by effluent discharge and other anthropogenic activities. The physicochemical parameters showed that station 1 was polluted by cumulative impacts while macroinvertebrate assemblages showed that station 2 was polluted by effluent discharge.

KEYWORDS: Bioindicators, Macroinvertebrates, anthropogenic activities, water quality, effluent DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.2</u> ZJPAS (2019), 31(5);9-17

INTRODUCTION:

Freshwater ecosystems are progressively more subjected to anthropogenic stressors in the forms of chemical and organic discharges, habitat alterations of the water channels and adjoining areas as well as land use changes and climate change (Goldschmidt, 2016). Surface water qualities of aquatic systems in watersheds have been significantly degraded due to anthropogenic activities (Anyanwu, 2012; Amah-Jerry *et al.*, 2017; Mohammed and Bamarni, 2019).

Emeka Donald Anyanwu E-mail: <u>ekadon@yahoo.com</u> **Article History:** Received: 12/04/2019 Accepted: 07/07/2019 Published: 17/10/2019 The use of macroinvertebrates in biological monitoring has shown consistency and reliability in relation to the use of other organisms because of their sensitivity to organic pollutants, 10

wide distribution as well as the ease and minimal cost of sampling (Kalyoncu and Gulboy, 2009; Setiawan, 2009). A number of factors such as water quality, substrate type, sediment and particle size, flow regime, nutrient availability, dissolved oxygen level as well as the prevailing conditions in the watershed determine the community structure of benthic macroinvertebrates (Ward *et al.*, 1995; Buss *et al.*, 2004).

Several studies in lotic environments have used benthic macroinvertebrates for biomonitoring and assessment of aquatic ecosystem health because of their potentials for long term anthropogenic impact monitoring programmes (Simboura et al., 1995; Emere and Nasiru, 2007; Spaak and Bauchrowitz, 2010; Arimoro et al., 2015). The organic pollution of water resulting from domestic and industrial discharges is a common source of perturbation on urban watercourses (Souto *et al.*, 2011). These discharges affect the physical and chemical characteristics of running water systems leading to in the benthic macroinvertebrates changes community structure (Hynes, 1970; Ward et al., 1995).

Ossah River is an urban river that receives industrial effluent from a vegetable oil processing factory and also subjected to other anthropogenic impacts (sand mining, bathing, swimming, washing of clothes and extraction of water for drinking). The objective of this study was therefore to evaluate macroinvertebrate community structure in relation to anthropogenic stressors and water quality.

2. MATERIALS AND METHODS

2.1. Study area and sampling stations

The study area was Ossah Ibeku Community, Umuahia North Local Government Area, Abia State, Southeast Nigeria while the studied section of Ossah River lie within Latitude $05^{\circ}29^{\circ}20.00^{\circ} - 05^{\circ}31^{\circ}40.00^{\circ}N$ and Longitude $07^{\circ}27^{\circ}50.40^{\circ} - 07^{\circ}28^{\circ}548.00^{\circ}E$ (Figure 1). The river was divided into three stations for the purpose of this study. Station 1 is the upstream and control station, located in Ahi Amanso, Ossah community. There are a lot of construction activities going on the area as a result stormwater from the sites discharge into the river. The station is relatively deep (69.3 cm) and the substrate is sandy. Direct activities observed in the station include sand mining, bathing, swimming, washing of clothes and extraction of water for drinking. Station 2 is located at Eziama Ossah, 510m downstream of station 1 and 410m downstream of the effluent discharge point. It is shallow (62.7 cm) and sandy with minimal activities like Station 3 is located at washing and bathing. Umuchime Ossah. 610m downstream of station 2. The station is by a bridge along a road under construction. The station was deep (79.5 cm) with a sandy substrate. Stormwater from the village discharge into this station during the rains; depositing sand on the edge of the river. Human activities observed include fishing, bathing, swimming, washing of motorcycles, tricycle (keke) and clothes as well as extraction of water for drinking.



Figure 1: Map of Umuahia, Abia State, Nigeria showing the sampling Stations of Ossah River.

2.2 Samples collection and analyses

The river was sampled monthly for physicochemical parameters and macroinvertebrate fauna between January and June 2018.

2.2.1 Water Samples

The water samples were collected in sterilized 11itre plastic bottles and transported in ice chests to the laboratory for analysis. Parameters like temperature, pH (Hydrogen ion concentration), Electrical Conductivity and total dissolved solids were determined insitu while Dissolved Oxygen, Biochemical Oxygen Demand, Nitrate and Phosphate were determined in the laboratory with the appropriate methods by American Public Health Association (APHA) (1998).

The Descriptive Statistic Package of Microsoft Excel was used to summarise the data

while one-way Analysis of Variance (ANOVA) was used to test for significant variations among the stations and the post-hoc analysis to identify the sources of significant variations used Tukey's pairwise comparisons (Hammer *et al.*, 2001).

2.2.2 Macroinvertebrate Samples

Macroinvertebrate samples were collected from the sediments using the modified kick sampling technique described by Victor and Ogbeibu (1985). The sediment upstream was disturbed by kicking with foot for about 5 minutes and the macroinvertebrates dislodged were washed into the net placed downstream of the disturbed point. Aquatic macrophytes along the banks of the river were also swept with the hand against the water current and net the macroinvertebrates dislodged were washed into the net (500μ) .

All the samples were preserved with 10% formaldehyde in a plastic container and taken to the laboratory for proper identification. The isolated macroinvertebrates were identified to the lowest possible taxonomic level with the aids of the following taxonomic keys: Pennak (1978), Brown (1994) and Merritt and Cummins (1996). The numbers were counted. Margalef (D) and Shannon (H) indices were used to determine species richness and diversity respectively while Evenness index (E) was used to assess the even distribution of the recorded macroinvertebrates.

3. **RESULTS**

3.1 Water Quality

The summary of physicochemical parameters recorded in Ossah River is presented in Table. 1. The water temperature levels were between 21.0 and 28.0°C. Temperature values were within acceptable limits. All the pH values were acidic ranging from 4.6 to 6.3 and outside acceptable limits for aquatic life (6.5-8.5). Station 1 recorded the lowest mean value (5.4). The electrical conductivity (EC) and Total Dissolved Solids (TDS) exhibited similar trend and the mean values decreased from station 1 to 3. EC ranged from 52.9 to 110.5µS/cm while TDS ranged from 25.7 to 55.3 mg/l. There was significant different (P < 0.05) in the three stations. The values of dissolved oxygen (DO) was between 3.2 and 6.4 mg/l; all the values were lower than the acceptable limit (>6mg/l) except one. The biochemical oxygen demand levels ranged between 1.5 and 4.2 mg/l. All the values were within the acceptable limit (3mg/l) except one. There was a significant difference (P<0.05) between Station 1 and the other two stations. The nutrients occurred in low values and below acceptable limits. Nitrate values ranged from 0.9 to 3.4mg/l. Stations 1 and 3 were significantly different (P< 0.05). A range of 0.5 to 2.7 mg/l was recorded for phosphate and Station 3 was significantly lower than Stations 1 and 2.

3.2 Macroinvertebrate composition, abundance and distribution

The community structure of macroinvertebrates recorded in Ossah River, Umuahia are presented in Table 2. This study recorded a total of 119 macroinvertebrate individuals comprising of five taxonomic groups and twenty (20) taxa. Percentage composition of showed that non-biting the taxa midge. Chironomus sp had the highest abundance (39.5%), followed by mayfly nymph, Cloeon sp. (16%) and Back swimmer, Notonecta sp (7.6%) while the least were leech (Hirudo medicinalis), scud (Gammarus pulex), horsefly larvae (Tabanus sp.), water scorpion (Ranatra linearis) and water strider (Gerris sp.) contributing 0.8%. Spatially, the highest number of individuals was recorded in station 2 (57) while stations 3 and 1 had 37 and 25 individuals respectively.

Assessment of pollution tolerance status showed that most of the macroinvertebrates recorded are the tolerant and very tolerant species (75%) while the sensitive and very sensitive species were 25%. Most of the pollution indicators were recorded in Stations 1 and 2 which witnessed most anthropogenic impacts including effluent discharge. However, non-biting midge (*Chironomus* sp.) recorded their highest number in Stations 2 and 3.

The diversity indices showed that a higher Shannon-Wiener index (H) was recorded in station 1 (1.923) compared to 1.717 and 1.798 recorded in stations 2 and 3 respectively (Table 3). Station 2 had the highest Magalef Species Richness (2.968) while stations 1 and 3 recorded 2.796 and 2.769 respectively. Evenness Index ranged between 0.4285 and 0.6843; with the lowest and highest values recorded in stations 2 and 1 respectively. 12

Parameter	Station 1	Station 2	Station 3	P-value	FMENV.
	x±SEM	x±SEM	x±SEM		2011
Water Temperature, °C	24.6±0.97	25.3±0.44	25.1±0.46	P>0.05	-
	(21.0-28.0)	(24.0-27.0)	(24.0-26.5)		
рН	5.4±0.22	5.6±0.21	5.6±0.19	P>0.05	6.5-8.5
	(4.6-6.0)	(4.8-6.3)	(4.8-6.2)		
Electrical	98.5 ± 4.52^{a}	73.8 ± 4.34^{b}	$58.4 \pm 2.34^{\circ}$	P<0.05	-
Conductivity, µS/cm	(80.7-110.5)	(56.4-84.4)	(52.9-65.9)		
Total Dissolved	49.4 ± 2.06^{a}	36.8 ± 2.33^{b}	29.1±71.24 ^c	P<0.05	-
Solids, mg/l	(41.5-55.3)	(26.8-42.1)	(25.7-33.1)		
Dissolved Oxygen, mg/l	5.1±0.29	5.7 ± 0.26	4.8±0.35	P>0.05	>6
	(3.8-5.7)	(4.7-6.4)	(3.2-5.5)		
Biochemical Oxygen	3.3±0.33 ^a	2.1 ± 0.20^{b}	2.1 ± 0.15^{b}	P<0.05	3
Demand, mg/l	(2.1-4.2)	(1.6-3.0)	(1.5-2.5)		
Nitrate, mg/l	2.6 ± 0.27^{a}	$2.0{\pm}0.21^{ab}$	$1.8{\pm}0.28^{b}$	P<0.05	50
-	(1.7-3.4)	(1.1-2.6)	(0.9-2.5)		
Phosphate, mg/l	2.0 ± 0.25^{a}	$1.8{\pm}0.20^{a}$	$0.7{\pm}0.08^{b}$	P<0.05	3.5
- •	(0.9-2.7)	(0.1-2.3)	(0.5-1.0)		

Table 1: Summary of Physico-chemical Parameters of Ossah River (with range in parenthesis)

(U.9-2.7) (U.1-2.3) (U.5-1.0) a, b, c = Means with different superscripts across the rows are significantly different at p<0.05; SEM= Standard Error of Mean; FMEnv. National Environmental (Surface and Groundwater Quality Control) Regulations

Table 2: Species composition, abundance and distribution of macroinvertebrate fauna encountered in Ossah River, Umuahia, Southeast Nigeria

Group	Family	Taxa	Station 1	Station 2	Station 3	Percentage	Pollution
Annalida	Hirudinea	Leech	0	1	0	0.8	Very Tolerant
Annenua	muunea	(Hirudo medicinalis)	0	1	0	0.8	very rolerant
Arachinida	Hydracarindae	(Hydrachnasp)	2	1	0	2.5	Sensitive
	Pisauridae	(<u>Invariacinia</u> sp.) Fishing spider	1	2	1	3.4	Very Tolerant
Mollusca	Pleuroceridae	Gilled snail (<i>Pleurocera</i> sp.)	0	0	2	1.7	Very Tolerant
	Physidae	Pouched snail (<i>Physis</i> sp.)	0	3	3	5	Very Tolerant
Crustacea	Gammaridae	Scud (<u>Gammarus pulex</u>)	1	0	0	0.8	Very Tolerant
Insecta							
Megaloptera	Sialidae	Alderfly larvae (Sialis sp.)	2	0	1	2.5	Sensitive
Diptera	Chironomidae	Non-biting Midge (Chironomus sp.)	1	30	16	39.5	Very Tolerant
	Tipulidae	Cranefly larvae (<i>Tipula</i> sp.)	1	2	0	2.5	Tolerant
	Tabanidae	Horsefly larvae	0	1	0	0.8	Tolerant
Coleoptera	Dytiscidae	Predacious diving beetle	3	0	1	3.4	Very Tolerant
Hemiptera	Nepidae	Water scorpion	0	1	1	1.7	Very Tolerant
		(Nepa cinerea) Water scorpion (Ranatra linearis)	0	0	1	0.8	Very Tolerant
	Gerridae	Water strider	1	0	0	0.8	Tolerant
	Notonectidae	(<i>Back swimmer</i>	0	9	0	7.6	Very Tolerant
	Corixidae	(<u>Ivoionecia</u> sp.) Water boatman	0	2	2	3.4	Very Tolerant
Ephemeroptera	Baetidae	(Closen sp.)	10	1	8	16	Very Sensitive
Coleoptera	Elmidae	(Clocon sp.) Riffle beetle	0	3	0	2.5	Sensitive
	Gyrinidae	Whirligig beetle	0	1	1	1.7	Tolerant
Odonata	Libellulidae	Dragonfly Larvae	3	0	0	2.5	Sensitive
	Total	inter onnu op	25	57	37		

Indices	Station 1	Station 2	Station 3
Number of Taxa (S)	10	13	11
Number of Individuals	25	57	37
Shannon (H)	1.923	1.717	1.798
Evenness (E)	0.6843	0.4285	0.5491
Margalef (D)	2.796	2.968	2.769

Table 3: Diversity, species richness and evenness indices of macroinvertebrate fauna recorded in Ossah River, Umuahia, Southeast Nigeria.

4. DISCUSSION

The recorded water temperatures were all within acceptable limits. Naturally, temperatures in water bodies vary daily and seasonally due to natural and anthropogenic activities that could affect surface water temperatures (Gebreyohannes *et al.*, 2015). Water temperature is an essential water quality parameter; it directly affects physiological activities of aquatic organisms. The oxygen need of aquatic animals tend to increase abnormally with every 10°C increase within the animal's tolerable temperature range (Halim *et al.*, 2018).

The pH is very important in water quality assessment because it influences other chemical reactions (Fakayode, 2005). pH values recorded were outside standard limits and could be attributed to geogenic influence (Anyanwu and Ihediwah, 2015) and anthropogenic activities (Amah-Jerry et al., 2017). Changes in pH can result from impacts of industrial pollutants; photosynthesis, respiration and decomposition also contribute to pH fluctuations due to their influences on CO_2 levels (Bellingham, 2012) while Radojevic and Bashkin (1999) observed that pollution result in extremes of pH in water. Consequently, changing the pH levels in every ecosystem affects all living organisms positively or negatively.

Total dissolved solids values were all within acceptable limit though higher TDS levels were recorded in Stations 1 and 2, indicating intense cumulative anthropogenic impacts in and around the stations. Clark (1994) observed that the most environmentally damaging impacts and associated social consequences sometimes may not be a product of a particular activity but as a result of complex interaction of many activities over time in the environment.

The electrical conductivity values were within acceptable limit and exhibited the same trend as TDS. Stations 1 and 2 were higher compared to station 3 and could be as a result of cumulative impact. Muhammad et al. (2013) reported that pure water is a poor conductor of electricity and the electrical conductivity of water increases with increase in ionic concentrations resulting from pollution. Pond et al. (2008) reported that increased dissolved ions could be responsible for poor benthic macroinvertebrate structure and declining mayfly communities' populations in streams receiving mining discharges.

All the recorded Dissolved Oxygen values were lower than acceptable limits except one. Murphy (2007) observed that animal and plant wastes as well as sewage are some of the ways by which organic materials can be discharged into natural water and the decomposition process of these organic materials by microbes require oxygen (Mahre *et al.*, 2007). The functioning and survival of most biological communities can be negatively affected when DO levels fall below 5mg/l while death of most fish could occur at DO levels lower than 2mg/l (Chapman, 1996).

Biochemical Oxygen Demand (BOD) is an important parameter of water used in determining the pollution load of freshwater bodies (Bhatti and Latif, 2011). The mean BOD value of Station 1 exceeded limit while Stations 2 and 3 were within the acceptable limit. The BOD values recorded in Station 1 were relatively higher and could be as a result of cumulative impact (Clark, 1994).BOD is an indication of organic pollutant in the river and therefore affects water quality (Nwankwo et al., 2014). Chapman (1996) observed that unpolluted waters usually have BOD values of 2 mg/l or less while values up to 10 mg/l or more are usually with rivers receiving associated effluents. especially close to discharge point. On the other hand, Radojevic and Bashkin (1999) reported that self-purification in rivers is more effective at BOD value of less than 4, but less effective at values greater than 4 mg/l.

Nitrate levels in water are often less than 1 mg/l; surface waters can have values up to 5 mg/l when influenced by human activities and values in excess of 5 mg/l are usually indicative of pollution from anthropogenic sources (Chapman, 1996). The recorded values were within acceptable limit

though Station 1 was significantly different from Station 3; which could be attributed to cumulative impacts.

Mandal et al (2012) observed that phosphate contamination comes from anthropogenic activities such discharge of detergent contaminated sewage, direct washing of clothes in water and runoffs laden with fertilizers and pesticides. Phosphate values recorded were within acceptable limit though Stations 1 and 2 were significantly different from Station 3. This could be attributed to cumulative impacts and effluent discharge. Phosphate values in most natural surface waters usually range between 0.005 and 0.020 mg/l (Chapman, 1996). Nutrients such as nitrogen and phosphates compounds have been implicated in the eutrophication of water bodies because they stimulate growth of algae and other photosynthetic aquatic life (Dubey et al., 2012).

The analysis of physicochemical parameters is an important method of getting reliable on-the-spot data on water quality and an insight into the nature of the contamination. Combination with additional biological evaluation is needed (Goldschmidt, 2016). On the other hand, since aquatic organisms spend most part of their life under certain conditions of the environment, biomonitoring provides for the quick integration of all impacts and enables a collective analysis of contributing factors as well as their complex interactions with reliability and cost-effectiveness (Valdecasas and Baltanás, 1990).

No new organism was recorded in this study (Olomukoro and Ezemonye, 2000: Olomukoro and Dirisu, 2012; Anyanwu and Jerry, 2017). The number of individuals was lower while the number of taxonomic groups and taxa was higher when compared 168 individuals, 4 taxonomic group and 7 taxa recorded in a suburban River in Umuahia (Anyanwu and Jerry, 2017). The species composition was dominated by group insecta as opposed to the group mollusca recorded by Anyanwu and Jerry (2017). Station 2 had the highest species richness while station 1 had the lowest and this could be as a result of the effluent discharged upstream of station 2. United Nations (2014) reported that although pollution occurs at discharge points along the river, its impacts are usually felt by downstream populations and ecosystems, because pollutants

are distributed throughout the river network. The extent of impacts depends on the self-purification capability of the river through natural processes of dilution by runoff and degradation by micro-organisms (Wen *et al.*, 2017).

Most of the species recorded were dominated by pollution tolerant groups especially Chironomus larvae. Chironomus spp are species indicators of organically polluted waters and can survive even in low oxygen waters (Mariantika and Retnaningdyah, 2014). The high number of Chironomus spp. recorded in station 2 is an indication of imbalance in the ecosystem resulting from instability in the water. Only certain types of organism like Chironomus sp. can thrive in such unstable environment because of their ability to cope with level of organic pollution (Mariantika and Retnaningdyah, 2014) and develop large populations (Kucuk, 2008). This finding was not in line with Anyanwu and Jerry (2017) that recorded the high species richness in station 3 instead station 2 that was most impacted; attributed to substrate stability. Station 3 recorded the lowest species richness but dominated by Chironomus larvae which could be attributed macroinvertebrate drifting from station 2. Naman et al (2016) reported that the passive or active downstream movement of aquatic invertebrates known as invertebrate drift is a basic process in streams ecology.

Station 1 had highest evenness value as in case of Anyanwu and Jerry (2017) while station 2 was the least because one of the species (Chironomus sp) recorded was dominated by many individuals. The Shannon diversity indices were of higher value than that of Anyanwu and Jerry (2017). Station 1 had the highest value while station 2 had the lowest as in the case of Anyanwu and Jerry (2017). This may be as a result of the effluent discharged in close proximity of the station 2. Kucuk (2008) reported that when effluents are discharged into waters, community structure and density change. The low diversity recorded as reflected in Margalef, Shannon-Wiener and Evenness indices may be attributable to lower number of species and environmental degradation due to anthropogenic impacts, apart from other biotic factors (Ravera, 2001).

5. CONCLUSION

Some physicochemical parameters (pH, DO and BOD) and macroinvertebrate fauna of Ossah River showed that the river was adversely

impacted by effluent from a vegetable oil processing factory in the vicinity and other anthropogenic activities in the watershed. The physicochemical parameters showed that station 1 was polluted by cumulative impacts from other anthropogenic activities while macroinvertebrate assemblages showed that station 2 was polluted by effluent discharge.

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Conflict of Interest

There is no conflict of interest and writing assistant was not used in the preparation of this manuscript.

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RESEARCH PAPER

Effect of Phosphorus Fertilization on Phytoremediation efficacy of Heavy Metals by Wheat and Bean Plants

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ABSTRACT:

A pot experiment was con ducted at the experimental farm of Halabja Technical College of Applied Sciences in July 2017 to July 2018, 2 km north of Halabja city in the longitude 45°59'40"E and latitude 35°10'28"N. To determine the impact of the use of phytoremedial techniques in the governorate of Halabja on contaminated soil with heavy metals. Soil samples were collected randomly from 15 sites within the solid waste dumping area and control sample, These samples were analyzed for chemical characteristics and concentration of certain heavy metals such as Lead (Pb), Cadmium (Cd), Nickel (Ni), Zinc (Zn) and Iron (Fe) using ICP-MS (inductively coupled plasma mass spectrometer. The summary of the main results was as follow maximum concentration of heavy metals from soil in bean plant were (Pb and Ni) were (55.4 and 50.1 %) for (TSP ₄₀), (Fe and Cd) were (49.7 and 44.5 %) for (TSP ₁₆₀) and uptake of Zn increased by (45.6 %) for (TSP ₁₂₀) compared to control treatment, while wheat plant extracted heavy metals (Pb, Cd, Ni, Fe, and Zn) by (60.6, 53.4, 37.1, 37.1, and 26.6 %) respectively for (TSP ₁₆₀) compared with control treatment. These results evidence that Phytoextraction of (Pb, Cd, Ni, Zn, and Fe) by (Bean) plant was much greater than that by (Wheat) from municipally polluted soil after adding phosphorus fertilizer to the soil. This study confirms that (Bean) as one of the plants that could be employed in phytoremediation of soil polluted by heavy metals.

KEY WORDS: Phytoremediation, Heavy metals, Soil pollution. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.3</u> ZJPAS (2019), 31(5);18-27 .

1.INTRODUCTION :

Pollution of the environment by toxic metals such as Pb, Cd, Ni, Zn, and Fe has become a worldwide crisis, affecting agriculture products and contributing to bioaccumulation and biomagnifications in the food chain. Recently, research groups have found out that certain toxic metals can remain in the environment for a long time and may eventually bioaccumulation to higher concentrations that could affect human health (Dipu *et al.*, 2011).

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Phytoremediation is a green solution to the problem of heavy metal contamination "Phytoremediation refers to the use of plants and associated soil microbes reduce to the concentrations or toxic effects of pollutants in the environments" (Greipsson, 2011). However the phytoremediation can be used for reduce or eliminate of heavy metals, radionuclides as well as for organic pollutants (like polynuclear aromatic hydrocarbons, pesticides and polychlorinated biphenyls) and other chemical materials in the polluted sites. Green plants have a great ability to uptake pollutants from the contaminated environment and accomplish their detoxification mechanisms bv number such as phytovolatilization, phyto-degradation, and phyto-

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accumulation (also called phyto-extraction) in this mechanism; plant roots sorb the pollutants along with other nutrient elements and water.

In addition, plants can growing fast and have high-biomass product like poplar, Jatropha willow might be used both and for and energy production phytoremediation (Abhilash *et al.*, 2012). Because of the accumulation of higher levels of heavy metals in the soil may restrict the plant growth. The aim of the study was determining the efficiency of two types of crops such as Wheat (Triticum aestivum-Aras) and Bean (Vicia faba-Somar) for clean-up polluted soil by heavy metals such as Pb, Cd, Ni, Zn, and Fe or reduce the level of these elements by using phytoremediation. There for these two

plants are the most strategical and economical plants.

2. MATERIALS AND METHODS

2.1 Descriptions of the study area of (MSW)

The municipal solid waste (MSW) site is located in the north west of Halabja city. It has a total area of $10000m^2$ in the longitude $45^{\circ}56'57.65''E$ and latitude $35^{\circ}12'28.08''N$ figure (1) and (2). The disposal place used for all kinds of municipal, industrial and hospital wastes. Also, different kinds of hazardous and recyclable waste including glass, plastic, batteries, and metal waste disposed into dump site.



Fig.1. Sites of Halabja Open Dump.



Fig. 2. Location map of the study area of Halabja Open Dump Site

20

2.2 Soil sampling and analysing

Soil sampling was carried randomly from 16 sites by using an auger at different directions and depths (surface layer 0-10, subsurface layer 10-20, and bottom layer 20-30) cm, within the solid waste dumping area in July 2017. Then labelled and sealed into plastic bags and transported to the lab. The samples were air dried in stainless steel trays and milled and sieved to obtain a <2 mm fraction for determine the chemical analysis then stored in polyethylene bags for laboratory analysis. The chemical analyses of the soil samples were done as shown in the table (1). These analyses performed as follows: The pH of soil extract (1:5) was measured by HANA pHmeter, Model pH211 microprocessor pH-meter using the procedure of (Estefan et al., 2013). The electrical conductivity was measured using ECmeter model BG43C according to (Estefan *et al.*, 2013). Soil organic matter was determined by Loss On Ignition method (LOI) according to (Margesin and Fchinner, 2005). Total Calcium Carbonate (CaCO₃) % as described in Rowell (1996). Total heavy metal concentrations in soil and plant samples (mg kg⁻¹) were analysed by the ICP-MS method (Masson *et al.*, 2010).

Table 1: Some chemical properties of the studied soils

Location No.	Depth (cm)	ОМ	Total CaCO ₃	EC mScm ⁻¹	рН	Pb	Cd	Ni	Zn	Fe
		%				(mg kg	⁻¹)			
	0-10	10.7	9.89	0.24	7.76	31.0	0.52	109	80.3	31372
1	10-20	11.0	7.89	0.23	7.95	36.5	0.63	118	85.5	34650
-	20-30	12.5	10.7	0.93	7.51	34.0	0.51	95.2	133	27808
	0-10	9.73	10.3	0.20	8.02	18.5	0.39	102	75.0	28381
2	10-20	9.53	11.4	0.18	8.12	12.9	0.41	100	63.5	28798
4	20-30	8.83	9.69	0.16	8.22	11.2	0.37	102	61.8	29008
	0-10	8.85	10.3	0.21	8.08	16.9	0.36	97.6	65.1	27715
3	10-20	9.27	11.8	0.45	7.81	14.3	0.42	105	67.9	30338
C .	20-30	9.69	10.9	0.67	7.80	12.2	0.36	100	64.1	28409
Control sample		2.55	27.7	0.10	8.22	3.10	0.08	11.1	14.2	10221
Minimum		8.83	7.89	0.16	7.51	11.2	0.36	95.2	61.8	27715
Maximum		11.0	11.8	0.93	8.22	36.6	0.63	118	133	34650
Mean		10.0	10.3	0.36	7.92	20.9	0.44	103	77.4	29609

2.3 Pot experiments

Soil samples were collected from landfill air dried in stainless steel trays and milled using a plastic hummer and sieved to obtain a 4 mm fraction then put in to 7 kg plastic pots. Each pot

(25cm height, 25cm top diameter, and 15cm bottom diameter) packed with the 6 kg of dry polluted soil. On 4/11/2017, 13 seeds of wheat

(*Triticum aestivum-Aras*) and 5 seeds of Bean (*Vicia faba-Somar*) were planted in each pot.

The experiment was designed by factorial CRD by using 5 levels of triple super phosphate 46% P_2O_5 fertilizer (0, 40, 80, 120, and 160 mg TSP kg⁻¹ soil). The plant samples (Wheat and Bean) were collected on 13/5/2018 and 3/6/2018 respectively.

2.4 Plant sampling and analysing

The plant samples (Wheat and Bean) were collected on 13/5/2018 and 3/6/2018 respectively. Plant grains were harvested, and then washed with distilled water to clean up from the soil then oven dried at 65 °C for 72 hours. After weighing and crushed with a stainless steel mill, and protected in a polyethylene bag in a form of powder in a dry place for analysis (Mekeague, 1978). 0.3gm of powdered dried samples (seeds) digested by using (4ml HNO₃ and 2ml H₂O₂) for bean and wheat seeds and using only 6ml H₂O₂ digested grain samples then stored in labelled test tube, at room temperature until elemental analysis (Masson *et al.*, 2010).

2.5 Statistical analysis

The statistical analyses were conducted by using both statistical analysis system (SAS) and software program and the Person's correlation coefficient. Analysis of variance (ANOVA) was used, and then analysis variance means were compared by the Least Significant Differences (LSD) at ($p \le 0.05$) level among treatments (Sas, 2002).

3. RESULTS AND DISCUSSION

3.1. Effect of different levels of (TSP) fertilizer on heavy metals concentration in wheat (*Triticum aestivum-Aras*) grains.

The application of triple supper phosphate (TSP) fertilizer has a significant effect ($P \le 0.05$) on heavy metals (Pb, Cd, Ni, Zn and Fe) uptake in the wheat grains table (2) and figure (3). TSP supply significantly increased (Pb, Cd, Ni, Zn and Fe) uptake in the wheat grains. Generally, phosphorus fertilizers that can derived from sedimentary rocks contains the highest concentration of most of heavy metal(loid)s like Th, As, U, Zn and Cd (Alloway, 2013). The concentration of metals in soil, soil pH, organic

matter, hydrous ferric oxides and density and type of soil colloids influence the heavy metal availablity (Chibuike and Obiora 2014).

application (TSP) significantly The increased Pb uptake by wheat grains was (18.71, 12.93, 7.471 and 6.265 mg kg⁻¹) in the (TSP 160, 120, 80, 40) treatments higher compared to (TSP 0.0) treatment. The highest value of Pb in wheat grains (18.71 mg kg⁻¹) was recorded in (TSP 160) treatment, while the lowest value $(5.254 \text{ mg kg}^{-1})$ was obtained in (TSP 0.0) treatment. These may be referred to the applied P fertilizer which contain a wide range of Pb (<5.2%) (EPA, 1999). The variation of Pb content of plants tissue is the presence of affected by geochemical anomalies, genotype ability to accumulate Pb, pollution and seasonal variation (Kabata and Pendias, 2001).

Cd uptake significantly increased by the effect of (TSP) fertilizer in wheat grains compared to control sample. Cd uptake were (0.191, 0.084, 0.048 and 0.040 mg kg⁻¹) in the (TSP 160, 40, 120, 80) treatments higher compared to (TSP 0.0) treatment. The highest value of Cd in wheat grains $(0.191 \text{ mg kg}^{-1})$ was obtained in (TSP 160) treatment, while the lowest value $(0.034 \text{ mg kg}^{-1})$ was recorded in (TSP0.0) treatment. The P fertilizer which products naturally contain the higher amount of Cd and Zn (Alloway, 2013). Increase mobility of Cd in soil due to formation soluble complexes with organic matter (Mölders, 1999).Cadmium is a very mobile and can accumulate in plant tissues in large amount without showing any phytotoxic symptoms. Therefore it is considered one of the most dangerous heavy metals to human health (Moustakas et al., 2001). The uptake of Ni significantly increased by the effect of (TSP) fertilizer in wheat compared to control sample. It means that the application of higher levels of phosphorus may cause the solubilisation of the nickel. The uptake of Zn in wheat grains ranged between $(474.88-886.26 \text{ mg kg}^{-1})$ was higher $(886.26 \text{ mg kg}^{-1})$ at (TSP 160) treatment, while the lowest value (474.88 mg kg⁻¹) was recorded from (TSP 0.0). The application (TSP) significantly increased Zn uptake by wheat grains. Wheat grain uptake of Zn was (886.26, 557.94, 548.01 and 511.65 mg kg⁻¹) in the (TSP 160, 120, 80, 40) treatments higher compared to (TSP 0.0)

treatment. The application of TSP fertilizer has a significant effect ($P \le 0.05$) on Fe uptake in wheat grains. Application of TSP fertilizer significantly increased Fe uptake in wheat grains. The highest value of Fe in wheat seeds (627.42 mg kg⁻¹) was recorded from (TSP 160), while the lowest value $(264.99 \text{ mg kg}^{-1})$ was recorded from (TSP 0.0) treatment. These results agree with (Ali et al., 2014) which reported that the application of P caused an increase in concentration of cationic metals such as Fe and Mn due to reducing the soil pH. In the present study results indicated that increase of heavy metals concentration cause decrease plant growth this is agree with (Ahmed and Abd-Alhamid, 2019, and Ahmed and Khoshnaw, 2019).

3.2 Effect of different levels of (TSP) fertilizer on heavy metals concentration in bean grains (*Vicia faba-Somar*) plant.

The application of (TSP) has significant effect ($P \le 0.05$) on the uptake of all studied heavy metals (Cd, Ni, Zn and Fe) by bean grains table (3) and figure (3). This may be due to increase in plant biomass this is agree with (Ahmed and Khoshnaw, 2019) reported that soil P application had significant influence on dry matter production of bean plants. This increase is attributed to adequate P supply which an essential nutrient element for plant growth due to its important role in plant biological processes like the creation and division of living cells in the transfer of genetic materials and protein synthesis, and ion transport across cell walls (Havlin, et al., 1999). But the application of (TSP) has no significant change on (Pb). The uptake of Pb by bean grain was (7.384, 6.99, 6.846 and 5.124 mg kg⁻¹) in the (TSP 80, 160, 40, 120) treatments and $(6.487 \text{ mg kg}^{-1})$ in (TSP 0.0) treatment. The highest value of Pb in bean grains (7.384 mg kg⁻¹) was recorded in (TSP 80) treatment in wheat plant, while the lowest value (5.124 mg kg⁻¹) was obtained in (TSP 120) treatment, this results disagree with (Ahmed and Khoshnaw, 2019) lead accumulated and only small portion of this metal was transported to the bean tissue. The Pb balance in different ecosystem shows that the input of this metal highly exceeds its output (Kabata and Pendias, 2001). The grain uptake of Cd significantly increased by the effect of (TSP) fertilizer in bean grains compared to control sample. Cd uptake were (0.153, 0.115, 0.107 and 0.067 mg kg⁻¹) in the (TSP 160, 120,

The uptake of Ni in bean grains ranged between (52.95-95.87 mg kg⁻¹) the highest value (95.87 mg kg⁻¹) was recorded from the (TSP 160) treatment, while the lowest value (52.95 mg kg⁻¹) was obtained from (TSP 40) treatment, it means that the synergistic relations exist between levels of (TSP) fertilizer and Ni uptake in bean grains. The bean grain uptake of Ni significantly increased by the effect of the applied (TSP) compared to control sample. Generally, the concentration of Ni in the solution of heavy metal contaminated soils is higher depending on the soil total concentration, source of Ni, and age of pollution (Alloway, 2013).

The application (TSP) significantly increased Zn uptake by bean grains. Bean grain uptake of Zn was (1585.01, 1486.28, 1115.46 and 646.46 mg kg⁻¹) in the (TSP 120, 160, 80, 40) treatments higher compared to (TSP 0.0) treatment. The uptake of Zn in bean grain was higher (1585.01 mg kg⁻¹) at (TSP 120) treatment, while the lowest value (646.46 mg kg⁻¹) was recorded from (TSP 0.0) treatment. Cd and Zn are movable and have the potential to pollute the food chain due to higher bioaccumulation factor (Saha et al., 2017). These results disagree with (Ahmed and Khoshnaw, 2019) reported that decrease the Zn content in plant body by increasing levels of P fertilizer.

Application of (TSP) fertilizers significantly increased Fe uptake in bean grains. The highest value of Fe in seeds (1580.45 mg kg ¹) was recorded from (TSP 160), while the lowest value (578.94 mg kg⁻¹) was recorded from (TSP 40), this result disagrees with those recorded by (Edmeades, 2003) stated that the application of high amount of P may lead to nutrient imbalance then decrease in iron concentration. The (TSP) supply generally increased (Pb, Cd, Ni, Zn, and Fe) concentrations in bean grains this may be due to decrease the soil pH by the effect of TSP fertilizer resulting increase the availability of heavy metals (Kabata-Pendias and Pendias, 2001).

Table 2: The effect of different levels of triple super phosphate (TSP) on heavy metals concentration (mg kg⁻¹) in grain and the heavy metals uptake (mg pot⁻¹)

by wheat plant.

Part of plant	TSP mg kg ⁻¹	Pb	Cd	Ni	Zn	Fe	Grains Dry Matter Weight	Pb	Cd	Ni	Zn	Fe
		Conc. (mg	Conc. (mg kg ⁻¹)					Uptake (m	ng pot ⁻¹)			
	0	1.215 ^a	0.008 ^b	1.026 ^c	109.8 ^a	61.27 ^a	4.325 ^d	5.254 ^c	0.034 ^b	4.437 ^c	474.88 ^b	264.99 ^c
	40	1.123 ^a	0.015 ^a	1.175 ^b	91.71 ^{ab}	57.34 ^{ab}	5.579 ^{cd}	6.265 [°]	0.084 ^b	6.555 ^b	511.65 ^b	319.89 ^{bc}
Wheat Grains	80	1.115 ^a	0.006 ^b	1.159 ^b	81.78 ^b	53.17 ^b	6.701 ^{bc}	7.471 [°]	0.040 ^b	7.766 ^b	548.01 ^b	356.29 ^b
	120	1.868ª	0.007 ^b	1.153 ^b	80.64 ^b	56.83 ^{ab}	6.919 ^b	12.93 ^{ab}	0.048 ^b	7.978 ^b	557.94 ^b	393.20 ^b
	160	1.669ª	0.017 ^a	1.373ª	79.06 ^b	55.97 ^{ab}	11.21 ^a	18.71 ^ª	0.191 ^ª	15.391 ^ª	886.26 ^ª	627.42 ^a

24

Table 3. The effect of different levels of triple super phosphate (TSP) on heavy metals concentration (mg kg⁻¹) in grain and the heavy metals uptake (mg

pot⁻¹) by bean plant.

Part of plant	TSP mg kg ⁻¹	Pb	Cd	Ni	Zn	Fe	Seeds Dry Matter Weight	Pb	Cd	Ni	Zn	Fe
	Conc. (mg kg ⁻¹)						(g)	Uptake (m				
	0	0.496 ^a	0.006 ^a	4.258ª	55.58ª	46.04 ^b	13.08 ^d	6.487 ^a	0.078 ^d	55.69 ^c	726.98 ^c	602.20 ^{cd}
Bean Seeds	40	0.506 ^a	0.005 ^{ab}	3.914 ^{ab}	47.78 ^b	42.79 ^b	13.53 ^d	6.846 ^a	0.067 ^d	52.95 ^c	646.46 ^c	578.94 ^d
	80	0.343 ^{ab}	0.005 ^{bc}	3.555 ^{bc}	51.81 ^{ab}	39.51 ^b	21.53 ^c	7.384 ^a	0.107 ^c	76.53 ^b	1115.46 ^b	850.65 ^{bc}
	120	0.177 ^b	0.004 ^c	3.134 [°]	54.75 ^{ab}	35.51 ^b	28.95 ^a	5.124 ^a	0.115 ^b	90.72 ^a	1585.01 ^a	1028.01 ^b
	160	0.274 ^b	0.006 ^a	3.757 ^{abc}	58.24 ^a	61.93 ^a	25.52 ^b	6.99 ^a	0.153 ^a	95.87 ^a	1486.28 ^a	1580.45 ^a



Fig. 3. The relationship between levels of applied phosphorus mg $P_2O_5 \text{ kg}^{-1}$ soil and heavy metals uptake mg kg⁻¹ in grain. Δ Wheat TSP

• Bean TSP

3.3.The efficiency comparison of wheat and bean plants on phytoremediation of the polluted soil

Phytoavailability of heavy metals depends upon different physico-chemical properties of the soil, such as organic C and pH, organic C responsible for the release higher quantity of cationic heavy metals and consequently results into more holding of heavy metals in the soil with lower availability to the plant (Singh et al., 2010). Variation in element concentrations between plant species indicated that the ability to accumulate trace elements differs between various types of plants and among various plants on the same species (Säumel et al., 2012). In general the application of (TSP) fertilizer accumulated higher amount of heavy metals in bean tissues compared to wheat plant and increase the concentration of (TSP) increased heavy metals uptake by wheat and bean plants. The overall results regarded that application of the higher amount of phosphorus cause an increase in the heavy metal concentration in the wheat and bean plant parts, probably due to the positive interaction between cationic metals and applied phosphorus in the soil. Because increased applied phosphorus lowered the soil pH and increased the extractable (Pb, Cd, Ni, Zn and Fe), causing these metals to be highly soluble in the soil. Similar results were testified by (Shuman, 1988).

These results disagree with (Ahmed and Khoshnaw, 2019) reported that application of the higher levels of phosphorus caused a decrease in the heavy metals concentration in the wheat plant parts, and with (Haldar and Mandal, 1981) which declared that the addition of P caused a significant decline in concentration of Fe. These results evidence that phytoextraction of (Pb, Cd, Ni, Zn and Fe) by bean plant was much higher than that by wheat plant from municipally contaminated soil after adding phosphorus fertilizer to the soil.

4. CONCLUSIONS

The application of the higher amount of phosphorus cause an increase in the heavy metal uptake in the wheat and bean grains, probably due to the positive interaction between cationic metals and applied phosphorus in the soil. The phytoextraction of (Pb, Cd, Ni, Zn, and Fe) by bean plant was much higher than that by wheat from municipally contaminated soil after adding phosphorus fertilizer to the soil.

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ZANCO Journal of Pure and Applied Sciences 2019

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RESEARCH PAPER

Heavy metals evaluation in soil of agricultural field around a pond of gas plant in the Kurdistan Region of Iraq.

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ABSTRACT:

This study was carried out to evaluate the limits of the heavy metals in soil around a produced water pond and the effect of the produced water on soil heavy metals in a gas facility in the Kurdistan Region of Iraq. The heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb) were analyzed from soil samples taken from different distances of (5, 10, 15, 20 and 25) meters from the pond. The experiment was designed in complete randomized design with 4 replications. The results showed that there were significant differences of mean values for some of the heavy metals in relation to the distances (Ni, Zn, Cu, Pb, Co). The limits of the heavy metals were then compared to maximum permissible limit (MPL) values of the heavy metals in soil by World Health Organization and Environmental Baseline Survey. It was evident that some of the heavy metals were within the normal range (Pb, Hg, Cd and Cu) and the others exceeded the normal range in the soil (Zn and Cr).

KEY WORDS:Heavy metal: Produced water: Soil, Oil and gas facility. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.4</u> ZJPAS (2019) , 31(5);28-35.

1. INTRODUCTION :

Heavy metal contamination and accumulation is a serious problem around the world due to the potential threat to food safety and its detrimental effects on human and animal health. (Morais et al., 2012) Heavy metals are natural constituents of soil, therefore they can be find naturally in soils and rocks with a variety of regular concentrations in soils, rocks and water (Bello. *et al.*, 2016).Human doings like industrial, agricultural, domestic, petroleum and other man made practices have effect to increase the levels of heavy metals to toxic levels when likened to those

Rawa Hatam Hama E-mail:<u>Rawahatam91@gmail.com</u> **Article History:** Received: 14/04/2019 Accepted: 21/07/2019 Published: 17/10/2019 effects from lithological processes(Pam. et al., 2013, Siti Norbaya Mat Ripin, 2014). Soil may become polluted by the addition of heavy metals hydrocarbons. spillage of over wastewater irrigation, disposal of metal wastes, leaded gasoline and paints, pesticides, fertilizers and sewage sludge (Yaylalı-Abanuz, 2011, Velea et al., 2009). It is important to evaluate the heavy metals in soil before cropping and harvesting crops. Heavy metal may pose a huge risk on human, crop, animal and water which may come from the oil and gas production activities such as drilling, construction, production, transportation and utilization. When using the contaminated soil to produce food by various crop plants, as the easy entry of these elements in the food chain which increases the risk to human health(Khudhur, et al., 2016). Heavy metals threaten human health through vegetable consumption and chronic low level intake of soil heavy metals through ingestion

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or inhalation (Abou-Shanab et al., 2007). Chronic exposure to Cd can have a harmful effect such as lung cancer, chronic effect of As include kidney and skin cancer and exposure to Pb may lead to gastrointestinal colic (Singh and Kalamdhad, 2011). Some heavy metals known as non-toxic heavy metal, uptake of excessive amount of those heavy metals are not known while but low intake of some heavy metals have adverse effect on human health such as zinc, the most important elements must disappeared from the soil are lead and cadmium, as they are very significant impact on human health(Toma., 2008)Heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition and pH, as many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor and activators of enzyme reactions e.g., informing enzymes/substrate metal complex (Emurotu and Onianwa, 2017). Plants are often sensitive both to the deficiency and to the excess availability of some heavy metal ions as essential micronutrient, while the same at higher concentrations and even more ions such as Cd, Hg, As are strongly poisonous to the metabolic activities (Tiwari and Lata, 2018). Heavy metals establish an indistinct group of inorganic chemical hazards, and those most commonly found at polluted areas are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soils are the main sinks for heavy metals released into the environment by man-made activities but different from organic contaminants which are decompose by microbial action, most metals do not undergo microbial or chemical degradation, and their total concentration in soils remain for a

long time after their introduction (ChiromaT.M. et al., 2014, Velea et al., 2009). However, changes in their chemical forms are possible (Mtunzi et al., 2015). The presence of toxic metals in soil can strictly prevent the biodegradation of organic pollutants (Zhou et al., 2016). From the foregoing, it is evident that considerable works have been on the contamination of soil done by anthropogenic sources in the world, but there is not any previous scientific work in the area of this scientific research, the primary objectives of the present study were to investigate the characteristic

levels of heavy metals and some physicochemical

the depth of 0- 20 cm, they were thoroughly ad Zn either mixed and put into a clean and labeled polyethylene bag and transferred to laboratory

2.2 Sample Preparation

to analysis.

The samples were air dried for 72 hours, then crushed and sieved through 2 mm sieve, in the gas facility's laboratory, to obtain a representative sample.

2.3 Sample Analysis

The samples were taken for heavy metal analysis by using Genius 9000 XRFSkyray instrument advanced laboratory at of Environmental sciences department, college of science, university of Salahaddin. The data was statistically analyzed using SPSS program. The comparison among the means of soil heavy metals were done using Duncan multiple comparison (Harter, 1960). The particle size distribution was determined by the hydrometer method (USDA, 1095), the soil texture was silty loam. Seven water samples in Produced Water Pond have been taken and analyzed for determination of some chemical properties as described by (Agoro et al., 2018) in the gas facility's laboratory. In the last 10 years, many oil and gas production, agriculture and other industrials companies have emerged in the Kurdistan Region of Iraq. Some of these companies have reportedly released a large amount of pollutants such as heavy metals into water and soil.

August 2018 to February 2019.

2.1 Sample Collection

properties of the produced water and its effect on soil heavy metal contamination, started from

20 soil samples were collected in August

2018 at a gas facility in Kurdistan/Iraq around

the Produced Water Pond, which uses to

collect produced water during the operation,

by means of complete randomized design

with 4 replications, 5 samples at different

distances by 5 meters for each sample at one

direction. The soil samples were collected by

using clean stainless-steel hand auger from

2. MATERIALS AND METHODS

30

3. RESULTS AND DISCUSSION

Heavy Metals

Samp

Table (1) describes the mean values of the heavy metals.

the results of statistical analysis indicated to significant effect of distance factor on some heavy metal content of the soil. The concentration of heavy metals series was arranged as follow:

Mn > Cr > Ni > Zn > Cu > Pb > Co > As > Fe > Cd > Hg in a successive form.

le No.	Mean of Cr	Mean of Mn	Mean of Fe	Mean of Co	Mean of Ni	Mean of Cu	Mean of As	Mean of Zn	Mean of Cd	Mean of Hg	Mean of Pb
Samp le No.	Mean of Cr	Mean of Mn	Mean of Fe	Mean of Co	Mean of Ni	Mean of Cu	Mean of As	Mean of Zn	Mean of Cd	Mean of Hg	Mean of Pb
D5	76.00 ^a	294.65 ^ª	1.97 ^a	7.42 ^b	60.43 ^a	18.28 ^c	3.62 ^a	49.38 ^a	0.13 ^a	0.03 ^a	6.50 ^b
D10	67.06 ^a	249.41 ^a	2.17 ^a	8.19 ^{ab}	51.45 ^b	18.30 ^{bc}	2.63 ^a	35.74 ^b	0.12 ^a	0.03 ^a	7.84 ^{ab}
D15	81.25 ^a	299.41 ^a	2.25 ^a	8.37 ^{ab}	51.17 ^b	24.17 ^a	3.63 ^a	50.11 ^a	0.15 ^a	0.02 ^a	12.22 ^{ab}
D20	69.43 ^a	320.29 ^a	2.30 ^a	8.72 ^{ab}	48.37 ^b	23.15 ^{ab}	2.79 ^a	48.51 ^{ab}	0.14 ^a	0.01 ^a	14.67 ^{ab}
D25	75.72 ^a	282.72ª	2.51 ^a	9.49 ^a	55.88 ^{ab}	17.39 ^c	3.02 ^a	48.65 ^{ab}	0.13 ^a	0.02 ^a	21.94 ^a

Table (1): mean values of heavy metals

The data reflects that Pb and Co are those heavy metals which were affected significantly by distance factor through all 25 meters, the mean value of Pb and Co is increasing by the increase of the distance, but comparing to Environmental Baseline Survey for heavy metals which has been done to the company by MapCom Company in 2010. The amount of Pb has not increased, so that the increasing of Co may be due to effect of the Produced Water Pond through evaporation of the produced water, and the silt loam soil allow the heavy metals to disperse through it and having the maximum adsorption capacity within the soil. While Zn and Cu have a significant difference and the mean has the highest value at 15 m and then decrease by increasing the distance, this may be because of medium adsorption capacity of the soil to Zn and Cu and it may collect in a distance and

cannot go longer as the capacity of the adsorption is limited and comparing to Environmental Baseline Survey (Velea et al.) (Table 2), Cu has not increased since the EBS has been done which means that there is not any effect on Cu in the soil by byproducts. In addition, Ni has the highest mean value in 5 m and the value decrease with increasing distance till arrives 20 m, which may be linked to limited affinity of Ni through soil. However, the mean value increases from the distance of 25 m, which may be attributed to using fertilizer and pesticide some years ago by the local farmers as it was an agricultural land (Aydinalp. and Marinova., 2003). This could also be related to burning of some byproducts through the plant flare and then deposit the heavy metals on the surrounding soil.

Elements	EBS Results
Cd	1.1
Cr	23
Cu	42
Ni	34
Pb	8.5
Hg	6.6
Zn	23.8

Tε	ıble	(2)	Environme	ntal Ba	iseline	Survey	for	Heavy	Metals.
		~ /							

However, heavy metals' mean values of the Cr, Mn, Fe, As, Cd, Hg did not affected significantly by the distance factor which may be due to limited amount of those heavy metals in the produced water and all the amount which is present in the soil may naturally existed from the soil formation. The produced water pH was in the range of (6.62 - 7.17), EC $(1369 - 2512 \mu S)$ TDS (975.3 - 1879 ppm), TSS (730 to 748 ppm) and total hardness was (90 - 180 ppm).

Comparison

Figure (1) showed linear charts of some heavy metals (Pb, Zn, Hg, Cd, Cr and Cu) mean values compare to standard maximum allowable limit of the heavy metal by World Health Organization (Table 4). Figure (1) (A,C,D and F) indicate that Pb, Hg, Cd and Cu despite of being present in the soil and surrounding the Produced Water Pond are within the normal range in accordance to the World Health Organization standard for heavy metals, and don't have a dangerous effect on leaching to underground water and on living organisms in the soil, while figure 1 (B and E) which are Zn and Cr have exceeded the normal range of the standard under effect of the produced water and other activities in surrounding soil.



A





С

D



Figure (1) compare heavy metals mean value to World Health Organization standard limit.

Elements	MPL value of soil(mg.kg ⁻¹)
Cd	0.8
Cr	50
Cu	36
Ni	100
Pb	85
Hg	1
Zn	35

Table (4) Guideline for the maximum permissible limit (MPL) values of the heavy metals in soil. (WHO, 1996)

4. CONCLUSION:

It is evident that there is a gradual buildup of some heavy metals in the soil around the gas plant area compared to Environmental Baseline survey which conducted 9 years ago and PEL of WHO. This reflects intense anthropogenic activities around the gas plant areas. Metal build up is gradual process, constant monitoring of the levels of these metals is required to safeguard farmlands

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and water bodies around these facilities. Also, burning byproducts have a role in increasing heavy metals. In addition, the heavy metals which were added to the soil in form of fertilizer and pesticide by local farmers some years ago, persist in the soil and increase the heavy metal levels. We recommend to performing a further research on effect of produced water on soil heavy metal by depth factor in future to detect the effect in the depth of the soil.

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RESEARCH PAPER

Association Study of HSD11B1 rs12086634 (T>G) Gene Polymorphism

with Polycystic Ovarian Syndrome in Erbil Province.

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ABSTRACT:

Background: Polycystic ovarian syndrome (PCOS) is one of the most prevalent gynaecological imbalances of endocrine hormones and metabolic disorders that affect women during puberty, with a prevalence of up to 17.8%.

Objective: The current study focused on the presence of rs12086634 T>G genetic variant of 11β -hydroxysteroid dehydrogenase type 1 (HSD11B1) gene polymorphism in Erbil province PCOS women.

Participants and methods: The present study was conducted on 104 PCOS women and 94 control women to investigate the association HSD11B1 rs12086634 (T>G) gene polymorphism with PCOS. The extracted genomic DNA from whole blood according to the protocol provided by the manufacturer company. HSD11B1 rs12086634 T>G gene polymorphism was detected by tetra primer-amplification refractory mutation system based polymerase chain reaction (T-ARMS-PCR) method.

Result: The obtained results suggest that the frequency of the HSD11B1 TG and GG genotypes in women with PCOS increased 3.60-fold and 4.39-fold compared to the control group (odds ratio [OR]; 3.60 and 4.39, 95% confidence interval [CI], 1.951-6.668 and 0.175-110.2; P-value <0.0001 and 0.22) respectively, the body mass index of women with G-allele was significantly greater than women with T-Allele (28.32 kg/ mt2 vs. 26.20 kg/ mt2, and p = 0.0256).

Conclusions: Our findings indicated and proved that the findings indicate that both homozygous and heterozygous genotypes of HSD11B1 rs12086634 T>G gene polymorphism was associated with PCOS in Erbil province.

KEY WORDS: Polycystic ovarian syndrome; HSD11B1; single nucleotide polymorphism; T-ARMS-PCR. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.5</u> ZJPAS (2019), 31(5);36-43 .

1.INTRODUCTION :

Polycystic ovarian syndrome (PCOS) is one of the most prevalent gynaecological imbalance of endocrine hormones and metabolic disorders that affect women during puberty (Legro *et al.*, 2013, Mohammed and Ameen, 2017), with an incidence of up to 17.8%. The PCOS is characterized by hyperandrogenism, irregular cycles and polycystic ovaries (Azziz *et al.*, 2016).

Abdullah Abubaker Shareef E-mail: <u>Abdullah.shareef@su.edu.krd</u> **Article History:** Received: 05/04/2019 Accepted: 10/06/2019 Published: 17/10/2019 these worsen the symptoms of hyperandrogenemia (Teede *et al.*, 2010, Baptiste *et al.*, 2010).

Despite the aetiological uncertainty of PCOS, it has been suggested that it is a multifactorial disorder, in which genetic factors (including CYP1A1, CYP19, HSD11B1, AR, SHBG, INSR, CAPN10, PPARG, ADIPOQ, FTO. FSHR, LHCGR, and AMHR2) interfere with environmental factors leading to develop genetic variations and subsequently genetic disorder (Jain et al., 2015, Azziz et al., 2016). One such candidate gene is HSD11B1 gene that is located at 1q32.2-41, consisted of six exons and five introns that encode 11β-hydroxysteroid dehydrogenase

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type 1(11β-HSD1) (Tomlinson et al., 2004, Dujic et al., 2012). This keto reductase catalyses the reduction of cortisone to its active form cortisol, hence amplify the activation of the glucocorticoid receptor in different tissues including liver and adipose tissues (Devang et al., 2018). The decrease 11β -HSD1 expression leads to impairment of cortisol recreation and also elevates the clearance rate of metabolic cortisol; the excessive level of adrenal androgen detected in some PCOS patients might be a resulted from the compensated activity of the hypothalamicpituitary-adrenal axis (Rodin et al., 1994, Draper et al., 2003). Previous studies on HSD11B1 gene polymorphism demonstrated that it also has a pathogenic role in metabolic diseases and showed a positive association with Type-II Diabetes Mellitus, insulin resistance, hyperandrogenemia, and hypertension (Nair et al., 2004, Gambineri et al., 2006, Hughes et al., 2008, Liu et al., 2008, Cooper and Stewart, 2009, Shimodaira et al., 2013, Devang et al., 2016).

In addition to nutrition and hormones, genetic factors play an effective role in the regulation of 11β -HSD1 expression. The G allele of rs12086634(T>G) **SNP** (single nucleotide polymorphism) was studied to have association with PCOS in south Indian women (Devang et al., 2018) and specifically with hyperandrogenism among PCOS lean Caucasian women (Gambineri et al., 2006), but these associations were not detected in all PCOS patients (Draper et al., 2006). Because of insisting of most studies to investigate these associations among different population groups, the current study was managed to investigate the role of HSD11B1 gene polymorphism in the predisposition of women at Erbil province.

2. MATERIALS AND METHODS 2.1. Participants

The current study was conducted on 104 PCOS women with the mean age 27.16 ± 0.73 and 94 control women with the mean age 27.36 ± 0.76 recruited from Erbil province general population during May 2018. PCOS patients were chosen based on the presence of at least two respective

features namely: chronic oligoanovulation, clinical signs of hyperandrogenism, and polycystic ovarian morphology on ultrasound, those features were selected depending on Rotterdam criteria (Eshre and Group, 2004).

2.2. DNA Analysis

The genetic analysis was performed at Salahaddin University-Erbil (SUE), College of Science, Biology Department. Five millilitres of peripheral blood were collected from both PCOS and control women. Blood samples were kept, in anticoagulant tubes at 4°C. Thermo Fisher DNA Blood Kit (Thermo Fisher Scientific, USA) was used to isolate genomic DNA from peripheral leukocyte. The isolation was made according to the manufacturer protocol. Quality and integrity of DNA were checked by NanoDropTM (Thermo Scientific, USA).

Genotyping of the SNP rs12086634 T>G HSD11B1 gene was performed by Tetraamplification refractory mutation systempolymerase chain reaction (TETRA-ARMS-PCR) technique, which is a rapid and cost-effective technique for SNP detection (Hashemi et al., 2012, Khoshnaw, 2018). Four primers were used, two of them were external primers (forward outer: 5'-TTTCTGCTGTATCACTGCAGGTGGTATC-3', reverse outer: 5'- CAGC TACAGTCAGGACC ACGTAACTGAG -3'), while the other two were internal allele-specific primers (forward inner specified for {G-allele}: 5'- CCTGCAAGAGATG GCTATATTAAGAAACCC -3', and reverse inner specified for {T-allele}: 5'- AGAATGGGAAAG ATCAACCCCAAAT -3') (Devang et al., 2016).

The PCR cycling program was 5 minutes at 95°C for initial denaturation, then the next 35 cycles of three repeated steps were: denaturation (95°C for 30 seconds), annealing (60°C for 1 minute), and extension (72°C for 1 minute), with a final step at 72°C for 10 minutes to extend all PCR fragments. Separation of the products amplified DNA amplicons were performed by horizontal gel electrophoresis on 2.5% agarose gel, and then visualized by UV-transilluminator after staining with ethidium bromide(Joseph and David, 2001).

2.3. Statistical Analysis

Statistical analysis was done using Graph Pad Prism 6 statistical software. Two sample t-test was used to compare the BMI between women with PCOS and healthy control, and G-allele carriers and TT-Genotype. Genotype and allele frequencies of cases and controls were analyzed using the Chi-square $(\chi 2)$ test and both genotype and allelic odds ratio (ORs) and 95% confidence interval (CI) were calculated to determine the association of HSD11B1 gene polymorphisms with PCOS. A *p*-value of less than 5% (p < 0.05) was set to be statistically significant.

38

3. RESULTS

An inspection of (Figure 1) indicates that a highly significant difference of BMI (p < 0.0001) was observed among PCOS women with a mean value (29.39 ± 0.7154 kg/mt²) when compared to healthy control women with a mean value (24.01 ± 0.4915 kg/mt²).



Figure 1: BMI comparison between PCOS and Control. PCOS: Polycystic ovary syndrome, BMI: body mass index.

Genotypes expressed as TT in normal homozygote, TG in the heterozygote, while GG in homozygote polymorphic genotype. In normal TT genotype, two bands of 385 bp and 185 bp were produced. In heterozygote genotype, three bands of 385 bp, 185 bp indicate for T-allele, and 255 bp indicate for G-allele, in homozygote genotype two bands of 385 bp and 255 bp were produced (Figure 2).



Figure 2: Agarose gel electrophoresis showing results of Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction of four PCOS cases illustrating an amplified 385-bp fragment of *HSD11B1* gene as a control, and two other fragments of 255-bp for T-Allele and 140 bp for G-Allele. Lane M: 100 bp DNA ladder, lane 1 and 4; TT-Genotype, lane 2; TG-Genotype, and lane 3; GG-Genotype.

The allele and genotype frequencies of the SNP rs12086634 detected by T-ARMS-PCR method and were compared between PCOS and healthy women (control). The G-allele frequency of rs12086634 was considerably higher in PCOS than control (27% vs 11.7%). Those patients carrying G allele of rs12086634 showed a higher

susceptibility to develop PCOS by 2.78-fold (OR 2.78; 95% CI, 1.620-4.771; P-value <0.0001). TG genotype was 3.607-fold higher (p=0.0001), and GG genotype was 4.394-fold higher (p=0.22) among PCOS women (Table 1).

Table 1: Distribution of genotypes and allele frequencies of *HSD11B1* rs12086634 gene polymorphism in the study population: PCOS women and controls.

Polymorphism	PCOS (PCOS (n=104)		Control (n=94)		95% CI	p value
	No.	%	No.	%			1
TT	49	47.0	72	76.6	1.0	-	-
TG	54	51.9	22	23.4	3.607	1.951-6.668	0.0001
GG	1	0.96	0	0	4.394	0.175-110.2	0.22
TG+GG	55	52.8	22	23.4	3.673	1.989-6.785	0.0001
T-Allele	152	73	166	88.3	2 7 9 0	1.620-4.771	0.0004
G-Allele	56	27	22	11.7	2.780		0.0001

PCOS: Polycystic ovary syndrome; OR: Odds ratio; CI: Confidence interval.

Regarding BMI, women with *HSD11B1* rs12086634 G-allele carriers (TG, and GG) polymorphic genotypes were significantly higher

BMI (28.32 \pm 0.7385 kg/mt² vs. 26.20 \pm 0.5863 kg/mt², p = 0.0256) when compared with wild type TT genotype (Figure 3).





4. DISCUSSION

Polycystic ovarian syndrome is one of the most prevalent gynaecological imbalance of endocrine hormones and metabolic disorders that affect women during puberty (Legro et al., 2013). The current understanding is that PCOS is not only a gynaecological condition but a metabolic syndrome with related disorders including; obesity, hormonal disturbance, dyslipidemia and, insulin resistance (Teede et al., 2010). It is a multifactorial disorder in which many genes with environmental factors together interact with it (Jain et al., 2015). Not just a single gene but alterations in the expression of a number of genes were studied, that inform us PCOS genetic abnormality deteriorates signal transduction pathways regulating the expression of these genes (Jakubowski, 2005).

40

The results of the current study showed the positive association HSD11B1 rs12086634 T > G gene polymorphism with PCOS. The polymorphic TG-genotype frequency was significantly higher while polymorphic GG-genotype frequency was not significantly greater in PCOS compared to control women. The conflicting results (positive and negative association) from different populations were reported. In the Caucasian population, a positive association of rs12086634 polymorphism that increased by 1.95 fold in PCOS have been reported women with (Gambineri et al., 2006). In the south Indian population, the same positive association was confirmed with a G-Allelic OR:1.95 (Devang et al., 2018). Whereas the conflicting results with no association were achieved such as the studies in non-Hispanic, Caucasian, and Dallas resident populations that were done by Chua et al., San Millan et al., and White et al. respectively (White, 2005, San Millán et al., 2005, Chua et al., 2012). However, in the study of Chua et al, and San Millan the number of case and control were not matched, the case numbers were higher than controls. San Millan et al. and White they did not follow the all criteria of Rotterdam consensus for diagnosis of PCOS in their study.

Regarding BMI, there was a significant difference in women with G-allele carriers (TG, and GG) genotypes when compared with women with TT genotype. The positive association of BMI marker indicates that this T>G polymorphism might increase the predisposition possibility of obesity in PCOS women. Our results

agree with previous reports which recommended that women with TG genotype are at high risk to develop obesity (Devang et al., 2018). This might be related to the overexpression of 11 β -HSD1 and increased levels of cortisol within the adipose tissue in obese cases (Masuzaki *et al.*, 2001, Kannisto *et al.*, 2004). The *HSD11B1* rs12086634 T>G polymorphism may increase the chance of individuals to visceral obesity by increasing the 11 β -HSD1 activity within visceral adipose tissue (Devang *et al.*, 2017).

Previous studies have reported the impact of genetic polymorphisms in several the development of PCOS. Eleven genetic loci were detected to have a role with PCOS such as: DENND1A, THADA, LHCGR, FSHR, YAP1, and RAB5/SUOX (Welt and Duran, 2014) . Previously, genetic polymorphisms in the thyroid peroxidase (TPO) gene, insulin (INS) gene, CYP17, Calpain-10 (capn10) gene, Peroxisome Proliferator Activated Receptors Gamma (PPAR- γ), follicle stimulating hormone receptor (FSHR) gene, and methylenetetrahydrofolate reductase (MTHFR) gene, were associated with PCOS women in Iraqi population (Nader and Aziz, 2014, AlFaisal et al., 2014, Mohammed et al., 2015, Ali, 2016, Ramadhan, 2018, Al Hayawi et al., 2018). Until now there were no studies to examine whether HSD11B1 gene polymorphism has any association with PCOS in Erbil population. It would be the first study in Erbil province for evaluation of association and frequency of this gene polymorphism among women with PCOS.

5. CONCLUSION

The present study demonstrated and confirmed that the SNP rs12086634 (T>G) of the *HSD11B1* gene is significantly associated with PCOS in Erbil province. Additionally the G-allele of this SNP represented a positive association with obesity.

ZANCO Journal of Pure and Applied Sciences 2019

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42

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ZANCO Journal of Pure and Applied Sciences 2019

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RESEARCH PAPER

Hypotensive Action of Pomegranate Seed Extract and Zinc Chloride in Hypertensive Rats

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ABSTRACT:

This study investigated the effects of pomegranate seed extract and supplementary zinc chloride (ZnCl2) on Dexamethasone (DEX) and NaCl induced hypertension in rats. For this purpose, female albino rats were divided into five groups: control and four DEX and NaCl induced hypertensive rats. The animals were given pomegranate seed extract, ZnCl2, or a combination of both for two weeks. Our results show a significant decrease in systolic blood pressure (SBP) in each of the treated groups, except the combination group. DEX and NaCl combination significantly decreased the total body weight in rats. The body weight was not changed with pomegranate seed extract or ZnCl2 administration. Besides WBC, RBC, HGB, HCT, and PLTs levels were not altered. There were no significant differences in the levels of serum K+, Ca2+, and Cl- ions among the treated groups. Nevertheless, the Na+ level in the serum increased significantly in rats of the combination group. Taken together, we conclude that pomegranate seed extract and ZnCl2 consumption can be beneficial for lowering blood pressure in hypertensive patients when consumed separately.

KEY WORDS: Pomegranate,Zinc chloride,Toxicity, Hypertension,Blood parameters . DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.6</u> ZJPAS (2019), 31(5);44-52 .

INTRODUCTION :

The most common cause of human mortality is cardiovascular diseases, and one of the main risk factors for heart diseases is hypertension (Sahebkar et al., 2017). There are many pharmaceutical drugs, dietary supplements, and herbal folk medicine that are used to manage increased blood pressure. In this study, we have sought to investigate the effectiveness of a herbal medicine and a dietary supplement on lowering blood pressure in experimental animals; and those are pomegranate seed extract and zinc chloride (ZnCl2).

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fruit has a nutritional, industrial, pharmaceutical values (Teixeira da Silva et al., 2013). It is composed of peel, aril, seed, and juice. Pomegranate juice is reported to have antioxidant effects on humans. The antioxidant activities are highly linked to the phenolic content of the pomegranate fruit, including anthocyanins, ellagic acid and its derivatives, and hydrolyzable tannins (Kalaycıoğlu and Erim, 2017, Jing et al., 2012, Hmid et al., 2016, Fawole and Opara, 2013). One of the major components of pomegranate seed is tocopherol, such as y-tocopherol. Additional components of the seeds are tocotrienols, phytophenols, triterpene, and phospholipids. Therefore, pomegranate seed considered a rich source of biolipids (Verardo et al., 2014). Pomegranate extracts have been reported to have antiparasitic (Fahmy et al., Al-Megrin, 2017), antibacterial (Silva et al., 2016, Labsi et al., 2016), anti-inflammatory(Achraf et al., 2018, Ambarwati et al.), and anticancer activities (An et al., 2015). It enhances male fertility (El Ghazzawy et al., 2011) and learning and memory of rats(Adiga et al., 2010), and it has anti-diabetic properties as well (Mestry et al., 2017).

Malondialdehyde (MDA), and protein carbonyls (CARB) are reduced in plasma after consumption of the pomegranate juice for 15 days, indicating less lipid peroxidation and less protein oxidation conditions. The antioxidant glutathione (GSH) increased after levels were pomegranate consumption (Matthaiou et al., 2014). Increased oxidative stress alters the balance between reactive oxygen species and nitric oxide (NO), which lowers NO bioavailability, that are the atherosclerosis preliminary steps in and cardiovascular diseases (Sahebkar et al., 2016).

It has been shown that Pomegranate peel extract, *Punica granatum* L., Lythraceae, has proapoptotic activities and subdue cancer cell proliferation in adult male Sprague-Dawley rats, possibly through suppression of Wnt/ β -Catenin signaling pathway (Ahmed et al., 2017). It is reported that pomegranate juice extract reduces epithelial to mesenchymal transition (EMT) in renal cell carcinoma (RCC) cell lines, through inhibition of the NF-kB and JNK pathways (An et al., 2015). Pomegranate also found to protect against oxidative stress in endothelial cells from Preeclampsia pregnant women.

Zinc supplements have protective antioxidant and anti-inflammatory properties. It has been revealed that it decreases hypoxia in obstructive sleep apnea rats. It protects against hypoxia induced cardiac dysfunction (Chen and Chen, 2016). ZnCl₂ demonstrates a protective role against cadmium chloride toxicity, which causes severe pathological changes in liver and kidneys in rats. Moreover, Zn balances out cadmium-induced oxidative stress, apoptosis and necrosis (Mahran et al, 2011). ZnCl₂ prevents oxidant-induced mitochondrial permeability transition pore (mPTP) opening through stimulation of PI3K/Akt signalling pathway, which is responsible for the inactivation of glycogen synthase kinase-3^β

(GSK-3 β) by phosphorylation at Ser9 site (Chanoit et al., 2008, Lee et al., 2009). Zinc can suppress apoptosis through suppression of caspase-3 and its antioxidant activities in cardiomyocytes. Zinc can activate ERK in SHSY5Y site and prevent apoptosis in neuronal cells (Chanoit et al., 2008). Although Zinc has anti-apoptotic activities, there are reports that it can participate in the induction of necrosis in pulmonary endothelial cells in response to oxidative stress. The necrosis process is likely via t-butyl hydroperoxide (tBH) and protein kinase (PK) C pathways (Tang et al., 2001, Kambe et al., 2015). Zinc also affects the expression and production of cytokines. It increases the expression of IL-2 and IFN-Y in Human Renal Proximal Tubule Epithelial (TH1). Zinc also downregulates the expression of TNF- α , IL-1 β , and IL-8 in the monocyte-macrophage cell line (Bao et al., 2003). The main aim of the current study is to obtain insight into the effectiveness of pomegranate seed extract as well as ZnCl₂ on lowering glucocorticoid dexamethasone (DEX) induced hypertension in rats. Moreover, we have investigated the possible toxicity of these two treatments on blood cell counts, animal weight, serum electrolyte levels, and blood glucose level.

2. MATERIALS AND METHODS

2.1 Animal housing

Female albino rats weighed 230- 244 grams, were divided into five groups. The animals were bred and housed at the Animal House of the Department of Biology, College of Science, Salahaddin University-Erbil. The rats kept under a controlled temperature of about $22\pm2^{\circ}$ C, with 12 hours of alternating cycles of light and dark. Before the treatments, the animals were fed a standard diet rat chow containing 0.5% NaCl, 22% protein, and 4-6% dietary fat and tap water ad libitum. The standard rat pellet contained wheat 66.6%, soya 25.6%, sunflower oil 4.4%, limestone 1.5%, salt 0.63%, methionine 0.158%, choline chloride 0.062% and trace elements 0.05% (Krinke, 2000).

2.2. Experimental design

The negative control group received a regular diet and tap water (n=6). The second group (n=7) received a combination of Dexamethasone (DEX)-containing water (0.5mg/L), and 4% NaCl containing diet. The (DEX+NaCl) combination is used to induce hypertension in experimental animals. The third group of animals (n=6) was given pomegranate seed (PG) extract Punica granatum (Damiana, Turkey) in addition to DEX+NaCl combination. The pomegranate seed powder was dissolved in water (2000mg/L). The fourth group (n=6) was given Zinc chloride (ZnCl₂) in addition to DEX+NaCl combination. The ZnCl₂ was prepared in a concentration of 420mg/L (50mg/Kg body weight) in water. The fifth group (n=6) was given a combination of PG, Dexamethasone, NaCl ZnCl₂. and (PG+ZnCl₂+DEX+NaCl) in concentrations similar to the other groups. The treatment lasted for two weeks.

2.3 Analytical methods

2.3.1 Weight of the animals

The animals were weighed at three time points during the treatment period of two weeks; prior to the treatment, at the end of the first week and at the end of the second week of treatment.

2.3.2 Systolic blood pressure measurement

Systolic blood pressure was measured by using tail-cuff plethymography in un-anaesthetizes rats, pre-warmed for 10 minutes at 37°C, in a thermostatically controlled heating cabinet. The tail pressure pulsations were detected with a pneumatic pulse transducer (ADInstruments PowerLab 2/25). Each blood pressure reading was obtained by averaging 2-4 individual reading values.

2.3.3 Blood collection and biochemical analysis

At the end of each experiment, the rats were anaesthetized with ketamine hydrochloride (100mg/mg). Blood samples were taken by cardiac puncture into tubes with or without enthylenediaminetetraacetic acid (EDTA) as an anticoagulant. The tubes with EDTA were used for haematological analysis. White Blood Cells (WBC), Red Blood Cells (RBC), haemoglobin (HGB), hematocrit (HCT), and platelets (PLT) counts were determined by using automated haematology analyzer (NIHON KOHDEN), Celtac α , MEK -6400 J/K). Blood glucose level was also measured by using glucometer (ACCU-Check Active, Roche, D-68298, Germany).

The blood samples without EDTA were centrifuged for 15min for sera collection. The sera were stored at -80 °C (Sony, Ultra low, Japan). Blood Gas Analyzer GASTAT-600 (TECHNO MEDICA, Japan) was used for the measurement of serum electrolyte concentrations (Na⁺, K⁺, Ca²⁺, and Cl⁻).

2.4 Statistical analysis

The statistical analysis was performed using Sigma Plot Statistical Package (Version 12.0) software. Pairwise multiple comparison tests were done using posthoc Bonferroni t-test. Data are shown as mean \pm SEM. The results were considered statistically significant at P< 0.05.

3. RESULTS

3.1 Dexamethasone and NaCl combination reduces weight

All the groups treated with DEX+ NaCl combination showed a significant decrease in the weight of rats after the first week of treatment compared to the untreated control group. This change was further detectable after the second week of the treatment Table (1). The maximum reduction is seen in $ZnCl_2+DEX+NaCl$ group.

Table (1): Change in animal weights during the period of the experiment (2 weeks) in Pomegranate and $ZnCL_2$ treated hypertensive rats. Pairwise multiple comparison tests were done using post-hoc Bonferroni t-test. Data are shown as mean±SEM. Treatment groups compared to the control group.

	Before starting	Week 1 of the	Week 2 of the
Treatments	the experiment	Experiment	Experiment
	(gm)	(gm)	(gm)
Control	233 ± 6.13	249 ± 8.51	252 ± 8.84
DEV N. CI	220 + 4.22	214 ± 4.25	204 ± 3.79
DEX-NaCI	239 ± 4.33	(P=0.021)	(P<0.001)
PG + DEX-	244 + 7.7	213 ± 7.64	$\textbf{208} \pm \textbf{7.14}$
NaCl	244 ± /./	(P=0.016)	(P<0.001)
ZnCl ₂ + DEX-	220 + 7 12	207 ± 6.2	199 ± 5.08
NaCl	230 ± 7.12	(P=0.004)	(P<0.001)
$PG + ZnCl_2 +$	222 + 7.55	$2\overline{11 \pm 8.07}$	203 ± 9.24
DEX-NaCl	202 ± 1.00	(P=0.009)	(P<0.001)

3.2 Each of pomegranate seed extract and zinc chloride reduces blood pressure

Pomegranate seed extract in PG+DEX+NaCl group reduced systolic blood pressure (mmHg) compared to blood pressure in DEX+NaCl group. Similar results were observed in ZnCl2 treated groups (ZnCl₂+DEX+NaCl) compared to DEX+NaCl group. However, the combination of pomegranate and ZnCl₂ showed no significant changes compared to the DEX+NaCl group (Figure 1).



Figure (1): Shows the effect of Pomegranate (PG) and/or ZnCL₂ administration on systolic blood pressure in Dexamethasone (DEX)-NaCl-induced hypertensive rats. Pairwise multiple comparison tests were done using post-hoc Bonferroni t-test. Data are shown as mean±SEM.

3.3 Blood glucose level is not altered by pomegranate nor zinc chloride

Blood glucose was not changed when the rats administered pomegranate seed extract. This finding was observed when PG+DEX+NaCl group was compared to the control and DEX+NaCl groups. However, there is a slight increase in blood glucose level in the groups that ingested zinc chloride, even though the results were not statistically significant (Figure 2). The average of glucose levels in DEX+NaCl group is 114±9.94, however in each of ZnCl₂+DEX+NaCl, and PG+ZnCl₂+DEX+NaCl groups the values are 124 ± 6.39 mg/dL 124±5.33 and mg/dL, respectively.



Figure (2): Pomegranate and/or ZnCL₂ administration does not show any significant changes in blood glucose level in Dexamethasone (DEX)-NaCl induced hypertensive rats. Pairwise multiple comparison tests were done using posthoc Bonferroni t-test. Data are shown as mean±SEM.

3.4 Hematological parameters are not changed by pomegranate nor zinc chloride

We did not observe significant changes in the levels of RBC, HGB, HCT, and PLTs compared to the controls in all treated groups. However, a slight decrease in the levels of WBC was observed in each of PG+DEX+NaCl ($3.6 \pm$ 0.68) and ZnCl₂+DEX+NaCl ($3.3 \pm$ 0.29) groups compared to the control ($5.08 \pm$ 0.44) and DEX+NaCl ($5.46 \pm$ 1.27) groups (see Table 2).

Table (2): Haematological parameters in Pomegranate and/or $ZnCL_2$ treated hypertensive rats. Pairwise multiple comparison tests were done using post-hoc Bonferroni t-test. Data are shown as mean±SEM.

	WBC	RBC	ИСР	нст	DI T
Treatments	(x	(x	HGB	нст	PLT
	ì		(g/dL)	(%)	(X10 ³ /µL)
	10 ³ /μL)	10%μL)			
	5.08 ±	6.54 ±	12.82	41.2 ±	000 1 (0
Control	0.44	0.23	+0.34	1.18	809 ± 69
	0.11	0.20	- 0.04	1.10	
	5 46 1	((2))	12 ()	41 1	
DEX-NaCl	5.46 ±	6.63 ±	13.6 ±	41 ±	632 ± 119
	1.27	0.21	0.23	1.04	
PG + DEX-	3.6 ±	6.54 ±	13 ±	39.9±	
NaCl	0.69	0.25	0.57	1.00	625 ± 84
NaCi	0.68	0.35	0.57	1.82	
ZnCl2+	3.3 ±	7.01 ±	14.15	45.13 ±	602 + 28
DEX-NaCl	0.29	0.21	± 0.25	0.82	002 ± 20
PG +					
10	4.23 ±	7.01 ±	14.32	43.93 ±	
$ZnCl_2 +$	0.60	0.22	1 0 5 2	1.74	564 ± 27
DEX-NaCl	0.69	0.32	± 0.52	1./4	

WBC: White Blood Cell count; RBC: Red Blood Cell count; HGB: haemoglobin concentration; HCT: Hematocrit; PLT: Platelet count.

3.5 Sodium ion concentration level is changed by pomegranate and zinc chloride combination

There were no significant differences in the levels of serum K^+ , Ca^{2+} , and Cl^- among the treated groups. Nevertheless, serum Na⁺ ion level increased significantly when the rats ingested a combination of pomegranate and ZnCl₂ compared with the control (see Table 3).

Table (3): Serum electrolyte concentrations in Pomegranate and/or ZnCL2 treated hypertensive rats. Pairwise multiple comparison tests were done using post-hoc Bonferroni t-test. Data are shown as mean±SEM.

* This group is significantly different compared to control group.

ZANCO Journal of Pure and Applied Sciences 2019

4. DISCUSSION

This study was designed to examine the effects of pomegranate seed extract and ZnCl₂, individually and in combination, on the blood pressure, weight, haematological and biochemical parameters of rats. Hypertension in the rats was induced via synthetic glucocorticoid, Dexamethasone (DEX) ingestion in combination with NaCl. DEX induction of hypertension was previously reported (Soto-Piña et al., 2016, Dubey et al., 2017). Dexamethasone induces increased blood pressure as a result of enhancing blood vessels' reactivity (contraction) to vasopressin (Iijima and Malik, 1988). Moreover, dexamethasone reduces nitric oxide (NO) synthesis and impairs relaxation of endothelial cells (Yin et al., 1992, Ong et al., 2009).

Short period NaCl induced hypertension is a result of vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) action, in response to high salt diet (Walkowska et al., 2015). High sodium diet, up to 8%, has been shown to increase superoxide formation, particularly in the kidney cortex. Superoxide radicals decrease NO bioavailability in the thick ascending loop; therefore, more NaCl reabsorption takes place which eventually causes high blood pressure in rats (Dobrian et al., 2003).

Our results show that each of pomegranate seed extracts and ZnCl₂ counteract the effects of Dexamethasone on blood pressure within two treatment. However, weeks of when а combination of both, pomegranate and ZnCl₂ were used, the hypertensive effect of Dexamethasone did not change, this might be due to the antagonistic effect of these two treatments together. It is possible that Na⁺ ion increase we observed only in pomegranate seed extracts and ZnCl₂ combination group has caused a decrease in the bioavailability of NO to lower the blood pressure. In fact, the phenolic compounds in pomegranate was observed to decrease amount of the zinc in kidney and heart tissues in rats (Aksu et al., 2017).

Pomegranate has been reported to increase nitric oxide activity; therefore, it is possible that the reduction in the systolic blood pressure we observed might be due partly to the enhanced NO action. In addition, pomegranate lowers protein and lipid oxidation via increasing the antioxidant molecules such as glutathione (GSH)(Gbinigie et al., 2017). Other studies have reported a possible link between the reduction of blood pressure and pomegranate intake (Sahebkar et al., 2017, Achraf et al., 2018); however, the data still is not conclusive. To our best knowledge, there is limited data on how pomegranate consumption affects cell signalling pathways, and whether there is any variation on the effects of pomegranate juice, skin, aril or the seed extract on the blood pressure.

Dexamethasone-treated animals showed а significant decrease in the total body weight compared to the control group. Dexamethasone is reported to acts in the hypothalamus to induce rapid transport of Intracerebroventricular neuropeptide Y (NPY) from cell bodies within the arcuate nucleus to terminal regions including the dorsomedial and ventromedial hypothalamic regions, thus controlling appetite (Chen and Romsos, 1996). This result was most prominent in rats that were treated with a combination of ZnCl₂ and Dexamethasone. However, the decrease in total body weight did not appear to be affected by pomegranate seed extract when the pomegranate+DEX+ NaCl-treated animals were compared with DEX+NaCl treated animal group. Therefore, it is most likely that the decrease in total body weight is due to Dexamethasone. Correlation between total body weight loss and Dexamethasone treatment has been reported previously (Dubey et al., 2017).

Even though our results show no change in blood cell components, other studies have reported that pomegranate juice can increase platelet count in men after 15 days of intake(Achraf et al., 2018). Previous studies proposed that pomegranate peel can assist with type 2 diabetes, by lowering blood glucose (Banihani et al., 2013); our study shows no significant changes in the levels of glucose after consumption of pomegranate seed extract. We did not observe changes in the levels of serum K+, Ca^{2+} , and Cl^{-} electrolytes or the haematological parameters (RBCs, HGB, HCT, and PLTs). A slight reduction of WBCs was observed in response to pomegranate seed extract treatments. A similar finding was detected in ZnCl₂ treatment group. In addition, serum Na⁺ electrolyte was significantly increased when a combination of pomegranate seed extract and ZnCl₂ were used together.

ZANCO Journal of Pure and Applied Sciences 2019

5. CONCLUSIONS AND SIGNIFICANCE

We conclude that pomegranate seed extract lowers systolic blood pressure (mmHg) in hypertensive rat models. In addition, the supplementary ZnCl₂ can significantly lower blood pressure as well. Whereas both treatments (pomegranate or ZnCl₂) do not cause any changes in blood glucose levels, RBCs, HGB, HCT, and PLT counts. They do not have any significant effect on body weight. However, there is a slight reduction in WBCs count in both pomegranate and ZnCl₂ treated groups. There were no significant differences in the levels of serum K^+ , Ca^{2+} , and Cl^- among the treated groups. Nevertheless, serum Na⁺ level in serum increased significantly when the rats ingested a combination of pomegranate and ZnCl₂ compared to serum Na⁺ level of the control group. This has a counter effect on the reduction of blood pressure. Therefore, we recommend not to combine pomegranate seed extract and ZnCl₂ together as a dietary supplement. Our data shows that pomegranate has a potential therapeutic effect in lowering blood pressure in hypertensive subjects.

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Conflict of Interest

The authors declare no conflicts of interest.

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RESEARCH PAPER

Pharmacognostic Review on Kurdish Plant Pterocephalus nestorianus

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ABSTRACT:

The genus *Pterocephalus*, one of the most important genera of Caprifoliaceae family, comprises about 30 species which are usually found in sunny, dry, rocky crevices, mostly in Europe and Western Asia. Nine species are native to the Kurdistan region of Iraq. *Pterocephalus nestorianus* Nab. is an important plant of this genera and locally known as *Lawa*. It is widely used in folkloric medicines in Kurdistan for treating inflammation and oral diseases. It is also widely used as ornamental plant and component of many herbal formulations, all around the world. The aim of the present study is to collect authentic literature on classification, geographic distribution, eco-cultural health, traditional uses, phytochemistry, biological (environmental) activities and pharmacological properties of *P. nestorianus*.

KEY WORDS: *Pterocephalus nestorianus*, Caprifoliaceae, Lawa, Phytochemistry, Pharmacology. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.7</u> ZJPAS (2019), 31(5);53-60.

INTRODUCTION:

Plant chemistry or phytochemistry has developed as a distinct discipline, someway in between natural product organic chemistry and plant biochemistry and is closely related to both (Ahamad et al., 2014, 2015). It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the understanding of the chemical structures of the secondary metabolites, their biosynthesis, turnover and metabolism, their natural distribution and their biological function in the organisms (Ahamad et al., 2014). In all these researches, it is necessary to find the right methods for extraction, separation, purification and, then, identification of the many different constituents present in each plant (Harborne, 1998). Historically, the first new "pure" isolated compounds were strychnine (1) from Strychnos

and colchicine (3) from Colchicum genus, followed by morphine (4), digitoxin (5), quinine (6) and pilocarpine (7), which can be considered the basis of the earliest commercial medicines, and aspirin (8), the first semi-synthetic drug derived from a natural remedy (Cragg and Newman, 2013). Furthermore, the accidental discovery of penicillin from the filamentous fungus *Penicillium notatum* and the findings over the therapeutic uses of penicillin G (9) in the first half of the last century, gave a substantial boost to the investigation of nature as the man source of novel bioactive compounds, ushering what is "Golden Age of Antibiotics" called the (Ahmadiani et al., 1998). The uses of medicinal plants are based on hundreds of years of beliefs, observations, and a rich medicinal history in Kurdistan (Alsamarkandi, 1985; Abdul and Hussain, 2018).

The genus *Pterocephalus*, one of the most important genera of Caprifoliaceae family, comprises about 30 species which are usually

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found in sunny, dry, rocky crevices, mostly in Europe and Western Asia (Iran, Turkey) (Shekha, 2007). Nine species are native to the Kurdistan region of Iraq (Shekha, 2007). These plants are widely used in folkloric medicines, all around the world, due to different biological activities such as anti-inflammatory, analgesic, antihepatotoxic, antioxidant, antibacterial, spasmolytic, hemostatic, antiseptic and astringent properties (Abdullah et al., 2017). The major bioactive compounds isolated and identified from different P. nestorianus including hydroxycinnamic acid iridoids, phenolic glycosides, esters. monoterpenoid glucoindole alkaloids, and lignans, triterpenoid saponins, and flavonoid C-glycosides (Abdullah et al., 2018). In Kurdish local medicine, P. nestorianus is considered to possess efficacious activities against various healing ailments; however, no systemic literature reviews available on phytochemistry and pharmacology of this plant. Folkloric medicines have become a part of eco-cultural health which directly contributes to the dynamic interaction between humans and ecosystems. This interaction provides important insights about health of the ecosystem and wellbeing of human populations. It is safe to say that studies related to folk medicine and medicinal plants maintain significant amount of knowledge about human and environmental health (Abdullah et al., 2016). Therefore, this review paper aims to collect authentic literature on classification, distribution. geographic traditional uses. phytochemistry and pharmacological properties of P. nestorianus.



Figure 1. Photography of P. nestorianus Nab. Plant

1. METHODOLOGY

P. nestorianus, locally known with the Kurdish name of "Lawa", is popularly employed

in the herbal medicine for the treatment of oral diseases and inflammation. A complete literature survey about *P. nestorianus* was carried out by thoroughly consulting the Chemical Abstracts, Biological Abstracts, textbooks, national and international journals, herbal databases from the Internet and other published research materials, SciFinder and PubMed.

1.1. Pterocephalus nestorianus Nab.

The genus Pterocephalus, belongs to the taxonomically complex very family of Caprifoliaceae, which contains about 30 species of perennial and annual herbs and shrubs; most of are widely distributed over them the Mediterranean region and the Middle East, with about 20% in Asia and Africa (Verlaque, 1984; Fahem et al., 2008). Nine species are native to the Kurdistan region of Iraq (Shekha, 2007). These plants are widely used in folkloric medicines, all around the world, due to different biological activities such as anti-inflammatory, analgesic, anti-hepatotoxic, anti-oxidant, antibacterial. spasmolytic, hemostatic, antiseptic and astringent properties (Abdullah et al., 2018).

1.2. Taxonomy (Hassler, 2016)

Plantae
Tracheophyta
Magnoliopsida
Dipsacales
Caprifoliaceae
Pterocephalus
nesrorianus

1.3. Geographic Distribution

Pterocephalus nestorianus Nab. is native throughout the world, but mainly distributed in Turkey, Iran, Iraq, as well as in Kurdistan – Iraq where, in particular, it is found in Bekher mountain, Zakho, Sharanish, Silavi Sharanish, Kani-masi, Gali-zanta, Aqra, Atrush, Lalish mountain, Duhok, Zawita, Swara-tuka, Sarsang, Gara, Matina, Sulav, Amadiya, Deralook, Rezan, Bekhma, Bradost mountain, Rowanduz, Gali Ali Beg, Shekhan, Aski-Kalak and Safeen Mountain (Shekha, 2007).

1.4. Traditional Uses

P. nestorianus is employed in the Kurdish herbal medicine for the treatment of oral diseases and inflammation. The dried aerial part powder is

directly applied onto oral mucus membrane three times a day for three consecutive days. For curing inflammations, aerial parts (100 g) are left in water (1 L) for 30 min; subsequently, the infusion is filtered and drunk, 2-3 cups a day (Zhang *et al.*, 2009; Abdullah *et al.*, 2017).

1.5. Phytochemical and Pharmacological Study of the Genus *Pterocephalus*

From the studies reported in the literature, it is interesting to note that many new and known compounds have been isolated from *Pterocephalus* species and their structures have been characterized by different techniques.

1.5.1 Iridoids and seco-iridoids:

Iridoids are basically a group of secondary metabolites that are present in different medicinal plants. Iridoids are monoterpenoids based on a cyclopentane-[C]-pyran skeleton (iridoid skeleton) (10). The name "iridoid" is derived from iridodial, which mainly consists of ten carbon atoms, though C11, and more rarely C10, is frequently missing. Oxidative cleavage at the C7, C8-bond of the cyclopentane moiety affords the so called secoiridoids (11).



The iridoids are separated in three groups: iridoid glycosides, secoiridoid glycosides and nonglycosylated iridoids. These glycosides occur as colorless often hygroscopic powders or as colorless crystals. They are readily soluble in water and ethanol and insoluble in chloroform and ether. They are often optically active, have a bitter taste and exhibit important pharmacological actions. So far, over 2500 iridoids have been isolated from natural sources and their structures have been identified, the vast majority of them differing only for the degree and type of substitution on the basic cyclopentane ring system. However, the most distinct chemical feature among iridoids is the biosynthetic pathway occurring in the different plant groups (Damtoft *et al.*, 1995).

Four new bis-iridoids, including an iridoid dimer contains a monoterpene pyridine alkaloid were isolated from the 95% aqueous ethanol extract of underground parts of *P. hookeri*. They have been called pterocenoids A-D (**12–15**). Their structures were elucidated by extensive spectroscopic methods, including 1D-NMR (¹H, ¹³C), 2D-NMR (COSY, NOESY, HSQC and HMBC) and mass spectrometry. All these bis-iridoids were found to possess secoiridoid/iridoid subtype skeletons and inhibited TNF-induced NF-kB transcriptional activity (Wu *et al.*, 2014).

Other bis-iridoids, named pterocephaline (16) and pterhookeroside (17), were reported from nature for the first time, along with the known iridoids cantleyoside (18) and cantleyoside methylhemiacetal (19).



⁽¹³⁾ $R_1 = H, R_2 = CH_3$ (14) $R_1 = CH_3, R_2 = H$ (15) $R_1 = H, R_2 = H$







In another work, loganin (20), loganic acid (21), sweroside (22), isoboonein (23) secologanin dimethyl-acetal (24), secologanin (25), and cantleyoside dimethyl acetal (26) were isolated from *Pterocephalus perennis*. These compounds were found to possess high anti-hepatotoxic, antibacterial and anti-inflammatory properties (Fahem *et al.*, 2008; Wu *et al.*, 2014; Wu Y-C *et al.*, 2014).



1.5.2. Flavonoids

Flavonoids constitute a class of secondary metabolites that has gained great interest for the large variety and number of its members, and some remarkable biological activities. Flavonoids are relatively low molecular weight bioactive polyphenols, which play a vital role in photosynthesising cells. Flavonoids have been known for a long time to exert diverse biological effects, which include, in particular, antitumor, anti-inflammatory, antiangiogenic, antiallergic, antioxidant and antiviral properties. The structural diversity of flavonoids is the result of a number of different reactions, such as hydroxylation, methoxylation, glycosylation, methylation, and acylation.

Flavonoids are characterized by a flavan nucleus which consists of a C6-C3-C6 carbon skeleton. The basic structural feature of flavonoids is a 2-(more rarely 3-) phenyl-benzo- γ -pyrane nucleus, consisting of two phenyl rings (A and B) linked through a heterocyclic pyran ring (C), as shown in (**27**).



The various flavonoids differ in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between the A and B rings. A variation in the C ring provides a subdivision in different classes. Thus, according to their molecular structure, they are divided into these classes: flavones (28), flavonols (29), isoflavones (30), flavanones (31), dihydroflavonols (32), anthocyanidins (33), catechins (34), chalcones (35) neoflavonoids (36).



In the past decade, only two flavonoid C-glycosides were isolated from *sanctus*, identified as luteolin-6-*C*- β -D-glucoside-7-*O*-methyl ether (**37**) and apigenin-6-*C*- β -D-glucoside-7-*O*-methyl ether (**38**) on the basis of spectroscopic studies (Fahem and Abdelaaty, 2006).



1.5.3. Terpenoids

Terpenoids are a large range of secondary metabolites widely distributed in nature. All terpenoids are formally based on the isoprene unit (39) and their carbon skeletons are build up from the union of two or more of these C_5 units. They comprise such different substances as the volatile mono-and sesquiterpene (C_{10}) and C_{15}) components of the essential oils, the less volatile diterpenes (C_{20}) , and the non-volatile triterpenoids (C_{30}) and derived steroids, as well as the carotenoid pigments (C₄₀) (Fahem and Abdelaaty, 2006; Harbone, 1998).



According to the reported literature (Jun et al., 1993; Yang et al., 2007), four novel triterpenoid saponins, named hookeroside A (40), hookeroside B (41) hookeroside C (42), and hookeroside D (43), together with two common bioactive triterpenoids, oleanolic acid (44) and its isomer ursolic acid (45), were isolated from *P. hookeri* by capillary zone electrophoresis and other chromatographic techniques. The structures were determined on the basis of chemical and spectral evidences. Triterpenoid saponins have drawn the attention of researchers because of their diverse bioactivities, including anti-inflammatory, anticancer, anti-microbial effects. Due to their significant pharmacological activities, plants rich in triterpenoid saponins are usually exploited as drug sources.



1.5.4. Lignans

Lignans form a rather large class of secondary metabolites synthesized by plants exhibiting a wide spectrum of important biological activities. fact, hepatoprotective, anti-inflammatory, In antimicrobial, antiviral. anticancer, cancer prevention and antioxidant effects are the main biological properties attributed to lignans in literature (Toshiaki, 2003). The "lignan" a group of dimeric phenylpropanoids with the basic skeleton 47, in which two C_6 - C_3 phenylpropanoid units (46) are attached by their central carbon C_8 .



Previous phytochemical investigations on Pterocephalus species led to the isolation and identification of several lignans, including (+)-1hydroxypinoresinol $4"-O-\beta-D-gluco-pyranoside$ (48), (+)-1-hydroxypinoresinol 4'-*O*-β-Dglucopyranoside (49), (+)-syrin-garesinol 4"-O- β -D-glucoside (50), (7*R*, 8S)-ervthro-7,9,9'trihydroxy-3,3'-dimethoxy-8-O-4'-neolignan 4'-O- β -D-glucoside (51), cedrusin 4'-O- β -D-glucoside (52), urolig-noside hexaacetate (53), translariciresinol $4-O-\beta$ -D-glucoside hexaacetate (54) (Graikou et al., 2006; Wu et al., 2014).

ZANCO Journal of Pure and Applied Sciences 2019



58

(48) $R_1 = R_2 = R_4 = H$ $R_3 = R_6 = OCH_3$ $R_5 = Glc$ $R_7 = OH$

(49) $R_1 = R_4 = R_5 = H$ $R_3 = R_6 = OCH_3$ $R_2 = Glc$ $R_7 = OH$

(50) $R_1 = R_3 = R_4 = R_6 = OCH_3$ $R_5 = Glc$ $R_2 = R_7 = H$



1.5.5. Essential oils

Essential oils contain hundreds of highly volatile components, including terpenoids, aldehydes, esters, carboxylic acids, ketones and alcohols. These mixtures are extracted from leaves, flowers, bark, roots or other parts of the plant. The odour and taste of an essential oil are mainly determined by the oxygenated constituents; moreover, the fact that they contain oxygen atoms gives them some solubility in water and considerable solubility in alcohol.

The composition of the essential oils from the aerial parts of *P. canus* and *P. gedrosiacus* (Hooshang *et al.*, 2011; Akhgar and Safavinia 2016), which grow in the wild in Iran, have been previously reported. The main constituents of the *P. canus* oil were naphthalene (42.4%) (55), tridecanoic acid (7.9%) (56), and palmitic acid (7.9%) (57), where's the characteristic components of the *P. gedrosiacus* oil were thymol (31.6%) (58), carvacrol (7.7%) (59), α -pinene



It has become more evident in studies that degradation of an ecosystem negatively influences human health; thus, that human health is linked to ecological context holds an important place in ecosystem services. The benefits of ecosystem services for people can be both material and quantifiable; for instance, while climate regulation and food supply is considered as material advantages, aesthetic and recreation is considered as quantifiable advantages.

2. CONCLUSIONS

In recent years, phytochemical investigation of herbal flora has received much attention of the scientists and pharmaceutical industries so as to know about novel herbal compounds which can be screened for their therapeutic potential to treat several health disorders without side effects. This genus could be a promising source for the development of novel strategies to cure fatal maladies. Further consideration, standardization and clinical trials of pharmacological potential of *P. nestorianus* is essential for its recommendation as a medicine at safer level. The information summarized above will serve as a reference tool for the research groups working in the area of alternatives of developing synthetic drug. However, there is a need to evaluate the therapeutic potential on modern scientific lines through clinical trials, phytochemicals and pharmacological studies. In addition, this study has showed that conducting studies on medicinal plants help people not only establish a relationship with the environment but also collect important knowledge about their possible contributions to human health.

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RESEARCH PAPER

Haemonchus contortus as a model in assessing activity of *Citrullus colocynthis* fruit extract to control benzimidazole-resistant parasitic nematodes

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ABSTRACT:

The present field and laboratory experimental study were designed to appraise the efficiency of crude aqueous methanol extract (CAME) of *Citrullus* (*C.*) *colocynthis* (bitter apple) fruits in combating benzimidazole-resistant digestive canal nematodes. To meet the requirements of the trial, the highly pathogenic gastric roundworm of ovine, albendazole-resistant *Haemonchus contortus* was used as a paradigm. The faecal egg count reduction test (FECRT) and egg hatch assay (EHA) revealed prevalence of resistance to the abovementioned dewormer. In reference to the RESO Computer Program, the calculated FECR% was 70.77, whilst the lower confidence interval was 49.7. The LC₅₀ value for albendazole, calculated via probit analysis, was 2.046 µg ml^{- 1} after carrying out EHA. Antinematicidal effectiveness of *C. colocynthis* CAME was assessed *in vivo* through administration of four doses (50, 100, 150, and 200 mg/kg⁻¹ B.W) utilizing FECRT as an indication for their efficacies. There was a dose dependent antinematicidal potency of *C. colocynthis* CAME. The ovicidal activity of the plant extract was also trialed *in vitro* via conducting EHA. The estimated LC₅₀ value was 0.3422 µg ml⁻¹ after performing EHA, whilst the calculated mean FECR% for the highest dose of CAME was 95.57, which is deemed effective. Moreover, administration of *C. colocynthis* CAME at 200 mg/kg⁻¹ B.W did not reveal untoward consequences in animals; accordingly, could be used as a substitute remedy in fighting antinematicidal resistant populations of gastrointestinal nematodes.

KEY WORDS: *Haemonchus contortus*, bitter apple, benzimidazole dewormer, resistance, alternative therapy DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.8</u> ZJPAS (2019), 31(5);61-70 .

INTRODUCTION:

Antinematicidal resistance (AR) is deemed a major barrier precluding the control of livestock pathogenic roundworms and a serious menace to the maintenance of population-extensive remedy policies being run to restrict rampancy of human parasitic nematodes in the under-developed countries (Waller, 2006; Gilleard, 2013). Unluckily, periodic mass therapy of humans and ruminants utilizing small synthetic antinematicidals, particular members in of benzimidazole

* Corresponding Author: Chreska Nooraldin Ahmed E-mail: <u>chreska 142@hotmail.com</u> Article History: Received: 02/03/2019 Accepted: 07/07/2019 Published: 17/10/2019 antiparasitics has led to development of AR as a result of mutation occurrence in chemotherapeutic targets of nematodes (Beech and Silvestre, 2010; Harhay *et al.*, 2010; Vercruysse *et al.*, 2011).

It could be said that attempts to delay prevalence of AR are too late (Waller, 2006). Thus, helminthologists now are satisfied that the conventional strategy based on allopathic (synthetic west drugs) dewormers to control pathogenic nematodes must be changed to be well-matched with the sustainability of best helminth management. In this regard, researchers and professionals in the domain of parasitology have adopted some substitutes such as immunization of vulnerable individuals, balanced foods to boost the defence system and biological control to overcome the dilemma of AR (Waller, 2006; Strain and Stear, 2001; Stear et al., 2007). Having said, these alternative approaches have not achieved any success pragmatically and still under assessment (Waller, 2006).

On the other hand, under the tent of nonsynthetic antiparasitics, ethnobotanicals are currently a promising domain of explorations expected to be reliable substitutes to contain spread of antinematicidal-resistant nematode infestations in the coming decades (Jabbar *et al.*, 2006; Hamad *et al.*, 2018). Thus, *Haemonchus* (*H.*) contortus parasite, a serious haematophagous gastric dweller nematode of ovine, resistant to the common broad-spectrum drug; benzimidazole was utilized as a paradigm to perform the trial.

Antinematicidal efficacy of aqueous methanol extract of indigenous *Citrullus* (*C.*) *colocynthis* fruits against the benzimidazole-resistant helminths were evaluated employing reliable parasitological techniques.

2. Materials and Methods

2. 1. Selection of experimental animals

Lambs (n=90) with the criteria mentioned below were chosen for the experiment:

Age: 12-24 weeks (Coles et al., 1992).

Dewormer administration record: not have been drenched for the last two months (Coles *et al.*, 1992).

Lower eyelid paleness: checked by FAMACHA Anaemia Guide Chart. Animals in anaemic stage 3, 4 or 5 were selected (Macedo *et al.*, 2010).

Egg per gram of feces (EPG): minimum 150 ova (Coles *et al.*, 1992).

H. contortus infestation: >90% among nematode populations (Bowman *et al.*, 2003).

The lambs chosen for the trial were marked and randomly allocated into six groups:

Group 1: Albendazole resistance detection group (n=15)

Group 2: *C. colocynthis* dosage (50 mg kg⁻¹ BW) drenched group (n=15)

Group 3: *C. colocynthis* dosage (100 mg kg-¹BW) drenched group (n=15)

Group 4: *C. colocynthis* dosage (150 mg kg-¹ BW) drenched group (n=15)

Group 5: *C. colocynthis* dosage (200 mg kg-¹ BW) drenched group (n=15)

Group 6: infested non-drenched group (n=15)

The tentative lambs did not administer any other therapy during the trial period.

2. 2. General clinical examinations

The selected animals for the experiment were inspected clinically. The clinical examinations included check of temperature, pulsation, auscultation, skin inspection for external parasites, lymph node checking, oral inspection, alimentary tract status and any abnormality in respiratory system (Kahn, 2005).

2. 3. Preliminary tests to detect natural infection with *H. contortus* and other nematodes

• Faecal examination

Faecal checks of all the experimental lambs were conducted during the assortment procedure (Soulsby, 1982; Coles *et al.*, 1992; Iqbal *et al.*, 2006a).

•Coproculture method

Coprocultures were also carried out to evaluate the contribution of a range of parasitic roundworms in entire natural helminth infections following MAAF (1986) during the choosing procedure. Faecal samples from each group of lambs were pooled and cultured in plastic containers. Amphotericin B (5 μ g g⁻¹) was added to prevent mycotic contaminations. The cultures were incubated for seven days at 27±1°C. After this time, the parasite larvae (L₃) were pulled together utilizing Baermann apparatus.

•Baermann technique

This assay was performed to collect the parasite larvae (L₃) from the coproculture method. Approximately 15g of the incubated faeces were wrapped up in medical gauze and placed in the Baermann apparatus funnel. Lukewarm water was added to stimulate larval motility to the end of collecting tube. The "Baermann" was set up overnight and a small volume of water was collected and poured in a plastic container. Then the water sample transferred to a petridish, Lugol's iodine was added to the culture (Iqbal *et al.*, 2006a) and 100 larvae were counted and recognized following MAFF (1986).

2. 4. Antinematicidal resistance investigations • Fecal egg count reduction test (in vivo assay)

Albendazole 5%, a member of benzimidazole group and manufactured by Dox-AL ITALIA SpA- Italy, was purchased from market. The lambs (n=15) in group 1 were exposed to albendazole at the standard dosage (5mg kg-¹ BW); while group 5 kept as infested untreated control. Coproculture of experimental lambs (group 1 and control) were performed at day 0 (pre-deworming) and day 14 (post-deworming) as mentioned previously. Eggs per gram of faeces (EPG) were counted utilizing Whitlock Universal

63

Egg Counting Slide (provided by JA Whitlock & Company, PO Box 51, EASTWOOD NSW 2122 AUSTRALIA). Pre-and post albendazole treatment EPG and contribution of all nematodes in the natural infestations were established. EPG were estimated by Whitlock slide utilizing the following formula:

EPG= Total eggs in chamber 1, 2 and 3/3 x 50 (dilution factor)

Faecal egg count reduction percentage (FECR %) was calculated using the formula below:

FECR % = [1- (mean EPG treatment/mean EPG control)] x100

RESO computer program (CSIRO Animal Health Research Laboratory, Private Bag 1, Parkville, Vic. 3052, Australia) was utilized to calculate the FECR data comprising arithmetic mean, variance of counts, FECR% and 95% confidence interval. Pursuant to Coles *et al.* (1992), resistance is built up if (i) the FECR% is less than 95% (ii) the lower limit of 95% confidence interval is less than 90% (iii) If just one of the two norms is met resistance is suspected. On the other hand, Gill (1996) has recommended that any negative values produced from FECR% and lower limit of confidence interval were deemed equal to zero, interpreting that the resistance is widely prevalent and has reached the serious level.

•Egg hatch assay (in vitro assay)

This assay was executed according to the standardized protocol that was accepted by World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) (Coles et al., 1992) to diagnose resistance against albendazole. Briefly, eggs were extracted from faeces, suspended in water and estimated. Albendazole 5% was dissolved in 0.3% Dimethylsulfoxide (DMSO) and stock solution was prepared as 50 μ g ml⁻¹. The stock solution was serially diluted (0.0244-50 $\mu g m l^{-1}$ in a multiwell plate. The control well received merely 1 ml solvent (0.3%DMSO). One ml (approximately 150 eggs ml⁻¹) of egg suspension was added to each well including control well. Plate was incubated at 27°C ±1 for 48 hours and 70% relative humidity. After incubation, two drops of Lugol's iodine was added. At least 100 of the unhatched eggs (dead and embryonated) and hatched larvae were counted to calculate the hatching inhibition percentage (Coles et al., 1992). The following

formula was used for evaluation of hatching inhibition (%):

Hatching inhibition (%) = P test/ P total x100

P test: number of unhatched or embryonated eggs.

P total: number of unhatched or embryonated eggs + Larvae (L₁).

 LC_{50} values were calculated for the eggs by probit analysis. Eggs with an LC_{50} value in excess of 0.1 µg ml⁻¹ were deemed as an indication of antinematicidal (albendazole) resistance as suggested by Coles *et al.* (1992).

2. 5. Evaluation of plant extracts against resistant *Haemonchus contortus*

• Extraction of Citrullus colocynths

C. colocynthis fruits were harvested naturally from Mamzainer plain, Kandinawa district, South of Hawler. After the separation of seeds, the tissues were dried in shade at room temperature. The herbaceous tissues were crushed to a powder using an electric grinding machine. The crushed tissues were solved in 70% aqueous methanol by cold maceration at 25-30 C° and the mixed materials were mixed 2-3 times every day by a stirrer. After 72 hours, the materials were filtrated through a piece of porous cloth and the filtrate was collected in a container. The abovesaid procedure was recurred thrice. The extract was evaporated to dryness at room temperature (Gilani et al., 2004). The crude aqueous-methanol extracts (CAME) was kept at 4°C until employed towards the parasitic nematodes.

• Fecal egg count reduction test (in vivo assay)

The animals in group 2, 3, 4 and 5 were exposed to CAME of *C. colocynthis* at doses; 50, 100, 150 and 200 mg kg-¹ BW correspondingly, whilst, group 6 exploited as infested non-drenched control. Fecal examinations and coprocultures of the tentative lambs were performed at day 0 (pretherapy) and day 14 (post-therapy) as mentioned previously. Pre- and post- CAME therapy EPG and parasite infestations were recorded. FECR % was estimated utilizing the undermentioned formula:

FECR% = [1-(mean EPG treatment/mean EPG control)] x100

•Egg hatch assay (in vitro assay)

This technique was conducted to assess the inhibitory potencies of different dosages of the CAME on egg hatching of the parasitic nematode. The test was carried out according to the protocol explained by Coles *et al.* (1992) with slight

changes by a number of investigators to be appropriate for evaluation of medicinal botanicals (Macedo et al., 2010). One gram of CAME was suspended in 10 ml of 70% acetone and this was deemed as mother solution (100 mg ml⁻¹) which was serially diluted in a 24 multiwell plate. The ova specimens were treated with 12 concentrations (100-0.048 mg ml⁻¹) of the extract. positive control, 0.025 mg ml⁻¹ For of albendazole5% was dissolved in 0.3% DMSO. The well of negative control got merely 1ml of 70% acetone. LC₅₀ was determined utilizing probit analysis. The rest procedure was similar as pursued in studies on antinematicidal resistance.

2. 6. Statistical analysis

The mean EPG for resistance detection in treatment and control groups (after 10-14 days of therapy with albendazole 5%) was analyzed through the RESO computer program. The degree of resistance prevalence was evaluated by this program via estimating FECR% and calculating lower limit of confidence interval.

The data procured from EHA for different concentrations of albendazole and *C. colocynthis* CAME to assess their ovicidal efficacy against *H. contortus* eggs, one way ANOVA was applied using Graph Pad Prizm (version 7). Tukey as multiple comparison test was utilized to compare among doses. All obtained data were expressed as Mean±SE. For computation of LC₅₀ (μ g ml⁻¹) at 95% confidence interval for avoiding 50% of egg hatching, probit analysis of LC₅₀ value on the EHA was applied.

For analysis of the data recovered from FECRT and evaluation the impact of various doses of *C. colocynthis* CAME on reduction of EPG, one way ANOVA was applied followed by application of Tukey test for comparison between doses.

3. Results

3.1. Identification of *H. contortus* infections in experimental animals

The larvae (L_3) of *H. contortus* were recognized following conduction of pre-treatment coproculture and Baermann apparatus (Picture 1).

3. 2. Composition of natural roundworm infestations in tentative lambs

The lambs comprised in the trial were principally infested with *H. contortus*; however, other parasitic nematodes were involved. Pre-treatment coproculture followed by Baermann apparatus demonstrated that *H. contortus*, through identification of L_3 , as the main contributor to the EPG (>90%) of the tentative groups of lambs (Table 1 & 2)



Picture 1 *Haemonchus contortus* larvae (L₃) after performing coproculture and Baermann technique

Table 1 Pre-treatment and post-treatment proportion of *Haemonchus contortus* larvae (L_3) and other nematode species in the lambs chosen for resistance studies based on pooled faecal specimens of group 1 and 6 (control)

		Pre-treatment	
Groups	<i>H. contortus</i> L_3 (%)	L_3 (%) of other nematodes	
Group 1 (Albendazole5% group)	95	5	
Group 6 Control (untreated)	93	7	

	Post-tre	atment	
	<i>H. contortus</i> L ₃ (%)	L_3 (%) of other nematodes	
Group 1 (Treated with albendazole5%)	92	8	
Group 6 Control (untreated)	96	4	

Table 2 Pre-treatment proportions of Haemonchus contortus larvae (L_{3}) and other nematode species in the lambs chosen for assessment of Citrullus colocynthis based on pooled faecal specimens of group 2, 2, 4, 5 and 6 (control)

2, 5, 4, 5 and 6 (control)					
Groups	<i>H. contortus</i> L ₃ (%)	L_3 (%) of other nematodes			
Group 2 (50mg kg- ¹ BW)	94	6			
Group 3 (100 mg kg- ¹ BW)	93	7			
Group 4 (150 mg kg- ¹ BW)	95	5			
Group 5 (200 mg kg- ¹ BW)	96	4			
Group 6					
Control (untreated)	92	8			

3. 3. Study of albendazole resistanceCoproculture

As apparent from the obtained data of pre-therapy coproculture of pooled faecal specimens of all experimental lambs, the albendazole and control groups had 95 and 93% *H. contortus* infestation, respectively, while infestation with other parasitic roundworms was 5 and 7% for albendazole and control groups, respectively (Table 1). Posttherapy (after 14 days), the albendazole and control groups had 92 and 96% *H. contortus* infestation, respectively, whilst infestation with other parasitic roundworms was 8 and 4% for albendazole and control groups, respectively (Table 1). Accordingly, it is an indication of development of resistance by the parasite against albendazole.

•FECRT

Mean EPG reduction, FECR% and lower confidence interval 95%, on day 14 post-treatment with albendazole depended on statistical analysis and RESO computer program, were 224.40 (control mean EPG= 767.80), 70.77 and 49.7, respectively. The aforementioned data had confirmed that the resistance was developed against the above drug.

•EHA

The LC₅₀ of albendazole was calculated to be 2.046 μ g ml⁻¹ (range 1.572-2.568), which was in excess to 0.1 μ g ml⁻¹ signifying development of resistance towards *H. contortus* (Coles *et al.*, 1992). Correlation between influences of various concentrations of albendazole and hatching inhibition (%) via carrying out EHA is demonstrated in figure 1.

3. 4. Antinematicidal potency of *C. colocynthis* CAME towards resistant *H. contortus*

•Coproculture

Post-therapy coproculture of pooled faecal specimens of all the lambs (four groups) exposed to different doses of *C. colocynthis* CAME is presented in table 3.

•FECRT

The results of antinematicidal efficacy of *C.* colocynthis CAME (four different doses) against albendazole-resistant parasite in the tentative lambs naturally infected with the predominant *H.* contortus (>90%) besides comparison between impacts of various doses of CAMEs on the egg reduction mean is statistically analyzed in table 4. There was a significant difference (P<0.05) between all doses. As evident from the procured data of coproculture and FECRT (Table 3 and 4), the two groups (4 & 5) exposed to effective doses *C. colocynthis* CAME (FECR% >80), very few *H*. *contortus* larvae (L_3) and other nematode larvae (group 4) and no larvae of this parasite and other parasitic roundworms (group 5) were detected. •**EHA**

The data obtained from analysis of variance (ANOVA) of EHA regarding ovicidal efficacy of various concentrations of *C. colocynthis* extract through computation of hatching inhibition (%)

had demonstrated different effects of various concentrations (dose-dependent ovicidal efficacy) which shown in figure 2. The estimated LC_{50} was 0.3422 µg ml⁻¹ (range 0.2733- 0.4275) at the level of 95% confidence interval



Figure 1 Correlation between the impacts of various concentrations of albendazole 5% and hatching inhibition (%)

Albendazole concentration (µg ml⁻¹)



Table 3 Post-treatment proportions of Haemonchus contortus larvae (L3) and other nematode specieson day 10-14 in the lambs chosen for assessment with different doses of Citrullus colocynthis CAMEbased on pooled faecal specimens

Groups	<i>H. contortus</i> L ₃ (%)	L_3 (%) of other nematodes	
Group 2 (50mg kg- ¹ BW)	92	8	
Group 3 (100 mg kg- ¹ BW)	94	6	
Group 4 (150 mg kg- 1 BW)	poorly recovered	poorly recovered	
Group 5 (200 mg kg- ¹ BW)	not found	not found	
Group 6 (control)	95	5	

Table 4 Egg per gram of faeces and percentages of faecal egg count reduction in animals on day 10-14 post-treatment with different doses of *Citrullus colocynthis* CAME

Groups	Mean EPG±SE	Mean FECR%	
Group 2 (50 mg kg- ¹ BW)	345.3±14.51 C	55.07	
Group 3 (100 mg kg- ¹ BW)	214.2±13.59 BC	72.13	
Group 4 (150 mg kg- 1 BW)	113.2±8.46 AB	85.28	
Group 5 (200 mg kg- ¹ BW)	$34.4\pm8.26~\text{A}$	95.57	
Group 6 (control)	767.8±72.95 D	-	
X 1 1 1 1 1 1			

Means sharing similar letters are statistically non-significant (P>0.05)

4. Discussion

Undeniably, AR has been emerged among parasitic nematodes of livestock in every country around the world (Waller, 2006; Hamad, 2014; Hamad et al., 2014; Hamad et al., 2017; Hamad et al., 2018). This problem has started to threat public health as well (Beach and Silvestre, 2010; Harhay et al., 2010). Having said, benzimidazoles are among the major broad-spectrum antinematicidals animal that human and roundworms have developed resistance against them seriously as compared to other classes of synthetic dewormers (Kaplan, 2004; Vercruysse et al., 2011). Hence, in this study, resistance to albendazole was detected among H. contortus populations in naturally infected sheep utilizing RESO computer program. The computed FECR%

was (70.77), whilst the lower confidence interval was (49.7) which signify that the AR was emerged (Coles *et al.*, 1992). LC_{50} value of albendazole (2.046 µg ml⁻¹) estimated by probit analysis after conduction of EHA also verified development of AR among the populations *H. contortus*. In this regard, Coles *et al.* (1992) have reported that LC_{50} value higher than 0.1 µg ml⁻¹ is an indication of benzimidazole group resistance. It can be concluded that AR level among the nematode individuals in ovine of the study area is above 25% and the nematodes are resistant to albendazole. It is evident that the AR could not be diagnosed by these traditional parasitological approaches if the resistance level is below 25% among the gastrointestinal roundworm individuals (Martin et al., 1989).

The livestock raisers are adapted to drench their animals 3-4 times with benzimidazole annually in the investigation zone (Veterinarian file in the investigation site). In this regard, Barnes *et al.* (1995) and Waller *et al.* (1995) have mentioned that recurrent annual administrations of an anthelmintic are closely related to the emergence and rampancy of resistance among gastrointestinal nematodes. This will conduce to incidence of mutation (loss of drug binding) at βtubulin isotype 1 (drug target) (Beech and Silvestre, 2010).

Although, other factors such as bad quality of dewormers, use of insufficient dosages by owners and storage conditions in developing countries have been incriminated as well (Coles *et al.*, 1995)

On the other hand, unavailability of reliable alternatives to synthetic chemotherapeutics nowadays, ethnobotanicals could be appropriate substitutes to control nematodiasis in humans and livestock particularly in under-developed countries (Jabbar et al., 2006; Sindhu et al., 2014). In continuation of previous limited herbological studies, albendazole-resistant H. contortus individuals were exposed to the indigenous medicinal plant, C. colocynthis fruit CAME utilizing in vivo and in vitro assays. Evidently, a number of bioactive phytochemical ingredients from bitter apple fruits such as alkaloids, glycosides, fatty acids, flavonoids and essential oils have been extracted (Rahuman et al., 2008; Hussain et al., 2014). Moreover, C. colocynthis fruit extracts have been investigated widely for their broad range pharmacological efficacies and medicinal uses, which comprise mosquito larvicidal activity against the early fourth instar larvae of Culex quinquefasciatus (Diptera: Culicidae) (Rahuman et al., 2008), antibacterial and anticandidal (Marzouk et al., 2009; Rasool and Jahanbakhsh, 2011), antioxidant anti-inflammatory/analgesic (Saba and and Oridupa, 2010) and other activities such as antidiabetic. antilipidemic, anthelmintic, anticancer...etc (Hussain et al., 2014).

It is noteworthy mentioning that the potency of *C. colocynthis* fruit extracts has not been studied *in vitro* and *in vivo* against antinematicidal-resistant alimentary tract roundworms of humans and livestock elsewhere, hence it could be said that this study is an original investigation in this field. The FECR% results post-administration of 50, 100, 150, and 200 mg

kg-¹ BW of the CAME were 55.07, 72.13, 85.28 and 95.57 respectively. In this connection, it should be pointed out to the recommendations of W.A.A.V.P (second edition) edited by Wood et al. (1995) proposing that any anthelmintic with FECR% (98) is reckoned highly effective; FECR% (80) and above is effective; whilst FECR% less than (80) is not recommended for employment. Accordingly, the two doses 150 and 200 mg kg $^{-1}$ BW of the extract are effective. The study has also revealed that the highest doses were more efficacious as compared to the lowest doses. This therapeutic activity phenomenon of was reported medicinal plants by many researchers in the field of phytotherapy (Iqbal et al., 2001; Iqbal et al., 2006b; Hamad et al., 2013; Hamad, 2018).

5. Conclusions

In accordance of the results of this research work, it may be extracted that the resistance percentage is above 25% among H. contortus communities to albendazole in sheep in Khabat district, Hawler governorate, where the The most probable reasons study conducted. behind the prevalence of albendazole resistance in the above area are the random and frequent annual use of benzimidazole members. On the other hand, the effective doses of C. colocynthis fruit CAME were 150 and 200 mg kg-¹ BW Consequently which enhanced in reducing EPG with percentage 85.28 and 95.57, respectively., these two doses could be employed in treating benzimidazole-resistant sheep carrying Н. contortus individuals. Furthermore, the EHA has revealed that the CAME of C. colocynthis fruit can prevent egg hatching but not embryonations.

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Conflict of interest

The researchers acknowledge that there is no conflict of interest concerning contents of the present research article.

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RESEARCH PAPER

Deep Improvement of Fine-Grained Soil Using Lime Piles

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ABSTRACT:

In the present study, the effects of lime piles on the geotechnical properties of fine-grained soil have been investigated in details, where the lime piles are used as sustainable deep improvement technique for the shear strength and compressibility of fine-grain textured soil. The sustainable development required using environmentally friendly materials and of low cost. Soil samples are obtained from the north of Babylon governorate and improved by lime piles, where a small-scale physical model prepared for this purpose. A suitable mesh, diameter, and depth of lime piles are suggested to measure the improvement in shear strength and compressibility of soil. Lime piles are prepared by mixing 15% of lime with dry soil and poured into the holes of 20 mm diameter and 500 mm length, and then lime piles left for 30 days as suggested curing period to allow the chemical reactions between soil and lime compounds. The results of tests detected that lime piles technique has significant effects on the chemical and engineering properties of silty clay soil samples. Also, the deep improvement of the soil causes increasing the undrained shear strength of soil by 23% and increasing the static and dynamic subgrade reaction of soil by 16 and 20% than that of natural soil sample respectively.

KEY WORDS: Lime pile, soil improvement, Geotechnical properties, Clayey soil, Cyclic subgrade reaction. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.9</u> ZJPAS (2019), 31(5); 71-79.

1. INTRODUCTION

The lime pile technique is one of the most methods used to improve the geotechnical properties of fine ground soil. It is developed before 3 decades in Europe and Japan and can be considered as sustainable development technique because of using environmentally friendly materials. The major strength gained by lime improved soil is mainly categorized into three reactions known lime hydration, ion exchange, and pozzolanic reactions. In addition, carbonation causes minor strength which increases with increasing of carbonic acid. Carbonic acid is resulted from the reaction of CO_2 of air with H_2O of pores water, or from any organic contaminant existed in the soil mass.

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Pozzolanic reactions essentially are cementation (CaSiO₂.2H₂O or CaAlO₃.2H₂O) and carbonation matters (Bergado et al., 1997). Broms (2000) simulated the reduction of soil moisture content is attributed to the slaking of quicklime and evaporation which are resulted in increasing the ground temperature. The decreasing of soil moisture content causes increasing the shear strength and decreasing the compressibility of soil. The increasing of soil strength by cement columns is achieved after 30 days of curing, while the increase of the soil strength by lime piles continues for more than one year (Broms, 1999). Furthermore, the clay fraction and then the clay minerals present in the soil have a significant impact on the undrained shear strength. The required lime content increases with increasing of the specific surface area of the clay minerals such as the montmorillonite.

Bergado et al. (1997) explained the lime behavior during insertion lime piles into the soil layers where the generated heat and expansion of
the lime will affect the behavior of soil. From the hydration of quicklime or when using calcium hydroxide as stabilizer will lead to increase of the pH value and dissolving of SiO_2 and AlO_3 presented in the clay particles. This process leads to ion exchangeable, flocculation, and pozzolanic reactions. Moseley and Kirsch (2004) concluded that the shear strength of soil is mainly obtained after 28 days of installation of lime piles. The shear strength of improved soil is increased by increasing the curing time due to achieving the soil strength of the long-term after 28 days of mixing. Therefore, the appropriate design of lime piles must be depended on the estimated shearing strength after three months.

In the soil improved by lime piles, the construction load is carried by the lime piles and the surrounding soil between the piles together. Settlement of lime piles and the surrounding soil can be computed according to the analytical approach of the Swedish Geotechnical Society (Carlsten, and Ekstrom, 1997). The settlement of the composite ground is determined by the soil profile into a property of layers, this calculation method is similar to conventional settlement calculation methods which use the average stiffness value of the composite ground. The finite element method is useful for these calculations (Kempfert, 2003).

Ashok and Reddy (2016) studied the effect of the lime pile technique on the properties of clay soil. The lime causes increasing the average of particle size and decreasing the clay content due to flocculation of fine particles. Also, lime piles caused high increase in the plastic limit and slight increase in the liquid limit. In addition, increasing the curing periods of lime piles leads to decreasing of optimum moisture content (OMC) due to increasing the desired water by the clay mineral particles undergoes to hydration reaction. The increasing of soil shear strength with lime (CaO_2) is achieved due to decreasing of soil moisture content by hydration and evaporation during the slaking of lime and ion exchange and pozzolanic reactions.

2. SUSTAINABLE IMPROVEMENT OF SOIL

Soil improvement is often considered to be a sustainable practice as it allows the use and development of inappropriate land. However, the most soil improvement methods involve a wide range of activities that result in environmental, social and economic impacts. The largest, most obvious impacts (particularly cost and duration) will usually be taken into consideration when selecting an appropriate technology to be used. However, potentially significant impacts can be observed such as dioxide emissions and consumption of raw materials. There are several technologies of soil improvement can be contributed to achieving a sustainable design. In this work, soil improvement of clay soil by using lime piles techniques is described as the main aspect and vital, so the principle of sustainability will be applied to this aspect. Several indicators that will be taken into consideration to reduce the environmental and economic impacts and which are included:

- 1) Use an environmentally friendly material to improve soft soil rather than other alternative techniques.
- Deep soft soil improvement contributes to sustainable development instead of using deep foundation, to reduce the environmental and economic impacts.
- 3) Using materials of low energy like lime leads to reduce carbon emission.
- 4) Using a contaminated site rather than of leaving it and using an alternate site.

The essential factors which control hardening properties of lime-improved soil are the type of lime where the quicklime is more effective than hydrated lime; lime content where the strength increases with increasing of lime content and curing time. Also, the type of soil is an important factor, where the normally consolidated clay is highly affected by improving with lime. For successful lime treatment, the clay fraction should not be less than 20% and the plasticity index should be more than 10%. Pozzolanic reaction and ion exchange are based on the clay mineral, where the clay minerals (kaolinite, illite, and montmorillonite) react with lime to give greater strength. Furthermore, the effectiveness of lime based on the pH value of soil where ion exchange is lowered with pH < 7, and the pozzolanic reaction is increased with pH>7. The long-term pozzolanic reactions have increased with increasing of pH value of soil, because the reactions are accelerated due to increasing of the solubility of SiO₂ and AlO₃ of soil particles (Bergado et al., 1997).

ZANCO Journal of Pure and Applied Sciences 2019

The activity of pozzolanic reactions increased by increasing the curing temperature, which causes increasing the soil strength. The rising of curing temperature up to 45°C leads to a significant increase in the solubility of SiO₂ and AlO₃ of soil particles and then increasing the pozzolanic reaction (Broms, 1999). Ion exchange and flocculation occurred immediately after some hours of mixing, where the calcium hydroxide is transformed again due to the presence of carbonic acid in the soil block (from the reaction of CO₂ of air with soil porewater). The reaction of lime with clay minerals causes exchangeable ions between calcium Ca^{+2} and ions of clay minerals. Consequently, this leads to increasing the electrical surface force of clay minerals, and transformation of soil structure which causes the flocculation and coagulation of clay particles into large spaces. The shear strength increased gradually by pozzolanic reactions due to reactions of calcium hydroxide with SiO₂ and AlO₃ present in clay particles resulting cementing materials or hydrated gel around soil particles such as (gel of calcium silicate hydrates and calcium aluminates). The stability of the pozzolanic reaction depends on the pH value of soil water and soil temperature (Bergado et al., 1996).

3. BEARING CAPACITY OF LIME PILES

Bergado et al. (1997) detected the bearing capacity of the single lime pile is either governed by the surrounding soil strength (soil failure) or by the lime column strength (column failure). In lime piles group, the ultimate bearing capacity is depended on both the shear strength of the surrounding soil (soil failure) and the strength of the lime column (column failure), but failure is governed by the bearing capacity of the block failure or by the local bearing capacity of the block along the edge when the spacing between columns is large. The short-term ultimate bearing capacity of a single column in the clayey soil which depends on the soil failure can be determined by the following equation:

$$Q_{ult,soil} = (\pi dH + 2.25\pi d^2) c_u$$
 (1)

where H and d are the length and diameter of the lime pile, and c_u is the undrained shear strength of the surrounding soil. The short-term ultimate bearing capacity of single lime pile which depends

on the column failure can be determined by the following equation:

$$Q_{ult,col.} = A_{col.} \left(3.5 c_{col.} + 3\sigma_h\right) \tag{2}$$

where A_{col} and c_{col} are the surface area and the cohesion of the lime pile, and σ_h is the horizontal stress acting on the lime column. The ultimate capacity of long-term is less than the short term by 65-85% due to creep of the column. The ultimate capacity of the lime piles group associated with the block failure is:

$$Q_{ult,g} = 2 c_u H(B+L) + (6 to 9)c_u BL$$
(3)

where B, L, and H are the width, length, and height of block failure of composite soil. The factor 6 is used for rectangular foundation L>Bbut factor 9 is used for the square foundation. The ultimate capacity of the lime piles group associated with local failure along the lime pile shaft is:

$$q_{ulti.} = 5.5 c_{av.} (1 + 0.2 \frac{b}{l})$$
(4)

where *b* and *l* are the width and the length of locally loaded area, and c_{av} is the average shear strength of improved area which affected by the relative area of the lime column.

4. SOIL SAMPLING AND MATERIALS

The soil specimens are obtained from the north of Babylon governorate (UTM: 33N515276, 44E28102). The disturbed and undisturbed intact soil specimens are obtained from 4 m below the ground surface. The water table encountered 2.5 to 3 m below the existing ground surface. An open pit of 10 m length and 5 m width was drilled at the site to get the soil samples. Then, the disturbed soil samples are placed in tight plastic containers and labeled, and transported to the laboratory to be used in the physical and chemical tests of the soil. The undisturbed soil samples extracted by Shelby tubes covered with wax and labeled to be used in testing the mechanical properties of soil samples.

The field unit weight is 19.3 kN/m³ (ASTM D2937-00) and the natural water content is 32% (ASTM D2216). Lime is a binder and transformed

ZANCO Journal of Pure and Applied Sciences 2019

into calcium hydroxide after mixing with water, and later is reacted with SiO_2 and AlO_3 existed in soil particles to produce hydrated silicate gel and hydrated aluminates gel. Those cementation gels lead to cover the clay particles and cementation the particles into close up together, which causes increasing the cohesion of lime-soil mixture (Bergado et al., 1997).

74

 $CaO + H_2O \rightarrow Ca (OH)_2 + Heat (300 mg of Ca/gm of Cao).$

5. LABORATORY WORK

In a present study, the impacts of soil improvement on geotechnical soil properties are investigated. The designations of tested soil samples are natural soil sample (Co) and improved soil sample CIo. Also, the remolded natural soil samples Cro to investigate the sensitivity of clay on the geotechnical properties of soil.

5.1. Deep Improvement Using Lime Piles

Deep soil mixing technique is used to install the lime piles by mixing the hardening binder of lime with the surrounding soil. Lime pile mechanism characterized by dry mixing and wet mixing. The dry mixing method involves the penetration of the hand auger to the required depth and then injection of the dry lime binder into the soft soil using compressed air. The dry lime is then mixed with the surrounding soil. In the dry method, no water is added therefore the natural moisture content soil should be at least 20%. The wet mixing method involves adding lime slurry to the soil mechanically, the mixing of lime slurry with the surrounding soil is easier than dry mixing method, especially when the soil moisture content is low and the initial shear strength of the soil is high (Kempfert, 2003).

Lime piles are used in the present study to increase the stability and strength of soil. Also, lime piles used to improve of clay soil samples. Soil samples firstly remolded in the container of physical model by tamping process according to the field unit weight and natural water content. Then holes are bored of 20 mm diameter (d) and spacing ratio 3d with depth 500 mm as shown in Figure 1. Lime is mixed with 15% of dry soil have been poured into the holes and mixed to ensure filling. Then, soil treated with lime piles left for 30 days for curing. The soil container was covered with two layers of polyethylene sheet and 10 cm of saturated sand to keep the moisture content of the soil and reduce the slaking and hydration reactions.



Figure 1: Lime pile installation process.

5.2. Chemical Tests

In the present study, the following chemical properties of soil samples are tested to measure the impacts of lime piles on the chemical composition of soil samples. The chemical properties tested according to ASTM (2003) are SO₃ (ASTM D516), SO₄ (ASTM D516), gypsum, Ca⁺² (ASTM D511), Cl⁻¹ (ASTM D512 A), pH value (ASTM D4972), TPH (atomic absorption spectrometer, AAS), TDS ASTM (D5907), Ec (ASTM D1125), CaCO₃ (ASTM D4373), and Mg⁺¹ (ASTM D511).

5.3. Physical Tests

Routine soil tests were carried out to characterize the physical properties of soil. The tests are the particle size distribution (ASTM D422), specific gravity (BS:1377, 1976, Test 6B), Atterberg's limits (ASTM D4318), and standard Proctor compaction test (ASTM D698).

5.4. Mechanical Tests Results

The tests included determining the shear strength parameters, modulus of elasticity, the coefficient of consolidation, and modulus of subgrade reaction. Two types of soil samples, undisturbed and remolded are used to investigate the compressibility characteristics of the natural soil, while remolded soil samples used to measure these characteristics of improved soil samples by an oedometer test according to ASTM (D2435). The shear strength of soil samples is measured by conducting unconfined compression test (UCT, ASTM D2166), direct shear test (DST, ASTM D3080), and unconsolidated undrained tests (UUT, ASTM D2850) on undisturbed and remolded soil samples before and after the improvement.

A comparison between static and cyclic modulus of subgrade reaction (Ks) due to deep improvement impacts are investigated by conducting static and cyclic plate loading tests. The tests are performed in a steel cube box of side 700 mm. The rigidity of steel box has been guaranteed by using rigid steel plates of 4 mm thickness. The loading is applied on a square steel plate of dimensions 125×125×10 mm. In the present study, Ks is determined according to ASTM (D1196). For verification the modulus of subgrade soil reaction under repeated loading test (loading, conditions unloading. reloading schedule) as per standard practice (Rao, 2011). Repeated loads are induced on plates with two cycles per second and number of cycles that applied at each load is equal to five hundred cycles to investigate the behavior of soil under cyclic loads. The model of cyclic plate loading test is shown in Figure 2.



Figure 2: Cyclic plate loading model.

6. RESULTS AND DISCUSSION

The results of chemical tests are given in Table 1. The pH values of the improved soil

specimen (CIo) increased slightly than that of Co, because lime is considered an alkaline medium. Three sulfate ions (SO_3) , gypsum, four sulfate ions (SO_4) , and chloride content (Cl^{-1}) are increased rapidly in the improved soil sample (CIo). This increase may be attributed to the chemical composition of lime which involves compounds. these The content of total hydrocarbon increased slightly in the soil sample CIo, where the chemical reactions of lime will produce carbonic acid resulted from the reaction of CO_2 of air and H_2O of pores water as an assistance factor in its reaction. The content of calcium carbonate (CaCO₃), calcium ion (Ca⁺²), and magnesium ion (Mg⁺¹) increased in improved soil CIo because lime has high concentrations of calcium carbonate. Finally, the electrical conductivity (Ec) and total dissolved salts (TDS) are highly increased in the improved soil sample due to the low value of salts in natural soil while the high content of salts such as sulfate compounds, chloride ions, calcium ion, and magnesium ion in lime.

 Table (1) Chemical analysis of soil samples.

Compound/Duonoutry	Soil Sample			
Compound/Property	Со	CIo		
SO ₃ (%)	0.1015	1.37		
$SO_{4}(\%)$	0.065	0.70		
Gypsum (%)	0.21	2.95		
Ca ⁺² (ppm)	680	820		
Cl^{-1} (%)	0.07	0.10		
pH value	7.2	7.42		
Total Hydrocarbon (%)	0.095	0.732		
TDS (ppm)	3742	200000		
Ec (ms/cm)	8.730	800		
$CaCO_3$ (ppm)	1700	2050		
Mg^{+1} (ppm)	413	498		

The impacts of deep soil improvement on the physical properties of soil are presented in terms of different physical indexes of soil. The results of physical properties of soil samples are given in Table 2. In addition, the particle size distribution curves and compaction test results are given in Figures 3 and 4.

 Table (2) Results of Atterberg's limits and specific gravity.

Soil sample	LL %	PL %	PI %	Gs	γ _{d,max} kN/m ³	W _{opt} %
Со	53	23	30	2.72	17	20
CIo	55	30	25	2.7	17.5	18

ZANCO Journal of Pure and Applied Sciences 2019

76



Figure 3: Particle size distribution curves.

The results of particle size distribution tests showed that the percentage of particles of size less than 0.005 mm in intact soil is 55%, but in the improved soil sample (CIo) is 40%, due to the flocculation of clay particles with lime, so the clay fraction is reduced. Also, the particle size of insoluble salts found within lime compounds are greater than that in the clay particles, therefore the soil condenses more quickly in a hydrometer test. Atterberg's limits are increased in the soil sample improved with lime piles. The plastic limit increased significantly due to cation exchange capacity (CEC). The cation exchange capacity led to abundant cations around clay particles, so it needs more water to obtain plastic properties while increasing of liquid limit is generally less than that of plastic limit. The non-absorbed water by clay minerals moved into the free water, and the quantity of this water is small, so increasing the liquid limit value is small.

In soil sample improved with lime piles, the specific gravity decreased slightly, because the lime has less density than that of soil. Also, the maximum dry unit weight is increased and the optimum water content is decreased. The reduction of soil moisture content is caused by evaporation and rising of soil temperature resulted from the chemical reactions between the lime and soil and dehydration affects the maximum dry unit weight. The second reason of decreasing the optimum water content is attributed to increasing demand for water cations and the clay mineral particles undergo to hydration reaction (Ashok and Reddy, 2016, Karkush et al., 2018). The dehydration, agglomeration, and flocculation of the clay particles led to an increase in the maximum dry unit weight as shown in Figure 4.



Figure 4: Results of standard Proctor tests.

From the results of oedometer tests, it's observed that the coefficient of consolidation (c_v) increased by 5%, the coefficient of permeability (k) increased by 13%, the compression index increased by 3%, but the constrained modulus (D) decreased by 6% for remolded soil sample (Cro) than that of natural undisturbed soil sample Co. This increase in the compressibility indexes is attributed to the loss of strength resulting from the destruction of molecular attraction forces between the clay particles which depends on the sensitivity of clay. In soil sample improved with lime piles, the coefficient of consolidation, the permeability, and compression index decreased by 16, 40, and 8% respectively than that of Co. Also, the constrained modulus (D) increased by 6% compared with the soil sample (Co). The lime will be flocculated the clay particles and causes a good compacted state of the soil. The permeability of the soil improved with lime has been as low as 1.4 times the permeability of the sample Co; this result is compatible with the result obtained by Ahnberg et al. (1994). The summary of oedometer test results is given in Table 3 and Figure 5.



Figure 5: Results of 1-D consolidation tests.

Table (3) Results of 1-D consolidation tests.

Soil Sample	c _v ×10 ⁻³ cm ² /sec	k×10 ⁻⁹ cm/sec	eo	e _f	Cc	Cs	D MPa
Co	1.31	6.08	0.8	0.61	0.145	0.034	21.02
Cro	1.38	6.92	0.8	0.6	0.149	0.035	19.54
CIo	1.11	4.3	0.81	0.65	0.134	0.033	25.47

The results unconfined compression test (UCT) and unconsolidated undrained test (UUT) showed that the undrained shear strength and the modulus of elasticity of remolded soil sample (Cro) are decreased by 7-8% than that of the undisturbed soil sample because of the soil disturbance that leads to the destruction of soil structure. The sensitivity of soil is about 1.075 and the soil sample can be classified as slightly or low sensitive clays (Skempton and Northey, 1952; Rosenqvist, 1953). A significant regain of strength occurred with elapsed time, where regain of soil strength in long-term phenomena depends on the sensitivity of clay. Consequently, the strength will usually increase because of the thixotropic regain of undrained shear strength as structural bonds destroyed by remolding. Improvement of soil by lime piles causes increasing the shear strength and modulus of elasticity of soil which may be attributed to the decreasing of soil moisture content by slaking and dehydration and partly by a reduction of plasticity index. Therefore; lime can play an important role to obtain certain shear strength with increasing of the specific surface area of clay minerals.

The results of DST indicated decreasing the shear strength of remolded soil sample (Cro) by 6 % and the angle of internal friction by 9% than that of the undisturbed soil sample due to soil disturbance and destruction of structural bonds by remolding of soil sample, while the cohesion and angle of internal friction of improved soil sample (CIo) increased by 14 and 5% respectively due to decreasing the moisture content by lime slaking and dehydration. Also, the soil-lime reactions produced cementation compounds such as calcium silicate gel which lead to increase the of soil. The agglomeration strength and flocculation of the clay particles make it come into a closer packing and then increasing the friction between particles. Thus, soil strength will usually increase due to the thixotropic regain of undrained shear strength as structural bonds destroy by remolding (Poulos and Davis, 1980). The results of UCT, DST, and UUT are given in Table 5. Also, the variations of stress with an axial strain of tested soil samples are given in Figures 6 and 7.



Figure 6: Stress versus axial strain from UCT.



ZANCO Journal of Pure and Applied Sciences 2019

78

Table (5) Results of shear strength tests.

G - 1	UCT		DST		UUT	
Soll	c _u kPa	E MPa	c _u kPa	φ degree	c _u kPa	E ₅₀ MPa
Со	85	12	74	22	102	6.0
Cro	79	11	70	20	94	5.6
CIo	105	12.0	84	23	130	7.5

The results of the plate loading test indicated increasing the modulus of subgrade reaction Ks_{static} and Ks_{dynamic} of soil samples (CIo) by 16 and 20% respectively in comparison with soil sample Co due to increasing the strength of soil and decreasing the compressibility of soil resulted from the reactions of lime which produce cementation matter among the soil particles. Thus, the microfabric of soil will be changed to allow the water-insoluble gel of calcium silicate coating the soil particles, which bring the soil particles closer together. Consequently, the stability of soil structure has resulted due to increasing the stiffness of the soil sample, as well as a modulus of subgrade reaction. In addition, the reaction of lime hydroxide with soil silicate aluminates to produce a cementation and substance around the soil particle consequently increases the soil strength and stiffness. The static and cyclic modulus of subgrade reaction resulted from loading a square plate of 125 mm side are listed in Table 6. The results of the static plate loading test are given in Figure 8.

Table (6)	Results	of static	and c	yclic p	late loa	ding test.
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	Soil	Ks _{static}	Ks _{dynamic}	% Reduction
_	Sample	MN/m ³	MN/m ³	Ks _{dunamic} /Ks _{static}
	Со	34.40	25.25	73
	Clo	39.750	30.3	76



Figure 8: Results of the static plate loading test.

7. CONCLUSIONS

Lime piles are considered a newly developed application for deep improvement of fine grain textured soils. Lime piles are mainly used for increasing the strength and stability of soil which is significantly affected by the chemical reactions between lime and minerals of soil and confinement of soil provided by lime piles. The conclusion drawn from this study can be summarized as follows:

- 1) The pH value, three sulfate ions (SO_3) , gypsum, four sulfate ions (SO_4) , chloride content; calcium carbonate $(CaCO_3)$, calcium ion (Ca^{+2}) and TDS are increased slightly in improved soil (CIo) as compared with Co.
- 2) PL and LL are increased in the improved soil, but increasing of PL is more than that of LL. In addition, the specific gravity and optimum water content decrease in stabilized soil by lime piles, but the maximum unit weight is increased in CIo.
- The compressibility and permeability are decreased in the improved soil sample since lime will be flocculated clay particles and causes good compaction. In addition, the constrained modulus (D) increased by 6% in the improved soil samples.
- 4) The undrained shear strength and the modulus of elasticity of remolded soil sample (Cro) decreased by 7% due to disturbance of the soil sample, where the soil sample classified as slightly sensitive.
- 5) The undrained shear strength and modulus of elasticity of the improved soil sample (CIo) are increased by 23 and 27% respectively in comparison with the natural soil sample (Co).
- 6) The direct shear test proved that the undrained shear strength and angle of internal friction have been increased significantly in soil samples improved by using lime piles.
- 7) Deep improvement of the soil leads to increasing the static and dynamic subgrade reaction of soil by 16 and 20% than that of natural soil sample respectively.
- 8) The cyclic modulus of subgrade reaction is less than the static modulus of subgrade reaction, due to the response of soil to cyclic loading.

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Conflict of Interest

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RESEARCH PAPER

Solving Linear Volterra Integro-Fractional Differential Equations in Caputo Sense with Constant Multi-Time Retarded Delay by Laplace Transform

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ABSTRACT:

In this paper presents Laplace transform methods for the first time to solve linear Volterra integro-differential equations of the fractional order in Caputo sense with constant multi-time Retarded delay. This method can be easily handling many linear Volterra problems and is capable of reducing computational analytical works where the kernel of difference and simple degenerate types. Analytical examples are presented to illustrate the efficiency and accuracy of the proposed methods.

KEY WORDS: Caputo fractional derivative, Integro-differential equation, Delay differential equations, Laplace transform, Difference and Simple Degenerate Kernels.
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1. INTRODUCTION :

The idea of this work is to solve linear Volterra Integro-Fractional Differential Equations (VIFDE's) in Caputo sense with constant multitime Retarded Delay (RD) in the general form:

$$\sum_{a}^{C} D_{t}^{\alpha_{n}} u(t) + \sum_{i=1}^{n-1} P_{i}(t) a^{C} D_{t}^{\alpha_{n-i}} u(t) + P_{0}(t) u(t-\tau)$$

= $f(t) + \lambda \sum_{j=1}^{m} \int_{0}^{t} \mathcal{K}_{j}(t, x) u(x-\tau_{j}) dx,$
 $t \in [0, h]$ (1)

For $\alpha_n > \alpha_{n-1} > \alpha_{n-2} > \alpha_{n-3} > \cdots > \alpha_1 > \alpha_0 = 0$, with initial conditions which are given: $u^{(k)}(0) = u_k$; $k = 0, 1, 2, \dots, \mu - 1$ and

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historical $\mu - th$ continuity differentiable functions $u(t) = \varphi(t)$ for $t \in [\bar{a}, 0]$, where $\bar{a} = -\max\{\tau, \tau_i: j = 1, 2, ..., m\}$ and $\mu = [\alpha_n]$. Connected, where u(t) is the unknown function which is the solution of equation (1) and $\mathcal{K}_i \in$ $C(S \times \mathbb{R}, \mathbb{R})$, and $S = \{(t, x): 0 \le x \le t \le b\}$ for $j = 1, 2, \cdots, m$ Given and $f, P_i \in$ all for all i = 0, 1, ..., n - 1 $C([0, b], \mathbb{R}),$ where $u(t) \in \mathbb{R}$, ${}^{C}D_{t}^{\alpha_{i}}u(t)$ is the α_{i} -fractional Caputo-derivative order of u on [0, b] and all $\alpha_i \in \mathbb{R}^+$ for $(i \neq 0)$, $n_{\alpha_i - 1} < \alpha_i \le n_{\alpha_i}$, $n_{\alpha_i} = [\alpha_i]$, for all i = 1, 2, ..., n. Furthermore, the $\tau_i \in \mathbb{R}^+$ for all j = 1, 2, ..., m are quantities called the delay or time-lags, (Miran B. M. Amin, 2016).

Laplace transform is a very useful method for solving certain initial and boundary value problems associated with differential equations and partial differential with constant coefficients also this method is used in solving different types of equations such as integral equations, integrodifferential equations and fractional differential equations and Delay Differential equations (Abdul, 1985; Daniel, 1997; Dr. Muna, 2008; I. podldubny, 1999; Shazad, 2002; Shazad, 2009; Shokhan, 2011; Talhat, 2016; Rostam, 2016), here we discuss the transformation Laplace operator techniques that how can be used for solving the LVIFDE's of RD that is expresses in equation (1). Before describing the Laplace transform technique, it is necessary to define and explain some important properties of Laplace method, and the way to drive Laplace transform for Delay functions and the Laplace transform of Caputo fractional derivative.

This paper is organized as follows: Section 2 presents Definition and some important property; section 3 Solve Linear Volterra Integro Differential Equation of Fractional Order with Constant multi-time Retarded Delay using Laplace Transforms Technique; our results illustrated throughout examples in section 4. Finally, section 5 includes a discussion for this method.

2. PRELIMINARIES AND PROPOSITIONS:

For this section we present the necessary information's from fractional calculus and Laplace operator, this information's are use in our suggested procedure to solve our problem, (1).

2.1 Fractional Calculus

For completeness, this part introduces the necessary definitions and important properties of fractional calculus theory, which are used throughout this paper. We begin by defining the function space $C_{\gamma}, \gamma \in \mathbb{R}$, which was used in development of the operational calculus for the differential operator. For more details, see (Anatoly et al., 2006; I. podldubny, 1999; Kelth, et al. 1974, Kenneth et al. 1993; SHAZAD, 2009): **Definition 1:**

A real valued function u defined on [a, b] be in the space $C_{\delta}[a, b]$, δ -any real number, if there exists a real number $\ell > \delta$, such that u(t) = $(t-a)^{\ell}u_c(t)$, where $u_c \in C[a, b]$, and it is said to be in the space $C^n_{\delta}[a, b]$ if and only if $u^{(n)} \in$ $C_{\delta}[a, b]$, *n*-positive integer number with zero. **Definition 2:**

Let $u \in C_{\delta}[a, b], \delta \ge -1$ with any positive arbitrary real number α . Then the RiemannLiouville fractional integral operator $_{a}J_{t}^{\alpha}$ of order α of a function *u*, is defined as:

$${}_{a}J_{t}^{\alpha}u(t) = \begin{cases} \int_{a}^{t} \frac{(t-\xi)^{\alpha-1}}{\Gamma(\alpha)} u(\xi) d\xi, & \alpha > 0\\ u(t) & \text{whenever} & \alpha = 0 \end{cases}$$

Definition 3:

Let $\alpha \ge 0$, and $m = [\alpha]$. the Riemann-Liouville fractional derivative operator ${}^{R}_{a}D_{t}^{\alpha}$, of order α and $u \in C_{-1}^m[\alpha, b]$ and defined as:

 ${}^{R}_{a}D_{t}^{\alpha}u(t)$ $= \begin{cases} D_t^m[_a J_t^{m-\alpha} u(t)], & \alpha > 0\\ u(t) & \text{whenever } \alpha = 0\\ u^{(m)}(t), \text{ If } \alpha = m(\in \mathbb{N}) \text{ and } u \in C^m[a, b] \end{cases}$

Definition 4:

The Caputo fractional derivative operator ${}^{C}_{a}D^{\alpha}_{t}$ of order $\alpha \in \mathbb{R}^+$ of a function $u \in C^m_{-1}[\alpha, b]$ and $m-1 < \alpha \leq m, m \in \mathbb{N}$ is defined as:

$$= \begin{cases} {}^{C}_{a}D^{\alpha}_{t}u(t) \\ {}^{a}_{t}J^{m-\alpha}_{t}[D^{m}_{t}u(t)], & \alpha > 0 \\ {}^{u}_{t}(t) & \text{whenever} & \alpha = 0 \end{cases}$$

$$(u^{(m)}(t), \text{ If } \alpha = m (\in \mathbb{N}) \text{ and } u \in C^m[a, b]$$

Note that:

i. For $\alpha \ge 0$ and $\beta > 0$, then ${}_{a}J_{t}^{\alpha}(t-a)^{\beta-1} =$ $\frac{\Gamma(\beta)}{\Gamma(\beta+\alpha)}(t-a)^{\beta+\alpha-1}.$

ii. For all $\alpha \ge 0$, $\beta \ge 0$ and $u(t) \in C_{\delta}[\alpha, b]$, $\delta \ge 0$ -1, then:

$${}_{a}J_{t}^{\alpha}{}_{a}J_{t}^{\beta}u(t) = {}_{a}J_{t}^{\beta}{}_{a}J_{t}^{\alpha}u(t) = {}_{a}J_{t}^{\alpha+\beta}u(t)$$

- iii. ${}^{R}_{a}D^{\alpha}_{t}\mathcal{A} = \mathcal{A}\frac{(t-a)^{-\alpha}}{\Gamma(1-\alpha)} \text{ and } {}^{C}_{a}D^{\alpha}_{t}\mathcal{A} = 0 ; \mathcal{A} \text{ is any}$ constant; $(\alpha \ge 0, \alpha \notin \mathbb{N})$
- iv. ${}^{R}_{a}D^{\alpha}_{t}u(t) = D^{m}_{t}{}^{a}J^{m-\alpha}_{t}u(t) \neq {}_{a}J^{m-\alpha}_{t}D^{m}_{t}u(t) =$ ${}_{a}^{C}D_{t}^{\alpha}u(t)$; $m = [\alpha].$
- v. Assume that $u \in C^m_{-1}[\alpha, b]$; $\alpha \ge 0, \alpha \notin \mathbb{N}$ and $m = [\alpha]$ then ${}^{c}_{a}D^{\alpha}_{t}u(t)$ is continuous on [a, b], and $[{}^{C}_{a}D^{\alpha}_{t}u(t)]_{t=a} = 0.$
- vi. Let $\alpha \ge 0$, $m = [\alpha]$ and $u \in C^m[\alpha, b]$, then, the relation between the Caputo derivative and R-L integral are formed:

$${}_{a}^{C}D_{t}^{\alpha}[{}_{a}^{\alpha}J_{t}^{\alpha}u(t)] = u(t) ; \quad a \le t \le b$$
$${}_{a}J_{t}^{\alpha}[{}_{a}^{C}D_{t}^{\alpha}u(t)] = u(t) - \sum_{k=0}^{m-1}\frac{u^{(k)}(a)}{k!}(t-a)^{k}$$

- vii. ${}^{C}_{a}D^{\alpha}_{t}u(t) = {}^{R}_{a}D^{\alpha}_{t}[u(t) T_{m-1}[u;a]],$ $(m-1 < \alpha \le m)$ and $T_{m-1}[u; \alpha]$ denotes the Taylor polynomial of degree m-1 for the function *u*, centered at *a*.
- viii. Let $\alpha \ge 0$; $m = [\alpha]$ and for power function $u(t) = (t - a)^{\beta}$ for some $\beta \ge 0$. Then:

ZANCO Journal of Pure and Applied Sciences 2019

$$\begin{aligned} & \stackrel{c}{a} D_{t}^{\alpha} u(t) \\ &= \begin{cases} 0 & \text{if } \beta \in \{0, 1, 2, \cdots, m-1\} \\ \hline \Gamma(\beta+1) & \text{if } \beta \in \mathbb{N} \text{ and } \beta \geq m \\ \hline \Gamma(\beta+1-\alpha) & \text{if } \beta \in \mathbb{N} \text{ and } \beta \geq m-1 \end{cases} \\ \text{We adopt Caputo's definition, which is a set of the set of the$$

modification of the R-L definition and has the advantage of dealing properly with initial value problem, for the concept of the fractional derivative.

Definition 5: (RUDOLF et al, 2000)

The Laplace transforms of a function u(t) of real variable $t \in \mathbb{R}^+$, denoted by U(s), is defined by the equation

$$U(s) = \mathcal{L}\{u(t); s\} = \int_{0}^{\infty} e^{-st} u(t) dt \quad ...(2)$$

and its inverse is given for $t \in \mathbb{R}^+$ by the formula, symbolically written as: $\mathcal{L}^{-1} \{ U(s); t \} = u(t)$ The Laplace transform has several properties

which are important for our work, as explained below, from Lemmas (1-4) and lemma (5-i, 5-ii) are can be founding in references (Abdul, 1985; Mariwan, 2013; Murray, 1965; Peter, 1985; Shokhan, 2011) and (I. podldubny, 1999; Kenneth et al. 1993), respectively:

Lemma 1:

The Laplace transform is related to the transform of the n - th derivative of a function, where U(s) is a Laplace of u(t):

$$\mathcal{L}\left\{\frac{d^{n}u(t)}{dt^{n}}\right\} = s^{n}U(s) - \sum_{k=0}^{n-1} s^{n-k-1} u^{(k)}(0)$$
$$= s^{n}U(s) - \sum_{k=0}^{n-1} s^{n} u^{(n-k-1)}(0) \dots (3)$$

Lemma 2:

The Laplace transform of the convolution of two functions is the product of their Laplace transforms. Thus U(s) and H(s) are the Laplace transforms of u(t) and h(t) respectively, then:

$$\mathcal{L}\{(u * h)(t)\} = \mathcal{L}\left\{\int_{0}^{t} u(t - x) h(x)dx\right\}$$

= U(s)H(s)(4)
If U(s) = \mathcal{L}\{u(t)\}, then:

$$\mathcal{L}\left\{\int_{0}^{t} u(x) \, dx\right\} = \frac{1}{s} \, U(s) \qquad \dots (5)$$

Lemma 3:

If U(s) is the Laplace of u(t) and t^n is a power function of order $n \in \mathbb{Z}^+$, then:

$$\mathcal{L}\{t^n u(t)\} = (-1)^n \frac{d^n}{ds^n} \mathcal{L}\{u(t)\}$$

$$= (-1)^n \frac{d^n}{ds^n} U(s) \qquad \dots (6)$$

Lemma 4:

Let U(s) be the Laplace of u(t) then:

$$\mathcal{L}\left\{\int_{0}^{t} t \ u(x) \ dx\right\} = -\frac{d}{ds}\left(\frac{1}{s} \ U(s)\right)$$
$$\mathcal{L}\left\{\int_{0}^{t} x \ u(x) \ dx\right\} = -\frac{1}{s}\frac{d}{ds} \ U(s)$$
...(7)

Lemma 5:

The Laplace transform of the R-L Fractional integral for order $\alpha \in \mathbb{R}^+$ using the convolution property (ii, 4), gives:

$$\mathcal{L}{J_t^{\alpha} u(t)} = \mathcal{L}\left\{\frac{t^{\alpha-1}}{\Gamma(\alpha)} * u(t)\right\}$$
$$= \mathcal{L}\left\{\frac{t^{\alpha-1}}{\Gamma(\alpha)}\right\} \mathcal{L}{u(t)} = s^{-\alpha} U(s) \quad \dots (8)$$

The Laplace transform of Caputo Fractional of order α $(m - 1 < \alpha \le m)$, $m = [\alpha]$ can be obtained as follows:

$$\mathcal{L}\{_{a}^{c}D_{t}^{\alpha}u(t)\} = \mathcal{L}\{J_{t}^{m-\alpha}D_{t}^{m}u(t)\}$$

$$= s^{-(m-\alpha)}\mathcal{L}\{u^{(m)}(t)\}$$

$$= s^{-(m-\alpha)}\left[s^{m}U(s) - \sum_{k=0}^{m-1}s^{m-k-1}u^{(k)}(0)\right]$$

$$= s^{\alpha}U(s) - \sum_{k=0}^{m-1}s^{\alpha-k-1}u^{(k)}(0) \qquad \dots (9)$$

Laplace transform of a constant delay function is explained in the following important new-lemma: Lemma 6: (New)

Let u(t) be a continuous differentiable function on a closed bounded interval $[0, b], b \in \mathbb{R}^+$ and let τ be a constant delay such that:

 $u(t) = \varphi(t)$, for $-\tau \le t < 0$... (10) Then the Laplace transform of a τ – delay function is given by:

 $\mathcal{L}\left\{u(t-\tau)\right\} = e^{-s\tau} \left[U(s) + Q(s,\tau)\right] \quad \dots (11)$ where

$$Q(s,\tau) = \int_{-\tau}^{0} e^{-sx} \varphi(t) dt$$

and $\mathcal{L}{u(t)} = U(s)$. If the historical function $\varphi(t)$ is defined by power function $t^n, (n \in \mathbb{Z}^+)$ we have:

$$\mathcal{L}\{u(t-\tau)\} = e^{-s\tau} U(s) + \sum_{p=0}^{n} (-1)^{n-p} p! \binom{n}{p} \frac{\tau^{n-p}}{s^{p+1}} - \frac{n!}{s^{n+1}} e^{-s\tau} \dots (12)$$
Proof:

By taking Laplace transform of τ -delay function $u(t-\tau)$, as in definition (1), and applying the change of variable by $t-\tau = x$ we obtain:

$$\mathcal{L}\{u(t-\tau)\} = \int_{0}^{\infty} e^{-st} u(t-\tau) dt$$

= $e^{-s\tau} \int_{-\tau}^{\infty} e^{-sx} u(x) dx$
= $e^{-s\tau} \left[\int_{-\tau}^{0} + \int_{0}^{\infty} \right] e^{-sx} u(x) dx \dots (13)$

Use by part integral method for solving first integral in (13) after instead u(x) by historical function $\varphi(x)$ which is defined $x^n, n \in \mathbb{Z}^+$, we get:

$$Q(s,\tau) = \int_{-\tau}^{0} e^{-sx} \varphi(x) dx = \int_{-\tau}^{0} e^{-sx} x^{n} dx$$
$$= e^{s\tau} \sum_{p=0}^{n} (-1)^{n-p} p! \binom{n}{p} \frac{\tau^{n-p}}{s^{p+1}} - \frac{n!}{s^{n+1}} \dots (14)$$

And the second integral part in (13) is the Laplace transform of u(x), thus

$$\int_{0}^{\infty} e^{-sx} u(x) dx = U(s) \qquad \dots (15)$$

Putting equations (14) and (15) into (13) we obtain:

$$\mathcal{L}\{u(t-\tau)\} = e^{-s\tau} U(s) + \sum_{p=0}^{n} (-1)^{n-p} p! {n \choose p} \frac{\tau^{n-p}}{s^{p+1}} - \frac{n!}{s^{n+1}} e^{-s\tau}$$

which completes the proof. Note that, in a more general way, for H.F. which is defined

$$\varphi(x) = \sum_{r=1}^{n} a_r x^{n_r}; \{R \in \mathbb{Z}^+, n_r \in \mathbb{Z}^+, a_r \in \mathbb{Z}^+\}$$

Then the formula (12) becomes: $f(u(t - \tau)) = e^{-ST} U(t)$

$$L\{u(t-\tau)\} = e^{-s\tau} U(s) + \sum_{r=1}^{R} a_r \left[\sum_{p=0}^{n_r} (-1)^{n_r-p} p! {n_r \choose p} \frac{\tau^{n_r-p}}{s^{p+1}} - \frac{n_r!}{s^{n_r+1}} e^{-s\tau} \right]$$

3. ANALYSIS OF THE METHOD:

In this section we try to find general solution form of linear VIFDE's with multi-time RD by applying the Laplace transform method in two different types of kernel: difference and simple degenerate kernel.

3.1 Difference Kernel Type:

Recall equation (1) with difference kernels and initial point (a = 0). Moreover, take $P_i(t)$ as a power function, say $C_i t^{\ell_i}$, $C_i \in \mathbb{R}$ and ℓ_i be any nonnegative integer numbers for all i:

$$\sum_{a}^{C} D_{t}^{\alpha_{n}} u(t) + \sum_{i=1}^{n} P_{i}(t) a_{a}^{C} D_{t}^{\alpha_{n-i}} u(t) + P_{0}(t) u(t-\tau)$$

= $f(t) + \lambda \sum_{j=1}^{m} \int_{0}^{t} \mathcal{K}_{j} (t-x) u(x-\tau_{j}) dx \dots (16)$

For all $\in I = [0, b]$; $\alpha_n > \alpha_{n-1} > \alpha_{n-2} > \alpha_{n-3} > \cdots > \alpha_1 > \alpha_0 = 0$, with initial conditions which are given: $u^{(k)}(0) = u_k; k = 0, 1, \dots, \mu - 1$ $(\mu = [\alpha_n])$ and historical $\mu - th$ continuity differentiable functions $u(t) = \varphi(t)$ for $t \in [\bar{a}, 0]$, where $\bar{a} = -max\{\tau, \tau_j: j = \overline{1:m}\}$. Let U(s), F(s) and $\mathcal{K}_j(s)$ be the Laplace transform of u(t), f(t) and $\mathcal{K}_j(t), j = \overline{1:m}$, respectively. Take the Laplace transform of both sides of equation (16):

$$\underbrace{\mathcal{L}\left\{ \stackrel{a}{a} D_{t}^{\alpha_{n}} u(t) \right\}}_{A} + \sum_{i=1}^{n-1} \underbrace{\mathcal{L}\left\{ P_{i}(t) \stackrel{c}{a} D_{t}^{\alpha_{n-i}} u(t) \right\}}_{B} + \underbrace{\mathcal{L}\left\{ P_{0}(t) u(t-\tau) \right\}}_{C} + \underbrace{\mathcal{L}\left\{ f(t) \right\}}_{D} + \sum_{j=1}^{m} \lambda \underbrace{\mathcal{L}\left\{ \int_{0}^{t} \mathcal{K}_{j} (t-x) u(x-\tau_{j}) dx \right\}}_{E} \dots (17)$$

Part A, using equation (9) and initial conditions, where $m_{\alpha_n} - 1 < \alpha_n \le m_{\alpha_n}$, we obtain

$$\mathcal{L} \{ {}^{C}_{a} D^{\alpha_{n}}_{t} u(t) \} = s^{\alpha_{n}} U(s) - \sum_{k=0}^{m_{\alpha_{n}}-1} s^{\alpha_{n}-k-1} u_{k} \dots (17, A)$$

for part *B*, first using equation (6) and then applying equation (9), we get for all $i = \overline{1:n-1}$:

$$\mathcal{L}\left\{P_{i}(t)_{a}^{c}D_{t}^{\alpha_{n-i}}u(t)\right\} = C_{i}(-1)^{\ell_{i}}\frac{d^{\ell_{i}}}{ds^{\ell_{i}}}\left(s^{\alpha_{n-i}}U(s)\right)$$
$$-C_{i}(-1)^{\ell_{i}}\frac{d^{\ell_{i}}}{ds^{\ell_{i}}}\sum_{k=0}^{m_{\alpha_{n-i}}-1}\left[s^{\alpha_{n-i}-k-1}u_{k}\right]...(17,B)$$

Where $m_{\alpha_{n-i}} - 1 < \alpha_{n-i} \le m_{\alpha_{n-i}}$, ℓ_i is the order of $P_i(t)$ for each $i = \overline{1:n-1}$. Part *C*, first using equation (6) and then applying the Lemma (6), (using 11 and 12) respectively, we obtain:

ZANCO Journal of Pure and Applied Sciences 2019

$$\mathcal{L}\{P_0(t)u(t-\tau)\}$$

$$= C_0(-1)^{\ell_0} \frac{d^{\ell_0}}{ds^{\ell_0}} \left[e^{-s\tau} (U(s) + Q(s,\tau)) \right] \dots (17,C1)$$
As a special case, where the historical function $\varphi(t)$ is $t^q, q \in \mathbb{Z}^+$, we have
$$\mathcal{L}\{P_0(t) \ u(t-\tau)\}$$

$$= C_0(-1)^{\ell_0} \frac{d^{\ell_0}}{ds^{\ell_0}} \left[e^{-s\tau} U(s) \right]$$

$$+ C_{0}(-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} \left[\sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau^{q-p}}{s^{p+1}} \right] \\ - C_{0}(-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} \left[\frac{q!}{s^{q+1}} e^{-s\tau} \right] \dots (17, C2)$$

where ℓ_0 is the order of $P_0(t)$ and q is the order of historical polynomial function. For part *D*, using the definition of Laplace transformation, we get:

 $\mathcal{L}{f(t)} = F(s) \qquad \dots (17, D)$ Part *E*, we apply equation (4) with Lemma (6), (11 and 12), respectively to obtain: for all *j* = 1,2, ... *m*

$$\mathcal{L}\left\{\int_{0}^{t} \mathcal{K}_{j}(t-x) u(x-\tau_{j})dx\right\}$$

= $\mathcal{K}_{j}(s) e^{-s\tau_{j}}\left[\left(U(s)+Q(s,\tau_{j})\right)\right] \dots (17, E1)$

As a special case, where the historical function $\varphi(t)$ is $t^q, q \in \mathbb{Z}^+$, we have:

$$\mathcal{L}\left\{\int_{0}^{t}\mathcal{K}_{j}\left(t-x\right) u\left(x-\tau_{j}\right)dx\right\}$$
$$=\mathcal{K}_{j}(s)\left[e^{-s\tau_{j}}U(s)+\sum_{p=0}^{q}(-1)^{q-p}p!\binom{q}{p}\frac{\tau_{j}^{q-p}}{s^{p+1}}\right.$$
$$\left.-\frac{q!}{s^{q+1}}e^{-s\tau_{j}}\right] \qquad \dots (17, E2)$$

Putting equations (17: A, B, C1, D, E1) into the equation (17) and after some simple manipulations, we get the following equation:

$$\sum_{i=1}^{n-1} C_i (-1)^{\ell_i} \frac{d^{\ell_i}}{ds^{\ell_i}} [s^{\alpha_{n-i}} U(s)] + C_0 (-1)^{\ell_0} \frac{d^{\ell_0}}{ds^{\ell_0}} [e^{-s\tau} U(s)] + \left(s^{\alpha_n} - \lambda \sum_{j=1}^m \mathcal{K}_j(s) e^{-s\tau_j}\right) U(s) = F^*(s) \dots (18)$$

where

$$F^{*}(s) = F(s) + \lambda \sum_{j=1}^{m} \mathcal{K}_{j}(s) \ e^{-s\tau_{j}} Q(s,\tau_{j}) + \sum_{\substack{k=0\\m\alpha_{n}-1}}^{m\alpha_{n}-1} s^{\alpha_{n}-k-1} u_{k} + \sum_{i=1}^{n-1} C_{i}(-1)^{\ell_{i}} \frac{d^{\ell_{i}}}{ds^{\ell_{i}}} \left[\sum_{\substack{k=0\\k=0}}^{m\alpha_{n-i}-1} s^{\alpha_{n-i}-k-1} u_{k} \right] - C_{0}(-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} [e^{-s\tau}Q(s,\tau)] \dots (19)$$

If historical function is power function $t^q, q \in \mathbb{Z}^+$, putting equations (17: *A*, *B*, *C*2, *D*, *E*2) into equation (17), we obtain the following equations (20) instead of (18):

$$\begin{aligned} F^{*}(s) &= F(s) \\ &+ \lambda \sum_{j=1}^{m} \mathcal{K}_{j}(s) \left[\sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau_{j}^{q-p}}{s^{p+1}} \right. \\ &- \frac{q!}{s^{q+1}} e^{-s\tau_{j}} \right] + \sum_{k=0}^{m_{\alpha_{n}}-1} s^{\alpha_{n}-k-1} u_{k} \\ &+ \sum_{i=1}^{n-1} C_{i}(-1)^{\ell_{i}} \frac{d^{\ell_{i}}}{ds^{\ell_{i}}} \left[\sum_{k=0}^{m_{\alpha_{n-i}}-1} s^{\alpha_{n-i}-k-1} u_{k} \right] \\ &- C_{0}(-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} \left[\sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau^{q-p}}{s^{p+1}} \\ &- \frac{q!}{s^{q+1}} e^{-s\tau} \right] \qquad \dots (20) \end{aligned}$$

Consequently there is an ordinary differential equation in U(s); solve it to find U(s). Finally, use the inverse of Laplace transform on U(s) to obtain the solution u(s) of linear VIFDE's for multi-time RD, (1). For a special case, if the $P_i(t)$ and $P_0(t)$ are only constants that is ℓ_i and ℓ_0 are equal to zero. Thus, equations (18), (19) and (20) become:

$$H(s)U(s) = F^{*}(s)$$
 ... (21)

where $F^*(s) = F(s)$

$$+\lambda \sum_{j=1}^{m} \mathcal{K}_{j}(s) \left\{ \begin{bmatrix} e^{-s\tau_{j}} Q(s,\tau_{j}) \end{bmatrix} \\ \left[\sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau_{j}^{q-p}}{s^{p+1}} - \frac{q!}{s^{q+1}} e^{-s\tau_{j}} \end{bmatrix} \right\} \\ + \sum_{k=0}^{m_{\alpha_{n}}-1} s^{\alpha_{n}-k-1} u_{k} + \sum_{i=1}^{n-1} C_{i} \begin{bmatrix} \sum_{k=0}^{m_{\alpha_{n-i}}-1} s^{\alpha_{n-i}-k-1} u_{k} \end{bmatrix} \\ - C_{0} \left\{ \begin{bmatrix} e^{-s\tau}Q(s,\tau) \end{bmatrix} \\ \left[\sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau^{q-p}}{s^{p+1}} - \frac{q!}{s^{q+1}} e^{-s\tau} \end{bmatrix} \right\} \dots (22) \\ \text{and} \end{cases}$$

$$H(s) = s^{\alpha_n} + \sum_{i=1}^{n-1} C_i s^{\alpha_{n-i}} + C_0 e^{-s\tau} - \lambda \sum_{j=1}^{m} \mathcal{K}_j(s) e^{-s\tau_j} \dots (23)$$

It has a unique solution providing that:

$$s^{\alpha_n} + \sum_{i=1}^{n-1} C_i s^{\alpha_{n-i}} + C_0 e^{-s\tau} \neq \lambda \sum_{j=1}^m \mathcal{K}_j(s) e^{-s\tau_j}$$

3.2 Simple Degenerate Kernel:

Laplace transform technique can be used to solve some kinds of Linear VIFDE's of constant multi-time Retarded delays which the kernel is not necessarily difference kernel, here we take the same equation (16) with all conditions on that equation except the kernel which is a simple degenerate kernel. Thus:

$$\sum_{a}^{C} D_{t}^{\alpha_{n}} u(t) + \sum_{i=1}^{n-1} P_{i}(t) \sum_{a}^{C} D_{t}^{\alpha_{n-i}} u(t) + P_{0}(t) u(t-\tau)$$

$$= f(t) + \lambda \sum_{j=1}^{m} \int_{0}^{t} \left[c_{j} t^{k_{j}^{1}} + d_{j} x^{k_{j}^{2}} \right] u(x-\tau_{j}) dx$$

$$\dots (24)$$

Since c_j , $d_j \in \mathbb{R}$ for all j = 1, 2, ..., mand $k_i^1, k_i^2 \in \mathbb{Z}^+$. Taking the Laplace transform of both sides of equation (24):

$$\underbrace{\mathcal{L}\left\{\stackrel{c}{a}D_{t}^{\alpha_{n}}u(t)\right\}}_{A} + \sum_{i=1}^{n-1}\underbrace{\mathcal{L}\left\{P_{i}(t)\stackrel{c}{a}D_{t}^{\alpha_{n-i}}u(t)\right\}}_{B} + \underbrace{\mathcal{L}\left\{P_{0}(t) u(t-\tau)\right\}}_{C} = \underbrace{\mathcal{L}\left\{f(t)\right\}}_{D} + \sum_{j=1}^{m}\lambda\underbrace{\mathcal{L}\left\{\int_{0}^{t}\left[c_{j}t^{k_{j}^{1}} + d_{j}x^{k_{j}^{2}}\right] u(x-\tau_{j})dx\right\}}_{E} \dots (25)$$

For parts A, B, C and D we obtain same equations as the section (3.1) respectively. Now

For part E: we apply equation (7) with Lemma (5), (11 and 12) respectively, and using Leibniz's formula for higher derivative of multiplication functions (Marc, 2005) then after some manipulating we obtain:

$$\mathcal{L}\left\{\int_{0}^{t} \left[c_{j}t^{k_{j}^{1}}+d_{j}x^{k_{j}^{2}}\right]u(x-\tau_{j})dx\right\}$$

$$= \frac{e^{-s\tau_{j}}}{s}\left\{\left[c_{j}\left(\sum_{r=0}^{k_{j}^{1}}r!\left(k_{j}^{1}\right)\frac{1}{s^{r}}\tau_{j}^{k_{j}^{1}-r}\right)+d_{j}\tau_{j}^{k_{j}^{2}}\right]$$

$$+ \left[d_{j}\sum_{r=0}^{k_{j}^{2}-1}(-1)^{r+k_{j}^{2}}\tau_{j}r\left(k_{j}^{2}\right)\frac{d^{k_{j}^{2}-r}}{ds^{k_{j}^{2}-r}}\right]$$

$$+ \left[c_{j}\sum_{r=0}^{k_{j}^{1}-1}(-1)^{r+k_{j}^{1}}r!\left(k_{j}^{1}\right)* + \left[s_{j}\sum_{r=0}^{k_{j}^{1}-r-1}(-1)^{r}\tau_{j}p\left(k_{j}^{1}-r\right)\frac{d^{k_{j}^{1}-r-p}}{p}\right)\right]\right\} U(s)$$

$$+ \frac{1}{s}\left\{c_{j}\left[\sum_{r=0}^{k_{j}^{1}}(-1)^{r+k_{j}^{1}}r!\left(k_{j}^{1}\right)\frac{1}{s^{r}}\frac{d^{k_{j}^{1}-r}}{ds^{k_{j}^{1}-r}}\right]$$

$$+ d_{j}\left[(-1)^{k_{j}^{2}}\frac{d^{k_{j}^{2}}}{ds^{k_{j}^{2}}}\right]\right\} H_{j}^{q}(s) \qquad \dots (26)$$

for all j = 1, 2, ..., m. Where, $H_i^q(s)$

$$= \begin{cases} e^{-s\tau_j}Q(s,\tau_j); \\ \text{if the (HF) any continuos differentiable function.} \\ \sum_{p=0}^{q} (-1)^{q-p} p! {q \choose p} \frac{\tau_j^{q-p}}{s^{p+1}} - \frac{q!}{s^{q+1}} e^{-s\tau_j} \\ \text{if } \varphi(t) = t^q. \\ \text{and } Q(s,\tau_j) = \int_{-\tau_j}^{0} e^{-sx}\varphi(x)dx \end{cases}$$

After some simple manipulations, for par (17: A, B, C, D, E and 26) then putting equations into equation (25) we obtain the general solution for (24):

$$\sum_{i=1}^{n-1} C_i \ (-1)^{\ell_i} \frac{d^{\ell_i}}{ds^{\ell_i}} (s^{\alpha_{n-i}} U(s)) + C_0 \ (-1)^{\ell_0} \frac{d^{\ell_0}}{ds^{\ell_0}} (e^{-s\tau} U(s)) \left[s^{\alpha_n} - \lambda \sum_{j=1}^m \frac{e^{-s\tau_j}}{s} \left\{ \left[c_j \left(\sum_{r=0}^{k_j^1} r! \binom{k_j^1}{r} \frac{1}{s^r} \tau_j^{k_j^1-r} \right) + d_j \tau_j^{k_j^2} \right] \right] \right\}$$

ZANCO Journal of Pure and Applied Sciences 2019

$$+ \left[d_{j} \sum_{r=0}^{k_{j}^{2}-1} (-1)^{r+k_{j}^{2}} \tau_{j}^{r} {k_{j}^{2} \choose r} \frac{d^{k_{j}^{2}-r}}{ds^{k_{j}^{2}-r}} \right] \\ + \left[c_{j} \sum_{r=0}^{k_{j}^{1}-1} (-1)^{r+k_{j}^{1}} r! {k_{j}^{1} \choose r} * \\ + \frac{1}{s^{r}} \left(\sum_{p=0}^{k_{j}^{1}-r-1} (-1)^{p} \tau_{j}^{p} {k_{j}^{1}-r \choose p} \frac{d^{k_{j}^{1}-r-p}}{ds^{k_{j}^{1}-r-p}} \right) \right] \right\} U(s) \\ = F^{*}(s) \qquad \dots (27)$$

If the historical function is any continuous differentiable function $\varphi(t)$, thus: $F^*(s) = F(s)$

$$\begin{aligned} &+\lambda \sum_{j=1}^{m} \frac{1}{s} \left\{ c_{j} \left[\sum_{r=0}^{k_{j}^{1}} (-1)^{r+k_{j}^{1}} r! \binom{k_{j}^{1}}{r} \frac{1}{s^{r}} \frac{d^{k_{j}^{1}-r}}{ds^{k_{j}^{1}-r}} \right] \\ &+ d_{j} \left[(-1)^{k_{j}^{2}} \frac{d^{k_{j}^{2}}}{ds^{k_{j}^{2}}} \right] \right\} e^{-s\tau_{j}} Q(s,\tau_{j}) \\ &+ \sum_{k=0}^{m\alpha_{n}-1} s^{\alpha_{n}-k-1} u_{k} \\ &+ \sum_{i=1}^{n-1} C_{i} (-1)^{\ell_{i}} \frac{d^{\ell_{i}}}{ds^{\ell_{i}}} \left[\sum_{k=0}^{m\alpha_{n-i}-1} s^{\alpha_{n-i}-k-1} u_{k} \right] \\ &- C_{0} (-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} [e^{-s\tau} Q(s,\tau)] \qquad \dots (27 A) \\ \text{If the historical function is } \varphi(t) = t^{q}, q \in \mathbb{Z}^{+} \\ \end{aligned}$$

$$F^*(s) = F(s)$$

$$+ \lambda \sum_{j=1}^{m} \frac{1}{s} \Biggl\{ c_{j} \Biggl[\sum_{r=0}^{k_{j}^{1}} (-1)^{r+k_{j}^{1}} r! \binom{k_{j}^{1}}{r} \frac{1}{s^{r}} \frac{d^{k_{j}^{1}-r}}{ds^{k_{j}^{1}-r}} \Biggr] \\ + d_{j} \Biggl[(-1)^{k_{j}^{2}} \frac{d^{k_{j}^{2}}}{ds^{k_{j}^{2}}} \Biggr] \Biggr\} \Biggl[\sum_{p=0}^{q} \binom{q}{p} (-1)^{q-p} p! \frac{\tau_{j}^{q-p}}{s^{p+1}} \\ - \frac{q!}{s^{q+1}} e^{-s\tau_{j}} \Biggr] + \sum_{k=0}^{m\alpha_{n}-1} s^{\alpha_{n}-k-1} u_{k} \\ + \sum_{i=1}^{n-1} C_{i} (-1)^{\ell_{i}} \frac{d^{\ell_{i}}}{ds^{\ell_{i}}} \Biggl[\sum_{k=0}^{m\alpha_{n-i}^{-1}} s^{\alpha_{n-i}-k-1} u_{k} \Biggr] \\ - C_{0} (-1)^{\ell_{0}} \frac{d^{\ell_{0}}}{ds^{\ell_{0}}} \Biggl[\sum_{p=0}^{q} \binom{q}{p} (-1)^{q-p} p! \frac{\tau^{q-p}}{s^{p+1}} \\ - \frac{q!}{s^{q+1}} e^{-s\tau} \Biggr] \qquad \dots (27 B)$$

Which is the Ordinary Differential Equation U(s) with variable coefficients and solving it to find U(s). Finally, use the inverse Laplace transform on U(s) to obtain the solution u(t) of Linear VIFDE's with constant multi-time RD, (27).

4. ILLUSTRATIVE EXAMPLES:

In order to show the efficiency of our proposed method and investigate its accuracy how to solve linear VIFDE's with constant multi-time Retarded delay (1), we present some examples.

Example (1): Consider linear VIFDE's of constant multi-time Retarded delay with constant coefficients on [0, 1]:

$${}_{0}^{c}D_{t}^{0.9}u(t) - \frac{1}{2} {}_{0}^{c}D_{t}^{0.5}u(t) + \frac{1}{2} u(t - 0.2) = f(t) + \int_{0}^{t} [(t - x)u(x - 0.3) + (t - x)^{2}u(x - 0.5)]dx$$
where

where

$$f(t) = \frac{2}{\Gamma(1.1)} t^{0.1} - \frac{1}{\Gamma(1.5)} t^{0.5} - \frac{1}{6} t^4 - \frac{1}{3} t^3$$
$$-\frac{1}{5} t^2 + t + \frac{3}{10}$$

1

1

1

with initial condition and historical function: u(0) = 1; with given historical function: $\varphi(t) = 2t + 1$.

Since here we notice that:

2

$$\begin{aligned} \mathcal{K}_1(t,x) &= (t-x) \; ; \; \mathcal{K}_2(t,x) = (t-x)^2 \\ \alpha_2 &= 0.9 \; ; \; \alpha_1 = 0.5 ; \; m_{\alpha_2} = m_{\alpha_1} = 1 ; \\ P_1(t) &= -1/2 \; ; \; P_0(t) = 1/2 \end{aligned}$$

$$\tau = \tau_0 = 0.2, \tau_1 = 0.3, \tau_2 = 0.5$$

which are constant different time delays. Taking Laplace transform to the equation mentioned above and finding $F^*(s)$ with H(s) from equations (22) and (23), we have:

$$\mathcal{L}{\mathcal{K}_{1}(t)} = \frac{1}{s^{2}}$$
; $\mathcal{L}{\mathcal{K}_{2}(t)} = \frac{2}{s^{3}}$

and

$$F^*(s) = \frac{2}{s^{1.1}} - \frac{1}{s^{1.5}} + s^{-0.1} - \frac{1}{2}s^{-0.5} - \frac{2}{s^4}e^{-0.3s}$$
$$- \frac{1}{s^3}e^{-0.3s} - \frac{4}{s^5}e^{-0.5s}$$
$$- \frac{2}{s^4}e^{-0.5s} + \frac{1}{s^2}e^{-0.2s}$$
$$+ \frac{1}{2s}e^{-0.2s}$$

with

$$H(s) = s^{0.9} - \frac{1}{2}s^{0.5} + \frac{1}{2}e^{-0.2s} - \frac{1}{s^2}e^{-0.3s} - \frac{2}{s^3}e^{-0.5s}$$

Thus:

$$U(s) = \frac{F^*(s)}{H(s)} = \frac{1}{s} + \frac{2}{s^2}$$

Then using inverse Laplace transform to obtain the exact solution u(t) for Linear VIFDDE's with constant coefficient.

$$u(t) = \mathcal{L}^{-1}\left\{\frac{1}{s} + \frac{2}{s^2}\right\} = 2t + 1$$

Which is the exact solution for our given problem.

Example (2): Consider linear VIFDE's of constant multi-time Retarded delay with variable coefficients on [0, 1]:

$$\int_{0}^{C} D_{t}^{1.5} u(t) + t \int_{0}^{C} D_{t}^{0.5} u(t) - 3t u(t-1) = f(t) + \int_{0}^{t} [(t-x)u(x-2) - e^{t-x}u(x-1)] dx$$

where

$$f(t) = \frac{2}{\Gamma(1.5)}t^{0.5} + \frac{2}{\Gamma(2.5)}t^{2.5} + e^t - \frac{1}{12}t^4 - \frac{7}{3}t^3 + 3t^2 - 3t - 1$$

with initial condition and historical function: u(0) = 0; u'(0) = 0; with historical function $\varphi(t) = t^2.$

Since here we have:

and

$$\mathcal{K}_1(t, x) = (t - x); \ \mathcal{K}_2(t, x) = e^{t - x}$$

 $\tau = \tau_0 = 1, \tau_1 = 2, \tau_2 = 1$

$$\tau = \tau_0 = 1, \tau_1 = 2, \tau_2 = 1$$

which are constant different time delays and $P_1(t) = t$; $P_0(t) = -3t$

variable coefficients. Taking Laplace are transform to the above equation and using equations (18) and (20) to obtain:

$$\mathcal{L}\{\mathcal{K}_{1}(t)\} = \frac{1}{s^{2}} \quad ; \quad \mathcal{L}\{\mathcal{K}_{2}(t)\} = \frac{1}{s-1}$$

Thus:

$$s^{1.5} U(s) - s^{0.5} U'(s) - 0.5 s^{-0.5} U(s) + 3e^{-s} U'(s) - 3e^{-s} U(s) - \frac{1}{s^2} e^{-2s} U(s) + \frac{1}{s-1} e^{-s} U(s) = F^*(s)$$

where

$$F^*(s) = \frac{2}{s^{1.5}} + \frac{5}{s^{3.5}} - \frac{2}{s^3}e^{-s} - \frac{6}{s^4}e^{-s} - \frac{2}{s^5}e^{-2s} + \frac{2}{s^3(s-1)}e^{-s}$$

So the equation becomes:

$$U'(s)[3e^{-s} - s^{0.5}] + U(s) \left[s^{1.5} - 0.5 s^{-0.5} - 3e^{-s} - \frac{1}{s^2} e^{-2s} + \frac{1}{s-1} e^{-s} \right] = F^*(s)$$

with $U(\infty) = 0$, (Rudolf, 2000), which is ODE of the first order, after solving it, the following is obtained:

$$U(s) = \frac{2}{s^3}$$

By taking the inverse of Laplace transform of U(s) the exact solution u(t) is obtained

$$u(t) = \mathcal{L}^{-1}\left\{\frac{2}{s^3}\right\} = t^2$$

Which is the exact solution for our given problem.

Example (3): Consider linear VIFDE's of constant multi-time Retarded delay with variable coefficients with degenerate kernel on [0,1]:

$${}_{0}^{C}D_{t}^{1.3}u(t) + \frac{2}{3}{}_{0}^{C}D_{t}^{0.4}u(t) - t u(t - 0.4) = f(t) + \int_{0}^{t} (2t + 3x^{2})u(x - 0.2) dx$$

where

$$f(t) = \frac{1}{\Gamma(1.7)} t^{0.7} + \frac{2}{3\Gamma(2.6)} t^{1.6} - \frac{3}{10} t^5 - \frac{11}{60} t^4 - \frac{8}{25} t^3 + \frac{9}{25} t^2 - \frac{2}{25} t^5$$

with initial condition and historical function: u(0) = u'(0) = 0; with historical function $\varphi(t) = \frac{1}{2}t^2$ and constant different time delay

and

$$\tau = \tau_0 = 0.4, \tau_1 = 0.2$$

$$P_1(t) = \frac{2}{3}$$
, $P_0(t) = -t$

By Applying Laplace transform for solving our problem, using equations (27) and (27 A) we obtain:

$$s^{1.3} U(s) + \frac{2}{3} s^{0.4} U(s) + e^{-0.4s} U'(s)$$

- 0.4e^{-0.4s} U(s) - $\frac{2}{s^2} e^{-0.2s} U(s)$
- $\frac{0.52}{s} e^{-0.2s} U(s)$
+ $\frac{3.2}{s} e^{-0.2s} U'(s) - \frac{3}{s} e^{-0.2s} U''(s)$
= $F^*(s)$

where

$$F^*(s) = \frac{1}{s^{1.7}} + \frac{2}{3s^{2.6}} - \frac{3}{s^4}e^{-0.4s} - \frac{0.4}{s^3}e^{-0.4s} - \frac{36}{s^6}e^{-0.2s} - \frac{11.6}{s^5}e^{-0.2s} - \frac{0.52}{s^4}e^{-0.2s}$$

So the equation becomes:

$$\begin{bmatrix} s^{1.3} + \frac{2}{3}s^{0.4} - 0.4e^{-0.4s} - \frac{2}{s^2}e^{-0.2s} \\ -\frac{0.52}{s}e^{-0.2s}\end{bmatrix}U(s) \\ + \begin{bmatrix} e^{-0.4s} + \frac{3.2}{s}e^{-0.2s}\end{bmatrix}U'(s) \\ -\begin{bmatrix} \frac{3}{s}e^{-0.2s}\end{bmatrix}U''(s) = F^*(s) \end{bmatrix}$$

with $U(\infty) = 0$, which is ODE after solving it for U(s) we obtain:

$$U(s) = \frac{1}{s^3}$$

By taking the inverse of Laplace transform of U(s) we get the exact solution

$$u(t) = \mathcal{L}^{-1}\left\{\frac{1}{s^3}\right\} = \frac{1}{2}t^2$$

5. DISCUSSION:

In this paper, through the Laplace transform methods for solving linear Volterra integrofractional differential equations of constant multitime Retarded delay type with variable coefficients introduced with some illustrating examples for each cases, we pointed the following:

The Laplace transform method which was improved here provided good results and effectiveness for various problems. The Laplace transforms was applied for difference kernel and simple degenerate kernel in general cases.

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ZANCO Journal of Pure and Applied Sciences 2019

88

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RESEARCH PAPER

Schedule Risk Analysis Using Monte Carlo Simulation for Residential Projects.

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ABSTRACT:

Scheduling is an essential part of construction project management. Planning and scheduling of construction tasks help engineers to complete the project on time and within the budget. Most of the construction project failed to finish within planned duration; one of the reasons is regarded to estimated project duration without considering uncertainties that may cause a delay in performing specific activities. Hence it is vital to develop a risk management process which deals with the risks of execution that affects the project duration. This study focused on Schedule Risk Analysis Using Monte Carlo Simulation for Residential Projects, by taking the construction of a residential house as a case study. The primary objective of this study analyzes the output of a project schedule risk simulation when Monte Carlo use to simulate the duration of individual activities of the project and compare the total project duration outputs graphically and through statistical analysis. Consequently, using the Critical Path Method (CPM) to determine the project duration, which is equal to 96 days. For deciding the activity duration, the researcher has made a form. The form consists of all house tasks and estimated quantity with three columns for estimating Optimistic Duration, Most Likely Duration, and Pessimistic Duration in accordance with the respondent's perception for establishing the project duration by using the Program Evaluation and Review Techniques (PERT) method, which is equal 103 days. Project duration with low risk equal to 103 days, with base risks equal to 107 days and with high-risk project duration equal to 111 days. The outcomes clearly show that it is extremely unlikely to complete the project within 98 days and there is 100% chance that the project will be completed in 115 days. The sensitivity analysis for residential construction house indicates that the project schedule is most sensitive to the activity of "Wall Ceramic Tiles", which can influence the completion date because of the correlation coefficient of this activity reached to 0.39 and top-ranked of all other activities.

KEY WORDS: Schedule Risk Analysis, Monte Carlo simulation, Residential Projects. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.11</u> ZJPAS (2019), 31(5);90-103 .

1. INTRODUCTION :

Development ventures are always exceptional, and risks raise from various distinctive sources. The accomplishment of development ventures is assessed dependent on such measures as creation quality, scope adequacy, social-ecological specialized usefulness, security necessities, planned completion time, and distributed spending plan (Ökmen and Öztaş, 2008).

Shamal Ali Othman E-mail: eng.shamal88@gmail.com Article History: Received: 28/102018 Accepted: 20/06/2019 Published: 17/10/2019 Today, viably overseeing risk is a fundamental segment of successful project management.

Appropriate hazard administration can help the project manager to alleviate both known and unforeseen risks on projects of different types (Carbone and Tippett, 2004).

The three-dimensional objective for any undertaking is outstanding, i.e., to finish the project on time, within budget, and with satisfactory performance or quality (Ganame and Chaudhari, 2015).

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Any development venture is relied upon to be done inside a predefined timeframe. Also, if the task gets late, it increases the cost of the undertaking and contractor may have to face a penalty for causing the delay. Consequently, it is crucial for both the proprietor and the contractor to pursue the task plan. Planning is an essential construction project management. part of Scheduling and Planning of construction activities assist engineers in completing the task on time and within the financial plan. In any case, construction activities possess uncertainties that may cause a delay in performing particular tasks or even increment the cost of the venture. Thus, it is essential to working up a hazard administration process which manages the dangers of execution that influence the project plan (Ganame and Chaudhari, 2015).

This study focus on risk management in construction schedule aims to determine project duration and the extension of project duration.

1.1 Scheduling

Construction scheduling and planning is a consistent examination of a construction project together with exhaustive information of development techniques, materials, and practices. Schedules are produced in a deterministic way, i.e., activity durations are given as solitary esteem, usually the most likely duration. There is a supposition that the duration is known with some assurance; in any case, the timetable regularly contains significant uncertainty, particularly for unsafe tasks. To address this issue, Program Evaluation Review Techniques (PERT) was developed (Nasir et al., 2003)

(PERT), in conjunction with the Critical Path Method, were developed in the 1958's to address the uncertainty in project duration for complex projects (Kerzner, 2017). The total project duration was determined by adding all the duration values of the activities on the critical path. The Monte Carlo simulation provides distribution for the whole project duration and is, therefore, more useful as a method or tool for decision making (Visser, 2016).

1.2 Schedule Risk Analysis

Different modeling strategies are utilized to portray or evaluate the size of a particular risk.

One of the most normally utilized strategies is Monte Carlo simulation, which is used for incorporating a hazard factor structure and likelihood dispersion model to evaluate uncertainty (Kerzner, 2017).

MCS is a statistical technique that could turn out to be progressively critical as a method for risk assessors to assess the uncertainty. Even though Monte Carlo simulation has been utilized since the 1940s, more powerful desktop computers have made it open and appealing for some new applications (Huang and Wang, 2009). Risky Project is a companion product that can be integrated with Microsoft Project Professional. Risky Project provides support for both qualitative and quantitative risk analysis techniques (Vajjhala, 2012).

Risky Project also provides support for performing Monte Carlo simulations. The availability of additional open source companion products such as the Monte Carlo simulation offers significant advantages for Microsoft Project Professional over using other software tools for project management. In this paper, Risky Project uses as a tool for the Monte Carlo simulation (Vajjhala, 2012).

1.3 Research Objectives

The primary objective of this study is:

- 1. Determine project duration by Critical Path method.
- 2. Collecting data and determine project duration by the PERT method
- 3. Analyze the output of a project schedule risk simulation when Monte Carlo use to simulate the duration of individual activities of the project and compare the total project duration outputs graphically and through statistical analysis.

1.4 Literature Review

Visser (2016), discussed the results of a project to investigate the output of schedule simulations when different distribution, e.g., triangular, normal, beta pert, are used to show the uncertainty in activity durations. The researcher used two examples to compare the output distribution; the first example is a network with ten activities in sequence, and the second is a network where some of the activities are performed in parallel. The result of this study was showed that there is no significant difference in the output distributions when different input distribution with the same mean and variance value is used.

Ganame and Chaudhari (2015), focused on risk identification, qualitative analysis, and quantitative analysis. The goals are to distinguish the key risk factors that influence the venture schedule and to determine the probability of finishing the undertaking inside the due date. Survey frames were conveyed with 31 industry professionals with a shifting background from 1 year to 17 years. The subjective investigation is finished by a probability impact (PI) framework. Furthermore, the quantitative study is finished by PERT and Monte Carlo simulation. @RISK by Palisade Corp. is utilized for Monte Carlo simulation.

Rui-Mei (2015), focuses on the application of Monte Carlo strategy in project management as pursue: first, manages the evaluated scale with Wideband Delphi technique and gets the interim of expectation in the wake of making rehashed tests in accordance with the most part, received likelihood models; that makes sense of the outstanding tasks at hand as indicated by the management capacities and authentic date.

Madadi and Iranmanesh (2012), in this paper, proposed one index, and one technique is proposed to fulfill this necessity. The essential hypothesis is, consuming exertion on activities and controlling them effectively results in activities risk decrease. In light of this theory, the proposed index and strategy are contrasted with the existing index in the literature. Looking at results demonstrates that the proposed index and strategy emphatically outperform another index

Sadeghi et al. (2010) in this article examined the inadequacies of the available techniques and proposes a Fuzzy Monte Carlo (FMCS) system Simulation for hazard investigation of development ventures. In this system, they build a fuzzy cumulative distribution function as the ideal approach to speak to uncertainty. To check the plausibility of the FMCS system and exhibit its fundamental highlights, the authors have built up a unique purpose simulation template for cost extend assessing. This layout is utilized to decide the expense of a roadway bridge venture.

Huang and Wang (2009) analyzed the traditional likelihood investigation strategy for duration risk in program assessment and audit procedure. Based on that, it simulates the task's length and inspects the danger of development plan by the Monte Carlo simulation technique. Monte Carlo technique had been utilized to simulate the duration of each activity and overall project to accurately determine the completion probability of the project under considering the changeability and randomness of duration for each activity. The outcome demonstrates that the MCS strategy is helpful, powerful, and productive. It gives a logical premise to extend essential leadership and backings the decider intensely.

Bennett et al. (2001) discuss the inference of a PERT simulation model, which consolidates the discrete occasion demonstrating the approach and a rearranged essential action recognizable proof strategy. This has been done trying to overcome the limitations and enhance the computing efficiency of traditional CPM/PERT analysis. In this paper, a case study was conducted to validate the developed model and compare it to traditional CPM/PERT analysis. the The established model illustrates checked upgrade in analyzing the risk of project schedule overrun and determination of activity criticality. Furthermore, the beta distribution and its subjective fitting techniques are discussed to complement the PERT simulation model. This new answer for CPM network analysis can provide project management with a convenient tool to assess alternative scenarios based on computer simulation and risk analysis.

Mizuno et al. (2000), in this paper, the researchers proposed a new scheme for the characterization of risky projects based on a performance by the project manager. To acquire the relevant data to make such an assessment, they a questionnaire from five first designed viewpoints within the projects: requirements, estimations, team organization, planning capability, and project management activities. Each of these viewpoints consisted of a number of detailed questions. Then compute the responses to the questionnaires as provided by project managers by applying logistic regression analysis. The coefficients of the logistic model from a set of the questionnaire responses were determined. The experimental results using actual project data in Company A showed that 27 projects out of 32

ZANCO Journal of Pure and Applied Sciences 2019

were predicted correctly. Thus, researchers would think that the proposed describing plan is the initial move toward foreseeing which ventures are dangerous at an early period of the improvement.

2. MATERIALS AND METHODS

2.1. Schedule Risk Analysis Steps:

A research process consists of some sequential steps. The three steps to successful risk analysis are described:

2.1.1 Baseline Scheduling

When appropriate techniques are picked, and the uncertainty of their outcome is evaluated, then the outcome might be contrasted with some normal result, or to a rule, standard or baseline level. Both the PERT and CPM methods calculate the shortest path of a project network, the critical path, based on the network logic and the activity duration estimates made by the project manager. However, since estimates are often, if not always, subject to a margin of error, people feel more comfortable with a range of possible project outcomes rather than with a single point estimate like the critical path length. Moreover, the blackand-white view of the CPM methods on the critical activities should be more refined since noncritical (critical) activities have the potential to become critical (noncritical) during the progress of the project (Vanhoucke, 2012).

2.1.2 Determine the activity duration ranges:

The activity durations that are used to determine the critical path are usually thought of as the best guess length of time needed to complete the project known the planned resources. Experienced engineer managers know that the work might take more or less time than the estimate they have assumed for the critical path method calculation. These times make up the low and high ranges for risk analysis (Hulett, 1996).

Once the ranges, often called "3-point estimates," are determined, the project manager must adopt a probability distribution shape for each risky activity. A probability distribution takes the three possible durations (low, most likely, and high) and expresses the relative likelihood of alternative outcomes within that overall range. That is, there are some potential durations which are more likely than others (Hulett, 1996).

Triangular distributions are often used in risk analysis because they are easy to specify (just needing three points and a straightedge) and to use in the analysis. Generally, triangular distribution is preferred when data submitted is judgmental in nature (Ganame and Chaudhari, 2015).

2.1.3 Monte-Carlo Simulation

Monte Carlo is a well-established method to represent a risk. Several commercial scheduling programs have a schedule risk analysis module. For some others, third-party software companies may have provided such capability. Not all network programs have risk analysis packages, however. The risk analyst using a network program that lacks the risk package will have to load the schedule into a network program that does (Hulett, 1996).

Monte Carlo Simulation methods have become famous amongst project managers and planners nowadays due to the availability of fast computers and software that is freely available, primarily as add-ins for spreadsheets like Microsoft Excel. The method is well documented and explained in various textbooks (Mun, 2006), (Cooper, 2005) and (Robert et al., 2010). Wood (2002) says Monte Carlo Simulation can be "applied in many diverse fields that require outcomes to be quantified statistically under conditions of uncertainty to aid in decisionmaking." Various programs software for performing simulations are available to model uncertainty in the cost or duration of activities. Two standalone software programs that use discrete event simulation are Arena and GoldSim. Some add-in for performing simulations with MS Excel is @Risk, Crystal Ball, SimVoi, and RiskAmp (Visser, 2016). The tool used for this study is the Risky Project software add-in for Microsoft Project 2016. The probability distribution for each activity is selected as a triangle to match the traditional assumptions in PERT.

2.2 Sensitivity Analysis

Sensitivity analysis helps to determine which individual project risks or other sources of uncertainty have the most potential impact on project outcomes. It correlates variations in project outcomes with changes in elements of the quantitative risk analysis model (Guide, 2017).

One typical display of sensitivity analysis is the tornado diagram, which presents the calculated correlation coefficient for each element of the quantitative risk analysis model that can influence the project outcome. This can include individual project risks, project activities with high degrees of variability, or specific sources of ambiguity. Items are ordered by descending strength of the correlation, giving the typical tornado appearance (Guide, 2017)

2.3 Case study

A case study adopted is scheduling duration for construction a residential house 125 m² which consist of two stories. Risk schedule process consists of a number of sequential steps:

2.3.1 First step:

According to the house plan, the researchers could find and sequencing each activity, which includes activities established based on work breakdown structure, as shown in Table 1. Critical Path Method network developed at a level of detail that illustrates the important project structure. The Microsoft project 2016 has been used to build the CPM schedule. Firstly, we make the new project file and setting the starting project time on April 1, 2018, with six days as a working day per week from Saturday to Thursday. Secondly, enter each task name and their predecessors with the relationship between each task.

2.3.2 Second step:

The gathering of information about project risk is usually an included procedure. Most associations do not have databases that incorporate data reasonable for quantitative hazard examination, so interview methods were utilized, and the information are typically evaluated judgmentally. The data to be gathered comprise of 3-point estimates of duration (optimistic, most likely, and pessimistic scenarios).

For determining the activity duration, the researcher has made a form. The form consists of all house tasks and estimated quantity with three columns for estimating Optimistic Duration, Most Likely Duration, and Pessimistic Duration in accordance with the respondent's perception. The researchers conducted an interview with expert engineers to estimate the three-point duration for each task according to their experience. After collecting data; the researcher calculates each task duration by using PERT calculations methods. The standard calculated values for PERT calculations (Mubarak, 2010) are as follows:

The weighted average activity time t:

$$t = (O + 4M + P)/6$$

where:

O = optimistic activity time (1 chance in 100 of completing the activity earlier under normal conditions)

M = most likely activity time (without any learning curve effects)

P = pessimistic activity time (1 chance in 100 of completing the activity later

under normal conditions)

The calculation process will have done by using PERT Add-in to Microsoft project 2016. The initial calculations apply to each project activity, and then the calculations result in an analysis of the entire project.

2.3.3 Third step:

The tool used for this study is the "Risky Project professional 7 software add-in for Microsoft Project 2016". By adding low and high estimates and distributions to each activity, simulation can be run. Monte Carlo simulations are utilized to assess the distribution of potential outcomes on probabilistic inputs. dependent Every simulation is produced by arbitrarily pulling a sample value for each information variable from its characterized probability distribution. These input sample values are then used to figure the outcomes (in Risky Project, it is projected schedule parameters: project duration, start and finish times, success date, and cost).

3. RESULTS AND DISCUSSION

ZANCO Journal of Pure and Applied Sciences 2019

3.1.Schedule Risk Analysis

By applying the Microsoft project 2016, the researchers built a schedule for determining the total duration of the project by critical path method. There is 32 sequencing activity, and the researchers estimate each activity average duration based on their experience as listed in Table 1, and it used as an entry table of Microsoft project 2016 which shows the work break structure code, duration, and predecessors for each activity. By entering the data from Table 1 to Microsoft project 2016, the network diagram of project and Gant chart of the project was drawn and determining the critical path, the total duration of project construction house by critical path method estimated 96 days. The Microsoft project outputs show in Figure 1 and Figure 2.

Table (1) Microsoft Project Entry Table

			WBS
WBS	Task Name	Duration	Predecess
Code		day	ors
A.1	Site Preparation	1	
A.2	Excavation	1	A.1
A.3	Concrete Foundation with	3	A.2
A.4	Solid Block Foundation with	3	A.3
A 5	curing Foundation Filling	1	A /
A.C	Ground Floor Hollow Block	2	A. 4
A.6	Wall with curing	3	А.Э
A.7	Ground Floor Slab with curing	12	A.6
A.8	First Floor Hollow Block Wall with curing	4	A.7
A.9	First Floor Slab with curing	12	A.8
A.10	Pent House Wall Hollow Block with curing	2	A.9
A.11	Pent House Slab with curing	9	A.10
A.12	Fencing block work	2	A.10[SS]
A.13	Rough Electrical Work (Cables layout)	3	A.11
	Rough Mechanical Work		A 11
A.14	(Plumbing - Pipes layout- ductwork)	3	A.11, A.12
A.15	Wall Ceramic Tile	12	A.14, A.13
A.16	Granit Façade Work	10	A.14
A.17	Flooring Tiles (Ground floor, first floor, and stair)	8	A.15, A.16
A.18	External and Garage Flooring Tile	2	A.17
A.19	Cement Plastering	5	A.17
A.20	Gypsum Plastering	3	A.19, A.18
A.21	Secondary Ceiling Work	5	A.20
A.22	Painting	2	A.21,
1 22		2	A.20
A.24	Door (Wood)	2	A.21
A.25		2 1	A.21
A.25	Door (Steel)	1	A.21
A.26	window (PVC)	2	A.21
A.27	Aluminum handrail for stair	1	A.21
A.28	Gate (2.8m*1.5m) steel	1	A.21
A.29	Finish electrical and Lighting Fixtures	4	A.22
A.30	Set Plumbing fixtures and trim	2	A.22
A.31	W.C and Bath Fittings	1	A.22
A.32	Kitchen furniture fixing	3	A.31, A.29, A.30, A.27, A.26, A.25,
			A.24, A.23, A 28

ZANCO Journal of Pure and Applied Sciences 2019







Figure 2: Gant Chart for Residential House

Secondly, determine the activity duration ranges. The researcher collected three points estimated duration for each task of the project by an interview with 26 civil engineers. The engineers asked to estimate Optimistic duration, most likely duration and Pessimistic duration for each task.

96

Number of the second	Task name	Duration	Optimistic	Expected	Pessimistic	
Site Preparation 1.42 0.5 1 4 Excavation 1.58 0.5 1.5 3 Concrete Foundation with curing 0.5 1.5 2 Solid Block Foundation with curing 0.71 0.25 0.75 1 Foundation Filling 0.71 0.25 0.75 1 Ground Floor Hollow Block Wall with curing 2.5 2 2.5 3 Ground Floor Hollow Block Wall with curing 7.42 6.5 7 10 First Floor Hollow Block Wall with curing 7.42 6.5 7 10 Pent House Wall Hollow Block with curing 1.08 0.5 1 2 Pent House Slab with curing 5.17 4 5 7 Pent House Slab with curing 5.17 4 5 1 Rough Mechanical Work (Plumbing - Pipes layout-4.17 3 4 6 Kottwork 10.17 7 10 14 Ground Floor and stair) 6 5 6 7 Wall Ceramic Tile 10.17 7 10 14 Grou		(t)	Dur.	Dur.	Dur.	
Excavation1.580.51.53Concrete Foundation with curing1.420.511.5Solid Block Foundation with curing1.420.51.52Foundation Filling0.710.250.751Ground Floor Hollow Block Wall with curing2.522.53Ground Floor Slab with curing7.426.5710First Floor Hollow Block Wall with curing2.522.53First Floor Hollow Block Wall with curing2.522.53First Floor Hollow Block Wall with curing1.080.510Pent House Wall Hollow Block with curing1.080.512Pent House Slab with curing5.174571Rough Electrical Work (Cables layout)0.540.250.511Rough Mechanical Work (Cables layout)4.173461Granit Façade Work4.1734611Flooring Tiles (Ground floor first floor, and stair)65671Secondary Ceiling Work (Supsum Plastering97911Secondary Ceiling Work (Stel)1.080.5122Door (Wood)2.581.533245Door (VCO)1.080.512236Door (VCO)2.581.533 </td <td>Site Preparation</td> <td>1.42</td> <td>0.5</td> <td>1</td> <td>4</td>	Site Preparation	1.42	0.5	1	4	
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Granit Façade Work 4.17 3 4 6 Flooring Tiles (Ground floor, first floor, and stair) 6 5 6 7 External and Garage Flooring Tile 2.17 1 2 4 Cement Plastering 3.33 2 3 6 Gypsum Plastering 9 7 9 11 Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1.5 3 Gate ($2.8m*1.5m$) steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Wall Ceramic Tile	10.17	7	10	14	
Flooring Tiles (Ground floor, first floor, and stair)6567External and Garage Plooring Tile2.17124Cement Plastering3.33236Gypsum Plastering97911Secondary Ceiling Work3.83245Painting6468Door (PVC)2123Door (Wood)2.581.52.54Door (Steel)1.080.512Window (PVC)1.080.51.53Gate (2.8m*1.5m) steel1.920.523Finish electrical and Lighting Fixtures3234Set Plumbing fixtures and trim1.580.51.53W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	Granit Façade Work	4.17	3	4	6	
first floor, and stair)2.17124External and Garage Flooring Tile 2.17 124Cement Plastering 3.33 236Gypsum Plastering97911Secondary Ceiling Work 3.83 245Painting6468Door (PVC)2123Door (Wood) 2.58 1.5 2.5 4Door (Steel)1.08 0.5 12Window (PVC)1.08 0.5 12Aluminum handrail for stair 1.58 0.5 1.5 3Gate ($2.8m*1.5m$) steel 1.92 0.5 23Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Flooring Tiles (Ground floor,	6	5	6	7	
External Flooring Tileand Garage 2.17 2 4 Cement Plastering 3.33 2 3 6 Gypsum Plastering 9 7 9 11 Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1.5 3 Gate $(2.8m*1.5m)$ steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	first floor, and stair)					
Flooring Tile 3.33 2 3 6 Cement Plastering 9 7 9 11 Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1.5 3 Gate (2.8m*1.5m) steel 1.92 0.5 2 3 Finish electrical and Lighting fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	External and Garage	2.17	1	2	4	
Cement Plastering 3.33 2 3 6 Gypsum Plastering 9 7 9 11 Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate ($2.8m*1.5m$) steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Flooring Tile		-			
Gypsum Plastering 9 7 9 11 Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1.5 3 Gate (2.8m*1.5m) steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Cement Plastering	3.33	2	3	6	
Secondary Ceiling Work 3.83 2 4 5 Painting 6 4 6 8 Door (PVC) 2 1 2 3 Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate $(2.8m*1.5m)$ steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Gypsum Plastering	9	7	9	11	
Painting6468Door (PVC)2123Door (Wood) 2.58 1.5 2.5 4Door (Steel) 1.08 0.5 12Window (PVC) 1.08 0.5 12Aluminum handrail for stair 1.58 0.5 1.5 3Gate $(2.8m*1.5m)$ steel 1.92 0.5 23Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Secondary Ceiling Work	3.83	2	4	5	
Door (PVC)2123Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate $(2.8m*1.5m)$ steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Painting	6	4	6	8	
Door (Wood) 2.58 1.5 2.5 4 Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate $(2.8m*1.5m)$ steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Door (PVC)	2	1	2	3	
Door (Steel) 1.08 0.5 1 2 Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate ($2.8m*1.5m$) steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Door (Wood)	2.58	1.5	2.5	4	
Window (PVC) 1.08 0.5 1 2 Aluminum handrail for stair 1.58 0.5 1.5 3 Gate ($2.8m*1.5m$) steel 1.92 0.5 2 3 Finish electrical and Lighting Fixtures 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Door (Steel)	1.08	0.5	1	2	
Aluminum handrail for stair 1.58 0.5 1.5 3 Gate ($2.8m*1.5m$) steel 1.92 0.5 2 3 Finish electrical and Lighting 3 2 3 4 Set Plumbing fixtures and trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Window (PVC)	1.08	0.5	1	2	
Gate (2.8m*1.5m) steel1.920.523Finish electrical and Lighting Fixtures3234Set Plumbing fixtures and trim1.580.51.53W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	Aluminum handrail for stair	1.58	0.5	1.5	3	
Finish electrical and Lighting Fixtures3234Set Plumbing fixtures and trim1.580.51.53W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	Gate (2.8m*1.5m) steel	1.92	0.5	2	3	
Fixtures3234Set Plumbing fixtures and trim1.580.51.53W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	Finish electrical and Lighting	2	2	2		
Set Plumbing fixtures and trim1.580.51.53W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	Fixtures	3	2	3	4	
trim 1.58 0.5 1.5 3 W.C and Bath Fittings 1.08 0.5 1 2 Kitchen furniture fixing 1.08 0.5 1 2	Set Plumbing fixtures and	1 50	0.5	1.5		
W.C and Bath Fittings1.080.512Kitchen furniture fixing1.080.512	trim	1.58	0.5	1.5	5	
Kitchen furniture fixing 1.08 0.5 1 2	W.C and Bath Fittings	1.08	0.5	1	2	
	Kitchen furniture fixing	1.08	0.5	1	2	

Table (2)First Response (R1)Expected Three PointDuration

Furthermore, based on the responded result, the researcher could find the average duration for each activity, table 3 illustrates how determined the site preparation duration.

Table (3) Site Preparation duration calculation

Response No.	Optimistic Dur.	Expected Dur.	Pessimistic Dur.
R1	0.25	0.5	1
R2	1	2	2
R3	0.5	0.75	1
R4	0.25	0.5	1
R5	0.5	1	2
R6	0.5	1	2
R7	0.5	1	4
R8	0.5	1	3
R9	1	2	3
R10	1	2	3
R11	1	2	3
R12	1	1	2
R13	0.5	1	2
R14	0.5	1	2
R15	0.5	1	3
R16	0.5	1	1
R17	0.5	1	1
R18	0.25	0.5	2
R19	0.25	0.5	1
R20	0.4	0.6	1
R21	0.5	1	1
R22	1	1	2
R23	1	1	2
R24	1	1	1
R25	1	2	5
R26	0.25	1	2
Total	16.15	28.35	53
Average	0.62	1.09	2.04

The standard calculated values for PERT calculations used to determine site preparation duration as follows:

t = (O+4M+P)/6t = (0.62+4*1.09+2.04)/6t = 1.17 day

Duration

The researcher preceded the same procedure

of individual project risks and other sources of

WBS	Task name	Duration (t)	Optimistic Dur.	Most likely Dur.	Pessimistic Dur
11 D S			day	day	day
Α	House				
A.1	Site Preparation	1.17	0.62	1.09	2.04
A.2	Excavation	1.7	1.04	1.62	2.7
A.3	Concrete Foundation with curing	3.68	2.96	3.48	5.19
A.4	Solid Block Foundation with curing	2.87	1.96	2.81	4.04
A.5	Foundation Filling	1.7	0.97	1.65	2.65
A.6	Ground Floor Hollow Block Wall with curing	4.74	3.23	4.75	6.19
A.7	Ground Floor Slab with curing	10.44	8.85	10.15	13.19
A.8	First Floor Hollow Block Wall	4.86	3.65	4.75	6.54
A.9	First Floor Slab with curing	10.28	8.73	10.04	12.81
A.10	Pent House Wall with curing	3.57	2.33	3.71	4.25
A.11	Pent House Slab with curing	6.61	5.35	6.46	8.46
A.12	Fencing block work	2.67	1.44	2.85	3.17
A.13	Rough Electrical Work (Cables layout)	4.44	3.25	4.37	5.92
A.14	Rough Mechanical Work	5.35	3.81	5.29	7.15
A.15	Wall Ceramic Tile	11.11	9	10.62	15.15
A.16	Granit Façade Work	8.36	6.31	8.19	11.08
A.17	Flooring Tiles (Ground floor, first floor, and stair)	7.37	5.46	7.19	10
A.18	External and Garage Flooring Tile	2.85	1.5	2.94	3.85
A.19	Cement Plastering	4.39	2.96	4.38	5.85
A.20	Gypsum Plastering	7.17	5.42	6.9	10
A.21	Secondary Ceiling Work	4.88	3.88	4.63	6.88
A.22	Painting	4.01	2.67	3.9	5.77
A.23	Door (PVC)	4.68	3.06	4.63	6.48
A.24	Door (Wood)	3.71	2.48	3.77	4.71
A.25	Door (Steel)	2.16	1.44	2.1	3.11
A.26	Window (PVC)	4.63	3.38	4.54	6.21
A.27	Aluminum handrail for stair	2.18	1.4	2.13	3.15
A.28	Gate (2.8m*1.5m) steel	2.54	1.71	2.44	3.79
A.29	Finish electrical and Lighting Fixtures	3.05	1.73	3	4.6
A.30	Set Plumbing fixtures and trim	2.55	1.6	2.48	3.77
A.31	W.C and Bath Fittings	2.1	1.29	2.06	3.1
A.32	Kitchen furniture fixing	4	2.88	3.85	5.69

for calculating each task duration, and the result demonstrated in table 4.

Table (4)AverageResponseEstimatedThreePointDuration

By entering the data to Microsoft project 2016 with PERT add-in and using the same CPM relationship between tasks project expected duration was determined, the result showed the house building will be finish with 103 working days and illustrated in figure 3.

Furthermore, quantitative risk analysis uses a model that simulates the combined effects

uncertainty to evaluate their potential impact on achieving project objectives. Simulations are typically performed using a Monte Carlo analysis. After calculating the average of responded optimistic most likely, and pessimistic time, all the three durations are inserted in Risky Project Professional 7 Add-In Microsoft Project 2016 of each responded. The output is defined, and triangle simulation is run for each responded data based on the following Monte Carlo parameters:

• The maximum number of simulation (600) sample.

Converged when mean and standard deviation changes by less than 0.15% and Over 20%
Low and high time and duration are calculated based on Percentile (10) and percentile (90).

The result of project durations by Monte-Carlo simulation for average response estimated duration with no risk equal to 103 days, project duration with low risk equal to 103 days, with base risks equal to 107 days and with high-risk project duration equal to 111 days as shown in Tabl

			1 401
Start Time		Duration	e 5.
No RISKS	Cur. Schedule	102.96 days	Tabl
With Risks	Low	102.72 days	e (5)
	Base	107 days	Risky
	High	110.65 days	Proje
			Outp

ut

Simulation.

Figure 4 and Figure 5 shows the descriptive, frequency statistics and percentage of completion of the construction project for various durations, the mean completion time of the project is nearly equal 107 with 576 number of the sample (iterate).

The 10% chance that duration will be less than 103 days and 90% chance that duration will be less than 111 days. The minimum and maximum completion times are 98 days and 115 days respectively shows that the percentage of completion of the construction project for various durations.



Figure 3: Estimation of Construction Duration by Using PERT

The results show that it is extremely unlikely to complete the project within 98 days. Moreover, there is a 100% chance that the project will be completed in 115 days. Table 6 shows the experience, education level and calculated total project duration by a PERT method for each response and Monte Carlo simulation result, the software run the result of each response data according to their estimation.

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Figure 4: Frequency and Cumulative Probability, Frequency and Cumulative.



Figure 5 : Descriptive, Frequency Statistics, and Percentage of Completion of The Construction Project for Various Durations

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Response	Experience	Educational level	PERT	duration with Risks Day			
Number	Year			Low	Base	High	
R1	15	BSc	79	78	82	85	
R2	10	BSc	80	79	81	84	
R3	10	MSc	84	84	87	90	
R4	8	BSc	85	83	84	86	
R5	17	BSc	85	83	85	87	
R6	21	BSc	88	90	93	97	
R7	21	BSc	88	86	89	92	
R8	5	BSc	89	90	92	95	
R9	17	BSc	90	89	91	93	
R10	8	BSc	91	92	95	96	
R11	30	Ph.D.	93	90	94	98	
R12	30	BSC	95	92	96	101	
R13	8	BSc	95	91	94	97	
R14	7	BSc	97	94	98	103	
R15	15	BSc	98	97	100	103	
R16	8	BSc	99	100	107	115	
R17	8	BSc	106	105	112	120	
R18	10	BSc	118	117	120	125	
R19	11	BSc	122	122	129	135	
R20	21	Ph.D.	123	125	131	137	
R21	37	BSc	136	134	137	141	
R22	30	BSc	138	142	144	146	
R23	9	BSc	143	141	148	155	
R24	15	BSc	148	149	151	153	
R25	7	BSc	152	146	155	165	
R26	10	BSc	153	153	164	176	

 Table (6) Respondents Profile and Perception of Estimated

 Duration

3.2 Sensitivity Analysis

The tornado diagram is a different bar chart that is utilized as a part of sensitivity analysis. The sensitivity analysis is a modeling technique that figures out which risks have the most impact on the project. As one of the tools used in the sensitivity analysis, the tornado diagram is used to compare the importance of different variables. The sensitivity analysis for residential construction house indicates that the project schedule is most sensitive to task number 15 which is Wall Ceramic Tile, which can influence the completion date

because of the correlation coefficient of ceramic wall tile equal to 0.39.

The project's second highest sensitivity is Gypsum Plastering, which can affect the completion date, as illustrated in Figure 6. Knowing that this is where the most schedule sensitivity lies and to find which activity is riskier, we may decide to pay delivery premiums for materials, and perhaps pay for additional overtime, etc., for the contractors to expedite construction.

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Task Name	Coefficient	Correlation between finish times
Task: Wall Ceramic Tile	0.39	
Task: Gypsum Plastering	0.33	
Task: First Floor Slab with curing	0.32	
Task: Pent House Slab with curing	0.29	
Task: Secondary Ceiling Work	0.29	
Task: Flooring Tiles (Ground floor, first floor and stair)	0.28	
Task: Ground Floor Slab with curing	0.28	
Task: Ground Floor Hollow Block Wall with curing	0.25	
Task: Painting	0.21	
Task: Cement Plastering	0.21	
Task: Rough Mechanical Work (Plumbing - Pipes layout- duct work)	0.20	
Task: Finish electrical and Lighting Fixtures	0.19	
Task: Excavation	0.16	
Task: First Floor Hollow Block Wall with curing	0.16	
	Task: Name Task: Wall Ceramic Tile Task: Gypsum Plastering Task: First Floor Slab with curing Task: Pent House Slab with curing Task: Secondary Ceiling Work Task: Flooring Tiles (Ground floor, first floor and stair) Task: Ground Floor Slab with curing Task: Ground Floor Slab with curing Task: Ground Floor Hollow Block Wall with curing Task: Cement Plastering Task: Rough Mechanical Work (Plumbing - Pipes layout- duct work) Task: Finish electrical and Lighting Fixtures Task: First Floor Hollow Block Wall with curing	Task NameCoefficientTask: Wall Ceramic Tile0.39Task: Gypsum Plastering0.33Task: First Floor Slab with curing0.32Task: Pent House Slab with curing0.29Task: Secondary Ceiling Work0.29Task: Flooring Tiles (Ground floor, first floor and stair)0.28Task: Ground Floor Slab with curing0.28Task: Ground Floor Slab with curing0.25Task: Ground Floor Hollow Block Wall with curing0.21Task: Cement Plastering0.21Task: Rough Mechanical Work (Plumbing - Pipes layout- duct work)0.20Task: Finish electrical and Lighting Fixtures0.19Task: First Floor Hollow Block Wall with curing0.16

Figure 5: Sensitivity to Finish Times of Other Tasks

4. CONCLUSIONS

This study develops the methodology for schedule risk analysis of building construction. The Residential House 125 m² construction project was investigated to recognize the risks that the project completion affect time. and furthermore to determine the probability of completing the project within the due date. Based on the researchers of other scholars, this paper analyzed the traditional probability analysis method for duration risk in PERT. Firstly, the researchers determined the project duration by the critical path method, which is equal to 96 days. Secondly, for determining the project duration by PERT method, a questionnaire form prepared, to estimate three durations for each activity by different expert engineers the average optimist, most likely and pessimistic duration of all responses was determined, the project duration equal to 103 days after calculated the responses data by PERT method.

Monte Carlo method was used to simulate the duration of each activity and the overall project to accurately determine the completion probability of the project under considering the changeability and randomness of duration for each activity. Project duration with low risk equal to 103 days, with base risks equal to 107 days and with highrisk project duration equal to 111 days. The results show that it is doubtful to complete the project within 98 days. Moreover, there is a 100% chance that the project will be completed in 115 days. In the end, for knowing that where is the most schedule sensitivity lies, the tornado graph was determined. The sensitivity analysis for construction Residential house indicates that the project schedule is most sensitive to Wall Ceramic Tile, which can influence the completion date because of the correlation coefficient of ceramic wall tile equal to 0.39 and top-ranked of all tasks.

The project duration should be determined by the PERT technique in conjunction with CPM to develop a probability distribution of possible completion dates and its quantified risks and opportunities. As a result, there are two primary types of risk analysis; qualitative and quantitative assessments.

A qualitative assessment is an evaluation that does not make use of schedule iteration techniques as used in this paper but instead relies on the experience and knowledge of key project stakeholders to identify items that have the potential to impact the schedule. A quantitative assessment makes use of probabilistic analysis techniques were considered in this paper, as the Monte Carlo simulation. The process requires building a logical activity network model where risks are identified from a variety of sources.

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RESEARCH PAPER

Office Building Adaptability through Buildings' Layers

(Office Buildings in Erbil city as case study)

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ABSTRACT:

A building has an average life span over fifty years through history, whereas adaptability in the building motivated by a number of factors, not least the desire to 'future need' or 'future change' for buildings against social, economic, and technological change. Adaptability is concerned with the capacity to adjust or be adjusted to suit new situations. A building as a composition separated in to a number of layers (site, structure, skin, space layout, services, stuff, social, surrounding) that together define building as a whole. Each layer is distinguished from others by special role it fulfills. According to previous studies, there are six types of adaptability that users 'demands; adjustable, versatile, refitable, convertible, scalable and movable, can be linked to building layers through building design strategies and characteristics. This research will examine adaptability through analysis of office buildings according to their layers, to show ratio of adaptability in both public and private office buildings and explore needs of users for each type of changes. The aim of the research is to bring needed clarity to the concept of adaptability by providing more detail insight into what constitutes adaptability is in Erbil office buildings and the factors that give rise to different level of adaptability. For analyses of case studies using meta-model methodology of Robert S. and Simon A (2016). It showed that the average percentage of adaptability is half of total characteristics of building and the need for types of change are increasing in both public and private office building. For feasibility of building the problem mostly in layers such as spaces ,space plan, services ,stuff and structure. Therefore this research provides researcher, designers and other practitioners with the knowledge and tools to raise client's awareness of building adaptability, helping to clarify their needs in future design.

KEY WORDS: Adaptability, Office, Building layers. DOI: <u>http://dx.doi.org/10.21271/ZJPAS.31.5.12</u> ZJPAS (2019), 31(5);104-120.

1. INTRODUCTION :

This research analyses the buildings that have an average life span, whereas the society can change radically and repeatedly during that time and architects are faced with task of giving form to a building which fulfill the needs of clients, for a period during which the composition of the building, associated rituals will go through major changes The word 'Flexibility' is often interchanged with ' adaptability ', due to their similar meaning. According to Collins Online Dictionary, the concept 'adaptability' refers to objects ability to change or be changed in order to suit new situations. Whereas 'Flexibility' refers to adaptability; in the case of a building, it is pliant and adaptable to new and changing situations. This research more concentrated on the concept of adaptability in office buildings of Erbil city.

Adaptability due to its types of change through different layers in office buildings are key words in the approach of designing for the unpredictable

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needs in future. The methods of movement in design, which sought to further integrate architecture with other disciplines, especially the social and technology since both of them change rapidly over time. Venturi tried to improve the connection between science, technology, humanities and social in order make the architecture a more human social art. While Brand (1994) says "The unit of analysis for us isn't the building, it's the use of the building through time. Time is the essence of the real design problem." (Brand, 1994). Therefore a strategy that solves this issue, with a capacity to accommodate change, is the idea of fluctuating space, where the dedicated functional spaces, are linked with more ambiguous territory - a sort of buffer zone in which many things can happen.Leaman et al (1998) reflects our own experiences, suggesting that adaptability is "now commonplace in the vocabulary of briefing, building design, building management". P. Russell and S. Moffatt in 2001, Adaptability refers to the capacity of buildings to accommodate substantial change. Robert S. and Simon A. (2016) argued that "the problem is temporary thus the solution as well'.

As a result this research will investigate the study of adaptability through buildings' layers for office building adaptation, since adaptability has always been tested through office design, due to high levels of flexibility needed for this program, to answer research questions through testing research hypotheses by using research methodology. The aim of research is to explore the aspect of adaptability that allow a building to adapt itself to the time and need of individuals (in changeable configuration), of determine its level implementation and its opportunity and constrain toward flexibility to adapt itself change by time.

2. CHANGE IN OFFICE BUILDINGS

Now, office buildings are developing and changing over time. Since office work continues to change within introduction of new technologies. In addition to building technology innovation with opened up new possibilities, the structural steel frame, the elevator and electric lights were important elements in the development of office buildings. "A study of the history of office buildings reveals how building technology enabled the evolution of the office building, and how new work-technology such as

telephones,typewriters, contributed to the development of office work" (Blakstad, 2001). Also, he mentioned two things in the history of office building, first office building has changed a lot during the years, each of them are products of their time. Second, there is a strong relationship between the office work which is performed and the office building. Buildings are physical structures that represent the social structure inside them. "Far the greatest rate of change comes right at the building as it does with everything that lives. It starts before the building is even complete." (Brand, 1994,p.164). On the other hand the organizational learning theorists such as "Gregory Bateson and Chris Agyris refer to it as 'single-loop learning', double-loop learning', and 'learning to learn', which consist of these three levels. Single-loop it respond to a simple feedback loop which define habit. Double-loop it is the kind of learning which threatens the habit it up level, in another word change habits. The third level is "learning to learn" changes how we change habits, raised floor is an example" (Brand, 1994, p167). As a result, the office work continues to change

As a result, the office work continues to change from the introduction of new technologies to a clear understanding of the task required become a significant motivating force. Designers can influence all aspects of the working environment good design is extremely, and has a strong influence on job satisfaction.(Researcher)

3. OFFICE INNOVATION

The development in business, organization and new work styles causes office building to have the ability to accommodate or adapt to this high level of changes. These changes appeared in the way office work which carried out in organization, changed with new ideas about management and work, existing new types of office work. In addition, technological innovations in both the building and the utility which are used by organization, and the structural changes in office's work, the time affect the requirements and standards for both building and working environments, also the most important change in the market, design, construction and management of buildings. (Researcher).

While, Frank Duffy 1992 has remarked on the absence of long view research in this area 'one of

the most dominant learn facts of 20th century life is the huge increase in the importance of the office. We have had a massive change from less than 10 percent to over 50 percent of people occupied in offices, and no one has studied physical evidence of that change from an organizational and social perspective.' He mentioned three wave of innovation, the first innovation which swept the world in a decade is 'open office'. The second wave of innovation is in office furniture, completed the revolution. The third element in innovation wave that making office environments forever liquid was the arrival of information technology. Information equipment kept taking more space, but office managers gradually realized that the computer equipment was going to keep needing replacement. The uses of specialist and technician have increased. (Brand, 1994).

In 1980s a new innovation was invented ,that is "smart building" which was based on the idea of

electronically integrating all the control of a building and offering tenants a full menu of builtin information services. This idea failed since integrating all the building components (climate control, security, lighting, and communication all were supposed managed by a bank of computers) only the specialist could understand it and a problem in one area could infect the other area. The new office building idea comes that the Frank Duffy has the story of what happened to it during 1980s in Scandinavia, Holland, and Germany where open office began. Guess what they don't like? They don't like open plan. And guess what they do like? They like windows that they can open, and door shut, and walls they can bang on. The new northern buildings, instead of being 120 feet deep, are 30 feet deep. They're like hotelsmillions of individual rooms, each with a window,' See figure (3-1)



Figure (3-1) Office buildings innovation through history diagram by researcher

Brand in 1994 claimed that best adaptation he

has ever seen of the open-office idea is partial retreat from it. People want acoustic privacy so they can talk on the telephone, but visual privacy is not as important-they like being able to see what's going on.

This have led to a very satisfactory compromise called "cave and common". Each office worker has a private office, often small, which opens into a generous open area surrounded by many other private offices. The open area has a kitchen, some couches, sometimes tables for sitting around informally, and sometimes a working library, or at least a rack of current periodical. You can shut the door of your cave and concentrate, or you can leave your door open and keep an eye and are on whose coming and

going in the common. The feeling is congenial and homey, and it encourages the casual encounters which, research keeps showing, are at the heart of creativity in offices.

4. HISTORY OF ADAPTABILITY

The start of architecture from the primitive hut. The architecture starts to develop into different types of building, as temples, market hall, offices, apartments, amphitheater ...etc. these all existed within the grow of civilization. Since the house is first building type in architecture become a sample of adaptability for other buildings types.

According to Sennett (2008) and Brand (1994) the vernacular process was simple, direct, and evolved out of a shared experience between owner and unlike todays complicated builder, multistakeholder process. For Brand (1994,p14) evolutionary forms will always adapt better than visionary solutions- as they are based on trial and error, embodying of understanding the culture, climate and convention. In general, from the history it become clear that pre-modern buildings without regarding the culture and geography their buildings benefited from simple. mature

construction techniques and spaces that can be typified as polyvalence .Figure (4-1)

At the turn of 20th century the idea of adaptability or flexibility became explicit in the modern movement. In the mid- 19th century during industrial revolution, this revolution increased pace of social change along with new building material types, type of buildings and spatial standards all together gave rise to urban condition and the demand for buildings to accommodate changes. Its principle clears enough in the works of architects such as Le Corbusier, Frank Lloyd Wright, Alvar Alto, Mies van de Rohe and later on the works of Louis Kahn and Stirling.(Robert S.III& Simon James A.,2016,p15).

The characteristics of modern buildings are simple, cubic forms, by structure and mechanism systems expressing the "truth". their structure composed of frame construction that separate the space plan and structure, allowing for the development of free plan, and the use of light weight cladding systems that separating exterior skin from the structure. In this style the architects should express contemporary technology through the use of new materials and new ways of building. Jencks (1973) stated that "what the building want to be". In addition, Hertzberger (2005) mentioned that the modern movement created over-specified solutions in monofunctional buildings and neighborhoods. One of the modernism bigger failure is mono-functional buildings as Pawley (2007) argues this sought to correct condition for use rather than usefulness itself" thus created demand for flexibility and now towards adaptability. Again Rabeneck et al. (1973) mentioned that "an artificial understanding of occupant's behaviors. Also Schneider and Tills (2007) modernist portrayal that the used is merely another design element that would perform the same function again and again with no possibility of changing or combining of the functions, not to mention doing things differently. Watkins(2000)
mentioned the better solution sit within traditional architecture, suggesting a need to reconnect architecture with its pre-modernist history. For Habraken (1998), modernism principle removed the idea of separation between stakeholders and physical form In general modernism's desire for using contemporary technology failed to understand user needs at the expense of technological fetishes.However; many of the negative aspects identified remain today as dominating perspective, suggesting a lack of evolution towards situating architecture in its real context.



Figure (4-1) The start of architecture through history to the concept of adaptability by researcher

5. DIFINATION OF ADAPTABILITY

At the moment the definition have changed subtly as "to make suitable for the requirements or conditions; adjust or modify fittingly' (Random House 2010). Olsson and Hansen (2010) found that "Stakeholders either used different terminology or the same terminology with different meanings, each of the projects tended to develop its own terminology.

Therefore, adaptability in this research paper is concerned with the capacity to adjust or be adjusted to suit new situations.

5-1 Five interpretations for adaptability:

In the book of Adaptable Architecture (theory and practice) by Robert Schimdt III and Simon Austin (2016,p43), which is the final conclusion of many research explorations on the adaptability. They found there are five interpretations within adaptability in the construction literature as follow:

- 1. Adaptive architecture or responsive structures: is the building's capacity to change with changing conditions through dynamic facades or transformable structures.
- 2. Adaptive reuse: this is referring to finds new uses for underutilized or vacant building by changes in social perception or desire. Adaptive reuse is commonly associated with converting office building to residential and vice versa, or other functions.
- 3. Accessibility for all or inclusive design: is particularly strong in designing homes or buildings to accommodate a diverse range of users and their changing capabilities throughout life. In the UK its driven by government policy such as lifetime homes, but in the Australian Standard (AS4299, 1995) define adaptability as "a move away from designing special accommodation for different community groups with different needs.

- 4. Increased control user (user referring customization) this is to separating parts of the building physically according to decisions levels between stakeholders. This development (e.g. shell and core construction) and a client- driven practicality to accommodate changing work conditions, that allow for spatial configurations at minimal disruption and cost.
- 5. *Climate adaptation:* is the most recent appropriation of adaptability to understand how building can adapt to significant changes with their surrounding environment, including the capacity to reduce their burden on the environment by lowering energy consumption.

Overall this research more concentrate on the adaptability of "Increasing user control in the office space".

5-2 Characteristics of Adaptability:

Robert Schimdt III and Simon Austin (2016,p45), give adaptability the following definition:

"The capacity of a building to accommodate effectively the evolving demands of its"

They suggest main theme for the adaptability of performance-based design; which they identify four underlying characteristics:

- 1. *The capacity for change:* this refer to either physically responsive or a passive accommodation to an internal or external change, the object of change might be structure, space or environment.
- 2. *Fitness for purpose:* this match between the building and its users.
- 3. *Value:* can be summarized as maximizing productive use, to fit both the use and the stakeholders' desires, at a minimum cost hence minimizing the effort (Time & Cost) of change is defining facet of adaptability.
- 4. *Time:* is described in two ways to indicate speed of change (e.g. quick transformations) and through life times

(such as future changes or extension of use). This maximizing the building's life, components or materials is a key feature.

6. ADAPTABILITY IN OFFICE SPACE

During the past years the importance of adaptability in office buildings has increased, mostly due to factors like rapid change, both in private and public organizations, new and innovative work place design and growing environmental concerns about building redundancy. Also it has always been tested through office design, due to high levels of flexibility needed for this programme.

Adaptable building can adopt new function and accelerate to adjust to fast development and thus be revitalized in uses and functions .Independency of building elements appears to be the most important principle key for enhancing adaptability. The building becomes more adaptable if each its feature more uncoupled from the other. According to Francis Duffy, co-founder of a British firm that specialized in advance office designs, a building over its lifetime changes not as a single entity, but rather as four separate layers: Shell, Services, Scenery and Set. Each layer has a unique time period for repair and replacement. As describes the differences in the table (6-1).

Table(6-1) Layers to be keep Independent w	ithin a
Building(Duffy,1990)	

Layers	Description	Average life time
1.Shell	Structure of buildings, including skin if load-bearing	>50 years
2.Services	Pies, ducts, cables, machinery, elevators, escalators.	~15 years
3.Scenery	Partitioning, ceiling, finishes.	~6 years
4.Set	Furnishing, furniture, computers	monthly

The challenge is to achieve functional independence, without loosing the independent features that enhance adaptability.

Brand (1994); showed that how office worker interested in changes monthly, yearly. Frank Duffy has remarked on the absence of long-view research in this area:" One of the most dominant facts of 20th century life is huge increase in the importance of the office. We've had a massive change from less than 10 percent to over 50 percent of people occupied in offices, and no one from an organizational and social perspective." Particularly worth examining is the history of the "open office," an innovation that swept the world in a decade.(Brand 1994,p167)

has studied the physical evidence of that change

In 1958 near Hamburg, Germany the idea of open office -- the scattering of desks and work groups around huge open floors- was a deliberate invention by a couple of professional who were brothers Eberhand and Wolfgang Schnelle were neither architects nor planning consultants: they were organizational designers. In their view organizations were severely restricted by small offices strung along lengthy corridor where was communication, nearly impossible poor flexibility, and the wrong size groups were always stuck in the wrong size space. So they thought in different way and started to threw away walls and straight lines and created what was called Büronlandschaft-"office landscape"-Later Americanized to "open office".

Over all adaptability in the office space focuses on the change which is the quality of a space that can be easily adapted to harmonize with the changes of use (function) that it undergoes and adaptable building using the

same amount of space more efficiently on average over entire life of building. Duffy claimed that all layer of building should be independent from each other because each layer has different period of time. And developing the concept of open offices by Duffy, Bernard which is first invented by German brothers since open office can adopt to many function through life time of building.

7.RESEARCH METHODOLOGY

7-1 .Meta-Models

The model is by (Robert Schmidt III and Simon Austin ,2016), show the relationships between design strategies, and six types of adaptability with, building characteristics, layers and guidelines.

Therefore, the first and second part of the metamodel are used as checklist to examine each case study for showing the ratio of adaptability in each building. In addition using radar chart (using Microsoft Excel for calculation, and Edraw max software to create radar chart) to visualize the adaptability ratio in each cases due to strategies through charateristics. The third part of the metamodel can be used by designers to check or establish pathways of interest or with the client to highlight the design intent or possible conflicting demands that need resolution.

7-2. The scenario planning

There is a tool called scenario-planning by Brand 1994 who claimed that 'the product of skilled scenario work is not a plan but a strategy, where a plan is based on prediction, a strategy is designed to encompass unforeseeably changing conditions. A good strategy ensures that, no matter what happens, you always have maneuvering room."

As a result, The scenario planning used to prepare the research questionnaire to find out user need due to types of adaptability in general for office design in future by using variable of metamodel

7-3. Research Case Study:

The study was conducted at office buildings in Erbil city. The data are gathered from private and public offices to show the highest and lowest ratio of adaptability of office buildings. Administrative staff, managers, employees who use building answer the questionnaires. The office building which are selected for this research are as below: Table (7-1).

	Public office buildings		Private office buildings
A1	Directorate of Erbil Holding no.2	B1	Darin Company
A2	Directorate of Erbil Holding no.1	B2	Ster Tower
A3	Board of Investment	B3	Jouhayna Center
A4	General Directorate of Erbil Retirement	B4	Ara Apartment
A5	Presidency Appellate Court of Erbil Region	B5	Gulan Park
A6	Directorate of Erbil Urban Planning	B6	Justice Tower

7-4. Sample size:

Through analysis of research methodology on adaptability; the number of buildings samples which have been assigned in the research, its range from 10 to 15 buildings. Therefore, number of building's sample in this research is 12 is midrange. The samples divided into two groups, which are public and private office buildings to find or differentiate the lowest and highest ratio of adaptability in the office buildings of Erbil city. For the questionnaires sample size (from, source: Rea, Louis, and Richard A. Parker. Designing and Conduction Survey Research: A Comprehensive Guide. 2nd ed.San Francisco, CA:Jossey-Bass,1997,page 121). Number of users for these 12 office buildings reaches 10.000, therefore for 95% level of confidence with±5% of error it need 357 users to respond the questionnaireAs a result, sample size for questionnaires used in this research paper is 360, which divided on 12 office buildings, each group of users in buildings have to fill 30 questionnaire forms.

8. RESULTS AND DISCUSSION

In practical studies, the percentage for adaptability of office building in Erbil city, its development due to (building characteristics, layers, strategies, and types of change) are explored; need of users for each type according to time and feasibility of buildings (public and private offices) also explored.

The average percentage of adaptability

approximately half of characteristics (32.5%).

Both groups A and B, mostly concentrated on the

modularity and increase & interactivity

strategies according to characteristics for those strategies. The weakest strategies are aesthetic and loose for each use them in different way. The radar chart showed that each building has been designed in different way. For building layers most architects tried to fulfill the client requirements instead making balance between all parameters of building, since time changes, need of users changes due to time and development of technology, therefore demand for changes in office building will increase. The problem mostly in layers as spaces, space plan, services, stuff and structure, each have different problem in each project.

As a result, the practical study showed the adaptability percentage in offices building of Erbil

ZANCO Journal of Pure and Applied Sciences 2019

city, and its development. The weakest and strongest point in strategies of office building both public and private. In order to improve design, use of strategies, characteristics, layers and type of changes increasing feasibility of office buildings which answered all research questions and hypotheses.

Table (8-1) Building cha	aracteristics mapped aga	ainst case studies by	researcher
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	Design Characteristics													
	Building analysis Public Offices Private Offices													
	Dunu	ing analysis	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5	B 6
	CAR1	Reversible	Х	Х		Х	Х	Х	Х	Х	Х	Х	Х	Х
	CAR2	Movable stuff	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	
	CAR3	Component accessibility	Х	X		X	Х	X	X	Х	X	X	Х	Х
	CAR4	Functional separation		Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х
	CAR5	Service zones	Х			Х	Х	Х		Х		Х	Х	Х
	CAR6	Configurable stuff			Х		Х	Х	Х	Х	Х	Х	Х	Х
	CAR7	Multifunctional components	Х			Х		Х						
	CAR8	Not precious		Х		Х		Х			Х			
	CAR9	'Extra' components	Х			Х	Х			Х			Х	
_	CAR10	Durability	Х	Х			Х	Х	Х	Х		Х	Х	Х
ysical	CAR11	Mature component		Х	Х	Х			Х	Х	Х	Х	Х	Х
Phy	CAR12	Efficient services										Х		
	CAR13	Good craftsmanship			Х		Х		Х	Х		Х	Х	Х
	CAR14	Overdesign capacity			Х	Х	Х			Х			Х	
	CAR15	Readily available materials	Х	Х	Х	Х	Х	Х	Х		Х	Х		
	CAR16	Standardized components												
	CAR17	Standard component locations			Х	Х	Х	Х		Х		Х	Х	Х
	CAR18	Off-site construction												
	CAR19	Simple construction method	Х	Х	Х		Х	X	X	Х	X	X		Х
	CAR20	Open space	Х			Х	Х	Х			Х		Х	
	CAR21	Support space				Х								
	CAR22	Oversize space	Х			Х	Х	Х		Х	Х			X
	CAR23	Typology Pattern						Х		Х	Х	Х	Х	
tial	CAR24	Joinable / divisible space		Х		Х	Х	Х	Х		Х		Х	Х
Spat	CAR25	Modular coordination						Х	Х	Х	Х			
	CAR26	Connects Buildings			Х		Х						Х	Х
	CAR27	Standard Room sizes		X		X	X			X		X		Х
	CAR28	Spatial variety	Х	Х		Х	Х	Х	Х	Х			Х	Х
	CAR29	Spatial Ambiguity			X				Х	Х	Х		Х	Х
	CAR30	Spatial zones	Х	Х	Х		Х	Х		Х	Х	Х	Х	Х

	CAR31	Spatial proximity	Х		Х	Х	Х	Х		Х		Х	Х	Х
	CAR32	Simple plan	Х	Х			Х	Х	Х	Х	Х	Х		Х
	CAR33	Standard grid		Х						Х	Х			
	CAR34	Simple form	Х	Х		Х	Х	Х	Х	Х	Х	Х		Х
	CAR35	Multiple ventilation strategies	Х	Х	Х	X	Х	X	Х	Х	Х	X	Х	X
	CAR36	Shallow plan depth							Х					
	CAR37	Passive climate control	Х		Х	Х	Х	Х	Х	Х	Х	Х	Х	X
	CAR38	Building orientation			Х				Х					
	CAR39	Good day lighting	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х
	CAR40	Space to grow into	Х	Х	Х	Х	Х	Х		Х			Х	
	CAR41	Phased				Х							Х	
	CAR42	User customization			Х	Х	Х		Х	Х	Х	Х	Х	Х
	CAR43	Multifunctional Spaces					Х	Х		Х			Х	
	CAR44	Use differentiation								Х	Х	Х	Х	Х
	CAR45	Mixed demographics							Х	Х		Х	Х	Х
	CAR46	Multiple/ mixed tenure								Х		Х	Х	X
	CAR47	Shared ownership						Х	Х	Х			Х	Х
	CAR48	Isolatable								Х		Х	Х	Х
	CAR49	Multiple access points	Х	X		X	Х	Х		Х		X	Х	X
	CAR50	Physical linkage	Х	Х	Х	Х	Х	Х		Х	Х	Х	Х	Х
	CAR51	Visual linkage			Х	X	Х	Х	Х	Х	Х	X	Х	X
	CAR52	Attitude and character		Х	Х	X	Х	Х				X	Х	X
cter	CAR53	Spatial Quality				Х	Х	Х					Х	
narao	CAR54	Building image	Х	Х	Х	Х	Х	Х		Х	Х		Х	Х
G	CAR55	Quirkiness		Х										
	CAR56	Time interwoven					Х	Х						
	CAR57	Good location	Х		Х	Х	Х	Х	Х	Х	Х		Х	Х
t	CAR58	Contextual		Х	Х	Х	Х	Х			Х	Х		
Context	CAR59	Circulation (neighborhood scale)					Х					Х	Х	Х
	CAR60	A communal place						Х				Х	Х	Х
		Total	24	25	25	37	39	38	26	40	29	34	41	37
	Ratio of Adaptability %		40	41.6	41.6	56.6	65	63.3	43.3	68	48.3	56.6	68	61.6



Figure (8-1) Case studies visualized. This supports; find out every project will require its own mix of strategies and characteristics

8-1 Comparison between Group A and B

The bar charts figure (8-1) and (8-2) illustrate total percentage of employees' needs and feasibility of their buildings for these changes, to show the difference between group A and B.

Both groups prefer changes during first years, but the average percentage in group B is 62.2% which is more than group A, 52.4%. Since most of the companies trying to apply their goals to increase their income, therefore they are going with daily needs and technologies. After these years the average percentage dropped dramatically as shown in bar charts of both groups. Moreover, both groups average percentage of needs for change are at the same range, group A is 53.3% and group B is 50.4%.

GROUP						Somewhat	Not
В	(1-5)	(5-10)	Yes	No	Feasible	feasible	Feasible
Average	62.2%	23.6%	50.4%	48.7%	19.8%	19.2%	10.1%
Median	63.3%	24%	48.3%	51.1%	18%	20.4%	9.4%

It's clear that the feasibility for each group's buildings have different average and median of feasibility level in their projects as shown in table (8-1) and table (8-2). For group A, somewhat level of feasibility has the most average and median percentage for change than other levels of feasibility. In contrast group B, both (feasible) and (somewhat feasible) nearly same percentage of average and median than (not feasible) level of feasibility.

Overall, this shows that both groups have same percentage for change during first five years. Whereas the feasibility percentage is different for each groups in some layers of building. But some of them have problem in space program lack of some spaces, proportion

of spaces, floor to floor heights and shape of spaces which have negative effect on layers such as spaces, space plan, services, stuff and structure. In group A they have problems in some layers such as site, surrounding and social, but these layers have very few or no affects.



Figure (8-1) Total result of employees' need (Public building offices) bye researcher

Table (8-1)Total average and median of employees' need (public building offices) by



Figure (8-2) Total result of employees' need (Private building offices) by researcher

 Table (8-2)Total average and median of employees' need (Private building offices) by researcher

GROUP						Somewhat	Not
Α	(1-5)	(5-10)	Yes	No	Feasible	feasible	Feasible
Average	52.4%	25.5%	53.3%	44.3%	17.9%	22.6%	10.9%
Median	53%	27.4%	54.9%	44.6%	16.4%	25.2%	10.4%

9. CONCLUSION

9-1. theoretical part

a)This research has dealt with concept of adaptability, which become an important issue in architectural design. The importance of adaptability in the office buildings has increased mostly due to factors as rapid change in technology, economic, and users' needs over time. b)From the previous study conclude that increasing level of difficulty in adaptation of building to fulfill user demands and buildings have to be studied in time for solving problem, since user needs change radically and repeatedly over times. As a result, aspiration on the adaptability is common but rarely given proper attention in design. Therefore, for increasing the proper attention of design towards adaptability to fulfill the requirements of user in office buildings of Erbil city, through this research on multilayering approach for office space adaptability in the offices of Erbil.

c) The methodology which are used in previous studies show that for increasing proper design attention towards adaptability, the architects, and researcher categorize building composition in to layers which rely on building characteristics and strategies for enhancing concept of building adaptability to respond to changes and fulfill users' needs over times. This research used final update of this methodology which is concept of meta-model" in the book of Adaptable Architecture, theory and practical by Robert Schmidt III and Simon Austin (2016).

9-2. practical study:

The following conclusions are the results of analysis and questionnaire as practical studies in this research field, in both public and private office buildings chosen in Erbil:

a)From the analytical parts of case studies get that office building which are designed in both public and private; the architects tried to fulfill the requirements of client due to the budgets of organization there are no balance between option (clients) and range (architects) and users (the one who use building) need to get high level of adaptability in office buildings. The ratio of adaptability for private office building is more than public office buildings, that tested first hypothesis.

b)From the radar charts which tested hypothesis number two in chapter two, concluded that even some characteristics due to strategies repeated in most projects but each of them have different shape of adaptability, which show form weakest to strongest strategy in each project and overall case study. It can be taken in consideration in future design and existing buildings to solve the problem toward adaptability to fulfill users' need.

c)The most problem faced in both groups during investigation of capability in adaptability of buildings for changes was the floor to floor height, lack of enough elevators, inadequate proportions of space, inadequate arrangements of socket points, lack of spaces in space program and its layout, shape of buildings, designed without take in concentration future needs, and problem in ventilations. The design of archive due to future grows demand of area and its protection was the most important spaces for all users.

d)The result for need of change over time due to type of adaptability for both groups (A&B) it was showed that during first five years need for changes was more than (6-10) years since during this time it show that which departments of organization grow rapidly for instance archive documentation need spaces, these types of spaces need to expand that's why demand for each type of changes are increasing and nearly its percentage same in both groups but in group B since the offices are commercial with rapid development in income for the company they try to cope with technology in order to increase income of company. The order of changes according to types of adaptability as adjustable, versatile, refitable, convertible and scalable alternatively, which tested hypothesis.

e)The problems which faced in level of feasibility of most buildings are in layers' space, space plan due to its layout in regards to circulations, and services, on the other hand social needs have high influence on the types of office to be either cellular or open office space.

f) It become clear that design for office building either open office or closed office depend on function and organization structure.

10. RECOMMENDATION

- For new buildings proposing that analyze space program layer and make a survey with users of building to anticipate the needs that they will face in future to take in consideration during design process.
- Recommending in new design buildings give archive space its importance and security since this space is the most important point for office.

ZANCO Journal of Pure and Applied Sciences 2019

- Municipalities should apply the metamodes in form of regulations to be confirmed by them upon new designed projects to get extra benefits on the user's level economy.
- For the existing building it recommends that analyze the building due to building characteristics to show ,which layers have more capability to change for solving problem in a logical way.
- The researcher suggests for architectural departments to shed light on the concept of adaptability and to be embedded within theoretical syllabuses
- Encourage young architects to build their capacity theoretically and practically about adaptability done by companies (Design and Construction)or universities.(Architectural departments).

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