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Mobility and Availability of Iron in A Calcareous Soil as Affected by Barley Cultivation and Iron Fertilizers

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PLASTIC pots containing 30kg calcareous soil sample (31% $CaCO_3$) for each were used to investigate iron mobility and availability in either rhizosphere or nonrhizosphere zone. Treatments include zero and 10 ppm Fe as $FeSO_4$ or Fe-EDTA applied to soil which was cultivated or not cultivated with Fe-efficient barley plant, *Hordeum vulgare* L.

The obtained results revealed that pH values of the cultivated soils were generally lower than those not cultivated ones by about 0.1 unit and the lowest pH values were recorded for the rhizosphere zone. Values of DTPA-extractable Fe were sharply decreased with increasing soil depth from 0-4 down to 16-20 cm, both in cultivated or not cultivated pots. However, extracted Fe was also decreased with increasing lateral distances only in not cultivated pots. In the control treatment, the highest Fe values were extracted from the rhizosphere zone and this could be due to the positive effect of the released root exudates from the intensive root hairs of barley. The vertical and horizontal Fe mobility were more pronounced in both cultivated and not cultivated soils that treated with Fe-EDTA compared with those treated with $FeSO_4$. Moreover, the highest straw and grain yield of barley, as well as Fe concentration were obtained from pots treated with Fe-EDTA. Active iron content in barley leaves showed similar trend to those obtained for total Fe. Thus, the graminaceous species , *i.e* barley plant particularly, the Fe-efficient cultivars can get Fe more available for uptake under calcareous soil conditions.

Keywords: Iron , Mobility , Availability , Calcareous soil , Barley plant (*Hordeum vulgare* L.)

Egypt has considerable areas of calcareous soils which are located in the northwestern littoral region and characterized by decreasing iron availability which is reflected on iron deficiency in most of higher plants grown on such soils. The availability of iron in soils is a function of a number of properties; including plant type and root exudates, soil pH, soil texture, $CaCO_3$ content, organic matter and the amount of Fe in the solid form, which is in equilibrium with those in the soil solution and interaction with other nutrients. Availability of iron and also its mobility in the bulk soil differ considerably compared to the soil surrounding the root which is affected by it and is called rhizosphere. The rhizosphere has

broadened, from a very narrow zone extending at most 1-2 mm from the root surface (Bais *et al.*, 2006), to one extending several centimeters as in the case of nutrient or water depletion profile (Darrah, 1993). Marschner & Romheld (1996) reported that the important examples of root-induced changes in the rhizosphere that affect micronutrient availability are the rhizosphere pH, reducing capacity of the roots, redox potential and root exudates. Rompre *et al.* (2005) stated that, in response to iron deficiency, root of graminaceous species *i.e.* barley release so-called phytosiderophores, which are highly effective to solubilize, by chelation the sparingly soluble Fe compounds. Release of organic acids, amino acids and phytosiderophores by Fe-deficient monocotyledonous plants roots was found to be fairly higher than those of Fe-sufficient ones (Awad *et al.*, 1994; Romheld & Marschner , 1990 and Zhang *et al.*, 1991). The current work is mainly conducted to evaluate mobility and availability of iron in a calcareous soil as affected by barley cultivation and different Fe treatments.

Material and Methods

A surface soil sample (0-30cm) represents a calcareous soil, clay loam, calcids, was collected from Burg El-Arab area. The soil sample was selected from cultivated region with crops suffering Fe-deficiency symptoms. The soil sample was air dried crushed and ground to pass through a 2mm sieve for analysis. Some physical and chemical properties of the soil are presented in Table 1.

TABLE 1. Some physical and chemical characteristics of the invistigated soil.

Particle size distribution ,%				Texture	CaCO₃,%		O.M,%	F.C
C.sand	F.sand	Silt	Clay		Total	Active		%
18.3	25.4	26	30.2	Clay loam	31	12.6	1.34	16.4

TABLE 1. Contd.

pH	ECe,dS.m⁻¹	Total micronutrients,ug/g				Chemically available micronutrients, ug/g			
(1.:2.5)	(paste ext.,)	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
8.2	1.6	14440	310	60	15	1.7	3.9	2.75	1.2

A pot experiment was conducted under greenhouse conditions, to study the horizontal and vertical iron mobility in soil with and without planting. Fifteen plastic pots, 35cm height and 32cm diameter, were packed with 30kg air dried soil sample for each. Each pot received the recommended N, P and K rates by ministry of Agriculture. Soil treatments were applied as follows: (i) control treatment, 3 planted pots without Fe addition, (ii) 3 planted pots and 3 not planted pots received 10ppm Fe as FeSO₄ and (iii) 3 planted pots and 3 not planted pots received 10ppm Fe as Fe-EDTA. Pots were planted with 5 barley plants (*Hordeum vulgar* L., Giza 125). Giza 125 was selected as the most Fe-efficient cultivar after primary experiment. Application of iron fertilizer was done at the

center of soil surface which was converted by mixing thoroughly within a circle of 4 cm diameter (Fig. 1). The seedlings were almost distanced 8 cm from the center and 10 cm from each other. There were three replicates for each treatment; the different treatments were irrigated twice a week with distilled water. Soil moisture was maintained between 60-80% of field capacity
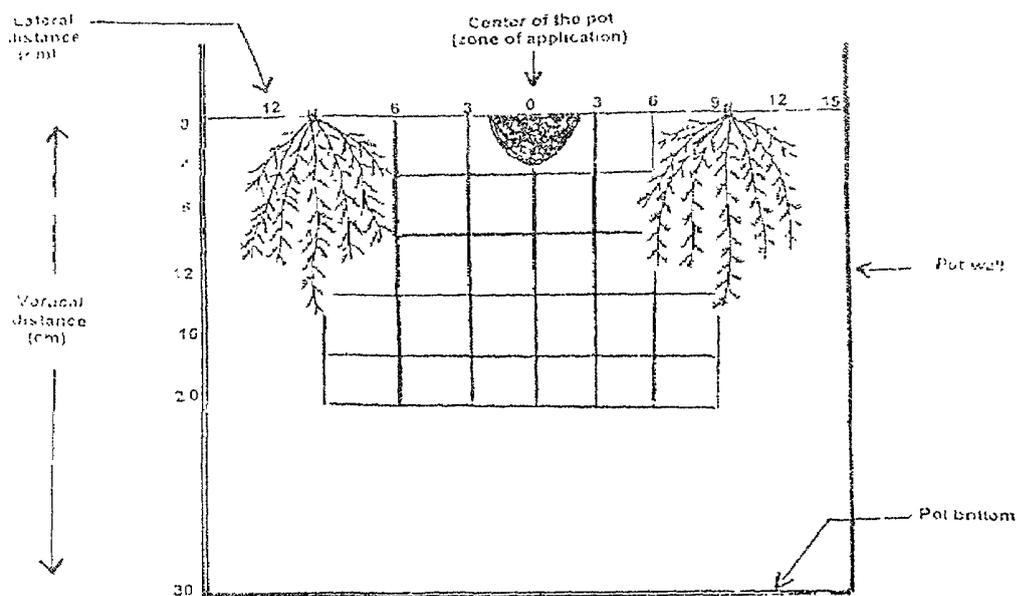

Fig. 1. Schematic diagram showing roots distribution of barley plants and sites of soil sampling.

After 60 days from planting, the 4 uppermost leaves from each pot were taken and mixed in three groups representing the different Fe treatments for determination of active iron (Jones, 1972). The plants were harvested (124 days after planting); the dry weight of straw, grain yield and weight of 1000 grain were recorded.

At the same time, soil samples were taken from each treatment at different horizontal and vertical distances. The distances were 0-3, 3-6 and 6-9cm from the center of surface layer and depths of 0-4, 4-8, 8-12, 12-16 and 16-20cm, respectively. Additional cultivated pots were included for showing pattern of root distribution in the studied soils to identify rhizosphere zone. Each soil sample was taken from a mixture of 5 samples. Soil pH and DTPA-extractable iron were determined.

Soil routine analysis was conducted according to the standard methods outlined by Piper (1950); Jackson (1973) and Lindsay & Norvell (1978). While active $CaCO_3$ was estimated as described by Yaalon (1957).

The ground plant materials were wet digested using $H_2SO_4-H_2O_2$ mixture and micronutrients were determined using atomic absorption spectrophotometer. The active Fe determination was done according to the method of Oserkowsky (1933) which was modified by Llorente et al. (1976).

Results and Discussion

Soil pH

Soil pH is an important factor in affecting iron mobility, so values of pH in barley root zone and the surrounding area were detected and given in Table 2. Generally, pH values of the cultivated soil slightly increased with increasing soil depth, showing highest values in the lower layers (16-20cm) from soil surface and away from plant root. Such increase was about 0.1 unit. Presence of barley root lowered soil pH values relative to initial one (7.6). The rhizosphere zone, which could be identified 6-9 cm laterally from pot center and 0-8 cm vertically, showed the lowest pH value comparing with those of the other non rhizosphere zones. This could be due to plant root exudates that contain organic acids as well as hydrogen ions especially in the soil root interface. It is worth to mention that the root-induced pH changes in the rhizosphere are maximal at soil pH between 5 and 6 and decrease in magnitude at both lower and higher bulk soil pH, (Nye, 1986 and Schaller, 1987). The lower pH in the rhizosphere zone is causally related to the enhanced H^+ secretion from roots in connection with cell extension, (Weisenseel *et al.*, 1979) or to the net excretion of HCO_3^- and OH^- due to imbalance between cation and anion uptake (Nye, 1986) . Also, the evolution of CO_2 by root respiration is responsible.

TABLE 2. Soil pH values at different horizontal and vertical distances as affected by iron sources and barley plant grown on the calcareous soil.

Fe source	Depth (cm)	With planting				Without planting			
		Distance from application zone (cm)							
		0-3	3-6	6-9	Mean	0-3	3-6	6-9	Mean
Control	0-4	8.18	8.12	8.09	8.14				
	4-8	8.18	8.15	8.09	8.14				
	8-12	8.18	8.17	8.13	8.16				
	12-16	8.19	8.19	8.16	8.18				
	16-20	8.20	8.21	8.21	8.21				
	Mean	8.19	8.17	8.12	8.17				
FeSO4	0-4	8.10	8.10	7.96	8.05	8.10	8.15	8.19	8.15
	4-8	8.16	8.11	8.03	8.10	8.17	8.19	8.18	8.18
	8-12	8.17	8.14	8.03	8.11	8.19	8.19	8.20	8.19
	12-16	8.17	8.16	8.11	8.15	8.20	8.20	8.21	8.20
	16-20	8.20	8.19	8.20	8.19	8.20	8.21	8.21	8.21
	Mean	8.16	8.14	8.06	8.12	8.17	8.19	8.20	8.19
Fe-EDTA	0-4	7.98	8.09	7.94	8.00	8.00	8.16	8.18	8.11
	4-8	8.11	8.10	8 00	8.07	8.17	8.19	8.19	8.18
	8-12	8.13	8.11	8.00	8.08	8.18	8.20	8.20	8.19
	12-16	8.19	8.15	8.09	8.14	8.20	8.20	8.20	8.20
	16-20	8.20	8.18	8.16	8.16	8.18	8.21	8.21	8.21
	Mean	8.12	8.13	8.04	8.10	8.14	8.18	8.19	8.17

Application of iron either as $FeSO_4$ or FeEDTA slightly lowered soil pH only near application zone, while did not markedly affect pH of the other zones. However, barley cultivation reduced pH of the studied soil by about 0.1 unit only and this could be attributed to the high buffering capacity of the calcareous soil.

Iron mobility and availability

Values of DTPA-extractable Fe from different vertical and horizontal distances in soil having high $CaCO_3$ content are shown in Table 3. Data show that Fe values were generally decreased with increasing soil depth from 0-4 cm down to 16-20 cm. Soil cultivation with barley plant was found to increase values of Fe extracted from different depths of the soil treated with $FeSO_4$ comparing with those not planted. Such increases recorded 10, 10.6, 32.2, 42.8 and 42.8% at 0-4, 4-8, 8-12, 12-16 and 16-20 cm, respectively. Increasing Fe extractability in the cultivated pots, particularly in the root zone could be due to the positive effect of barley root exudates containing organic acids, amino acids and phytosiderophores. The effect of decreasing pH due to H^+ ions secretion is also considered, that the solubility of Fe^{3+} is highly pH-dependent and it decreases 1000 fold for each unit increases in pH (Lindsay, 1979). As for the horizontal Fe mobility in both cultivated and non cultivated soils under $FeSO_4$ application, it is obvious that extractable Fe values were sharply reduced with increasing distance from application zone. The average of increases in Fe values of the different lateral distances of the cultivated soil compared with non cultivated one were 18.4, 16 and 15.2% at 0-3, 3-6 and 6-9 cm, respectively.

On the other hand, extractable Fe values from different depths of the tested calcareous soil treated with Fe-EDTA were sharply decreased with increasing soil depth from 0-4 cm to 16-20 cm. Increases in Fe extractability from different depths due to barley cultivation were observed. Such increases recorded 19.4, 26.8, 35.0, 37.6 and 37.6% at 0-4, 4-8, 8-12, 12-16 and 16-20 cm depth, respectively. As expected, results show that the extractable Fe values at different horizontal distances in both cultivated and non-cultivated soils treated with Fe-EDTA were reduced with increasing distance from application zone. However, the average of increases in Fe values of the different lateral distances of the cultivated soil compared with non cultivated one were 31.6, 18.6 and 12.3% at the 0-3, 3-6 and 6-9 cm lateral distances, respectively.

Note worthy mentioning that the vertical and horizontal Fe mobility were more pronounced in both cultivated and non cultivated soils treated with Fe-EDTA under high $CaCO_3$ content as compared with those supplied with the mineral source of iron ($FeSO_4$). The superiority of Fe-EDTA treatment compared to $FeSO_4$ one was indicated by 66.6, 66.8 and 68.9% increases in Fe extractability from the cultivated soil at the lateral distances of 0-3, 3-6 and 6-9 cm, respectively, while those for non cultivated soil were 49.8, 63.2 and 73.2%, respectively. The effect of phytosiderophores on mobilization of iron was also studied by Takagi *et al.* (1988);Treeby *et al.* (1989) and Romheld (1991), who stated that phytosiderophores acted as natural chelating material and was more effective in Fe mobilization in soil compared with the synthetic chelators, *i.e.* DTPA, EDTA and EDDHA.

TABLE 3. Values of DTPA-extractable Fe (ug/g) from different horizontal and vertical distances as affected by iron sources and barley plant grown on the calcareous soil.

Fe source	Depth (cm)	With planting				Without planting			
		Distance from application zone (cm)							
		0-3	3-6	6-9	Mean	0-3	3-6	6-9	Mean
Control	0-4	1.70	1.80	2.10	1.87				
	4-8	1.56	2.04	2.00	1.89				
	8-12	1.60	1.56	1.90	1.69				
	12-16	1.70	1.50	1.90	1.70				
	16-20	1.54	1.42	1.80	1.59				
	Mean	1.64	1.66	1.94	1.75				
FeSO$_4$	0-4	35.60	3.90	3.20	14.23	32.50	3.50	2.80	12.93
	4-8	7.30	3.40	3.00	4.57	6.60	3.00	2.80	4.13
	8-12	3.50	3.00	2.50	3.00	2.00	2.60	2.20	2.27
	12-16	3.50	2.10	2.10	2.57	2.00	1.70	1.70	1 80
	16-20	3.50	2.10	2.10	2.57	2.00	1.70	1.70	1 80
	Mean	10.68	2.90	2.58	5.39	9.02	2.50	2.24	4.59
Fe-EDTA	0-4	57.60	6.80	5.10	23.17	48.00	5.60	4.60	19.40
	4-8	13.10	5.00	5.00	7.70	8.80	4.90	4.50	6.07
	8-12	6.10	4.60	4.70	5.13	4.60	3.30	3.50	3.80
	12-16	6.10	3.90	3.50	4.50	3.10	3.30	3.40	3.27
	16-20	6.10	3.90	3.50	4.50	3.10	3.30	3.40	3.27
	Mean	17.80	4.84	4.36	9.00	13.52	4.08	3.88	7.16

Barley yield and Fe content

Apparently, application of Fe-EDTA resulted in a marked increase in dry weight of straw and grain yield comparing with those treated with FeSO$_4$ or control treatment (Table 4). The obtained results confirm the previous findings that Fe-EDTA remains more available for plant root comparing FeSO$_4$ under such conditions. Similar trend was found for wheat by Basyouny (1996). Although containing the studied soil DTPA-Fe less than the critical Fe level, 3.8 ppm, as determined by Osman (1983) in calcareous soils of Egypt, barley plants absorbed and utilized significant amounts from soil Fe in either soluble Fe^{2+} or in Fe^{3+} form. As previously mentioned phytosiderophores released by barley roots could be principally responsible in getting Fe more available for barley root uptake beside the role of other organic acids and amino acids present in plant root exudates.

TABLE 4. Straw, grain yield and Fe concentration of barley plant grown on calcareous soil as affected by Fe treatment.

Fe source	Straw, g/pot	Grain, g/pot	Weight of 1000 grain, g	Total Fe, ug/g	Active Fe, ug/g	
				Straw	Grain	Dry leaves
Control	41.1	5.13	23.6	78.7	62.2	15.9
FeSO$_4$	44.0	5.86	24.2	111	86.5	18.2
Fe-EDTA	55.8	6.14	24.8	147	100	25.8
L.S.D$_{0.05}$	4.76	n.s.	n.s.	11.9	6.23	2.30

On average, the increases in Fe concentration in straw and grains of barley plants treated with Fe-EDTA compared to those treated with FeSO$_4$, were 32.2 and 63.4%. If chelate was absorbed by barley roots or not, the fact that Fe EDTA is superior for increasing plant content compared to FeSO$_4$. Similar findings were also obtained by Christine (1992) and Basyouny (1996). However, Fe concentrations in straw or grains of the untreated barley plants with iron were found to be quite or at least causing no deficiency symptoms, such Fe concentrations recorded values above the critical Fe level for barley (50 ppm) as reported by Brown (1956). Barley root exudates, particularly phytosiderophores were the main reason responsible in providing the non treated plants with enough Fe for plant growth. Moreover (Marschner et al., 1986) reported that barley roots have a specific uptake system of ferreted phytosiderophores. This indicates the ecological importance of such system for adaptation of grass species to calcareous soils with low iron availability.

The concentrations of the so-called active iron in barley plant leaves at 60 days age as affected by Fe treatments are shown in Table 4. Several authors used the term of active iron such as Elgala & Maier (1964); Terry & Abadia (1986) and Koseoglu & Acikgoz (1995). Marschner (1995) mentioned that localization and binding state of iron in leaves could be reduce the content of total iron in chlorotic leaves making it similar or even higher than that in green leaves whereas a proportion of iron might be precipitated in the apoplasm. In the present investigation active iron fraction was extracted to compare with total Fe concentration of barley plants although neither the composition nor the location of this extracted iron, however, are known.

Under Fe stress, enhancing release of phytosiderophores from roots of graminaceous species occurred in soils, particularly in calcareous one can get Fe more available for uptake and this could be benefit in reducing Fe fertilization. On the other hand, attention must be given towards intercropping cultivation, i.e. dicotyledonous species together with the monocotyledonous ones under such conditions.

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حركة وتيسر الحديد فى أرض جيرية وتأثرها بزراعة الشعير والتسميد بالحديد

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أجريت تجربة اصص لدراسة حركة وتيسر الحديد فى منطقة انتشار الجذور والمنطقة الأبعد عن المجموع الجذرى لنباتات الشعير المزروع فى أصص سعة ٣٠ كجم تربة جيريه (٣١٪ كربونات كالسيوم) ، وكانت المعاملات تحت الدراسة عبارة عن صفر و ١٠ ملجم حديد/ كجم تربة فى صورة حديد مخلبى Fe – EDTA أو معدنى $FeSO_4$ لأصص مزروعة بالشعير ولأصص أخرى مماثلة غير مزروعة وتتلخص أهم النتائج فى الاتى:

- كانت قيم الـpH فى الأصص المزروعة بالشعير اقل ــ بصفة عامة ــ من مثيلتها الغير مزروعة بمقدار ٠,١ وحدة، وكانت اقل قيم الـpH فى منطقة الريزوسفير.

- نقصت قيم الحديد الميسر كيميائيا مع زيادة عمق التربة، كما نقصت أيضا مع زيادة المسافة جانبيا عن مركز الأصص (منطقة إضافة سماد الحديد) وكانت أعلى القيم للحديد الميسر كيميائيا فى منطقة الريزوسفير.

- ازدادت حركة وتيسر الحديد فى الأرض الجيرية المستخدمة مع إضافة الحديد المخلبى Fe – EDTA مقارنة بالمعدنى $Fe\,SO_4$ او الكنترول (بدون إضافة Fe) .

- كانت أعلى القيم لمحصول الحبوب والقش لنبات الشعير المسمد بالحديد المخلبى وكذلك تركيز الحديد فيه والبير تركيز الحديد النشط فى أوراق النبات اتجاها مشابها للحديد الكلى.

وخلاصة القول ان النباتات، أحادية الفلقة مثل الشعير و خاصة الأصناف عالية الكفاءة من حيث الاستفادة من الحديد يمكنها أن تجعل الحديد أكثر تيسرا تحت ظروف الأرض الجيرية .