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ASSESSMENT OF AVAILABLE COBALT IN SOME IRAQI CALCAREOUS SOIL USING DIFFERENT EXTRACTION METHODS

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ABSTRACT

The present experiments were conducted to study the best technique for extracting available Cobalt from some calcareous virgin soils . The study was carried out on twelve surface samples (0- 30) cm collected from different sites of Kurdistan region in Iraq . The results indicated that the values of chemically extracted available Cobalt varied with the type of the extracting solution and soil . The obtained data showed that the extraction with (0.005 M DTPA + 0.01M $CaCl_2$ + 0.1 M TEA) at pH 7.3 was the best for the determination of available Cobalt in the studied calcareous soils.

Key words *:calcareous soil, cobalt, extraction*

1.INTRODUCTION

Scientists differed concerning Cobalt status either as essential mineral for plant growth or not . Legumes and some other plants have a Cobalt requirement independent of nitrogen fixation, although the amount required is small compared to that for the nitrogen-fixation process. (Brady and Ray,2000). Cobalt is essential for the growth of symbiotic microorganisms such as *Rhizobia* , free living N_2-fixing and blue algae and is also important in the synthesis of vitamin B_{12} in ruminant animals. (Havlin *et al.*, 2005) . Extraction of available forms of Cobalt for plant nutrition is an important issue to be studied in different types of soil .

In order to assess the proper extraction solution for Cobalt from the soil different extracting solutions and methods have been developed. All procedures are based on the assumption that water soluble , exchangeable , organically bound , occluded in Fe and Mn oxides ,definite compounds and silicates (residual fraction) species of metal exist in soils (Kabata , 2000).

Tessier *et al.* (1979) were among the first to develop the procedure of sequential extraction to determine the speciation of particular trace metals in soils . Recently three stages of sequential extraction procedures were proposed by the Commission of the Bureau of European Communities References (BCR) . This procedure was developed and improved by SMT (Standers Measurements and Testing) (Formerly BCR) and it will facilitate comparability of data in the European Union . In this technique, metals were divided into three fractions by the application of following chemicals: (1) CH_3COOH ,(2) $NH_2OH.HCl$,(3)H_2O_2 and CH_3COONH_4 (Thomas *et al.*, 1994; Rauret *et al.*, 1999) .

The DTPA is designed for the use on higher pH soils and has been found to be highly correlated with plant availability for some of the nutrient trace elements such as zinc and manganese (Hopkins and Ellsworth, 2005) .

In an experiment conducted in Spain the extractable of Cd , Ni , pb , Cr , and Co from eight contaminated soils were evaluated using ammonium chloride, calcium chloride ,strontium chloride and DTPA extraction .The amounts of metal extracted were related to the total metal contents . Results showed a high availability of metal extraction (Moral *et al.*, 2002) .

The aim of this investigation was to select the proper method for extracting available cobalt from some calcareous soils of Kurdistan region of Iraq.

2.MATERIALS AND METHODS

2.1. Sampling : Representative composite soil samples were taken from 12 sites in Kurdistan region of Iraq (Table 1) (Fig1) .The collected soil samples were air dried , passed through 2 mm sieve and subjected to physical and chemical analyses.

2.2.Physical and chemical analysis

2.2.1. : The Particle size distribution was

Table (1) Location name, location of sites and some field observation

Location	Name of locations	Site locations	Descriptions
Bazian	Gurgaie –shamar area	About 3.700 m from right side of the main street.(Bazian).	Natural pastures area for sheep grazing
	Kochak nakhshena area	About 10 km from right side of Takia district.	Natural pastures area for sheep grazing
	Takia area	About 500 m from right side of Takia district	Natural pastures area for sheep grazing
Qaradakh	Bakhan area	About 5 km from north of Qaradagh district.	Natural pastures dense area (Alfalfa cultivated) for sheep and goats .
	Qaraman area	About 8 km from north of Qaradagh district	Natural pastures dense area (Alfalfa cultivated) for sheep and goats.
	Tafan area	About 2 km from south of Qaradagh district	Natural pastures dense area for sheep and goats.
Arbat	Bareka area	About 1.5 km from east of Arbat district.	Natural pastures dense area for sheep.
	Kharaba area	About 5 km from northeast of Arbat district	Natural pastures dense area for sheep.
	Darbandn- faqara area	About 12 km from southeast of Arbat district	Natural pastures dense area for sheep and goats grazing .
Pshdar	Zoghan area	About 700 m from east of Sangasar district.	Natural pastures dense area for cattle grazing.
	Sangasar area	About 500 m from east of Sangasar district center.	Natural pastures dense area cattle and sheep
	Dashtewan area	About 1 km from east of Sangasar District .	Natural pastures (clover and alfalfa) for cattle grazing

Fig. (1): Location of the studied soil samples

determined according to the international pipette method as described by Black , (1965) .

2.2.2: Field capacity and wilting point were calculated according to empirical equations submitted by (Karim , 2000) .

2.2.3 :Electrical conductivity at 25ºC : (EC_{25}) was measured in soil paste extract as described by (Hesse ,1971) . Soil pHs and $pH_{:2:1}$0.0.1M $CaCl_2$ of the soils were measured as described in (Jackson , 1973) .

2.2.4 : Total carbonate was determined by back titration as described in (Rowell , 1996) and active calcium carbonate was determined using Drouineau procedure as described in(Kozhekov and Yakovleva ,1977) .

2.2.5 :Total Cobalt: Total Cobalt was determined by wet digestion method as described in(Jackson,1973) .

2.3.The extraction of Cobalt from these soils was done as follows:

2.3.1: Water soluble Cobalt extraction : In this method the ratio of 1: 50 soil / distilled water was used as described in (U . S. D .A ,Handbook - 60,1969) .

2.3. 2(0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA)method : Cobalt was extracted using extracting solution which prepared by 0.005M DTPA (Diethylene triamine penta acetic acid) with 0.01M calcium chloride , and 0.1 M (TEA) Triethanolamine , the pH was adjusted at 7.3 according to (Lindsay and Norvell, 1978) .

2.3.3 Extraction with NaOAC: Cobalt was extracted with 1 N NaOAC at pH 8.2 , according to (Jackson, 1973) ,

2.3.4. Sequentional extraction method

A sequential extraction procedure was performed according to the procedure recommended by the Standards Measurements , and Testing programme of the European Union (SM and T- formerly BCR) , for the determination of Cobalt in soils. The chosen extraction scheme is an operationally and standardized procedure , in which the reagent used at each stage is intended to release metals associated with particular soil phase such as acid soluble , reducible , oxidisable , and residual , as described by (Tokalioğlu *et al.,* 2001) .Cobalt determined in the obtained extracts by Atomic Absorption Spectro- photometer (model Varian Australia Pty Ltd Mulgrave , Victoria ,2005) .

2.4. Biological experiment

Four soil samples (Takia , Bakhan , Darban - faqara , and Dashtewan) were selected from 12 locations to represent different clay , CEC and $CaCO_3$ content for the study of Biological experiment . The experiment was performed in a complete randomized design (CRD) and in Triplicate during 2006 / 2007 . Each plastic pot was filled with 500g gravel, and four Kg air-dried soil . Alfalfa (*Midcago sativa*) was used as indicator plant . Each pot was fertilized with a complete nutrient solution . Five different rates of cobalt (0, 0.25 , 0.5 , 0.75 and 1) mg kg^{-1} were applied to the soils as Co $(NO_3)_2$ .6 H_2O . Ten seeds were germinated in each pot , and then four plants were left after germination. The water content of the pot was adjusted to 75 % of field capacity throughout the experimental period. Alfalfa shoots were harvested after appearance 10 % of blooms (Saeed, 1989) .

Harvested shoots were weighed and dried at 65 º C for 48 hrs to determine the dry matter yields . Dried plant materials were digested using 1:1 (H_2SO_4 : H_2O_2) . (Schuffeelen and Van Schuwenburg 1961) . The Cobalt concentrations in plant digests were determined using Atomic Absorption spectrometer (model Varian Australia Pty ltd Mulgrave , Victoria , 2005).

3.RESULTS AND DISCUSSION

Some physical and chemical properties of the studied soils are shown in (Table 2) .The results in (Table 3) show the seven extraction methods used for the determination of mobility of Cobalt in soil samples, and their correlations with biological indices .

Natural soils showed that there was positively significant correlation coefficient between Cobalt extracted by DTPA method and Cobalt uptake by the plant (r = 0.955*) . This indicates that the (DTPA + $CaCl_2$+ TEA) method can extract the highest amount of Cobalt in slightly alkaline soils . This might suggest that the chelating agents were more promising for assessing readily available micronutrient cations in soils . This is in agreement with (Abdullah , 2006) and (Hopkins and Ellisworth, 2005) .

It was found that there was a negatively significant correlation coefficient among mixed extraction solution (H_2O_2 + NH_4OAc) with dry matter yield and Cobalt uptake by the plant (r =- 0.955*, - 0.95*) respectively . This indicates that the mixed extraction (H_2O_2 + NH_4OAC) method can extract the highest amount of oxidizable Cobalt or / and precipitated and it is not a suitable method for the determination of available Cobalt in the studied soils .

In the Cobalt loaded soils, it was found that

Table (2):Some selected chemical and physical properties of the studied soils;

Soil No	Soil location	Texture	Saturation %	pH		dS . m $^{-1}$		gm. kg $^{-1}$			(A/T) x100	CEC Cmol.kg^{1} soil
				pHs	$pH_{1:2}$ 0.001M $CaCl_2$	ECs	$EC_{1:2}$ 0.001M $CaCl_2$	O.M	Total carbonate	Active lime		
1	Gurgaie-shamar	SCL	39.53	7.57	8.38	0.43	0.17	13.22	404.3	175.5	43.40	25.11
2	Kochak-nakhshena	SCL	42.53	7.61	8.16	0.45	0.19	15.99	387.2	170.8	44.11	28.03
3	Takia	SCL	59.11	7.28	8.12	0.44	0.18	17.04	377.9	143.3	37.92	31.21
4	Bakhan	SC	49.88	7.30	7.41	0.41	0.20	27.12	232.7	70.9	30.46	41.12
5	Qaraman	SCL	61.77	7.41	7.72	0.37	0.16	22.52	245.1	116.5	47.53	36.44
6	Tafan	SCL	57.51	7.59	7.70	0.44	0.22	23.12	250.9	98.1	39.09	35.66
7	Bareka	SCL	56.76	7.69	8.32	0.65	0.40	16.64	383.8	160.7	41.87	30.21
8	Kharba	SCL	43.25	7.49	8.22	0.54	0.29	15.93	392.8	157.1	39.99	28.04
9	Darban faqara	SCL	59.86	7.73	8.31	0.34	0.14	17.43	406.2	173.8	42.78	26.22
10	Zoghan	SC	56.26	7.03	7.32	0.54	0.16	26.03	157.7	59.9	37.98	41.10
11	Sangasar	SC	50.23	7.09	7.39	0.61	0.28	25.11	162.2	67.5	41.61	39.98
12	Dashtewan	SC	50.01	7.06	7.40	0.69	0.34	26.70	144.6	53.4	36.93	40.89

SC -------- Silty Clay SCL -----Silty Clay Loam. (A/T) x100 = (Active lime / Total carbonate) x 100

Table (3) The amount of total and extracted Cobalt with different extracting solution ($mgkg^{-1}$)

Sample No.	1:50 Soil :water	NaOAC	DTPA+$CaCl_2$ +TEA	Sequential extraction procedure method				Pseudo total	Total Co (Digestion)
				Co Extracted with CH_3COOH	Co Extracted with $NH_3OH.HCl$	Co Extracted with H_2O_2+ CH_3COONH_4	Co Extracted with 3HCL+HNO_3		
1	0.09	0.12	0.17	10.22	3.97	8.53	8.04	30.76	32.04
2	0.22	0.24	0.28	14.97	4.85	8.07	5.69	33.58	35.22
3	0.19	0.28	0.48	13.09	6.21	7.12	8.85	35.27	36.36
4	0.45	0.52	0.82	22.32	10.96	6.87	3.66	43.81	44.34
5	0.41	0.51	0.68	19.67	8.71	6.56	5.91	40.85	41.08
6	0.34	0.37	0.47	18.21	9.32	6.11	5.76	39.40	40.16
7	0.14	0.26	0.36	13.06	4.85	7.81	10.16	35.88	36.42
8	0.12	0.21	0.68	11.96	4.86	7.54	8.86	32.22	33.84
9	0.01	0.06	0.82	9.33	8.32	8.32	6.85	32.82	34.52
10	0.56	0.75	1.76	24.04	13.05	5.59	2.75	45.43	46.53
11	0.51	0.60	1.87	22.98	12.11	5.75	3.17	44.01	44.88
12	0.63	0.67	2.36	24.96	12.22	4.55	4.61	46.34	47.02

Table (4): The correlation coefficient (r) for the relationship between the amounts of Cobalt extracted by different chemical methods and biological indices.

Chemical extraction methods ($mg.kg^{-1}$)	Non-application of Cobalt in pots			Application of Co fertilizer											
	gm. pot^{-1}	µgCo.gm^{1}plant		Dry matter yield gm. pot^{-1}				Co concentration in plant µgCo . gm $^{-1}$plant				Uptake of Co by plant Uptake µgCo. gm $^{-1}$ plant			
	Dry matter	Co Conc. in plant	Uptake of Co by plant	0.25	0.5	0.75	1	0.25	0.5	0.75	1	0.25	0.5	0.75	1
Water soluble	N.S	N.S	N.S	0.96*	N.S	0.95*	N.S	0.98*	0.98*	0.99**	0.99**	0.95*	N.S	N.S	N.S
NaOAC	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.96*	0.93*	0.98*	0.99**	N.S	N.S	N.S	N.S
DTPA	N.S	N.S	0.955*	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.95*	N.S	N.S
CH_3COOH	N.S	N.S	N.S	0.96*	0.95*	0.95*	0.94*	0.96*	0.97*	0.99**	0.99**	N.S	N.S	N.S	N.S
$NH_2OH.HCl$	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
H_2O_2+ H_3COONH_4	-0.95*	N.S	-0.95*	-0.97*	-0.95*	-0.97*	-0.96*	-0.96*	-0.99**	-0.97*	-0.96*	-0.96*	-0.95*	-0.95*	-0.95*
Aqua regia($_3HCl+_1HNO_3$)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

N.S Correlation is not significant. * Correlation is significant at 0.05 levels. ** Correlation is significant at 0.01 level

soluble Cobalt was significantly correlated to dry matter yield (r = 0.96 *, 0.95*) for the levels the plant (r = 0.98 * ,0.98 * ,0.99 ** , 0.99**) at levels of (0.25 , 0.5 , 0.75 , 1) µg Co .gm, $^{-1}$ respectively and Cobalt uptake (r = 0.95*) at a level of 0.25 µg Co gm $^{-1}$. This indicates that the water soluble method has the ability to extract soluble Cobalt from these soils and a good indication for intensity factor.

A positive significant correlation was found between ammonium acetate (NaOAc) and acetic acid (CH_3COOH) extracting solution , and Cobalt concentration in plant (r = 0.96* , 0.93 * , 0.98* , 0.99**) , and (r = 0.96*,0.97*, 0.99*, 0.99*) at a levels of (0.25 ,0.5 , 0.75 , 1) µg Co.gm $^{-1}$ respectively (Table 4).

Also, we found that there were positive significant correlation coefficients between acetic acid CH_3COOH method and dry matter yield (r = 0.96 *, 0.95*, 0.95*, 0.94*) at a level of added Cobalt (0.25 , 0.5 , 0.75 , 1) µg.gm^{-1}, respectively .

This indicates that NaOAC and acetic acid methods had the ability to extract exchangeable Cobalt from these soils but the NaOAC method extracted the lowest exchangeable Cobalt, while the CH_3COOH method extracted the highest amounts of exchangeable Cobalt . This might be due to that the acetic acid CH_3COOH method is acidic extraction which reduce the pH . Hydrogen ions can displace metallic cations from the colloidal complex at low pH .Whereas NaOAC reduce acidity and increase the soil pH . Consequently , Na can not easily displace Ca, Mg, and K from soil colloidal surfaces. This is in agreement with (Kabata ,2000) .

It was found that there were several significant correlations among DTPA and Cobalt uptake by plant (r = 0.95*) at a levels of (0. 50) µg.gm $^{-1}$ added Cobalt .This indicates that DTPA can extract available Cobalt at level (0.50) µg.g m^{-1} .On the other hand negative significant correlation coefficients were obtained among (H_2O_2 + NH_4OAC) method with dry matter yield (r = - 0.97*, -0.95*, - 0.97*, - 0.96*) , Cobalt concentration in plant (r = - 0.96* , - 0.99* , - 0.97* , - 0.96*) and Cobalt uptake by plant (r = - 0.96*, - 0.95* , - 0.95* , - 0.95*) at a levels of (0.25, 0.50 , 0.75 , 1) µg.gm $^{-1}$ added Cobalt , respectively. This indicates that mixed extracting solution (H_2O_2+NH_4OAC) can extract high amounts of non-available Cobalt. The efficiency of the various extracting solutions for the determination available Co content could be arranged : (8.8 mol.L^{-1}H_2O_2 + 1.0 mol.L^{-1} NH_4 OAC at pH 2) < 1: 50 water soluble < 1 N NaOAC at pH 8.2 < (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA) at pH 7.3 < (0.11 mol. L $^{-1}$ CH_3COOH) at pH 2.85 .

The abovementioned results indicated that the (0. 005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA) at pH 7.3 method was the best extraction method for the determination of available Cobalt in slightly alkaline and calcareous soils because it has been found to be significant correlated with dry matter yield ,cobalt concentration in plant and cobalt uptake by plant at different levels and NH4OAC is not adapted or not successful method to evaluate availability of cobalt in studied soils due to negative correlations.

These finding could help to select the best extraction method for available Cobalt from calcareous soils .

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تقدير الكوبلت الجاهز في بعض الترب الكلسية في العراق بأستخدام طرق ومحاليل إستخلاص مختلفة

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ملخص

أجريت هذه التجريه لدراسة اختيار أنسب طريقة ومحلول لاستخلاص الكوبلت الجاهز من بعض الترب الكلسية البكرة والتي تم انجازها على بعض الترب السطحية لأثناعشر تربة من مناطق مختلفة من إقليم كردستان العراق مستخدما محاليل استخلاص مختلفة و من خلال إجراء تجربة بيولوجية مستخدما نبات ألجت . بينت النتائج بأن قيم الكوبلت الجاهز المستخلص كيميائيا اختلفت مع اختلاف نوع محلول الاستخلاص و الترب و من كفاءة محاليـل الاستخلاص المختلفـة المستخدمـة لتقديـر الكوبلت الجاهـز تبيـن بأن طريقة استخدام محلـول

(0.005M DTPA + 0.01M $CaCl_2$ + 01M. TEA)عند pH=7.3 كان أنسب طريقة لاستخلاص الكوبلت الجاهز من هذه الترب الكلسية.

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