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Effect of wheat (*Triticum aestivum* L.) rhizosphere on fractionations of copper in some sewage sludge amended soils

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Abstract. Our purpose was to quantify the effect of rhizosphere processes on the fractions of Copper (Cu) in 10 soils amended with sewage sludge under greenhouse conditions by using a rhizobox. For amended soils, 1% (w/w) of sewage sludge was added to soil samples and then amended soils were incubated at field capacity, for 1 month. After incubation, soils were put in rhizobox and seeds of wheat were planted. Plants were harvested after 8 weeks and rhizosphere and bulk soils were separated. Fractions of Cu in the rhizosphere and bulk soils were determined. The results showed that Cu extracted using several extractants in rhizospheric soils were significantly ($P<0.01$) lower than in bulk soils. In the rhizosphere of amended soils the average of residual Cu, Cu associated with iron-manganese oxides, Cu associated with organic matter, Cu associated with carbonates and exchangeable Cu were 18.8, 2.10, 1.00, 0.37 and 0.24 mg kg^{-1} respectively, whereas above fractions in the bulk soils were 18.1, 2.43, 0.80, 0.42 and 0.30 mg kg^{-1} respectively. This study illustrated that Cu-fractions in the wheat rhizosphere were different compare to bulk soils in sewage sludge amended soils.

Key words: Copper; Rhizosphere; Sewage sludge; Cu-fractions

Introduction

Rhizosphere is commonly defined as the zone where root activity influences significantly the biological and chemical properties of the soil (Zoysa et al., 1999).The conditions at the rhizosphere are considerably different from those existing some distance from the root system (Nye, 1981). For this reason many researchers have focused on this zone in addressing issues concerning metal fractionation and bioavailability using various kinds of cultivation devices (Youssef and Chino, 1989; Wang et al. 2001; Tao et al. 2003).

Application of sewage sludge to agricultural soils has many advantages, which include providing a whole array of nutrients for plant growth (e.g., micronutrients and organic matter), increasing beneficial soil organisms, reducing the need for fertilizers and pesticides, and improving soil physical and biological properties (Su and Wong, 2003). Land application of sewage sludge has been limited by its enriched heavy metal contents including Cu (McCartney et al. 2001). In order to evaluate the amount of copper in the soils amended with sewage sludge, it is essential to understand its bioavailability which depends on its form in the soil, rather than on the total amount accumulated (Allen, 1997).

The dependence of metal bioavailability on its chemical speciation is well documented. On the other word, the complexity of the soil–plant relationship may induce changes in the properties of the soil rhizosphere, consequentially the metal fractions in it (Levesque and Mathur, 1986). Some of researcher investigated the effect of rhizosphere on Cu-availability. Wang et al. (2002) stated that fractionation differences were observed for Cu between the wheat (*Triticum aestivum* L.) rhizosphere and bulk soil. Tao et al. (2003) investigated chemical forms of Cu in the rhizosphere and bulk soil of maize plant using rhizobox cultivation and sequential extraction techniques. Their results demonstrated that there were continuous changes in Cu fractions within the maize rhizosphere. This study was conducted to evaluate fractions of Cu in bulk and rhizosphere amended soils with sewage sludge.

Materials and Methods

Ten soil samples were collected from Chaharmahal-Va-Bakhtiari province, in central Iran. Particle size

Table 1. Selected properties of studied soils

Soil No.	clay	silt	$CaCO_3$	OC	pH	EC	CEC	Available Cu	Total Cu
	(%)					(dS m^{-1})	($cmol_c$ kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
1	55	40	28.7	0.72	7.8	0.13	20.9	1.18	24
2	53	44	35.6	0.30	8.1	0.13	19.3	1.50	23
3	49	39	29.4	0.51	7.9	0.12	22.5	0.86	19
4	46	42	26.4	0.71	7.8	0.14	21.6	1.12	18
5	41	42	32.2	0.54	8.1	0.13	16.0	1.07	18
6	37	44	32.5	0.80	7.6	0.16	15.6	0.85	17
7	25	33	41.0	0.47	7.7	0.21	11.5	0.54	15
8	38	55	23.1	1.19	8.1	0.24	17.9	1.30	21
9	48	46	11.3	1.16	7.8	0.25	18.5	0.89	21
10	49	46	14.8	0.97	7.9	0.23	17.9	1.41	25

Available Cu: Cu extracted using DTPA-TEA.

distribution, Electrical conductivity (EC), pH, Organic carbon, equivalent calcium carbonate and cation exchange capacity were determined by standard methods. Total and available Cu was determined using 4 M HNO_3 (Sposito et al. 1982) and DTPA-TEA (Lindsay and Norvell, 1978) respectively.

Value of pH in sewage sludge was 7.5. Electrical conductivity was 2.25 dS m^{-1}. Amount of organic carbon was 38%. Also, the available and total concentrations of Cu were 41 and 78 mg kg^{-1}, respectively.

A homemade rhizobox (Wang et al. 2001) was used to plant wheat. The dimension of the rhizobox was 200mm × 130mm × 200mm (length × width × height). The rhizobox was divided into three sections: a central zone or rhizosphere zone (30mm in width), which was surrounded by nylon cloth (300 mesh), and left and right nonrhizosphere zones (bulk soil) (50 mm in width). One percentage (w/w) of sewage sludge was added to soils, and then amended soils were incubated at field capacity, for 1 month. After incubation, soils were air-dried. Three seeds were sown per rhizosphere zone. Plants were grown under greenhouse conditions. The aboveground parts of the plants were first harvested as shoots. The rhizobox was then dismantled. The rhizosphere soil was sieved gently to remove the roots by keeping the root mass intact as much as possible. The soils taken from rhizosphere soil zone and two bulk soil zones of the left and right were mixed separately for further analysis.

In the bulk and the rhizosphere soils exchangeable Cu (EXCH-Cu), Cu associated with carbonates (CARB-Cu), Cu associated with manganese-iron oxides (OXIDS-Cu) and Cu associated with organic matter fractions (OM-Cu) were determined based on that employed by Tessier et al. (1979) and residual fraction (RES-Cu) was determined using 4 M HNO_3 (Sposito et al. 1982).

The significant differences of cu fractions, between rhizosphere and bulk soils were determined by using paired-samples t-test.

Results and Discussion

Selected chemical and physical properties of the studied soils are shown in Table 1. These results showed that there was a wide variation in the selected chemical and physical properties of the soils. Clay contents in all soils averaged 44 and ranged from 25 to 55%. The soils were alkaline and low in EC and organic matter. The OC ranged from 0.30 to 1.19%. The equivalent calcium carbonate contents varied from 11.3 to 41.0%. The CEC ranged from 11.5 to 22.5 $cmol_c$ kg^{-1}. The total Cu in all soils ranged from 15 to 25 mg kg^{-1}. Available Cu ranged from 0.54 to 1.50 mg kg^{-1}.

The results of the sequential extraction are presented in Table 4. The distribution of various fractions of Cu was similar for wheat rhizosphere and bulk soils. In the rhizosphere soils the mean of RES-Cu, OXIDS-Cu, OM-Cu, CARB-Cu and EXCH-Cu were 18.8 (83.54% of total), 2.10 (9.33% of total), 1.00 (4.43% of total), 0.37 (1.64% of total), and 0.24 (1.06% of total) mg kg^{-1} respectively, whereas in the bulk soils the mean of above Cu-fractions were 18.1 (82.23% of total), 2.43 (10.91% of total), 0.80 (3.61% of total), 0.42 (1.90% of total) and 0.30 (1.35% of total) mg kg^{-1} respectively. Sequential extraction or fractionation of Cu in soils is a useful technique for determining chemical forms of Cu in soils. Such information is potentially valuable for predicting bioavailability in agricultural and polluted soils (Tao et al. 2003). The results of table 5 showed that concentrations of Cu-fractions in the rhizosphere were significantly ($P<0.05$) different from concentrations of Cu-fractions in the bulk soils. In sewage sludge amended soils, the Cu associated with organic matter and residual fractions were increased 23.45 and 3.86% respectively, in the rhizosphere soil. Exchangeable Cu, Cu associated with iron-manganese oxides and Cu associated with carbonate decreased 20.0, 13.58 and 11.90% respectively, in the rhizosphere soils.

The decrease in exchangeable fraction was also reported by Youssef and Chino (1989). An explanation for the change in exchangeable Cu is the process of plant uptake (Tao et al., 2003). The exchangeable Cu may provide some indication of the form of Cu that is most

Table 2. Cu concentration (mg kg^{-1}) in different fractions in the rhizosphere (R) and the bulk (B) soils

Soil No.	EXCH-Cu		CARB-Cu		OXIDS-Cu		OM-Cu		RES-Cu	
	R	B	R	B	R	B	R	B	R	B
1	0.25	0.31	0.37	0.43	2.56	3.03	1.13	0.91	23.8	21.7
2	0.22	0.30	0.22	0.29	2.06	2.45	0.74	0.75	20.3	19.7
3	0.20	0.27	0.33	0.40	1.81	2.32	0.64	0.54	17.4	16.7
4	0.23	0.30	0.16	0.23	2.44	2.61	1.31	0.78	17.5	17.0
5	0.25	0.29	0.67	0.69	1.82	1.93	0.64	0.35	16.1	15.8
6	0.22	0.28	0.37	0.40	1.58	2.14	0.72	0.52	16.4	14.7
7	0.24	0.29	0.57	0.55	1.69	1.82	0.62	0.48	13.1	12.8
8	0.30	0.34	0.22	0.38	2.57	2.64	1.34	1.22	19.6	19.6
9	0.24	0.31	0.46	0.46	2.30	2.78	1.46	1.10	19.5	18.7
10	0.24	0.30	0.34	0.36	2.22	2.53	1.39	1.31	24.7	24.6
mean	0.24b	0.30a	0.37b	0.42a	2.10b	2.43a	1.00a	0.80b	18.8a	18.1b

Means in final row for different fractions followed by the different letters are significantly different ($P<0.05$).

available for plant uptake (Sparks, 1983). Decrease in the Cu associated with iron - manganese oxides, can be interpreted by reduction of oxides by organic acid secreted from the plant roots (Godo and Reisenauer, 1980). While the increase in fraction organically bound forms, may be mainly owing to the abundance of organic materials in rhizosphere. Moreover organic matter can redistribute Cu from soluble and exchangeable forms to Cu associated with organic matter and the residual fraction (Clemente et al. 2006). Clemente et al. (2006) reported that a humification of sewage sludge organic matter during incubation experiments tended to promote the formation of fulvic acids having a high capacity for Cu complexation. Such kinds of organic matter may result in slightly increased Cu availability (Sadovnikova et al. 1996). But the effect of sewage sludge on Cu mobility will depend also on the organic matter decomposition rate.

Conclusions

This study illustrated that Cu fractions were in the order of residual > associated with iron-manganese oxides > associated with organic matter > associated with carbonates > exchangeable in the wheat rhizosphere and bulk soils. Also, the results showed that concentrations of Cu-fractions in the rhizosphere were significantly different from concentrations of Cu-fractions in the bulk soils. The rhizosphere is a small zone but important environmental in soils with quite different chemical properties. The processes involved in the rhizosphere are rather intricacy, and many factors can influence the environmental behaviors of elements in the rhizosphere.

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