

*Full Length Research Paper*

# **Heavy metal accumulation in *Melilotus officinalis* under crown *Olea europaea* L forest irrigated with wastewater**

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This study was conducted to investigate heavy metal accumulation in *Melilotus officinalis* under crown *Olea europaea* L forest in Rey town (Tehran, Iran), irrigated with wastewater and well water. Zn, Pb, Cr and Ni were determined at two sites. Heavy metal total concentrations ( $\text{mg kg}^{-1}$ ) in clay soils of pH 7.95 - 7.93 from site irrigated with wastewater were 187.3 for Zn, 78.4 for Pb, 82.83 for Cr and 46.00 for Ni in the 0 - 15 cm soil depths. The concentrations increased significantly in the forest compared with control soils. Heavy metal concentration in plants increased in site irrigation with wastewater. Zn, Pb and Ni exceeded their permitted limits in soils and Pb, Cr and Ni exceeded their permitted limits in roots of plants irrigated with wastewater. It was concluded that the use of wastewater in urban forest enriched the soils with heavy metals to concentrations that may pose potential environmental and health risks on the long term.

**Key words:** Heavy metals, *Olea europaea* L, *Melilotus officinalis*, wastewater, irrigation.

## **INTRODUCTION**

Heavy metals contribute to environmental pollution mainly because they are non-biodegradable, non-thermo-degradable and generally do not leach from the topsoil. Unlike petroleum hydrocarbons and litter that visibly buildup on soils, heavy metals can accumulate unnoticed to toxic concentrations (Bohn et al., 1985) that affect plant and animal life. The duration of contamination by heavy metals may be for hundreds or thousands of years, even after their addition to soils had been stopped. Metals added in small concentrations find specific adsorption sites in soil where they are retained very strongly, either on inorganic or organic colloids (Sauve et al., 2000). Wastewater use occurs either indirectly, when partially and untreated effluent is discharged into rivers that supply water for agriculture, or directly at municipal farms when partially treated sewage effluent is conveyed into some gardens. Past experience had shown that these developmental projects, created with the aim of

producing socio-economic benefits, have also produced adverse environmental impacts (FAO, 2000) such as land degradation. In suburban areas, the use of industrial or municipal wastewater is common practice in many parts of the world (Singh et al., 2004). The studies implicated land disposal of wastewater as the chief source of Cu, Zn, Cd and Pb enrichment of pasturelands. However, it has not been clear whether vegetable production sites irrigated with wastewater have also been enriched with heavy metals in the same magnitude or not. Although no cases of heavy metal poisoning due to the ingestion of vegetables irrigated with wastewater have been reported in humans, heavy metals remain important cumulative poisons (Kitagishi and Yamane, 1981). Soils, as filters of toxic chemicals, may adsorb and retain heavy metals from wastewater. But when the capacity of soils to retain toxic metals is reduced due to continuous loading of pollutants or changes in pH, soils can release heavy metals into groundwater or soil solution available for plant uptake. The amount of heavy metals mobilized in a soil environment is a function of pH, clay content, organic matter content, cation exchange capacity and other soil properties making each soil unique in terms of pollution

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management (Kimberly and William, 1999). With the exception of Mo, Se and As, heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes (Smith, 1996). Heavy metals are capable of forming insoluble complex compounds with soil organic matter and according to Sauve et al. (2000) solid-solution partitioning of Cd, Cu, Ni, Pb and Zn is dependent on soil solution pH, total metal content and soil organic matter.

The objectives of this paper is to determine the total concentrations of Zn, Pb, Cr and Ni, and estimate their annual loading rates in soil and pasture plants under crown forest at Rey town production sites where wastewater has been applied for 7 years. This would provide knowledge that guides future research into the protection of the environment and domestic animals from exposure to heavy metals with potential to cause health problems. Although total concentrations of heavy metals in soil poorly indicate their availability for plant uptake (Kimberly and William, 1999), existing permissible limits of heavy metals in soils are based on total concentrations. Thus, the information would be useful from a policy point of view. The objective of this study was to evaluate the impact of long-term land application of wastewater on soil and possible accumulation of heavy metals in the soil-plant system.

## MATERIALS AND METHODS

Two sites, irrigated with wastewater and well water are in the urban fringe area of Tehran city (Rey town), 5 km south of Tehran (35.37°N latitude and 51.31°E longitude, elevation of 1005 a.s.l.). Rey town has relatively mild-cold winters and semi-arid continental climatic zone. Average annual rainfall is about 232 mm (for the period 1993 - 2003, in Mehrabad Station) with maximum during winter the highest precipitation falls in March and the lowest in August. Average annual temperature is 13.3°C. The forest of *Olea europaea* L located near river that contaminated by domestic and industrial effluent from Tehran and used for irrigation during 7 years. The crops commonly grown under crown *O. europaea* L include *Medicago sativa*, *Melilotus officinalis* and *Trifolium pratense* as forage crops. These crops grow wildly in study area and can be used for forage consumption. Crops grown at the two sites (irrigated with wastewater and well water) are furrow irrigated, during the dry month (Mid April- Mid November). The river flows through industrial and residential areas of Tehran receiving waste discharges at various points along its length. The soil is clay (according to US soil taxonomy) with 45% clay, 30% sand and 25% silt.

Wastewater and well water were collected from the sites monthly from May to November 2005 (In each month 7 days and each day 3 samples, at three intervals 0700, 1300 and 1900 hours to make a composite sample of each day). These samples were brought to the laboratory, filtered through Whatman 42 mm filter paper, and stored at 4°C (OMA, 1990). Each sample was analyzed for pH, electrical conductivity (EC) and concentrations of macro and micronutrients using standard procedures (OMA, 1990). Nitrogen and phosphorus were analyzed calorimetrically (Jackson, 1973). Zn, Pb, Cr and Ni were estimated by the aqua regia method of Jackson (1973) followed by a measurement of concentrations using an Atomic absorption Spectrophotometer (model: Philips AA-10).

Soil samples were collected from wastewater irrigated forest as well as from the well water irrigated (control) sites at Rey town. A

composite sample, made up of five sub-samples collected using a Dutch auger along zigzag paths (Zigzag sampling) to achieve randomness from each site. The samples were collected at 0–15 and 15–30 cm depths. Soils were collected in July 2005. After transportation to the laboratory, soil samples were air dried, crushed, and passed through 2-mm-mesh sieve and stored at ambient temperature before analysis of soil properties and concentrations of heavy metals. The texture of the studied soils at two sites was clay. Soil pH from all production sites was measured with a pH meter using the water method (McNeal, 1982). Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Organic C was determined by the modified Walkley Black method (Houba et al., 1989) with additional heat applied under reflux. Soil samples were digested for heavy metal analysis using the aqua regia digest method (Baker and Amacher, 1982). One gram soil for each sample, in duplicates, was transferred into a 100 mL digestion flask to which 10 mL of aqua regia (a mixture of concentrated HCl and concentrated HNO<sub>3</sub> in the ratio of 3:1) was added before covering the digestion flask with a watch glass and allowing the mixture to react overnight (for at least 12 h). The next day, the mixture was heated progressively and boiled under reflux for 2 h after which the digestion flask was cooled. The cooling column was rinsed with 15 mL of distilled water recovering rinse water in the digestion flask. The mixture was separated using a centrifuge at 1500 rpm for 5 min after which a supernatant solution was collected into a 50 mL volumetric flask before diluting to the mark with hot 2 M HNO<sub>3</sub>. The soil extract was analysed for Zn, Pb, Cr and Ni using an atomic absorption spectrophotometer (model: Philips AA-10).

In this study, two afforestations area (*O. europaea* L.) have been selected. *M. officinalis* was common species grown under crown forest. The first stand (20000 m<sup>2</sup>) was irrigated by municipal effluent and the second (10000 m<sup>2</sup>) by well water. The irrigations were carried on 8-day durations for 8 months/year (during April-November). Water, soil and plant samples (roots, leaves and stems of *M. officinalis*) were taken from all the sites. In each site, 50 plants were randomly selected. Leaves and stems and roots were separated and washed with tap water, rinsed with distilled water and dried at 80°C for 24 h. Dry mass of the leaves and stems and roots was recorded after oven drying the samples for 72 h at 80°C. Dried plant samples were grounded and retained for mineral analysis. Data were statistically analyzed using an SPSS package (Lindaman, 1992). Initially, normal distribution of the data was performed using the Shapiro-Wik's test. The difference between the concentrations of nutrient elements in the wastewater and well water, soils, leaves, stems and roots in the treatment and control site were assessed by the independent-sample t-test. All statistical analyses were carried out with the program SPSS 12.5 for Windows.

## RESULTS AND DISCUSSION

Heavy metal concentrations in irrigation water showed significant variations at two sites (Table 1). Soil pH and conductivity were higher at site irrigated with wastewater (Table 2). Organic matter content also showed a similar trend. The value was highest at site irrigated with wastewater and lowest at site irrigated with well water (Table 2). The t-test showed significant variations in different soil properties due to sites. All heavy metals; Zn, Pb, Cr and Ni were higher at site irrigated with wastewater. The highest mean concentrations of Zn, Pb, Cr and Ni were recorded at site irrigated with wastewater (Table 3).

Both sites showed significant effects on heavy metal concentrations in *M. officinalis* (Table 4). Concentration of Pb was highest in *M. officinalis*, followed by Zn, and then

**Table 1.** Main characteristics of municipal effluent and well water (mean $\pm$ SE).

Variations	pH	EC	Zn (mg/L)	Pb (mg/L)	Cr (mg/L)	Ni (mg/L)
Wastewater	7.63 $\pm$ 0.01 <sup>a</sup>	1.91 $\pm$ 0.02 <sup>a</sup>	3.3 $\pm$ 0.06 <sup>a</sup>	0.1 $\pm$ 0.02 <sup>a</sup>	0.1 $\pm$ 0.005 <sup>a</sup>	0.08 $\pm$ 0.007 <sup>a</sup>
Well water	7.32 $\pm$ 0.50 <sup>b</sup>	0.59 $\pm$ 0.008 <sup>b</sup>	0.73 $\pm$ 0.01 <sup>b</sup>	0.03 $\pm$ 0.02 <sup>b</sup>	0.04 $\pm$ 0.002 <sup>b</sup>	0.028 $\pm$ 0.005 <sup>b</sup>
WHO standard*	6.5-8.5	3	3	0.01	0.05	0.5

\*Source Hach, 2002.

**Table 2.** Effect of application of wastewater on soil physico-chemical properties.

Parameter	Soil depth					
	0-15 cm		15-30 cm		30-60 cm	
	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>
Type	Clay	Clay	Clay	Clay	Clay	Clay
pH	7.95 $\pm$ 0.016 <sup>a</sup>	7.68 $\pm$ 0.032 <sup>b</sup>	7.93 $\pm$ 0.075 <sup>a</sup>	7.67 $\pm$ 0.017 <sup>b</sup>	7.95 $\pm$ 0.014 <sup>a</sup>	7.71 $\pm$ 0.017 <sup>b</sup>
EC (dSm <sup>-1</sup> )	0.89 $\pm$ 0.05 <sup>a</sup>	0.69 $\pm$ 0.07 <sup>b</sup>	0.85 $\pm$ 0.068 <sup>a</sup>	0.64 $\pm$ 0.008 <sup>b</sup>	0.81 $\pm$ 0.085 <sup>a</sup>	0.63 $\pm$ 0.008 <sup>b</sup>
OM%	0.83 $\pm$ 0.13 <sup>a</sup>	0.37 $\pm$ 0.045 <sup>b</sup>	0.41 $\pm$ 0.038 <sup>a</sup>	0.24 $\pm$ 0.045 <sup>b</sup>	0.29 $\pm$ 0.06 <sup>a</sup>	0.11 $\pm$ 0.03 <sup>b</sup>

T1: irrigation with wastewater; T2: irrigation with well water. Values with different superscripts within column indicate significant ( $p < 0.01$ ) difference. Values are mean of three replications with  $\pm$ SE.

**Table 3.** Accumulation of heavy metal in soil irrigated with wastewater and well water (mean $\pm$ SE).

Soil depth	ZN (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Ni (mg/kg)
<b>0-15</b>				
Wastewater	187.3 $\pm$ 10.72 <sup>a</sup>	78.4 $\pm$ 2.01 <sup>a</sup>	82.83 $\pm$ 2.51 <sup>a</sup>	46.00 $\pm$ 1.52 <sup>a</sup>
Well water	94.66 $\pm$ 2.9 <sup>b</sup>	50.00 $\pm$ 2.04 <sup>b</sup>	34.66 $\pm$ 0.88 <sup>b</sup>	27.37 $\pm$ 1.54 <sup>b</sup>
<b>15-30</b>				
Wastewater	129.61 $\pm$ 3.17 <sup>a</sup>	53.04 $\pm$ 1.03 <sup>a</sup>	61.33 $\pm$ 0.88 <sup>a</sup>	36.41 $\pm$ 0.57 <sup>a</sup>
Well water	49.16 $\pm$ 5.08 <sup>b</sup>	23.02 $\pm$ 1.04 <sup>b</sup>	27.24 $\pm$ 1.15 <sup>b</sup>	20.24 $\pm$ 0.57 <sup>b</sup>
<b>FAO standard*</b>	150	50	100	30

Values with different superscripts within column indicate significant ( $p < 0.01$ ) difference. Values are mean of three replications with  $\pm$ SE.

\*Source FAO, 2000.

**Table 4.** Heavy metal concentrations in edible portion (leaves and stems) and roots of *Melilotus officinalis*.

Variations	ZN (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Ni (mg/kg)
<b>Roots</b>				
Wastewater	21.7 $\pm$ 1.45 <sup>a</sup>	30.5 $\pm$ 1.38 <sup>a</sup>	3.21 $\pm$ 0.2 <sup>a</sup>	3.81 $\pm$ 0.29 <sup>a</sup>
Well water	11.67 $\pm$ 0.71 <sup>b</sup>	13.33 $\pm$ 0.25 <sup>b</sup>	0.68 $\pm$ 0.32 <sup>b</sup>	1.21 $\pm$ 0.17 <sup>b</sup>
<b>Leaves and stems</b>				
Wastewater	13.3 $\pm$ 0.5 <sup>a</sup>	20.21 $\pm$ 0.31 <sup>a</sup>	0.40 $\pm$ 0.052 <sup>a</sup>	1.48 $\pm$ 0.17 <sup>a</sup>
Well water	9.17 $\pm$ 0.51 <sup>b</sup>	11.12 $\pm$ 0.31 <sup>b</sup>	0.21 $\pm$ 0.044 <sup>b</sup>	0.89 $\pm$ 0.17 <sup>b</sup>
<b>Permissible limits of SPEA standard</b>	50	0.5	1	2

Values with different superscripts within column indicate significant ( $p < 0.01$ ) difference. Values are mean of three replications with  $\pm$ SE.

Ni and Cr. The lowest concentration was observed for Cr. The trend of metal concentrations in the plants would be a function of trace metal content in the soil and their ab-

sorption in plant tissues.

The results of the field study showed that continuous application of treated and untreated wastewater led to

elevated levels of heavy metals in the soil and edible portion and root of *M. officinalis*. The concentrations of all the heavy metals showed spatial and temporal variations, which may be ascribed to the variations in heavy metal sources and the quantity of heavy metals discharged through the wastewater. The application of wastewater increased soil pH in comparison with well water irrigated sites. Past research (Zaranyika et al., 1993; Oloya and Tagwira, 1996) has indicated that the wastewaters applied for irrigation at the Pension, Crowborough and Mukuvisi sites have in most cases neutral to alkaline pH (6.5 – 8.0) in addition to the high concentrations of basic cations such as Ca, Mg and K. Oloya and Tagwira (1996) also found out that the pH of wastewater-irrigated soils in Harare ranged from 6.2 to 8.0, while the highest pH in the virgin soil was 6.4. The concentrations of Cr, Ni, and Zn in soil observed during the present study were also higher than those reported at Zimbabwe (Mapanda et al., 2005). Zn, Cr and Ni concentration was lower than permissible level in well water (Table 1). The elevated levels of heavy metal in wastewater may be due to effluents discharged from various heavy-metal-based industries such as the fabric printing (Cu, Cd, Zn, Ni, and Cr), battery (Pb, Mn, As, and Cd), and paint industries (Pb, Cd, Zn, Cu, and Pb). The higher Pb content in soil and plant irrigated with well water is due to cars near two areas. Our results of total Cr and Pb in soil generally agreed with those ranges found by Flores-Delgadillo et al. (1992), Siebe and Cifuentes (1995) and Ramirez-Fuente et al. (2002). Absorption and accumulation of heavy metals in plant tissue depend upon many factors, which include temperature, moisture, organic matter, pH, and nutrient availability (Sharma et al., 2007). Soil properties influencing heavy metal availability varied significantly between the depths. The site irrigation with wastewater showed higher organic matter content than the site irrigated with well water. Organic complexing molecules of low molecular weight (LMW) serve as carriers of micronutrients (Chen and Aviad, 1990); the presence of organic matter has been reported to increase the uptake of Zn in the wheat plant (Rupa et al., 2003). On the other hand, the binding metal–organic matter can be strong, with a high degree of irreversibility, which could make more difficult any future attempt of soil remediation (Poggi-Varaldo et al., 2001; Poggi-Varaldo and Rinderknecht-Seijas, 2003). The high nutrient input from irrigation water at these sites could result in relatively high growth rates and relatively high uptake of heavy metals as a result. Long-term application of N is known to be associated with an increase in plant uptake and bioavailability of heavy metals (Nambiar and Ghosh, 1984). It seems that many soil factors such as pH, organic matter, nitrogen bioavailability, soil moisture, and temperature have interacted to impact on uptake (sharma et al., 2007). The uptake and accumulation of Zn, Pb, Cr and Ni in *M. officinalis* were higher at site irrigated with wastewater. It can be predicted that LMW organic molecules act as a good carrier for Zn, Pb, Cr and Ni. The mean

concentrations of all the heavy metals in *M. officinalis* at site irrigated with wastewater were considerably higher than the values reported by Singh et al. (2004) in vegetable crops. This may be ascribed to the considerable variation in heavy metal uptake by different types of vegetables. In the present study, samples of *M. officinalis* only were collected, whereas Singh et al. (2004) analyzed a range of vegetable crops having different heavy metal absorption potentials. In the present study plant were harvested at maturity; no such information is available for the study of Singh et al. (2004), but if harvest occurred earlier, clearly less heavy metal absorption would have taken place. The comparison of mean values of heavy metals recorded in the plant material during the present study with SPEA standard showed that Pb, Cr and Ni concentrations were higher in roots of plant irrigated with wastewater and mean concentrations of Zn were below the permissible limits. This study clearly showed that consumption of edible portion of *M. officinalis* by the animal will not pose health hazards due to Zn, Cr and Ni contamination.

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