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Additional Information

1 **EFFECTS OF Fe-EDDHA CHELATE APPLICATION ON THE EVOLUTION**
2 **OF SOIL EXTRACTABLE Fe, Cu, Mn, AND Zn**

3
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9
10 **ABSTRACT**

11
12 Up to date, soil application of synthetic chelates is the most effective mean of
13 controlling iron (Fe) deficiency chlorosis in many crops. The responses of three
14 representative soils (I, II and III) of a large orchard area (Ribera Alta del Júcar) to the
15 application of three commercial Fe-EDDHA chelates (Sequestrene 138 Fe G-100,
16 Group Carla Val F.E.A 6 Superior and Ferrishell plus) at 100 (D1) and 200 µg Fe/kg of
17 soil (D2) were analyzed. Extractable concentrations of Fe, Cu, Mn and Zn were
18 determined at 7, 14, 21, 42 and 71 days after the application. Data were subjected to
19 multifactor ANOVA to analyze the effects of time, dose, soil and chelate type on Fe,
20 Cu, Mn and Zn concentrations. Soil type affected the recovery percentage of Fe by
21 DTPA extraction. The extractable Fe increased to 40-60 mg Fe/kg of soil by the D1
22 dose and to 70-100 mg Fe/kg of soil by the D2 dose for soils I and II. However, in the
23 case of soil III, recovery increased to 60-80 mg Fe/kg of soil for D1 and 100-140 mg
24 Fe/kg of soil for D2. As the pH of the three soils was similar, this recovery difference is
25 attributed to the differing textural compositions of the soils. The extractable

26 concentrations of Fe increased in the sandy loam soil in contrast to the clay loam soils.
27 The Fe-EDDHA formula did not affect significantly extractable Fe concentration.
28 Increases in the extractable Cu and Mn were observed after Fe- EDDHA soil
29 application. These increases could be due to changes in the redox potential that alters
30 the form and solubility of some metals, possibly affecting the metal-chelate equilibrium.
31 In the case of Zn, the variation in Zn concentration is hardly appreciable, with Fe
32 preventing effective Zn chelation. No difference in effectiveness has been found
33 between the Fe-EDDHA formula brands used in this experiment.

34

35 INTRODUCTION

36

37 Iron deficiency is frequently found in crops growing in calcareous soils in semiarid
38 climates. This deficiency appears even though the soils are well provided with Fe.
39 Plants need at least 0.6 mg Fe /kg soil, whereas average soil content is over 20000
40 mg/kg (1). Deficiencies are induced by external factors related with Fe absorption,
41 transport or metabolism mechanisms such as a high pH, high HCO_3^- concentration in
42 soil solution, interactions with other elements, etc. (2, 3, 4, 5, 6).

43 In Spain, a number of crops are affected by this deficiency, especially citrus (*Citrus*
44 *spp.*) groves, vineyards (*Vitis vinifera*, L.), peach orchards (*Prunus persica*., L), and
45 herbaceous crops such as the strawberry. It is estimated that more than 82000 ha are
46 supplemented with Fe fertilizers, with approximately 280000 ha of crops showing
47 deficiency symptoms (7). The market for synthetic chelates used in agriculture is
48 increasing, being Spain the main consumer.

49 To date, in calcareous soils, there are two chelates that show stability: di(o-hydroxy-
50 phenylacetic) acid (EDDHA) (8) and recently, ethylene diamine di(o-hydroxi-p-

51 methylphenyl-acetic) acid (EDDHMA) with a similar structure (9). There are many
52 commercial chelates with more than 200 different formulae in the Spanish market (10).
53 This fact is important because it can affect the effectiveness of the chelate. Results of a
54 study of 80 commercial formulae of EDDHA and EDDHMA used in 1998 and 1999
55 have recently been published (11) concluding that a lack of rigour exists in the
56 information displayed on the label (chelate agent, % soluble Fe, % Fe-chelated, and the
57 pH range in which the chelation fraction is stable).
58 Studies have been carried out on the interaction of Fe chelates with soil components and
59 its effect on the availability of Fe (12). However, the effect of the application of Fe
60 chelates on the availability of other micronutrients has not been reported. The aim of
61 this study is to evaluate the stability of three commercial chelates, and additionally to
62 estimate the effect of Fe-EDDHA (6%) chelate interaction on the extractability of Cu,
63 Mn, and Zn.

64

65 **MATERIALS AND METHODS**

66

67 Three commercial Fe-EDDHA (6%) chelates, greatly demanded in the Valencia
68 agricultural area, were used: 1) Sequestrene 138 Fe G-100; 2) Group Carla Val F.E.A. 6
69 SuperiorTM, and 3) Ferrishell plus. Three representative soils of the Ribera Alta del
70 Jucar area, from orchards plots showing chlorosis symptoms, were used (Table 1). Soil I
71 was from a four-year-old peach orchard, soil II was from a two-year-old mandarin plot.
72 Both of them were drip irrigated with groundwater obtained from the same well (Table
73 2). Soil III was from an orange orchard in full production, flood irrigated with water
74 from the Jucar-Turia channel. In each plot a representative soil sample was taken from
75 0-25 cm depth and, after air drying, passed through a 2-mm stainless steel sieve.

76 TABLE 1. Properties of the soils used in the incubation experiment. Data are expressed
 77 on dry weight basis. Organic carbon was determined by Walkey-Black wet oxidation
 78 method, inorganic carbon was determined by calcimeter method and active lime by
 79 Droineau ammonium oxalate extraction.
 80

	SOIL I	SOIL II	SOIL III
Sand %	28.9	40.3	62.1
Silt %	36.4	28.3	23.8
Clay %	34.7	31.4	14.1
Textural class	Clay loam	Clay loam	Sandy loam
Organic C (g/kg)	6.7 ± 0.3	8.9 ± 0.3 ⁽¹⁾	7.2 ± 0.3
Inorganic C (g*/kg)	487 ± 10	123 ± 1	553 ± 10
Active lime (g*/kg)	128 ± 18	28 ± 1	36 ± 15
C.I.C (mmol _c /kg)	56 ± 6	101 ± 1	9 ± 10
pH (1:2,5 soil:water)	8.58 ± 0.01	8.15 ± 0.01	8.52 ± 0.01
DTPA-Fe (mg/kg)	4.4 ± 1.1	4.2 ± 0.4	8.8 ± 1.6
DTPA-Cu (mg/kg)	0.9 ± 0.1	1.3 ± 0.1	3.7 ± 0.5
DTPA-Mn (mg/kg)	23 ± 8	19.1 ± 8.9	18.2 ± 10.9
DTPA-Zn (mg/kg)	2.0 ± 0.9	3.4 ± 1.0	3.5 ± 0.7

- 81 • * Expressed as CaCO₃ equivalent
 82 • ⁽¹⁾ Each value represents the mean of two replications ± one standard deviation.
 83

84 TABLE 2. Electrical conductivity (EC), and ionic concentrations of waters used in the
 85 experiment
 86

	SURFACE WATER	GROUNDWATER
EC (dS/m)	0.73	0.59
Na ⁺ (mmol _c /L)	1.4	1.1
Mg ²⁺ (mmol _c /L)	2.9	1.9
K ⁺ (mmol _c /L)	0.17	0.08
Ca ²⁺ (mmol _c /L)	5.4	4.6
Cl ⁻ (mmol _c /L)	1.6	1.6
SO ₄ ²⁻ (mmol _c /L)	6.6	2.0
HCO ₃ ⁻ (mmol _c /L)	3.9	6.7
NO ₃ ⁻ (mmol _c /L)	0.11	0.41

87

88 To see the interaction of the chelates with the water solution, a trial was carried out
89 comparing the stability of the three chelates in distilled water and in the irrigation
90 waters used. The experiment design was carried out in a similar way to that adopted by
91 Alva (12). In each treatment, 0.1 g Fe-EDDHA chelate was mixed with water up to 40
92 mL, this trial being carried out in duplicate. The mixture was placed in an orbital shaker
93 for 24 h at 70 rpm and subsequently left to rest 30 min. 1 mL of the remaining liquid
94 was sampled and diluted to 25 mL, for later analysis of Fe, Cu, Mn and Zn by AAS.
95 The sampling was repeated 9 d later without replacing the water: 1mL samples were
96 taken from each one of the treatments for the analysis of Fe, Cu, Mn, and Zn.

97 To analyze the interaction of chelates with soil, an experiment was performed with a
98 factorial design of tree soils, tree chelates and two doses, D1 and D2 (100 and 200 μ g
99 Fe/kg of soil). For each soil a control was used without adding chelate. Each treatment
100 was carried out in duplicate. 85-g subsamples were homogeneously mixed with the Fe-
101 EDDHA (6%) chelate in percolation tubes. All the samples were brought to field
102 capacity moisture with the irrigation water employed in each field. The samples were
103 covered with polyethylene film to avoid evaporation. The humidity was controlled
104 periodically by weighing and, if necessary, water was added to soil.

105 The total incubation time was 71 d. From each treatment, 10-11 g soil samples were
106 taken from each tube with a spatula after 7, 14, 21, 42, and 71 d of incubation.
107 Extractable micronutrients were determined from each sample using the extraction
108 method proposed by Lindsay and Norvell (13), which uses DTPA as a chelate agent.
109 The extracts were analysed by atomic absorption spectrometry at 248.3 nm (Fe), 324.8
110 nm (Cu), 279.5 nm (Mn), and 213.9 nm (Zn) (14).

111 Statgraphics software (STATGRAPHICS® Plus Version 4.0. program) was used to
112 analyze the data. Multifactor ANOVA was used to study the effect of the water and

113 chelate type on Fe, Cu, Mn and Zn concentrations and also was applied to analyze the
 114 effect of soil, dose and time on extractable Fe, Cu, Mn, and Zn.

115

116 **RESULTS AND DISCUSSION**

117

118 *Solubility in Water*

119

120 Water and chelate type effects on Fe, Cu, Mn, and Zn concentrations are shown in Table
 121 3. All the chelates had higher soluble Fe concentration than values given by
 122 manufacturer (6%). The chelate with the highest levels of soluble Fe was Sequestrene,
 123 followed by G. Carla Val. There were significant differences in Fe concentration
 124 according to the type of water employed, with distilled water showing lower Fe
 125 concentration. The Ferrishell + chelate contained higher levels of Cu, Mn, and Zn.

126 TABLE 3. Main effects of type of chelate and water on Fe, Cu, Mn, and Zn
 127 concentrations in water solutions. Table of averages.

FACTOR		Fe % *	Cu mg/kg *	Mn mg/kg *	Zn mg/kg *
CHELATE	Ferrishell +	6.16 ^a	176 ^c	718 ^b	171 ^c
	G. Carla Val	6.70 ^b	0 ^a	739 ^b	64 ^b
	Sequestrene	7.51 ^c	66 ^b	334 ^a	45 ^a
WATER	Surface water	7.10 ^b	79 ^a	604 ^b	85 ^a
	Groundwater	6.97 ^b	81 ^a	555 ^a	101 ^b
	Distilled	6.30 ^a	82 ^a	632 ^c	94 ^{ab}

128 Values followed by the same letter are not significantly different at the 5% level according to least
 129 significative difference Fisher's test. There not significative interaction water by chelate type

130 *Concentrations are expressed respect to chelate weight

131 Groundwater → irrigation water used with soils I and II

132 Surface water → irrigation water used with soil III

133

134

135

136 *Extractable Micronutrients Evolution in the Soil*

137

138 Results from one way ANOVA applied to data did not show any significant differences
 139 in Fe, Mn and Cu soil extractable concentrations due to chelate type effect. The chelate
 140 type used did not affect the extractable concentration of nutrients, except for Zn, which
 141 indicates that there were no differences in effectiveness among the tested chelates. A
 142 multifactor ANOVA was performed in order to analyze time, dose (control, D1, and
 143 D2) and soil type effects. For extractable Fe, Cu, and Zn (Table 4), there were
 144 significant differences between soils. The chelate dose increased significantly the
 145 extractions for all the nutrients (Table 5), with the exception of zinc. Also significant
 146 was the time factor for all the nutrients, decreasing extraction, with exception of iron.

147 TABLE 4. Main effects of chelate application on extractable micronutrients. Variance
 148 analysis results.

Treatment main effect	Extractable Fe	Extractable Cu	Extractable Mn	Extractable Zn
Factors				
A. Soil	***	***	NS	**
B. Dose	***	**	***	NS
C. Time	NS	***	***	*
Interactions				
A x B	**	*	NS	NS
A x C	NS	***	**	*
B x C	NS	NS	NS	NS

149 Level of significance: NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

150 A. 3 Soils: I, II, and III

151 B. 3 Doses: Control, D1, and D2

152

153

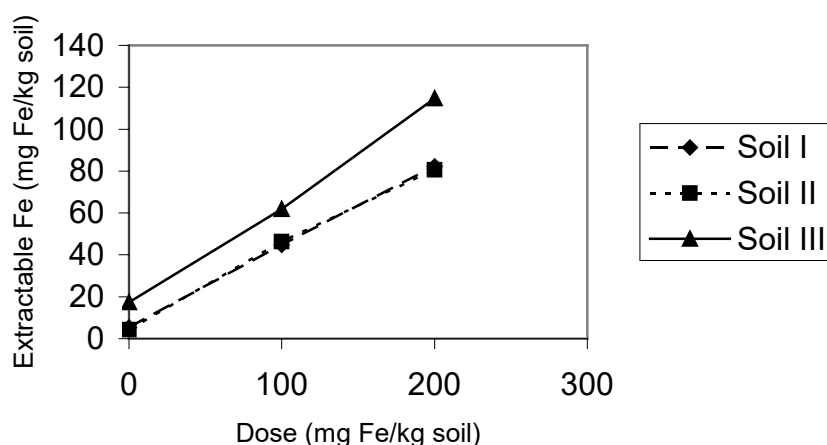
154 TABLE 5. Main effects of treatments on extractable Fe, Cu, Mn, and Zn. Table of
 155 averages.

EFFECT		Extractable Fe	Extractable Cu	Extractable Mn	Extractable Zn
		mg/kg	mg/kg	mg/kg	mg/kg
SOIL	Soil I	43.0 a	0,77 a	20.4 a	2.78 a
	Soil II	43.3 a	1.22 b	28.1 b	5.28 b
	Soil III	66.2 b	4.59 c	29.2 b	4.66 b
DOSE	Control	9.0 a	1.95 a	20.1 a	4.08
	D1	51.0 b	2.15 ab	23.6 a	4.37
	D2	92.5 c	2.48 b	34.0 b	4.27
TIME	7		2.63 bc	44.6 c	5.13 bc
	14		3.07 c	40.1 c	4.3 abc
	21		2.30 b	25.1 b	5.74 c
	42		1.69 a	11.6 a	3.3 ab
	71		1.26 a	8.0 a	2.72 a

156 Values followed by the same letter are not significantly different at the 5% level according to least
 157 significative difference Fisher's test.

159 Since the soils used in the experiment are calcareous, all the non-chelated Fe³⁺ forms
 160 precipitate. Soil type affected the recovery percentage of soluble Fe (Fig. 1). The D1
 161 dose (100 mg Fe/kg of soil) increased extractable Fe up to 45-46 mg Fe/kg of soil
 162 (average recovery value of 40%), while the D2 dose (200 mg Fe/kg of soil) raised it to
 163 82-81 mg Fe/kg of soil (average recovery value of 40%), for soils I and II. In the case of
 164 soil III, the recovery percentage increased respectively for dose D1 and D2 to 62 mg
 165 Fe/kg of soil (44%) and 115 mg Fe/kg of soil (49%). Losses in soluble Fe were also
 166 found by Follet and Lindsay (15) who obtained a recovery percentage of 87%
 167 immediately after fertilization with Fe-EDDHA. The recovery percentage of Fe varied
 168 depending on the composition of the soil, being higher for the coarser textured soil. The
 169 effectiveness of Fe chelates as Fe sources and carriers in soil can be severely limited by
 170 sorption of Fe chelates or chelating agents in the solid phase (16), and by the

171 decomposition of the chelating agent by microorganisms (17) or light (18, 19). The
 172 factors that affect sorption include the type of chelating agent, time, pH, salt
 173 concentrations, and soil texture (20). Clay soils were found to be implicated as major
 174 sorbants of chelating agents by Wallace and Lunt (21). In our experiment, DTPA extract
 175 recovery differences cannot be attributed to soil pH, as the pH of the three soils are
 176 similar. This behaviour can be explained by the differing textures of the soils, with
 177 increasing Fe concentration in the sandy loam soil in contrast to the clayey soils. The
 178 texture influence on the recovery percentage of Fe in solution was also found by Barona
 179 *et al.*(22). However, the brand of the Fe-EDDHA formula was not significant in the
 180 variance analysis for any of the three soils.



181
 182 Figure 1. Effect of chelate dose on extractable Fe. Soils I and II have clay loam texture
 183 and soil III has sandy loam texture.

184
 185 In relation to the influence of Fe-EDDHA chelate on the extraction of Zn, Cu and Mn,
 186 Cu was affected by the soil, the dose and time (Table 4). The addition of Fe-EDDHA
 187 chelate increased the Cu in the soil, due to the chelating effect. Chelating agents added
 188 to soils and nutrient solutions increase the metal solubility and mobility and frequently
 189 improve cationic micronutrient availability to plants (12, 20). The extent of metal
 190 chelation depends on the simultaneous equilibrium of chelating agents with all
 191 competing cations, and on the stability of the chelates formed. In soil III, there is also a

192 higher Cu concentration than in the case of soils I and II (Figure 2). EDDHA appears to
 193 have some limited potential as a chelator for Cu in alkaline conditions. Cu^{2+} solubility
 194 in soils is generally too low to allow effective chelation even when Cu-EDDHA itself is
 195 added (2, 23). However, EDDHA is likely to be one of the most effective chelators for
 196 Cu^{2+} in reducing conditions, whereas it is one of the least effective chelators in well-
 197 oxidised conditions (20). A possible explanation of the increase on Cu solubility may be
 198 that the soil reaches more reducing conditions as soil microbiological activity increased
 199 as a consequence of adding water and organic compounds. This may be the case of
 200 fertirrigation where reducing conditions may be reached in the wet soil bulb.

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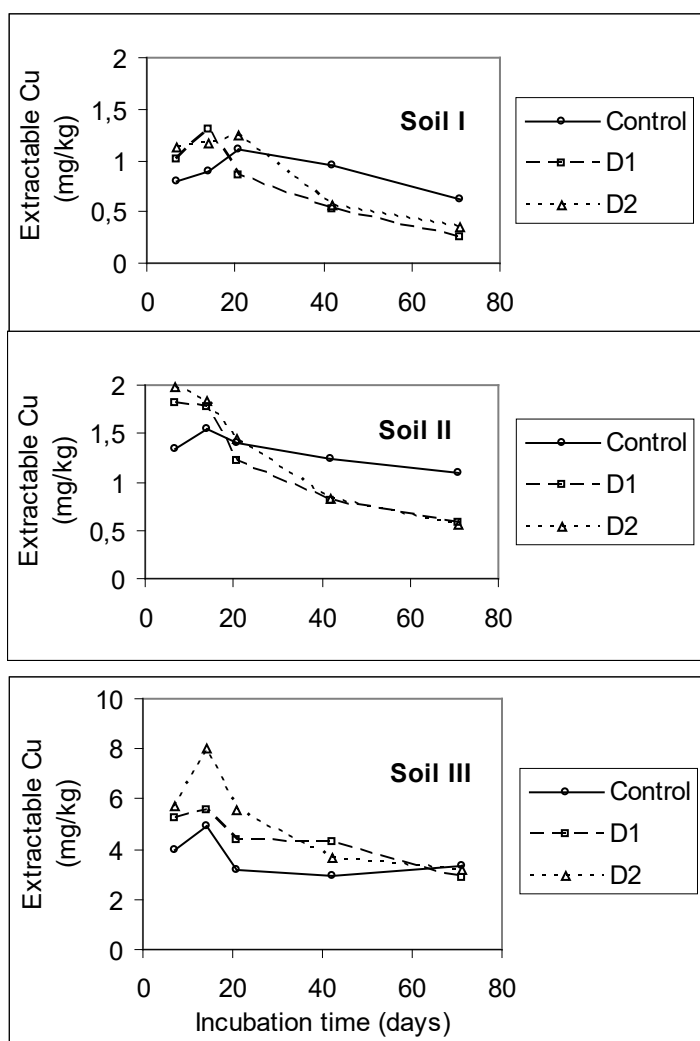


Figure 2.- Extractable Cu concentration in soils I, II and III, with time

217 Observing the evolution of extractable Cu with time (Figure 2) there was a maximum at
218 day 14 of incubation. This initial increase is attributed to the combining effect of high
219 Cu affinity to EDDHA in reducing conditions and the increase in Cu mobility due to
220 changes in the redox potential, which alters the forms and solubility of a variety of
221 metals, possibly affecting to a greater extent the metal-chelate equilibrium (20). Then,
222 the quantity of extractable Cu diminished with time. However, the available Fe
223 remained rather constant. In soils, the persistence of soluble chelates in solution through
224 time provides some assurance that the degradation of EDTA, DTPA, CDTA, HEDTA,
225 and EDDHA is slow (24, 25, 26). These results could be due to the evolution of well-
226 oxidized conditions and a greater effect of the variation of redox potential against the
227 chelation effect. The chelation effect is made evident by the existence of a greater
228 concentration of soluble Cu in the three soils with dose D2 of Fe-EDDHA in contrast to
229 dose D1.

230 The evolution of Mn in the three soils showed a high level of variability, with a clear
231 tendency to decrease with time (Figure 3) (Table 5). Dose increased the extractable Mn
232 specially at the first 14 days of incubation, except in soil I. This effect is associated with
233 a higher solubility, as in the case of Cu, as the redox potential moves towards reducing
234 conditions. Since soil I was the one that had greater active lime, increase in Mn
235 solubility as a consequence of reducing conditions was contrarrested by lesser solubility
236 in higher carbonate medium.

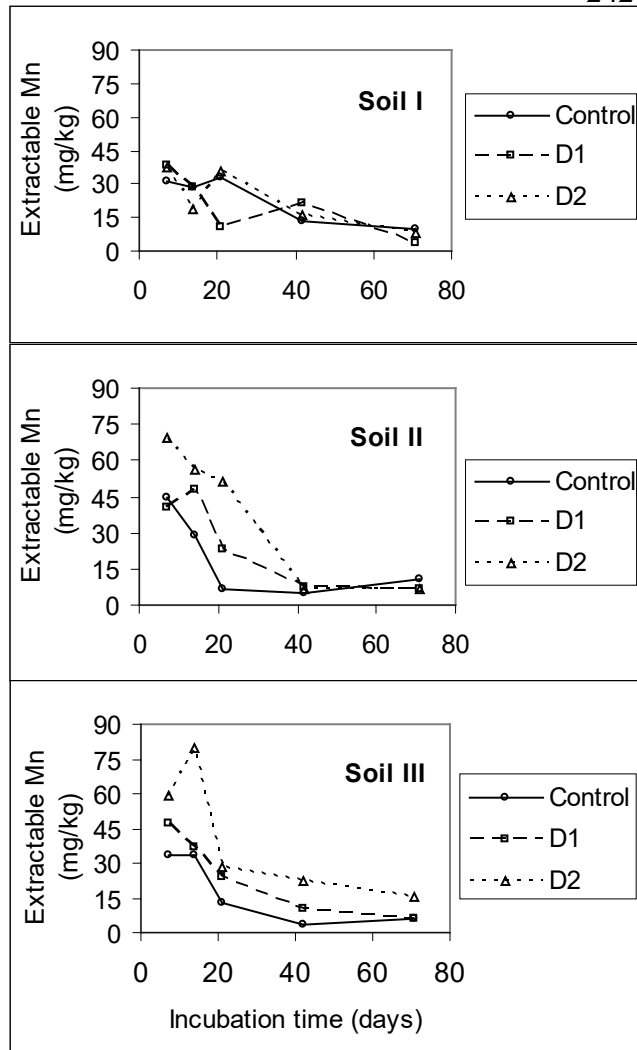
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260 Figure 3.- Extractable Mn concentration in soils I, II and III, with time.

261 In the case of Zn, the evolution was similar in the three soils, with no great differences
 262 among them. A lower chelation effect has been observed with Zn, as the doses have not
 263 shown differences. Chelation of Zn by EDDHA is negligible with Fe competition
 264 preventing effective Zn chelation at any soil pH (20). With time, there is a decreasing
 265 trend in the quantity of extractable Zn, both in the treatments and in the control.

266

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