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Effects of Waste Water Application on Heavy Metals (Mn, Fe, Cr and Cd) Contamination in a Black Locust Stand in Semi-Arid Zone of Iran

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Abstract: A case study was undertaken to assess the long-term effects of irrigation with municipal waste water on heavy metals contamination of soil and leaf of black locust (*Robinia pseudoacacia* L.) trees. For this purpose, a field study was conducted at two sites irrigated by waste water and well water in the suburban areas of Tehran, Iran. Samples of irrigation water, soil and tree leaf were collected and were analyzed for Mn, Fe, Cr and Cd concentrations. Results indicated that municipal waste water had higher significantly ($p<0.01$) amount of Mn, Fe and Cr compared to well water. These heavy metals in waste water were upon the internationally recommended (WHO) maximum permissible limits set for land use. Heavy metals accumulation of soil was significantly greater in waste water-irrigated site and in depth of 0-15 cm. The mean of heavy metals concentration in soil was below the standard for all heavy metals. Fe and Mn concentrations in leaf of trees irrigated with waste water were significantly greater than those in well water but without risk. Cr and Cd were not detected in leaves and also Cd in water and soil samples. It was concluded that the use of waste water in irrigation might enriched soils with heavy metals to concentrations that may pose potential environmental and health risks in the long-term. Hence regulations about the utilization of waste water in irrigation should consider for control heavy metals content that may be added to soil, in order to minimize the risk of negative effects to ecosystem health.

Key words: Afforestation, irrigation, *Robinia pseudoacacia*, heavy metals, waste water, soil pollution

INTRODUCTION

In suburban areas, the use of municipal and industrial waste water is common practice in many parts of the world (Sharma and Ashwath, 2006; Singh and Agrawal, 2008). Waste waters can be used for the restoration of degraded land and the growth of vegetation having commercial and environmental value (Madejón *et al*., 2006). In Iran, huge section of useful water of major metropolitan cities converts to the municipal waste water (Tajrishi, 1998). Since the deficiency of access to adequate water for irrigation is a matter of increasing concern and limiting factor to develop plantation, therefore municipal waste water could be utilized as an important source of water for expansion of tree plantation in and around the city and industrial complexes (Al-Jamal *et al*., 2000; Kalavrouziotisa and Apostolopoulos, 2007; Salehi *et al*., 2007). Beside, woody species by utilize waste water may uptake heavy metals through extensive root systems and accordingly reduces the toxicity of soil and plays an important role in safeguarding the environment (Stewart *et al*., 1990; Bhati and Singh, 2003).

Again, waste waters carry appreciable amounts of toxic heavy metals (Brar *et al*., 2000; Yadav *et al*., 2002; Salehi and Tabari, 2008) and concentrations of heavy metals in waste waters vary from city to city (Rattan *et al*., 2002; Aghabarati *et al*., 2008). Important sources of heavy metals in waste water are urban and industrial effluents. Heavy metals are extremely persistent in the environment; they are nonbiodegradable and nonthermodegradable and thus readily accumulate to toxic levels (Sharma *et al*., 2007). Long-term use of waste waters on lands often results in the build-up of the elevated levels of heavy metals in soils (Rattan *et al*., 2002; Larchevêque *et al*., 2006). Hence waste water irrigation is known to contribute significantly to the heavy metals content of soils (Nyamangara and Mzezewa, 1999; Nan *et al*., 2002; Singh *et al*., 2004; Mapanda *et al*., 2005). When the capacity of the soil to retain heavy metals is reduced due to repeated use of waste water, soil can release heavy metals into ground water or soil solution available for plant uptake (Sharma *et al*., 2007). In fact, the main problem for utilizing waste water in plantations is existence of the heavy metals, because these materials

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are accumulate in soil and absorbed in plant organs. High concentration of heavy metals affects mobilization and balanced distribution of the fundamental elements in plant organs via the competitive uptake (Schat and Ten Bookum, 1992). Thus, if waste water is to be recycled for irrigation the problems associated with using it need to be known (Emongor and Ramolemana, 2004).

A number of previous studies from developing countries have reported heavy metal contamination in waste water and waste water-irrigated soil (Cao and Hu, 2000; Nan *et al*., 2002; Singh *et al*., 2004; Mapanda *et al*., 2005). However, there are very few empirical data from Iran for heavy metal contamination of soil and irrigation water and its transfer to trees.

Black Locust *(Robina pseudoacacia)* is native to the southeastern United States, but has been widely planted and naturalized elsewhere. *R. pseudoacacia* has nitrogen-fixing bacteria on its root system, for this reason it can grow on poor soils, therefore it can improve fertility of soil. In Iran it often planted alongside streets, in green space and parks, especially in large cities, because it tolerates pollution well (Mossadegh, 1993). The use of municipal effluent in growing *R. pseudoacacia* in suburban areas could be beneficial for the economic disposal of waste water, defers ecological degradation by containing the pollutants in the soil and growth of vegetation having aesthetic and environmental value. The present study was carried out around Tehran, Iran, where waste water has been commonly used for irrigation of peri-urban crops for many years. The objective of the present report is to quantify concentration and accumulation of Mn, Fe, Cr and Cd in irrigation water, soil and leaf of black locust (*Robinia pseudoacacia* L.) trees from site having long-term use of waste water for irrigation of land.

MATERIALS AND METHODS

Site description: The study site is located in Shahr-e Rey, 5 km south of Tehran-Iran (Latitude 35°37' N, Longitude 51° 23' E, 1005 m above sea level). The climate of the site is semi-arid with mild-cold winters and 7 months (Mid April-Mid November) dry season. Average annual rainfall and average annual temperature are 232 mm and 13.3°C, respectively. The highest rainfall is in March and the lowest in August. The warmest month occurs in August and the coldest in January. Experiment was conducted at two even-aged (15 years) artificial stand of *R. pseudoacacia.* The first stand (4 ha) was being irrigated with municipal waste water and the second (1 ha) by well water since they were planted. The irrigation was applied daily based on tree water-use and the potential evapo-transpiration, which varied seasonally in response to the climate. The soils of two fields were clay-loam, with properties showed in Table 2.

Plant and soil sampling: The examination was made in October 2006. For the sampling of leaf and soil, four plots were randomly identified in each field. Plots were 30×30 m, with tree spacing of 3×4 m. In each plot, four trees were selected and in the growing season leaf samples of *Robinia pseudoacacia* trees taken from the top of crown and the part affected by sunlight (Letacon, 1969; Habibi Kaseb, 1992). This collection provided 16 leaf samples in each treatment. At the end of the sampling, one representative leaf sample from each plot (by mixing of four samples of each plot) was taken (decreasing of samples quantity for chemical analysis). Soil samples were collected under each selected tree at a depth interval of 15 cm down to 60 cm (Yadav *et al*., 2002; Tzanakakis *et al*., 2003) by digging profiles. This collection provided 48 soil samples in each treated field from three depths (0-15, 15-30 and 30-60 cm). At the end of soil sampling three representative soil samples of three depths from each plot were taken by mixing of samples of each layer in each plot (decreasing of samples quantity for chemical analysis) according to Habibi Kaseb (1992). Municipal waste water and well water were sampled daily (3 days in each month) from early June to late November, at three times per day (morning, noon and evening) to make a composite sample of each day.

Laboratory analysis: Concentrated HNO_3 was added to the water samples to avoid microbial utilization of heavy metals (Sharma *et al*., 2007) and then they were brought to the laboratory in resistant plastic bottles to avoid adherence to the container wall. Water samples were filtered through 42 mm filter paper and stored at 4°C to minimize microbial decomposition of solids (Yadav *et al*., 2002; Bhati and Singh, 2003). Some parameters were measured separately, pH and EC by the procedure described using OMA (1990) and Heavy Metals (Mn, Fe, Cr and Cd) of water samples were estimated by the aqua regia method of Jackson (1973) followed by a measurement of concentrations using an Atomic Absorption Spectrophotometer (AAS).

The soil samples air-dried, crushed, passed through a 2 mm sieve and were analyzed for various physico-chemical properties. Soil texture was determined using the hydrometer method according to Bouyoucos (1965). Soil pH and Electrical Conductivity (EC) were determined in 1:2 soil:water suspension by pH and EC meters (Hati *et al*., 2007). Soil Organic Carbon (SOC) content was determined by the Walkley-Black method (Nelson and Sommers, 1996). Calcium carbonate ($CaCO_3$) was measured with a calcimeter. Heavy Metals (Mn, Fe, Cr and Cd) soil content was extracted after digestion with 3:1 concentrated $HCl-HNO_3$ and measured by Atomic Absorption Spectrophotometer (Gascó and Lobo, 2006).

Samples of leaf were washed using tap water, rinsed with distilled water, oven dried at 80°C for 24 h (Singh and Bhati, 2005), ground in a stainless steel mill and retained for chemical analysis. For determination of heavy metals (Mn, Fe, Cr and Cd), the leaf samples were wet digested as per Jackson (1973) and were estimated using an Atomic Absorption Spectrophotometer (AAS).

Statistical analysis: Average leaf heavy metals and soil physico-chemical properties of two irrigation treatments (irrigation by municipal waste water and irrigation by well water), compared using independent-samples t-test (Pelosi and Sandifer, 2003). Heavy metals data of soil were analyzed for differences due to depth in the profile using one-way ANOVA. Furthermore, the variations in EC, pH and heavy metals of municipal waste water and well water were also tested using independent-samples t-test. All the data were analyzed using the SPSS statistical package (Lindaman, 1992).

RESULTS AND DISCUSSION

Physico-chemical properties of waste water and well water: The quality of municipal waste water and well water was assessed for irrigation with respect to their pH, EC and content of heavy metals (Table 1). Results indicate that the waters were alkaline in reaction. The pH of the waste water in various months ranged from 7.51-7.75 and for well water 6.69-7.62. The tolerance limit of pH for irrigation water ranged from 6.0-9.0 (Patel *et al*., 2004), Thus, pH of all the water samples is within the permissible limit. The electrical conductivity of waste water ranged from 1.78-2.12 dS m^{-1}. The pH and EC of municipal waste water was significantly ($p<0.01$) higher than those of well water. The concentration of heavy metals tended to be higher in municipal waste water. In water samples, Fe, Mn and Cr content were 0.73, 0.51 and 0.044 mg L^{-1}, respectively in groundwater samples, whereas, corresponding values for waste water were 3.30, 5.01 and 0.104 mg L^{-1}. On an average, waste water contained 8.67, 9.82 and 26 times higher amounts of Fe, Mn and Cr, respectively compared to groundwater. The concentration of these heavy metals in waste water was upon the internationally recommended (WHO) maximum permissible limits set for land use, which could prove to be toxic to soil and plant. Cd heavy metal was not detected in water samples.

Soil properties: Data of Table 2 indicated that application of municipal waste water were resulted an increase (0-60 cm soil layer; mean of soil layers) in pH, EC, C, organic matter and $CaCO_3$ of waste water-irrigated soil as compared to well water- irrigated soil. Increase in pH and EC of soil in the waste water-irrigated site may have been due to alkaline nature of municipal waste water (Mitra and Gupta, 1999; Munther, 2001). After pH and EC, soil organic carbon is the most important indicator of soil quality and plays a major role in nutrient cycling (Rattan *et al*., 2005). Increase in C content might be due to municipal waste water application (Kumar *et al*., 1998). Baddesha *et al*. (1985) also reported an increase in C of soil followed by the continuous use of waste water for irrigation.

The concentration of Mn, Fe and Cr (Cd was not detected in soil samples) was higher in all depths of soil in waste water-irrigated field (Fig. 1). As a matter of fact, high concentration of heavy metals in waste water leads to increase them in soil (Huerta *et al*., 2002; Nan *et al*., 2002; Mapanda *et al*., 2005). In this study, with waste

Table 1: Characteristics of municipal waste water and well water

Parameters	Municipal waste water	Well water	WHO*
pH	7.630±0.01[a]	7.320±0.05[b]	6.5-8.5
EC (dS m^{-1})	1.910±0.02[a]	0.590±0.008[b]	3.0
Fe (mg L^{-1})	6.330±0.12[a]	0.730±0.01[b]	3.0
Mn (mg L^{-1})	5.010±0.11[a]	0.510±0.09[b]	1.0
Cr (mg L^{-1})	0.104±0.005[a]	0.044±0.002[b]	0.05
Cd (mg L^{-1})	nd	nd	-----

Different superscripts in row indicate significant ($p<0.01$) difference. Values are mean of eighteen replications, (3 days *6 months) with±SE; nd: not detected, *World Health Organization: Hach (2002)

Table 2: Soil properties of two fields (0-60 cm)

Soil properties	Clay (%)	Silt (%)	Sand (%)	Texture	pH	EC (dS m^{-1})	C (%)	Organic matter (%)	$CaCO_3$ (%)
Waste water irrigated soil	30.27	36.00	34.55	Clay loam	8.17 (0.03)[a]	1.28 (0.04)[a]	0.718 (0.032)[a]	1.23 (0.05)[a]	20.20 (0.57)[a]
Well water irrigated soil	26.04	33.78	35.00	Clay loam	7.94 (0.10)[b]	0.763 (0.036)[b]	0.585 (0.062)[b]	1.00 (0.107)[b]	18.55 (0.45)[b]

Values are mean of four replications with±SD in parentheses; Different superscripts in column indicate significant ($p<0.01$) difference

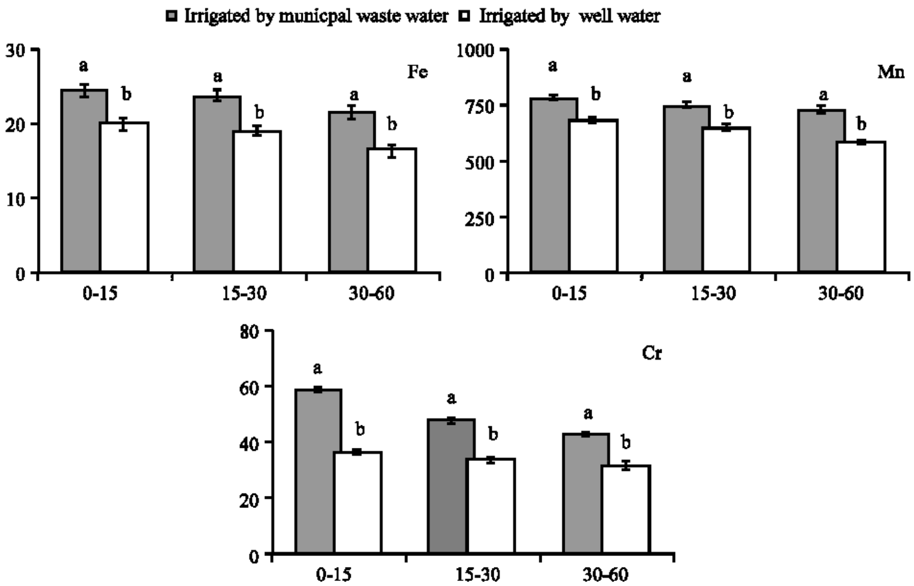

Fig. 1: Comparison of heavy metals in similar depths (0-15, 15-30 and 30-60 cm) between soils irrigated with waste water and well water (Fe in g kg^{-1} and Mn and Cr are in mg kg^{-1}); Error bars are ± SE

Table 3: Critical range of heavy metals in soil

Heavy metals	Critical range
Fe	5-50 (g kg^{-1})
Mn	200-10000 (mg kg^{-1})
Cr	500 (mg kg^{-1})

Fe and Mn: Salardiny (1992), Cr (EPA)

Table 4: Concentration of heavy metals in leaf of *R. pseudoacacia* irrigated with waste water and well water

Heavy metals	Waste water	Well water	p-value	Range*
Fe (mg kg^{-1})	110±4.56^a	91.87±3.59^b	<0.05	40-200
Mn (mg kg^{-1})	46.56±4.48^a	31.56±1.38^b	<0.05	20-100
Cr (mg kg^{-1})	nd	nd	-----	--------
Cd (mg kg^{-1})	nd	nd	-----	---------

Values are mean of four replications with±SD; different superscripts in rows indicate significant difference; nd: not detected; *Salardiny (1992)

water irrigation, concentration of heavy metals (Mn, Fe and Cr) did not reach to harmful range (Fig. 1, Table 3). In general, the effects of waste water irrigation on heavy metals accumulation of soil, depends on various factor such as concentration of heavy metals of waste water, period of irrigation, soil properties (pH, texture, cation exchange capacity ...) (Hodji and Jalalian, 2004). Generally, 10 to 50 years is needed so that the heavy metals levels precede the standard levels (Smith *et al.*, 1996). This is while that Ramirez-Fuentes *et al.* (2002) and Smith *et al.* (1996), respectively with studies of 4 and 17 years, showed that heavy metals concentration in waste water-irrigated soil did not vary markedly during these periods.

In our examination concentration of heavy metals decreased with soil depth in both sites (Fig. 2). These results are in agreement with the findings obtained later (Bansal *et al.*, 1992; Yadav *et al.*, 2002). Since, the soil surface was richer in heavy metals than the underlying layers, greater accumulation in the topsoil probably was due to soil texture (the soil texture in both areas was loam-clay, as a result penetrability is decreased and accumulation of heavy metals are often observed at upper lyres), low mobility of heavy metals in soil (Afyoni *et al.*, 1998) and surface application of municipal waste water and their retention in soil micelles (Singh and Bhati, 2005).

Heavy metals content in leaf of black locust trees: Results showed that only Fe and Mn elements were found in leaf samples, showing significantly ($p<0.01$) greater in trees irrigated with waste water than well water. Cr was detected in water and soil samples but not in leaf samples (Table 4). This may be due to the low dynamic of heavy toxic metals, whereas it was likely accumulated in lower parts of the plant, such as root and stem (Baldantoni *et al.*, 2004), Cd also was missing in leaf samples. Since main problem of the waste water-irrigated plantations is existence of heavy and toxic metals (Aucejo *et al.*, 1996; Bozkurt and Yarilga, 2003; Paula *et al.*, 2006), present results showed that irrigation with waste water was not led to toxicity in leaf of *R. pseudoacacia* trees. Generally, little quantity of micro elements in plants is not hazardous but is necessary; however, disturbance in plant nutrition may occur when absorption and accumulation of these elements increase in soil and plant (Woolhouse, 1983; Toze, 2006). In our study micro elements of Fe and Mn existed in waste water was favored for Black locust trees. The ratio of concentration Fe to Mn (2.45±0.66) in the leaf of waste water trees did not differ with its respective value

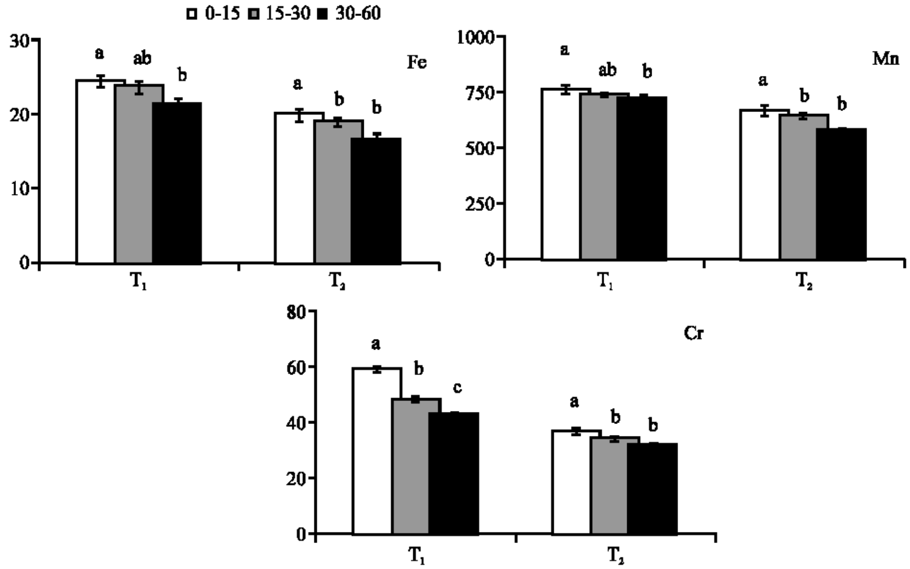

Fig. 2: Comparison of heavy metals among different depths (0-15, 15-30 and 30-60 cm) of soil in each irrigated field (Fe in g kg^{-1} and Mn and Cr are in mg kg^{-1}); T_1: soil irrigated by waste water, T_2: Soil irrigated by well water; Error bars are±SE

(2.99±0.22) (mean±SD) in the trees of well water treatment. These ratios suggested that the application of waste water did not have a negative impact on the balance of elements of trees leaf (Wheeler *et al*., 1992; Malkanathi *et al*., 1995).

CONCLUSION

Fe, Mn and Cr concentration in waste water was found above the permissible limit set by WHO for irrigation of land. Irrigation by waste water has increased the some heavy metals concentration in soil and plants of receiving field and although, these heavy metals were not still hazardous but long term and continuous use of waste water may lead to build-up of heavy metals in waste water-irrigated soils and plants in quantities above the maximum permitted concentrations. Hence, utilization of waste water in irrigation should be based on an accurate and controlled management and programs need to be monitored periodically in properties of water, soil and plant, in order to minimize the risk of negative effects to ecosystem health. Because of differences in conditions of climatic, vegetation, social, cultural and also changes in qualities of soil and sewage among the different regions and even through a time period in a region, just utilizing the world wide guidelines would be a mistake and in the long-term would damage the soil and water resources, therefore local researches need to be carried out.

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