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Full Length Research Paper

Effect of biosolids application on soil chemical properties and uptake of some heavy metals by *Cercis siliquastrum*

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The objective of this study is to investigate the effect of three kinds of biosolids usage on soil chemical properties and uptake of heavy metals by *Cercis siliquastrum* leaves. The experiment was carried out in a completely randomized block design with seven treatments including: no fertilizer as a control (C), sewage sludge (SW), cow manure (CM), municipal solid waste compost (MC), mixture of sewage sludge and cow manure (50% SW + 50% CM), mixture of sewage sludge and municipal solid waste compost (50% SW + 50% MC) and mixture of cow manure and municipal solid waste compost (50% CM + 50% MC) at three levels of 0, 2.5 and 5 kg/shrub and three replicates in calcareous sandy loam soil at the botanical garden of Mobarekeh steel company. After 180 days, soil samples were collected from 30 cm depth and leaves assemblage. Results showed that the treatments had significant effect ($P < 0.05$) on organic matter content, pH, electrical conductivity and cation exchange capacity of the soil. Total diethylen-triamine pent acetic acid (DTPA) extractable concentrations of Zn, Fe and Pb increased significantly ($P < 0.05$) in biosolid treatments. Between unwashed and washed metals concentrations of leaves, significant differences were shown, so it appeared that the source of metals on leaves was mainly airborn.

Key words: Biosolids, soil chemical properties, heavy metals, *Cercis siliquastrum.*

INTRODUCTION

Land application of organic amendments like sewage sludge, municipal solid waste compost and animal manure is an excellent way of recycling both the nutrients and the organic matter contained in them. Apart from the agricultural use, this practice is becoming one of the most promising ways for the reclamation of soils with low organic matter content. However, the potential health risks associated with the presence of pathogens, heavy metals and organic pollutants are well known, as well as the short and long term effects that these contaminants have on soil, from the agronomic point of view (Sastre et al., 1996; Albiach et al., 2000; Vasseur et al., 2000).

Municipal solid waste (MSW) is largely made up of kitchen and yard waste, and its composting has been adopted by many municipalities (Otten, 2001). Composting MSW is seen as a method of diverting organic waste materials from landfills while creating a product, at relatively low-cost, that is suitable for agricultural purposes (Eriksen et al., 1999; Wolkowski, 2003). The quality of MSW compost is dependent on many sources of variation including the composting facility design, feed-stock source and proportions used, composting procedure, and length of maturation. In addition to the differences between MSW composts, when it is applied to different types of field soils, there are further variabilities in plant response (Hargreaves et al., 2008). The metal concentrations in the sewage sludge and municipal solid waste compost depend on several factors such as origin and

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Abreviations: **MSW**, Municipal solid waste; **EC**, electrical conductivity; **CEC**, cation exchange capacity; **OM**, organic matter; **SW**, sewage sludge; **CM**, cow manure; **MC**, municipal solid waste compost; **DTPA**, diethylen-triamine pent acetic acid; **MAC**, maximum acceptance concentrations.

Table 1. Some chemical and physical properties of soil profiles.

Texture	pH (1:2)	EC (dS/m)	CEC ($Cmol^+/Kg$)	OM (%)	Depth (cm)
Sandy loam	7.86	0.75	9.26	0.7	0 - 30
Sandy clay loam	8.11	0.72	10.51	0.53	30 - 60
Sandy loam	8.09	0.56	10.33	0.41	60 - 150
Sandy loam	7.93	0.52	8.20	0.51	0 - 30
Sandy loam	8.07	0.89	11.39	0.72	30 - 60
Sandy clay loam	8.11	2.72	11.39	0.49	60 - 150
Sandy clay loam	8.1	0.40	11.75	0.52	0 - 30
Sandy loam	8.05	1.13	11.93	0.58	30 - 60
Sandy loam	8.24	2.22	9.97	0.55	60 - 150

treatment processes of them (Hue and Ranjith, 1994).

Biosolids not only improve soil fertility and increase plant production but also change heavy metals availability. When using organic materials as soil amendments, the effect of their organic matter on heavy metals availability cannot be separated from other collateral effects on the soil properties, such as pH, redox potential, cation exchange capacity, soluble salts, etc. (Walker et al., 2003). Ortiz and Alcaniz (2006) reported that adding sewage sludge to the soil usually triggers a reduction in pH, which may also provoke an increase in the solubility of certain metallic elements. So the addition of heavy metals to the soil and their subsequent transfer to the food chain is one of the potential risks associated with this practice. This risk is increased by the effect that the addition of organic matter may have on the solubility of the metals. It has been shown that organic matter is one of the factors that govern the solubility of metals in the soil (McBride et al., 1997).

The calcareous soils in arid and semi arid regions of Iran are low in organic matter content and productivity. Farmers are extensively using sewage sludge as a cheap fertilizer and there are no regulations controlling sludge and compost quality or limiting rate application of them. Also, many research projects have studied the bioavailability of heavy metals in the soil, although most of them have been carried out on acidic soils or under laboratory or greenhouse conditions (Stacey et al., 2001; A´lvarez et al., 2003). Although valuable data have been obtained for neutral or acidic soils, less knowledge has been achieved in the context of restoration with sewage sludge, municipal compost and animal manures on soils rich in carbonates.

Thus, the aim of this experiment is to study the effects of application of three sources of organic matter: sewage sludge, municipal solid waste compost and cow manure applied to a calcareous soil on pH, electrical conductivity (EC), cation exchange capacity (CEC), percentage of soil organic matter (OM%) and uptake of heavy metals by *Cercis siliquastrum* leave.

MATERIALS AND METHODS

The experiment was conducted at the botanical garden of Isfahan Mobarekeh steel company of Iran. This garden is located approximately 80 km south west of Isfahan city in central Iran and lies between longitudes 51°25´ 19.5˝ and 51°25´ 25.2˝ E and latitudes 32°15´31.15˝ and 32°15´37.6˝N. The climate of the area is arid and the annual precipitation is 140 mm. The soil of the experimental plots was Typic Haplocalcids (USDA, 2006). This experiment was carried out in a completely randomized block design with seven treatments including: no fertilizer as a control (C), sewage sludge (SW), cow manure (CM), municipal solid waste compost (MC), mixture of sewage sludge and cow manure (50% SW + 50% CM), mixture of sewage sludge and municipal solid waste compost (50% SW + 50% MC) and mixture of cow manure and municipal solid waste compost (50% CM + 50% MC) and three replicates in calcareous sandy loam soil. Starting in January 2009, several rates (0, 2.5 and 5 kg/shrub) for each treatment of biosolids were applied in state of localized fertilization in distance of 50 cm from each shrub to plots of 38 × 24 m size and then done in ten irrigation periods.

Sampling and analysis

In 2009, before preparation of the treatments, soil samples were collected from the 0 - 30, 30 – 60 and 60 - 150 cm depth increments to determine soil chemical and physical properties (Table 1). Soil pH and EC were measured in a 1:2 (w/v) ratio of soil to water by a glass electrode. Particle size analysis was made using the hydrometer method. CEC was determined as described by Rhoades (1982) and OM by standard methods (Black 1965). Three biosolids were tested: aerobically digested municipal SW from the city of Isfahan, WC and CM. Also, biosolids pH and EC were measured in a 1:5 (w/v) ratio of biosolids to water. CEC and OM were determined like soil. Selected chemical properties of biosolids are given in Table 2.

In September 2009, soil samples were collected from 30 cm depth and analyzed like explained. About 200 g (fresh weight) of well developed leaves of *C. siliquastrum* were selected and collected. Plant samples were then divided into two subsamples. One subsample was thoroughly washed with running distilled water

Table 2. Some chemical properties of organic biosolids.

Property	Unit	Sewage sludge	Municipal solid waste compost	Cow manure
pH	-	7.0	8.1	7.9
EC	ds/m	6.0	10.2	12.3
CEC	$Cmol^{+}/kg$	40.2	44.3	21.5
OM	%	48.7	42. 5	38.5
Fe	mg/kg	36943	46264	14788
Zn	mg/kg	1460	588	66.3
Pb	mg/kg	110	100	55

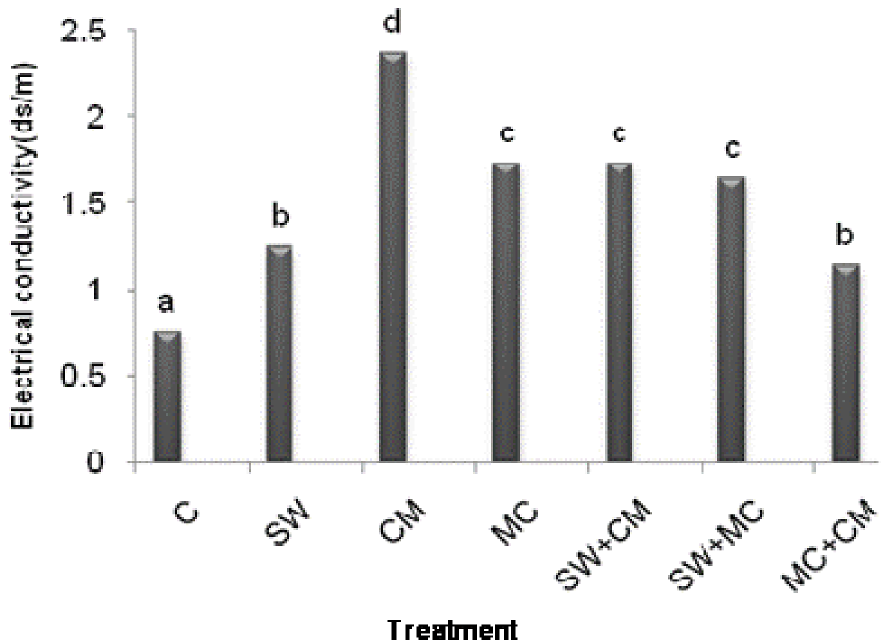

Figure 1. Effect of biosolids application on soil electrical conductivity.

to remove dust particles, the other remained untreated. All samples were dried in an oven at 70°C during 48 h, then milled, crushed to pass a 1.5 mm sieve and one gram digested with HCl (2 M). Total heavy metals in soils and biosolids were digested in nitric acid. The metal (Fe, Zn and Pb) contents of these solutions were determined by flame atomic absorption spectrophotometry (FAAS, BUCK model VGP 210). The plant available fractions of metals were determined by using diethylen-triamine pent acetic acid (DTPA) buffered at pH 7.3. The metal contents of these solutions were determined by FAAS (Lindsay and Norvell, 1978). Also metals transfer factor from soil to plant was obtained:

TF= $C_{plant}/C_{Total\text{-}soil}$

Where, TF, Transfer coefficient of metals from soil to plant, C_{plan}, metal concentration in washed leaves (mg/kg) and $C_{Total\text{-}soil}$, metal concentration in soil (mg/kg).

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 17 software (SPSS Inc.).

Data were also subjected to analysis of variance (ANOVA) and differences between means were determined using the Duncan and T test.

RESULTS

Effect of biosolids on some chemical soil properties

Application of the biosolids increased the EC of soil significantly compared to control soil (Figure1). Among treatments only SW, SW + CM and SW + MC had significant ($P < 0.05$) effects on soil pH. Sewage sludge reduced the pH of soil, whereas municipal solid waste compost increased the pH slightly but it was not significant (Figure 2).

Maximum OM% of soil was attributed to SW + CM treatment (Figure 3). All treatments had significant effects on soil CEC compared to control soil. Among treatments, sewage sludge had maximum CEC and did not show

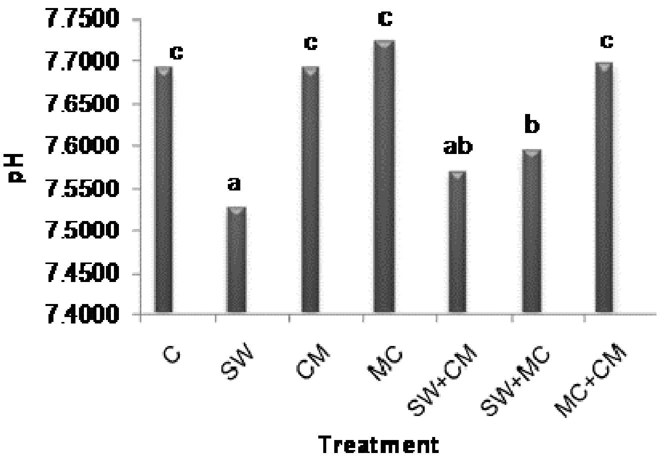

Figure 2. Effect of biosolids application on soil pH.

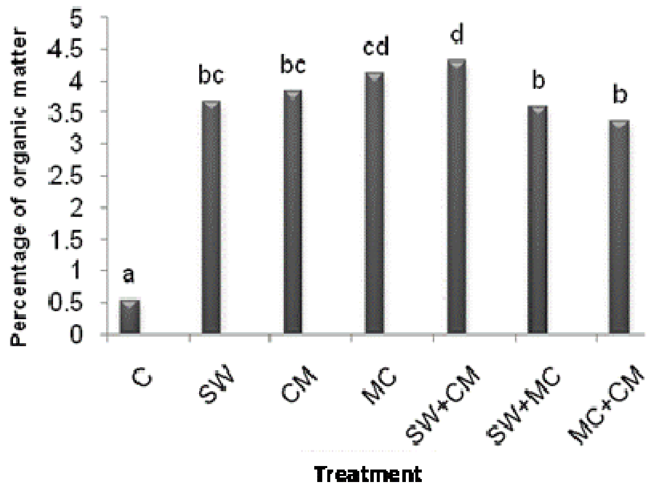

Figure 3. Effect of biosolids application on percentage of organic matter.

significant difference between SW, CM, MC and SW+CM (Figure 4).

Effect of biosolids on metals concentrations in soil

Total concentrations

Biosolids application significantly increased the total concentrations of metals in soil (Table 3).

DTPA-extractable concentrations

Table 3 gives a summary of the variation in the DTPA-extractable concentrations of metals in the soil. Biosolids application significantly increased the DTPA extractable soil Zn, Fe, Pb and maximum rates of them showed in

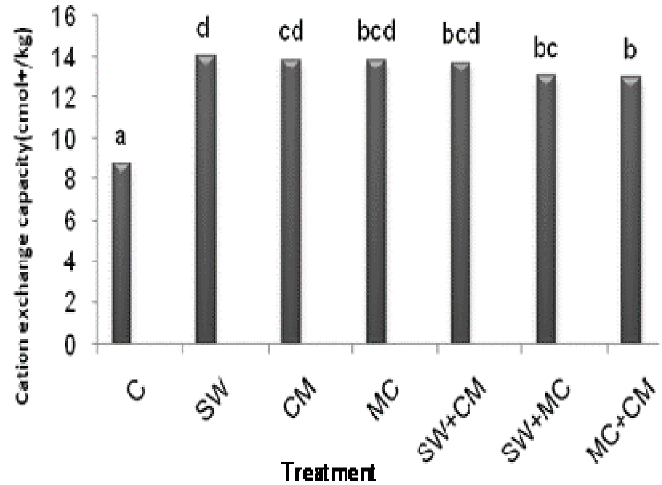

Figure 4. Effect of biosolids application on soil cation exchange capacity.

mixture of cow manure and compost, sewage sludge and compost treatments, respectively.

DTPA-extractable concentrations of Pb showed nearly close and significant correlations with total concentrations in soil (R^2 = 0.63), but this relationship was better described by quadratic equations for Pb and Zn. The R^2-values were 0.74 and 0.7, respectively (Figures 5, 6 and 7).

Effect of biosolids on metals concentrations of plant's leaves

Unwashed leaves

Unwashed leaves concentrations of Zn and Fe showed significant difference among treatments (Table 4), but biosolids application did not significantly affect unwashed leaves Pb concentration.

Washed leaves

Washed leaves concentrations of Fe and Pb were not significantly different among treatments (Table 5), whereas Zn showed significant difference with washed leaves of control soil. Maximum concentrations of Zn and Fe in washed leaves were found in cow manure treatment and for Pb at sewage sludge treatment.

Relationships between total and DTPA-extractable metals, soil properties and washed leaves of *C. siliquastrum*

The plant uptake of heavy metal from soil is influenced by soil properties, such as pH and organic matter (McLaughlinet al., 1999; Evangelou et al., 2004). Results

Table 3. Means comparitions effect of biosolids on total and DTPA-extractable concentrations of Zn, Fe and Pb.

Treatment	Total concentration (mg/kg)			DTPA-extractable concentration (mg/kg)		
	Zn	Fe	Pb	Zn	Fe	Pb
Control	49.2[a]	18855[a]	18.33[a]	0.87[a]	9.94[a]	0.75[a]
Sewage sludge	275[d]	2208[d]	30[b]	18.85[cd]	27.48[c]	2[b]
Cow manure	245.83[bcd]	21629[cd]	30[b]	15.23[bc]	21.87[bc]	2.59[bc]
Municipal Compost	176.81[bc]	20327[abc]	46.67[c]	18.68[cd]	25.5[c]	2.97[c]
Sewage sludge +cow manure	250[cd]	21669[cd]	31.67[b]	13.14[b]	18.57[b]	2.42[bc]
Sewage sludge+compost	168.47[b]	20910[bcd]	30[b]	17.12[c]	17.41[b]	2.38[bc]
Cow manure+ compost	234.29[bcd]	19474[ab]	25[ab]	21.45[d]	16.91[b]	1.87[b]

Treatments having the same letters within a column are not significantly different with the Duncan test at 5%.

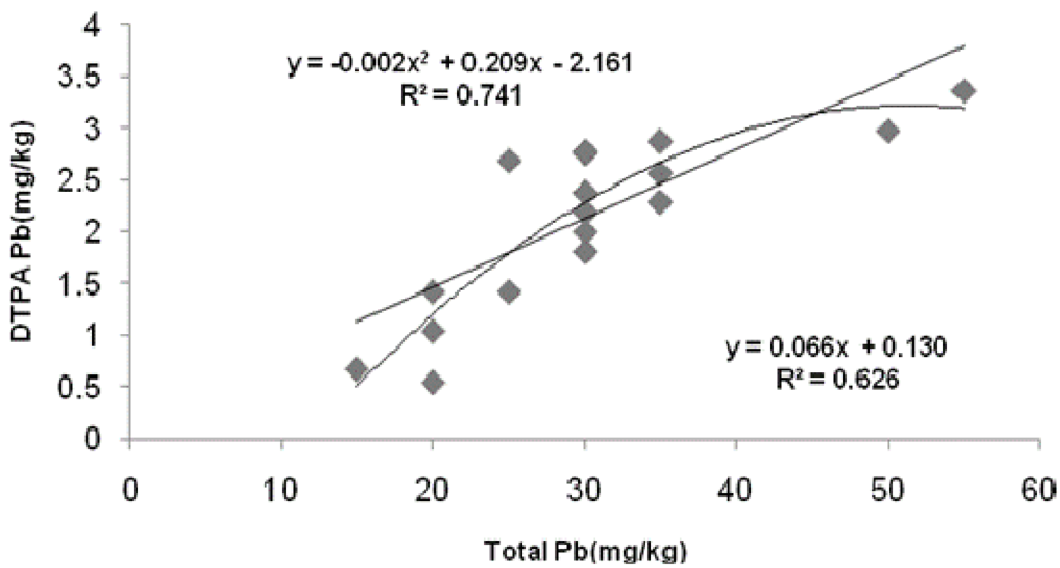

Figure 5. Relationships between total and DTPA-extractable Pb.

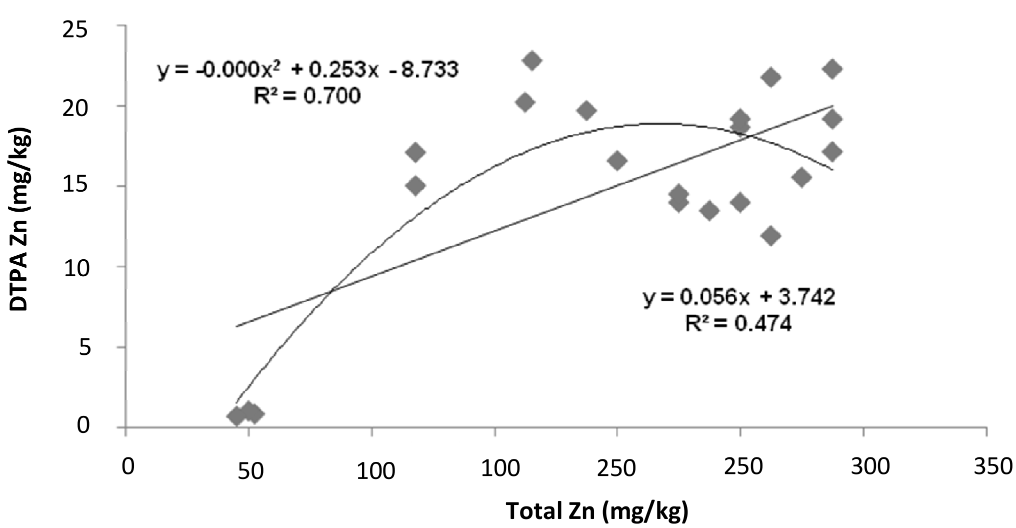

Figure 6. Relationships between total and DTPA- extractable Zn concentrations in soil.

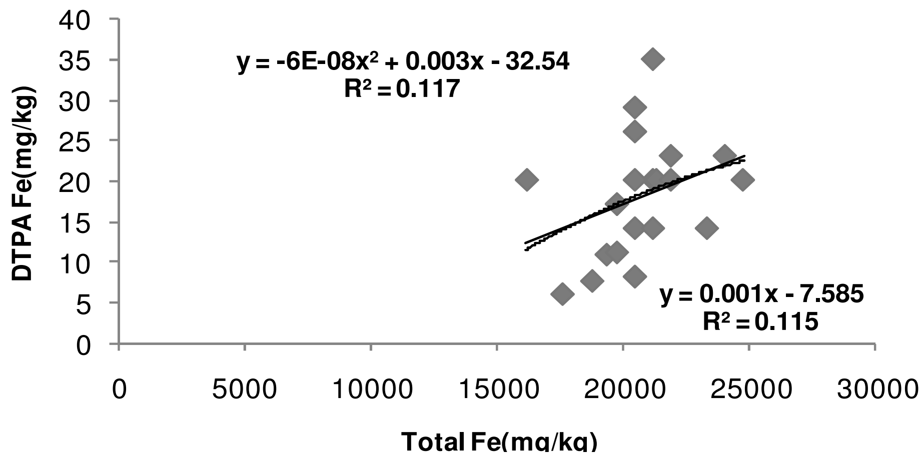

Figure 7. Relationships between total and DTPA-extractable Fe concentrations in soil.

Table 4. Means comparition of Zn, Fe and Pb concentrations (mg/kg) on unwashed leaves of *C. Siliquastrum.*

Treatment	Zn	Fe	Pb
Control	14.14^{a}	363.87^{ab}	6.21^{a}
Sewage sludge	18.77^{ab}	381.63^{ab}	10.55^{a}
Cow manure	20.45^{b}	382.06^{ab}	8.43^{a}
Municipal compost	17.51^{ab}	402.72^{b}	6.22^{a}
Sewage sludge + cow manure	20.03^{b}	332.33^{ab}	8.73^{a}
Sewage sludge + compost	20.04^{b}	403.86^{b}	7.14^{a}
Cow manure + compost	16.24^{ab}	252.03^{a}	6.35^{a}

Treatments having the same letters within a column are not significantly different with the Duncan test at 5%.

Table 5. Means comparition of Zn, Fe and Pb concentrations (mg/kg) on washed leaves of *C. Siliquastrum.*

Treatment	Zn	Fe	Pb
Control	12.04^{a}	224.19^{a}	3.6^{ab}
Sewage sludge	17.93^{b}	228.86^{a}	7.14^{b}
Cow manure	18.35^{b}	267.05^{a}	5.55^{ab}
Municipal Compost	14.99^{ab}	250^{a}	4.22^{a}
Sewage sludge +cow manure	17.93^{b}	264.95^{a}	5.89^{ab}
Sewage sludge+compost	17.93^{b}	225.14^{a}	4.22^{a}
Cow manure+ compost	14.56^{ab}	252.03^{a}	3.63^{a}

Treatments having the same letters within a column are not significantly different with the Duncan test at 5%.

of Pearson correlation analysis among soil properties, Zn, Fe and Pb of soil and washed leaves are shown in Tables 6, 7 and 8.

Zinc

A significant positive correlation was found between total Zn and soil CEC, OM and concentration of zinc on washed leaves. Also, DTPA-extractable of zinc had a positive correlation with its total concentration, CEC and soil organic matter (Table 6).

Between Zn and Fe, total concentrations in the soil showed significant correlation ($P < 0.01$). Also DTPA-extractable concentration of Zn had positive correlation with Fe and Pb DTPA- extractable concentrations (Table 10).

Table 6. Correlation matrix of soil properties, Zn in soil and Zn in leaves of *C. siliquastrum.*

Property	1	2	3	4	5	6
Total Zn (1)	1					
DTPA- Zn (2)	0.74**	1				
Zn in washed leaves (3)	0.64**	0.35	1			
CEC (4)	0.7**	0.71**	0.47**	1		
pH (5)	-0.30	-0.13	-0.56**	-0.39	1	
OM percentage (6)	0.71**	0.80**	0.38	0.87**	-0.24	1

*, **Correlation are significant at the 0.05 and 0.01probability level (two-tailed), respectively.

Table 7. Correlation matrix of soil properties, Fe in soil and Fe in leaves of *C. siliquastru.*

Property	1	2	3	4	5	6
Total Fe (1)	1					
DTPA- Fe (2)	0.34	1				
Fe in washed leaves (3)	-0.04	0.01	1			
CEC (4)	0.40	0.56**	0.22	1		
pH (5)	-0.58**	-0.03	-0.10	-0.39	1	
OM percentage (6)	0.26	0.57	0.19	0.87**	-0.24	1

**Correlation are significant at the 0.05 and 0.01probability level (two-tailed), respectively.

Table 8. Correlation matrix of soil properties, Pb in soil and Pb in leaves of *C. siliquastrum.*

Property	1	2	3	4	5	6
Total Pb (1)	1					
DTPA- Pb (2)	0.79**	1				
Pb in washed leaves (3)	0.09	0.08	1			
CEC (4)	0.52	0.66	0.43	1		
pH (5)	0.06	-0.06	-0.46	-0.39	1	
OM percentage (6)	0.51	0.77	0.22	0.87	-0.24	1

*,**Correlation are significant at the 0.05 and 0.01probability level (two-tailed), respectively.

Table 9. Correlation matrix of total concentration of Zn, Fe and Pb in soil.

Metal	Zn	Fe	Pb
Zn	1	0.55**	0.19
Fe	0.55**	1	0.09
Pb	0.19	0.09	1

*,**Correlation are significant at the 0.05 and 0.01probability level (two-tailed), respectively.

Iron

Correlation between total and DTPA-extractable of Fe concentration in the soil was not significant but total concentration of iron and soil pH had a significant negative correlation ($r = -0.58$). Also Fe DTPA-extractable concentration positively correlated ($P < 0.01$) with soil CEC and OM. Concentration of Fe on washed leaves of *C. siliquastrum* had no significant correlation with other parameters (Table 7). Between Fe and Zn, total concentrations in the soil was a significant correlation ($r = 0.55$). Also DTPA-extractable concentration of Fe had a positive correlation with Zn and Pb DTPA- extractable concentrations (Table 10).

Lead

Total and DTPA-extractable of Pb concentration had a significantly positive correlation ($P < 0.01$) and both of them correlated with soil CEC and OM significantly. A significant negative correlation ($r = -0.46$) was found between concentration of Pb on washed leaves of *C. siliquastrum* and soil pH (Table 8). Pb total concentration had no significant correlation with total concentrations of Fe and Zn in the soil (Table 9), but DTPA-extractable

Table 10. Correlation matrix of DTPA- extractable concentrations of Zn, Fe and Pb in soil.

Metal	Zn	Fe	Pb
Zn	1	0.53*	0.59**
Fe	0.53*	1	0.8**
Pb	0.59**	0.8**	1

*,**Correlation are significant at the 0.05 and 0.01probability level (two-tailed), respectively.

concentration of Pb had positive correlation with Zn and Fe DTPA-extractable concentrations (Table 10).

DISCUSSION

Sewage sludge application decreased soil pH slightly. This mainly was because of the high buffering capacity of this calcareous soil. The result was inconsistent with results of previous studies (Karami et al., 2009; Harding et al., 1984). Increased soil pH is regarded as a major advantage when MSW compost is used (Mkhabela and Warman, 2005). For example, increases in soil pH from 6.1 to 7.6 (Hernando et al., 1989), 5.8 to 6.4 (Maynard, 1995), 5.3 to 6.6, and 6.0 to 6.6 (Zheljazkov and Warman, 2004a), 4.9 to 5.8 and 5.1 to 5.9 (Shanmugam, 2005), and 5.8 to 6.7 and 6.1 to 6.5 (Zhang et al., 2006) have been reported. These increases were usually proportional to the application rate. The increase in the pH of soil may be due to the mineralization of carbon and the subsequent production of OH^- ions by ligand exchange as well as the introduction of basic cations, such as K^+, Ca^{2+}, and Mg^{2+} (Mkhabela and Warman, 2005).

The EC of the soil increased significantly with biosolids application and maximum value of EC was attributed to cow manure treatment because of the elevated EC of the applied manure. Differences between EC of treatments perhaps have been because of the different methods of the production of biosolids. For instance, at the separate processes of sewage from waste water, the major part of dissolved salts exit from sewage sludge, whereas with evaporation of water from manure fertilizers, concentration of salts increases (Koopmans et al., 2004). Other researchers reported that manure fertilizers have remarkable salts and frequent application in agriculture soil causes the accumulation of salt in soil (Eghbal et al., 2004).

The increase in organic matter and CEC after biosolids application could be explained by the large amount of organic matter in them and the high CEC of the organic matter. Although organic amendments application significantly increased the total concentrations of metals in soil (Table 3), the total Pb concentrations were below the respective limits of maximum acceptable concentrations (MAC) in agricultural soils for these metals in countries such as Germany, Canada and Poland (Pais and Benton Jones, 1997). However, the concentrations of total Zn exceeded the MAC for Zn in England (150 mg/kg) in sewage sludge treatment.

The addition of biosolids significantly increased the DTPA extractable soil Zn, Fe and Pb concentration compared to control due to higher metal contents (Table 3). Some authors also reported similar findings (Jordao et al., 2003; Karami et al., 2009 and McGrath et al., 2000). One aspect which should be taken into account is that the addition of sludge not only results in an increase in the total concentrations of metals, but also in the quantity of organic matter, which may have a direct effect on their solubility and bioavailability (McBride et al., 1997). A number of authors have agreed that the presence of organic matter increases the DTPA-extractability of the metals. It is probable that the organic matter existing in the sludge was the main reason why higher dosages of sludge were accompanied by a greater DTPA-extractability of practically all the metals studied (Ortiz and Alcaniz., 2006).The increase in Zn availability could be explained by the fact that this element in organic substrates is associated with the more soluble fractions of organic matter, such as non humic fractions of MSW compost (Sanchez-Monedero et al., 2004). Also Bell et al. (1991) found that DTPA-extractable metal content in fertilizer treated soil was higher than values in control treatment, which might be due to the presence of impurities usually found in fertilizer samples.

DTPA-extractable concentrations of Pb and Zn correlated significantly with the total concentrations of these metals in soil (Figures 5 and 6). It is worth noting, however, that for Zn, Fe and Pb, this increase in the DTPA-extractable fraction did not imply a higher concentration of these metals in the leaves. It is important to remember that the host soil had a high carbonate content and a basic pH. These conditions do not favour the solubilization or the bioavailability of the majority of the metals studied (Miller et al., 1995; Kunito et al., 1999; Martınez and Motto, 2000; Abollino et al., 2002). Jahiruddin et al. (1985) reported that in calcareous soils with high pH values, the formation of soluble organometallic complexes can be inhibited. Also the high CEC of this soil (Table 1) was probably one of the main factors limiting the solubility of the metals.

Table 11. Means transfer coefficients of Zn, Fe and Pb from soil to washed leaves of *C. siliquastrum.*

Treatment	Zn	Fe	Pb
Control	0.25	0.01	0.02
Sewage sludge	0.07	0.01	0.24
Cow manure	0.08	0.01	0.18
Municipal Compost	0.09	0.01	0.1
Sewage sludge + cow manure	0.07	0.01	0.19
Sewage sludge + compost	0.11	0.01	0.14
Cow manure + compost	0.06	0.01	0.15

Table 12. Mean heavy metals concentrations (mg/kg) in leaves of *Cercis siliquastrum* and comparison of unwashed and washed leaves by paired t-test.

Metal	Unwashed	Washed	T-Test
Zn	18.17 ±3.11	16.25 ±2.99	***
Fe	359.79 ±77.07	236.11 ±41.8	***
Pb	8.30 ±3.3	4.89 ±1.72	***

*** $P < 0.001$ significance.

In this study, whereas total and DTPA-extractable concentrations of Zn, Fe and Pb had no significant correlation with concentration metals of washed leaves of *C. siliquastrum* (Table 6, 7 and 8) and transfer coefficients of metals from soil to plant were low (Table 11), source of metals on leaves did not show anthropogenic activities. But because between unwashed and washed metals concentrations of leaves significant differences were shown (Table 12), it appears that the source of metals on leaves were airborn. It thus appears that the risk of transferring heavy metals into leaves could be considered less significant compared to the positive effects of biosolids applications to soil. Some of these effects increase in soil organic matter with a consequent increases on CEC, as well as increases in plant availability and uptake of micronutrients such as Zn and Fe.

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