Evaluation of Some Chemical Extractants for Testing Zn Availability to Barley Grown on Calcareous Soil

A.M. Falatah, A.S. Modaihsh, W.A. Al-Mustafa and M.O. Mahjoub

Department of Soil Science, College of Agriculture, King Saud University. P.O.Box 2460, Riyadh 11451, Saudi Arabia

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Abstract. A greenhouse experiment was carried out to evaluate the potential of utilizing NH_4HCO_3 -DTPA, DTPA, EDTA and $MgCl_2$ soil tests for assessing Zn availability to barley plants grown in 32 highly calcareous soils in Saudi Arabia. Zinc extracted by the four soil tests was in the order: EDTA> NH_4HCO_3 -DTPA> DTPA> DTPA> MgCl₂ and was significantly correlated with $_{P}H$, CaCO₃%, yield of dry matter, zinc concentration in plant tissue and plant Zn-uptake. The variability of extractable-Zn and the concentration of Zn in plants were best estimated using the NH_4HCO_3 -DTPA followed by the DTPA soil test. Only these two soil tests were able to adequately separate the 32 calcareous soils to responsive and non-responsive groups to Zn-fertilization based on yield response and Zn-tissue concentration combined criteria. Zn critical levels in soils as determined by NH_4HCO_3 -DTPA soil test using the visual method, Cate and Nelson graphical method and Chi-square method were very similar and ranged between 0.82 and 0.85 ppm. The respective Zn critical levels using the DTPA soil test were 0.40-0.52 ppm indicating that the NH_4HCO_3 -DTPA soil test was superior compared to other soil tests under investigation. Extractable Zn by NH_4HCO_3 -DTPA and DTPA, from soil receiving ZnSO4, were $18.4\pm2.1\%$ for NH_4HCO_3 -DTPA and $16.9\pm2.9\%$ for DTPA test. Both extractants provide a measure of the residual value of Zn fertilizer in calcareous soils situated in semi-arid region.

Introduction

Zinc deficiency is common in many soils, especially alkaline and calcareous soils of semi-arid and arid regions [1,2,3]. Precise and accurate soil test values have been the objective of many researches. One of the problem in soil testing programs is to define the level of extractable Zn below which fertilizer recommendations should be made. A number of soil factors such as soil pH, CaCO₃, organic matter and texture have variable effect on the availability of Zn [3,4].

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However, even with recognizing the above factors associated with Zn- deficiency, soil critical levels are a necessary part of soil testing and differ from one region to another with each soil extractant [3]. Plant species and varieties within a species differ in their susceptibility to Zn deficiency, due either to the requirement or to the ability to extract the element from the soil and therefore different Zn critical levels were determined for different crops using the same soil Zn test [3,5].

In recent years, multiple-element extractants have received renewed interest because instrumentation like inductively coupled agron plasma (ICAP) can efficiently analyze a large number of plant nutrients simultaneously in single solution [6]. Validation of new multiple element extractant generally relies on a relatively precise relationship between test value obtained with an earlier and a new solution. The incentive to adopt a new extractant is the saving in time and cost analysis. Multiple-element extractants may enable determination of other plant nutrients at little additional cost, thereby possibly improving the diagnostic value of soil testing.

Soils in the Arabian Peninsula, in general, are saline or calcareous, thus prone to Zn deficiency [3,7]. As agricultural soils in Saudi Arabia are being intensively used for growing not only major field crops but also a multitude vegetables and fruits, correct appraisal of their micronutrients status is very important. Barley is considered as one of the main forage crops in the Kingdom, therefore, a study was conducted to evaluate the potential use of NH_4HCO_3 -DTPA (AB-DTPA) soil test for assessing Zn availability to barley in highly calcareous soils of Saudi Arabia and to evaluate the ability of the AB-DTPA soil test to separate Zn deficient from non deficient soils when compared with DTPA, EDTA and MgCL₂.

Materials and Methods

Thirty two surface soil samples (0-25cm) representing a wide area in the central and eastern regions of Saudi Arabia were used. These soil samples have not received Zn or other micronutrient fertilizers recently. The soils had wide range of total and extractable Zn and varied contents of organic matter, clay, CaCO₃ content and pH (Table1). According to the general nature of their parent material, the soils are defined into relative course texture alluvium and belong to the Aridisol and Entisol order [8]. The NH₄HCO₃-DTPA extractable levels of P. K, Cu, Fe, Mn and Zn were determined [6] along with other soil chemical and physical properties (Table1).

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Parameter		Range	Mean	S.D*	Method
Clay%		03.10-16.20	9.880	3.020	Hydrometer
Silt%		10.30-23.30	16.940	2.970	Hydrometer
Sand%		60.50-86.60	73.180	5.180	Hydrometer
pН		07.21-08.45	7.750	0.370	1:2 soil:water
OM%		00.15-01.01	0.610	0.300	$Cr_2O_7^{2-}Oxidation$
CaCO ₃ %		11.50-50.10	29.690	9.950	Acid neutralization
Р	µg∕g	00.90-13.20	5.010	3.360	NH4 HCO3-DTPA
Fe	μg/g	01.80-05.81	3.360	2.120	NH4 HCO3-DTPA
Mn	μg/g	00.81-05.28	2.710	2.850	NH₄ HCO₃-DTPA
Zn	µg∕g	00.30-02.10	1.040	0.520	NH4 HCO3-DTPA
Total Zn	μg/g	22.00-77.00	53.880	12.560	HClO ₄ /HNO ₃

Table 1. Selected chemical and physical properties of the 32 soils used in the study

S.D* =Standard deviation.

The greenhouse study was carried out to evaluate the NH4HCO3-DTPA (AB-DTPA), DTPA, EDTA and MgCL₂ as soil test for available Zn. The experiment used a randomized complete block-design with three replications. Two treatments were applied to each of the soils and consisted of (1) 0 rate of Zn and (2) a Zn fertilizer treatment. Ten mg Zn kg⁻¹ of Zn as $ZnSO_4.7H_2O$ were applied to each soil. To ensure that no other plant nutrient would be lacking, 125 mg N kg⁻¹, 60 mg P kg⁻¹ and 30 mg K kg⁻¹ were added to each pot as urea, triple superphosphate and potassium sulfate, respectively. The nitrogen fertilizer was added in two equal split applications, the first application was added before sowing and the second after 20 days from germination. Phosphorus and potassium fertilizers were added as single application before sowing. Fe and Mn were also added at a rate of 10 and 5 mg kg⁻¹ as Fe EDDHA chelate and MnSO₄.7H₂O respectively. The fertilizers and treatment additions were mixed with 6 kg soil in 7 kg plastic pots. Twenty seeds of barley (Hordeum vulgare) were planted in each pot. After emergence plants were thinned to ten plants / pot and grown in controlled climate using a 14 hr day length, irradiance of 6000 µw/ cm and 20-22°C.. The moisture in each pot was brought to 0.3 bar moisture tension with distilled water and the pots were weighed every 3 days and watered to original moisture content. Plant tops were harvested 8 weeks after germination, placed in paper bags, dried for 48 hr in forced air dryer at 65°C and weighed. After harvest, the soils from each pot were air-dried, passed through a 2.0 mm sieve and thoroughly mixed to be reanalyzed for AB-DTPA and DTPA extractable-Zn.

The plant material was ground in a Wiley mill to pass a 20 mesh stainless steel screen. The ground material was homogenized, ashed by slowly heating for 10 hr to 400

°C, held at this temperature for 12 hr then digested using equal parts of 4N HCl₄ and concentrated HNO₃ [9]. Zn in the digest was determined by Perkin- Elmer atomic absorption spectrophotometer model 2380. Zn in duplicate samples was extracted from each soil using AB-DTPA, [6], DTPA[5] EDTA [10] and 2N MgCl₂ [11] and Zn in the extract was determined as above.

Results and Discussion

Table 2 presents ranges, means and standard deviations of extractable Zn in the 32 investigated calcareous soils for the four soil Zn chemical methods. The amount of extractable Zn decreased in the order: EDTA> NH, HCO3-DTPA>DTPA> MgCl3. EDTA extracted about twice as much Zn as NH4HCO3-DTPA and about three times as much as the DTPA or MgCl₂. The amounts of Zn extracted by these extractants were in the range of those obtained by other workers for calcareous soils [2,4,12.13,14,]. Zn extracted by all four chemical methods was significantly correlated with clay content, soil PH, organic matter and CaCO₃ content (Table 3). These soil properties were shown by other investigators to be the most important factor influencing Zn-availability in soils [4,15,16,17]. Simple correlation coefficients between these soil properties and Zn extracted by NH₄HCO₃-DTPA and DTPA methods were generally higher than those for Zn-EDTA and least for Zn-MgCl₂. Variability of extracted Zn among the 32 calcareous soils was quantitatively described using stepwise regression procedure with the clay, organic matter, CaCO, contents and soil PH as independent variables. The multiple linear regression equations for predicting extractable Zn by the four soil test and the respective coefficients of determination are shown in Table (3). The results show that soil properties that affected extractable Zn most vary with the method of extraction. Soil Zn extracted by EDTA was largely determined by organic matter (O.M) content whereas both O.M. and clay fraction affected Zn extracted by DTPA to the greatest degree. NH₄HCO₃-DTPA-Zn was largely affected by O.M and soil PH, while MgCl₂ extractable Zn was largely affected by PH and to a less extent by CaCO₃ content.

The correlation coefficient (r) for both NH_4HCO_3 -DTPA and DTPA models were much higher than that for EDTA model and lowest for $MgCl_2$ (Table 3). This shows that variability of extractable-Zn is best described by NH_4HCO_3 -DTPA and DTPA soil test.

Table2. Extracted 2h hi 52 Saudi Calcal cous soils by unferent son tests					
Extraction		Range	Mean	S.D*	
NH4HCO3-D	ſΡΑ μg/g	0.300-2.100	1.040	0.520	
DTPA	µg∕g	0.120-1.550	0.680	0.420	
EDTA	μg/g	0.960-4.300	1.920	0.890	
MgCl ₂	μg/g	0.110-1.200	0.570	0.290	

Table2. Extracted Zn in 32 Saudi calcareous soils by different soil tests

S.D* =Standard deviation

The NH₄HCO₃-DTPA model, however, takes into account the effect of soil PH on soluble Zn. Theoretically, the activity of Zn decreases 100-fold for every unit increase in PH [18]. The NH₄HCO₃-DTPA model predicts that extractable-Zn by this method would be reduced by 40% in a soil of 0.6% O.M when the pH increases from 7.0 to 8.0. Because NH₄HCO₃-DTPA reaction with soil Zn is affected by other competing metal species, one can not expect extractable-Zn to reflect the change in Zn solubility with PH exactly. It is evident from the data, however, that as PH increases extracted Zn decreases.

The regression equations and related correlation coefficients between the four soil Zn tests were calculated. The r values were significant at the 0.01 level, but the highest r obtained was between the NH₄HCO₃-DTPA and DTPA soil tests (r=0.683).

 Table 3. Simple correlation coefficient(r) and multiple linear regression equations at(R²) for the correlation between extractable Zn by different soil Zn tests* and soil properties

Soil Zn test		Clay%	0.M%	pH	CaCO ₃ %	Regression equation	R ²
NH₄HCO₃-DTPA	µg/g	0.416*	0.746**	-0.743**	-0.673**	Y=4.92+.73 O.M56 pH	0.60**
DTPA	µg∕g	0.608**	0.713**	-0.695**	-0.603**	Y=.22+.79 O.M+.04 clay	0. 59**
EDTA	µg∕g	0.507**	0.700**	-0.614**	-0.509*	Y=.64+2.11 O.M	0.49**
MgCl ₂	µg∕g	0.382*	0.557**	-0.566**	-0.362*	Y=5.9474pH+.01 CaCO ₃	0.37*

*Significant at the 0.05 level of propability.

**Significant at the 0.01 level of propability.

Yield of dry matter, Zn-uptake and Zn concentration in barley were variabily affected by Zn-addition (10 ppm Zn as $ZnSO_4$) in the 32 investigated soils. Table 4 shows ranges, means and standard deviations of these parameters in unfertilized and fertilized soils. Inspection of the data shows that Zn-addition increased the yield of dry matter of barley by more than 100% in 6 soils (NH₄HCO₃-DTPA extractable-Zn= 0.3-0.6 ppm). In six other soils the increase was more than 50% (NH4HCO3-DTPA extractable -Zn=0.60-0.85 ppm), the rest of the soils showed increases of 0.40 to 38.2%. Zn-uptake by barley also increased over 100% in 10 soils (NH₄HCO₃-DTPA extractable- Zn +0.3-0.72 ppm). In three other soils Zn-uptake increased by more than 50% (NH₄HCO₃-DTPA extractable-Zn +0.73-0.83ppm) and in the rest of the soils the increase was 0.9 to 26.2%.

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Zn rate (µg/g)	Range	Mean	S.D		
	Y	field of dry matter g/po	t		
0	0.583-2.211	1.177	0.540		
10	1.022-2.219	1.540	0.380		
	2	Zinc concentration µg			
0	14.00-62.00	35.438	15.910		
10	20.00-65.00	40.250	14.010		
		Zinc uptake µg/g			
0	08.20-135.8	49.570	40.870		
10	30.30-137.0	64.147	36.500		

Table 4. Effect of Zn-Fertilization(10µg/g) on yield of dry matter, Zn concentration and Zn uptake by barley in 32 Saudi calcareous solls.

S.D* =Standard deviation.

The yield of dry matter, Zn-uptake and Zn-concentration in barley were significantly correlated with Zn extracted by the four extractants (Table 5). Simple correlation coefficients, however, were higher for Zn-extracted by NH_4HCO3 -DTPA and DTPA methods.

Table 5. Simple correlation coefficient(r) between extractable Zn by different soil Zn tests and plant parameter of barley.

Plant parameter		NH ₄ HCO ₃ -DTPA	DTPA	EDTA	MgCl ₂	
dry matter	g/pot	0.9643**	0.7868**	0.5520**	0.5892**	
Zn concentration	ppm	0.9438**	0.8629**	0.6822**	0.6329**	
Zn uptake µg/pot		0.9752**	0.8248**	0.5496**	0.5551**	

** Significant at the 0.01 level of probability.

 variables to greater extent than do the other extractants. It should be mentioned that even with addition of soil variables to the regression equation for the DTPA, EDTA and MgCl₂ soil tests, the r^2 for the NH₄HCO₃-DTPA soil test was still considerably higher than the other Zn-extractants.

with extractable 20(A) by unite ent son tests					
r ² simple	Regression equation	R ² multiple			
0.951**	Y=6.3+19.84 AB-DTPA+14.5 OM+0.38 clay	0.962			
0.680**	Y=20.43+19.14 DTPA+17.76 OM-0.31 CaCO3	0.881			
0.362*	Y=24.34+29.4 OM-0.42 CaCO ₃ +2.96 EDTA	0.786			
0.368*	Y=25.13+27.07 OM+13.37 MgCl ₂ -0.46 CaCO ₃	0.814			
	r ² simple 0.951** 0.680** 0.362* 0.368*	r² simple Regression equation 0.951** Y=6.3+19.84 AB-DTPA+14.5 OM+0.38 clay 0.680** Y=20.43+19.14 DTPA+17.76 OM-0.31 CaCO ₃ 0.362* Y=24.34+29.4 OM-0.42 CaCO ₃ +2.96 EDTA 0.368* Y=25.13+27.07 OM+13.37 MgCl ₂ -0.46 CaCO ₃			

Table 6. Regression equation and coefficient of determination (R²) for the correlation of Zn uptake(Y) with extractable Zn(X) by different soil tests

**Significant at the 0.01 level of propability. *Significant at the 0.05 level of propability

Critical levels of Zn for barley

The ability of the four soil tests to separate Zn deficient from nondeficient soils according to crop response is illustrated in Fig.1. Zn deficiency in barley which gives a significant yield response at the 5% level of probability is illustrated by the black bars (Fig.1). The open bars indicate sufficient available soil Zn for normal plant growth. Zn deficiency in small grain crops are in the range 15-20 ppm [1]. Inspection of the data shows, however, that all 13 soils that produced plants with <25 ppm Zn responded to Zn fertilization under the conditions of the experiment and therefore separation of the 32 soils into responsive and non-responsive groups will be based on yield response and Znconcentration in plant (25 ppm Zn) at the same time. The NH₄HCO₄-DTPA and DTPA and critical Zn levels for barley were visually determined to be 0.82 ppm and 0.52 ppm (Fig. 1a and Fig. 1b) respectively. Both soil tests efficiently separated the soils into responsive and non-responsive groups. At 0.82 ppm NH₄HCO₃-DTPA extractable-Zn, 31 of the 32 soils were separated correctly with only one soil (No.8) testing below the critical level while showing no yield response to Zn fertilization. Zn-concentration in barley growing in these soil was higher than 25 ppm Zn (34 ppm). Similarly, at 0.52 ppm DTPA extractable-Zn, 30 of the 32 soils were separated correctly with only two soils (soil No. 9 and soil No.13) testing below the critical level while showing no yield response to Zn fertilization. Again Zn-concentration in barley grown in these two soils was higher than 25 ppm, namely 37 and 41 ppm respectively.

When Cate and Nelson [19] graphical method (Fig.2) was used similar critical value of 0.83 ppm of Zn was obtained for the NH_4HCO_3 -DTPA soil test while lower critical value of 0.40 ppm of Zn was obtained for the DTPA soil test (Fig. 2a and Fig. 2b, respectively). A more objective approach using an interaction Chi-square statistical

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procedure developed by Keisling and Mullinix [20] gave a very close critical level of 0.85 ppm Zn for NH_4HCO_3 -DTPA but lower value (0.40 ppm Zn) for DTPA soil test. The "transition zones" described by Keisling and Mullinix (1979) for soil Zn levels were found to be 0.6-0.95 ppm for NH_4HCO_3 -DTPA and 0.35-0.60 ppm Zn for DTPA. These data also show that NH_4HCO_3 -DTPA soil test extracts higher level of Zn than DTPA. Similar results were obtained by Havlin and Soltanpour [4]. They showed that NH_4HCO_3 -DTPA soil test gave the same Zn- critical level for corn (0.90 ppm) using the visual method or the Chi-square method. The Zn-critical values for DTPA soil test were reported to be 0.7 and 0.6 ppm for the visual and the Chi-square methods respectively.

On the other hand, EDTA and MgCl₂ soil Zn tests were not able to correctly separate the soils to responsive and non-responsive groups. This is because seven soils showed almost the same critical level of 1.9 ppm for EDTA (Fig. 1c) and 0.6 ppm for MgCl₂ (Fig. 1d). When Cate and Nelson [19] graphical method was used, lower Zn critical levels were obtained namely 1.4 ppm (Fig. 2c) and 0.45 ppm (Fig. 2d) respectively. The Chi-square method gave Zn critical levels of 1.58 and 0.45 ppm for the EDTA and Mg Cl₂ soil test respectively.

Extractibility of fertilizer Zn (data are not shown) by AB-DTPA test for 32 soils had over a 8- fold range (3.8 to 31.2%) and over a 12- fold range (2.2 to 26.4%) with DTPA soil test, respectively, after 8 weeks of crop growth. Simple correlation coefficient between initial soil Zn extracted by AB-DTPA and DTPA with final soil Zn at harvest was 0.798 and 0.780 (p < 0.01), respectively. Using the same source of fertilizer Zn, Havlin and Soltanpour [4] reported 57 \pm 8% extractibility of Zn by AB-DTPA soil test after 8 weeks of ray grass crop growth in Colorado calcareous soils. On the other hand, Follett and Lindsay [21] found that 44% extractibility of Zn by the DTPA soil test after 14 weeks of crop growth.

These data indicate that the AB-DTPA and DTPA soil tests provide a measure of the residual value of Zn fertilizer in Saudi Arabia calcareous soils. However, predictable recoveries of field applied fertilizer Zn on other micronutrients would require long-term field studies.

The results obtained indicated that both NH_4HCO_3 -DTPA and DTPA soil Zn tests were adequately able to separate the 32 highly calcareous soils into responsive and nonresponsive groups to Zn-fertilization. The NH_4HCO_3 -DTPA soil test, however, was superior to DTPA soil test in estimating plant Zn (Table 6) and in extracting higher amounts of Zn from soils. The critical Zn levels estimated by the visual, Cate and Nelson [19] and Chi-square [20] when using the NH_4HCO_3 -DTPA soil test were very similar. These results and the fact that NH_4HCO_3 -DTPA soil test can successively be used to determine P and K in addition to the miconutrients (Fe,Mn,Cu) increase the potential of utilizing this soil test over the other extractants.



Fig. 1. Barely response of 32 Saudi Arabia calcareous soils to Zn as a function (a) NH₄HCO₃-DTPA, (b) DTPA, (c) EDTA and (d) MgCl₂ soil test levels.



Fig. 2. Cate and Nelson (1965) graphical method plots for (a) NH₄HCO₃-DTPA, (b) DTPA, (c) EDTA and (d) MgCl₂ extractable Zn.

Conclusion

Results obtained from this study show that NH_4HCO_3 -DTPA soil test was superior to the other soil tests for assessing Zn availability to barley in highly calcareous soils of Saudi Arabia. The amount of zinc extracted by the four soil tests was in the order: EDTA> NH_4HCO_3 -DTPA> DTPA>MgCl₂ and was significantly correlated with PH, CaCO₃%, yield of dry matter, zinc concentration in plant tissue and plant Zn-uptake. NH_4HCO_3 -DTPA followed by the DTPA not only could mirror the variability in soil extractable-Zn but also gave an accurate assessment for plant available zinc. Since NH_4HCO_3 -DTPA soil test is routinely used to determine P and K successfully, the results described in this paper would encourage other workers to adopt this test to distinguish between Zn deficient and non deficient soils.

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عبد الرزاق محمد فلاته، عبد الله سعد المديهش، وجيه على المصطفى، و محمد عثمان محجوب قسم علوم التربة ، كلية الزراعة، حامعة الملك سعود الرياض، المملكة العربية السعودية (قدم للنشر ٢٦/١٥/١٩ و قبل للنشر في ١٤١٧/٧/١٣)

ملخص البحث: أجريت تجربة في الصوبة الزراعية لتقييم قدرة مستخلصات التربة التالية ,NH4HCO3-DTPA : DTPA, EDTA, MgCl على إحتبار مدى تيسر الزنك لنباتات الشعير النامية في ٣٢ تربة جيرية من ترب المملكة العربية السعودية. أوضحت النتائج أن كميات الزنك المستخلصة كانت على النحو التــالي-EDTA> NH4HCO: DTPA>DTPA>MgCl أن هذه المستخلصات مرتبطة معنوياً مع كل من كربونات الكاسيوم، تفاعل التربيسة، محصول المادة الجافة ، تركيز الزنك في النبات وكذلك الكمية الكلـــــية المتصـة منــه، إلا أن مستخـــلص DTPA يليه DTPA كانا أفضل المستخلصات التي تعكس الإختلاف في كمية الزنك المستخلصة و تلك الممتصة بواسطة النبات؛ و بواسطة هذين المستخلصين فقط أمكن تقسيم الترب تحت الدراسة بصورة مرضية الى ترب تستجيب و أخرى لا تستجيب للتسميد بالزنك. تبين من الدراسة أن إستخدام مستخلص NHaHCO2-DTPA لإستخلاص الزنك من الترب لتحديد المستويات الحرجة بطريقة أعــراض النقص أو بإستخدام طريقةNelson Cate and مربع كاي تعطى قيماً متشابحة جداً تتراوح ما بين ٨٢, و ٨٥, جزء في المليون أما مستخلص DTPA فقد أعطى قيماً تتراوح ما بين ٤, و ٥٢, جزء في المليون مما يدل على أن محلول NHaHCO3-DTPA استخلص كميات أكبر من الزنك. النتائج المتحصل عليها برهنت أن مستخلص NH4HCO3-DTPA يتفوق على مستخلصات التربة الأخرى تحت الدراسة.وتبين أيضاً أن الزنك المستخلص بواسطة NH4HCO3-DTPA يستخلص قدراً مسب التربية. المسمدة بكبريتات الزنك يتراوح بين ١٨,٤ %٧. ± من الكمية المضافة بينما يصل هذا القدر إلى ١٦.٩ %2.9 + بإستخدام مستخلص DTPA و هذا يعطى مؤشراً على أن كلا المستخلصيه يمكن استخدامهما لتقدير كميات الزنك المتبقية بعد إضافة الاسمدة المحتوية على الزنك للترب الجيرية الواقعة في نطاق المناطق شبه الجافة.