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EFFECT OF IRRIGATION WITH URBAN SEWAGE AND AQUEDUCT WATER ON HEAVY METALS ACCUMULATION AND NUTRITIONAL VALUE OF LUCERNE (*Medicago sativa* L.)

Gholamali Akbari[1], Mandana Dadresan[1], Fardin Khazaei[2] and Hossein Sadeghi[2]
[1]Department of Agronomy, Faculty of Agriculture, Aboureihan Campus, University of Tehran, Iran
[2]Seed and Plant Certification and Registration Research Institute (SPCRI), Karaj, Iran
E-Mail: f_khazaie2006@yahoo.com, m_dadresan@yahoo.com

ABSTRACT

This research was carried out to study heavy metals accumulation and nutritional value of Lucerne in three areas of Tehran's south in 2007 based on completely randomized block design in the form of split plot with 3 replications. The main factor in three levels was included sewage canals in Salehabad, Talebabad and Dehkheir areas; sub factor in two levels was involved sewage and aqueduct water (control). Heavy metal accumulation especially Cd in foliar parts of Lucerne at first harvest was 10 times more than second harvest while there was not found significant difference at Lucerne's second harvest irrigated by sewage water and planted at three different locations. Probably concentration of most accumulated nutrients in roots after first harvest to emerging new leaves at next harvest has decreased and resulted in low nutrient transition from roots to foliar organs. Application of wastewater irrigation compared to Aqueduct irrigation resulted in more Fe and Mn accumulation in foliar parts meanwhile different locations does not have significant effect on heavy metal accumulation. Overall agglomerated rates of Fe, Ni, Pb, Mn, Cr, Cd and Zn in stem and leaves of Lucerne was lower than critical rates in livestock food, so livestock nutrition by second harvest of Lucerne irrigated by waste water cannot be resulted in any nutritional damages. Thus the 2nd to 5th harvest of Lucerne can be devoted for livestock food supply and also first harvest can be considered for seed production. Results of this study demonstrated that sewage irrigation did not cause toxicity in the leaves. However, long-term sewage irrigation may result in the accumulation of some heavy metals in the soil and go through the plants more than permitted concentrations. The highest and lowest rates of Nutrient accumulation in Lucerne were attained from Talebabad and Dehkheir areas, respectively.

Keyword: lucerne, heavy metals, sewage, irrigation, pollution.

INTRODUCTION

Municipal wastewater is an important alternative source of water for irrigation. Using sewage irrigation for many countries specially those who are located in warm and dry regions has been prevailed. Around 80% of Iran's land is located in dry region and economizing in water resources by re-using of sewage can be useful for overcoming the water limitation problem. Evaluation of increasing urban sewage according to the annually growth population can be considered as an important issue for optimum use of wastewater. Excessive use of waste water will be resulted in heavy metal accumulation in soil (Hoodji, 2001). However, apart from plant nutrients, it may contain various potentially toxic elements and organic matters with highly harmful effects on human and animal health. Municipal wastewater contains relatively high amounts of sodium, which can be accumulated in the soil during irrigation with this wastewater and display toxic effects on the plants. If this wastewater is not disinfected or treated in stabilization ponds, it is highly contaminated with microorganisms. Therefore, the utilization of municipal wastewater for the irrigation of crops is associated with a number of risks. Very serious risks are those of crop yields reduction, crops quality deterioration, crops contamination with pathogens and intestinal helminthes. It is, however, possible to achieve high yields of crops without deterioration of their quality by using treated wastewater for the irrigation of crops under controlled conditions (Josef Zavadil, 2009).

Heavy metal accumulation in plants has multiple direct and indirect effects on plant growth and alters many physiological functions (Wool Hause, 1983) by forming complexes with O, N and S ligands (Van Assche and Clijsters, 1990). They interfere with mineral uptake (Yang *et al*., 1998; Zhang *et al*., 2002; Kim *et al*., 2003; Shukla *et al*., 2003; Drazic *et al*., 2004; Adhikari *et al*., 2006) protein metabolism (Tamas *et al*., 1997) membrane functioning (Quariti *et al*., 1997; Azevedo *et al*., 2005) water relations (Kastori *et al*., 1992) and seed germination (Iqbal and Siddiqui, 1992; Al-Hellal, 1995) There may not always be a strong relationship between the concentrations of trace elements in soil and plants (Siegel 2002), but there always exists a strong relationship between their concentrations in irrigational water and plants (Ahmad and Goni 2009; Sharma *et al*., 2006). Thus, the deposition of heavy metals in water bodies can doubly increase the human intake through food chain as well as through drinking water. Most of the surface discharge sources contaminate soil and water bodies under limited spatial range, aerial emissions being prone to long range transport, contaminate wider range of ecosystems especially down wind to emission sources (J. PANDEY, 2010).

Urban wastewater irrigation can be used as a nutritional element for plants, as well as fertilizers for the plants and also it will be resulted in water saving (Ramirez-Fuente *et al*., 2002 and 2002 Ratton *et al*., 2005).

It was reported that application of wastewater irrigation increased soil nutrient elements and thus increased soil fertility and convenient access to high concentration of nutrients which resulted in increment of growth and biomass of plants (Gardiner *et al*., 1995; Soumlnmez and Bozkurt., 2006; Yang *et al*., 2010 and Keller *et al*., 2002). Wastewater application as plant irrigation should be done based on proper management and consistent control (Bozkur and 0 Yarilga, 2003).

Nutritional elements in wastewater makes higher yield in crops. Transmitted heavy metals to the soil are a function of the clay, organic matter, and cationic exchange capacity (Azimi *et al*., 2006; Cobb *et al*., 2000; Taha *et al*., 2004 and Walker *et al*., 2003). It was reported that sewage irrigation caused cadmium adsorption for plant and animal as toxic rates. Also significant relationship between heavy metal concentration in sewage and its absorption in plant and fruit was observed (AL Enezi *et al*., 2006). This fact should not be ignored that wastewater irrigation can play a role as a factor for ecosystem disrupting in order to its high elements concentration (Yadav *et al*., 2002; Brar *et al*., 2000). Hence plants as first cycle of food chain have considerable influence on living things organisms, so influent agent of metal pollutants to food chain should be considered more than another food levels. In lands under wastewater irrigation in various countries, with heavy metal concentration in the assembly plant, average cadmium concentration was reported of heavy elements 0.07, chrome 0.16, lead 0.43 and zinc 39.2 milligrams per kilogram in brown rice (McGrath *et al*., 1997; Ngole *et al*., 2009 and Zheng *et al*., 2007). Nowadays, local research into non-conventional water resources such as wastewater in irrigated lands has become very important. Relying only on instructions provided in other regions of the world were proven wrong and resulted in irreparable long term damage to soil and water resources. Results in a research about industrial wastewater showed that none of the concentrations of elements Ni, Cd, and Pb were above the limit, but the limitations were noted for wastewater salinity, pH and high concentrations of some positively-charged ions (known as cations), and negatively-charged ions (known as anions) (Ramulu and Sree., 2002 and Zavadil, 2009).

This study, taking into account the cases discussed above, focuses on the effects of minerals' accumulation on the nutritional value of bean plants irrigated with wastewater in three regions of Tehran.

MATERIALS AND METHODS

For evaluation the effects of irrigation with sewage water and pure water, the experiment was conducted on the area south of Tehran (Rey) in 2007. A split-plot design based on randomized complete blocks with three replicates was used.

The main factor in three levels of sewage canals in Tehran were monitored for different soil and climates including: 1 - SHOOR river wastewater in the lands of SALEHABAD region (Agro-Industry JAMARAN), 2 - FIROOZABAD sewage canal (in agricultural lands of DEHKHEIR village), 3 - SORKHEH HESAR canal (in agricultural lands of TALEBABAD village). The type of irrigation method was selected as sub factor and was monitored at two levels: 1 - Wastewater, 2 - aqueduct water. One liter sample of wastewater was taken to measure the amount and concentration of heavy metals, Ec, PH and other cases and then submitted to the laboratory. Irrigation water requirements of plants were based on irrigation with wastewater combined (the three canals) with aqueduct water. Also entrance channels for the combined irrigation adjusted equally with adjustable valves. Soil samples from a sampling depth of 30 centimeters, transferred to the laboratory in air-dried and then crushed-in porcelain mortar, and were sieved to a thickness of two millimeters. To prepare extracts of minerals extracted from soil, a mixture was used of two acid including normal HCL 0.05 and sulfuric acid 0.025. For the study of dry-weather soil, five grams of air-dried soil was weighted exactly with the lab scale 0.01 grams and purred in a 100-ml glass jar, and mixed with 20 ml solution extractor for 15 minutes. The material was then filtered using filter paper 42 and was collected in a 50-ml beaker and then brought to the amount of 50 ml by mixing with solution extractor. Then, using the atomic absorption model B1100, the minerals were measured. Water samples were filtered by filter paper 42, and the suspended particles separated, as the minerals were measured with atomic absorption equipment. Samples of the plants were looked over for chemical analysis. Furthermore, samples were observed for one hour at normal temperatures and then in a 70°C oven for 72 hours to remove the plant's moisture completely. Finally, one gram of plant powder was placed in an urn, and positioned into an electric furnace for an entire day at temperatures of 500°C. Afterwards, ash was dissolved in five milliliters of 20-percent hydrochloric acid solution and filtered using filter paper 42. Extracts of the minerals were collected in a 50ml beaker, and filled with distilled water to 50 ml to check their atomic absorption. The mix was measured in samples of soil and plant dry powder, respectively 10 and 50 times of read numbers base of milligram element per kg. Densitometer apparatus was used to calculate the elements in water samples, according to mg per liter. The raw data was then compared with LSD test, after being run through the SAS software for statistical analysis.

RESULTS AND DISCUSSIONS

The impact of sewage water irrigation on heavy metal accumulation in Lucerne at different harvest stages

First harvest

The results showed that there was not significant difference between application of aqueduct water and wastewater irrigation and three different soils (Talababd, Dehkheir and Salehabad regions) from the respect of minerals accumulation (Fe, Ni, Pb, Mn, Cr and Cd) except Zn (Table-1).

The results of some researches indicated that the Zn, Cu, Cd and Cr concentrations in plant tissues has increased with sludge application (Frost *et al*., 2000; Pinamonti *et al*., 1997).

There was not seen significant difference between application of Aqueduct water and wastewater irrigation and also their interactions with different locations from the aspect of heavy metal accumulation in Lucerne foliar parts (Table-1). Lucerne storage roots probably have impeded heavy metal transition to foliar parts. This can be explained by the low heavy metal concentrations in the sludge and the high pH and lime content of the experimental soil.

Second harvest

Application of wastewater irrigation compared to Aqueduct irrigation resulted in significant accumulation of Fe and Mn in foliar parts meanwhile different locations does not have significant effects on heavy metal accumulation (Table-3). A meaningful different of Plant Total dry weight at first and second harvest in various planting area was observed (Table-4). Plant height, total dry weight and stem number during two harvest stages were affected significantly by Aqueduct water and wastewater irrigation (Table-4). Also Cd accumulation rate was influenced by various planting areas (Table-5).

Mn and Zn accumulation rates at first and second harvest and also Cd accumulation rate at second harvest were lower than permissible value and thus they cannot be resulted in Lucerne damages. Also it was noticed that Ni, Pb and Cr agglomeration rate at first and second harvest and also Cd gathering rate at first harvest were higher than permissible rate (1.5- 8 times) in Lucerne and can be resulted in sever damages. It was found heavy metal accumulated rates in sewage water was 2-4 times more than its concentration in aqueduct water that indicates more toxicity in Lucerne plants which irrigated by municipal water irrigation.

The agglomeration rates of Fe, Ni, Pb, Mn, Cr, Cd and Zn in stem and leaves of Lucerne was lower than critical rates in livestock food, so livestock nutrition with Lucerne irrigated by waste water cannot be resulted in any nutritional damages. Zavadil J (2009) reported irrigation with the secondary-treated wastewater did not result in a statistically significant increase in nitrates content or in other potentially harmful element contents assayed in the consumable parts of the vegetables and potato tubers.

Wastewater effects on vegetative and reproductive characteristics of Lucerne plant

Data made clear that heavy metal accumulation at first harvest was 10 times more than second harvest. By hence Lucerne total dry weight was affected by three different soils and sewage water irrigation. Plant height, total dry weight and stem number during two harvest stages was affected significantly by Aqueduct water and wastewater irrigation treatments (Table-4). The different between aqueduct water irrigation and sewage irrigation at three different locations were not significant. By the way created limitation via heavy metal agglomeration in foliar and underground parts through sewage irrigation might hindered plant height, sprout number per plant and dry weight accumulation (Tables 4 and 5).

Sing *et al*., (2008) by using wastewater irrigation for plants reported decrement in stem length, root length and leaf area. The present results are not in agreement with results of A. A. Azimi *et al*., (2006), which noted not significant increment of cadmium in radish roots by using wastewater irrigation.

Wahid and Ghani (2008) in their study concluded the use of soil containing heavy metals in the planting of vetch bean reduced the absorption of essential nutrients for plant growth, due to interference. So, the amount of dry weight, the leaf number and leaf area of vetch bean besides other biological factors reduced significantly.

Moreover Aiman *et al*., (2009) reported that sewage with heavy metals decreased shoot mass, root length and leaf area. They concluded that heavy metals can potentially be poisonous for plants and cause growth retardation and interference enzyme activity, due to absorption, transport and deposition in the plant stem.

Heavy metal accumulation and transition at three different locations

Accumulation and transition rates of elements in Salehabad region at first harvest (2-3 times more than second harvest) for Lucerne was nearly similar to Dehkheir region. Also agglomeration of heavy metals in Salehabad region at first harvest was 1.5-3 times more than second harvest. Accumulation and transition status of heavy metals from soils to Lucerne leaves in Talebabad region showed trends similar to Dehkheir and Salehabad regions at first and second harvest. Furthermore it was noticed that accumulated elements in Talebabad was 20-30% higher than Salehabad (Table-6).

The highest and lowest rates of Nutrient accumulation in Lucerne were attained from Talebabad and Dehkheir areas, respectively. It seems that less dynamic of heavy metals properties have decreased its transfer from root to shoot by passing the time. So the first harvest of these plants can be devoted as seed production and other harvests from 2-5 harvest can be considered as livestock nutrition in order to standard heavy metals agglomeration.

CONCLUSIONS

According to results and comparison of irrigation with sewage water at three different locations, the first harvest of these plants can be devoted as seed production and other harvests can be considered as livestock nutrition in order to standard heavy metals accumulation. During some years Lucerne can produce a large quantity of heavy metals in its deep storage roots that can be resulted in soil pollution and bioenvironmental problems in long term especially for other rotations so it is suggest after 4-5 years

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Lucerne planting the residuals moved from the field and land is cultivated.

Table-1. Variance Analysis of heavy metals accumulation in foliar parts of Lucerne at first harvest (ppm).

		Mean of squares						
SOV	**Degree of freedom**	**Cd**	**Mn**	**Cr**	**Pb**	**Ni**	**Zn**	**Fe**
Replication (block)	2	1.972 ns	221.267 ns	6.157 ns	51.336 ns	20.782 ns	36.132 ns	1749.502 ns
Main factor (a)	2	60.875 ns	304.894 ns	84.681 ns	16.151 ns	18.302 ns	595.292 ns	2411.42 ns
Error (a)	4	7.087	855.461	64.731	62.241	14.093	675.723 ns	7050.863
Sub factor (b)	1	0.180 ns	5.227 ns	1.176 ns	79.380 ns	5.120 ns	634.867 ns	10172.135 ns
Interaction A*b	2	28.012	550.304 ns	14.821 ns	91.812 ns	3.312 ns	1657.394 ns	37257.602 ns
Error (b)	6	74.663	897.305	10.352	56.674	8.756	1444.098	24029.928
Variance coefficients		22.01	22.56	22.94	28.79	24.80	26.07	21.16

*, **: Respectively significant at 5% and 1% Ns: not significant

Table-2. Variance Analysis of heavy metals accumulation in foliar parts of Lucerne at second harvest (ppm).

		Mean of squares						
Degree of freedom	**SOV**	**Fe**	**Zn**	**Ni**	**Pb**	**Mn**	**Cr**	**Cd**
2	Replication (Block)	3535.40 ns	75.071 ns	7.069 ns	13.905 ns	25.748 ns	442.672 ns	4.037 ns
2	Main factor (a)	13983.127 ns	251.350 ns	6.634 ns	255.485 ns	5.571 ns	320.282 ns	1.136 ns
4	Error (a)	5096.728	692.585	6.992	258.600	7.896	270.653	4.533
1	Sub factor (b)	43149.428*	177.976 ns	7.867 ns	145.630 ns	0.376 ns	1061.069*	4.705 ns
2	Interaction a*b	8603.121 ns	311.694 ns	16.041 ns	354.821 ns	27.891 ns	190.977 ns	1.402 ns
6	Error (b)	3402.360	555.671	6.314	95.711	15.479	128.839	2.949
	Variance coefficients	58.58	20.17	21.34	28.57	29.46	28.26	25.34

*, **: Respectively significant at 5% and 1% Ns: not significant

Table-3. Mean comparison of heavy metals accumulation at different harvest stages (ppm).

Plant organ	Heavy metals	control	Sewage water	Mean	Total
Foliar parts	Fe	212.889[a]	114.967[b]	163.925	327.856
First harvest	Mn	52.611[a]	37.256[b]	44.9335	89.867
Foliar parts					
Second harvest	Cd	2.65[b]	8.85[a]	5.75	11.50
Mean	-	89.3833	53.691	71.5361	-

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Table-4. Variance analysis of vegetative and reproductive traits of Lucerne plant.

Mean of Squares				
Degree of freedom	**SOV**	**Stem height**	**Total dry weight**	**Stem number**
2	Replication (Block)	9.55 $^{n.s}$	17.23 $^{n.s}$	17.17 $^{n.s}$
2	Main factor (a)	89.55 $^{n.s}$	378.85 **	17.17 $^{n.s}$
4	Error (a)	35.72	12.68	6.83
1	Sub factor (b)	144.50 *	159.61 *	26.89 *
2	Interaction a*b	38 $^{n.s}$	34.27 $^{n.s}$	5.39 $^{n.s}$
6	Error (b)	19.67	21.73	3.39
	Variance coefficients	9.19	12.76	25.10

Table-5. Means of heavy metals accumulation in Lucerne at different areas (ppm).

Different harvests	Region	Irrigation type	Ni	Pb	Mn	Cr	Cd	Zn	Fe
First harvest	Talebabad	Aqueduct water	5.3	6.7	38.5	63.5	3.2	59.1	192.3
		Sewage water	2.7	1.8	52.2	10.4	49	72.2	328
	Salehabad	Aqueduct water	1.9	1.4	13.6	0.84	0.41	22.9	58
		Sewage water	8.6	23.7	57.3	7.3	4	88.1	395.3
	Dehkheir	Aqueduct water	4.3	17.1	57.9	9.1	11.4	117.6	342.9
		Sewage water	6.8	23.9	59.7	11.1	6.2	70.1	256.9
Second harvest	Talebabad	Aqueduct water	5	6.7	43.8	4.1	0.7	76.7	145
		Sewage water	6.3	12.5	41	2.4	0.8	87.3	127.2
	Salehabad	Aqueduct water	10.1	-	38.5	3.8	0.5	86.1	139.4
		Sewage water	5	12	37.5	4.5	1	75	120
	Dehkheir	Aqueduct water	-	-	-	-	-	-	-
		Sewage water	6.1	21.3	52.1	2.2	1.1	78.5	126

Table-6. Transition rate of heavy metals from soil to foliar parts of Lucerne at different areas.

Experimental area and standard rate transition	Ni	Pb	Mn	Cr	Cd	Zn	Fe
Talebabad (First harvest)	0.2	0.05	12.7	0.52	1.75	5.8	32.8
Standard rate transition	-	-	-	-	-	-	-
Talebabad (Second harvest)	0.6	0.35	10	0.12	0.29	7.04	12.7
Standard rate transition	-	-	-	-	-	-	-
Salehabad (First harvest)	0.8	0.5	9.2	3.65	1.2	5.3	30.1
Standard rate transition	-	-	-	-	-	-	-
Salehabad (Second harvest)	0.5	0.3	6.04	2.25	0.52	4.5	9.16
Standard rate transition	-	-	-	-	-	-	-
Dehkheir (First harvest)	0.6	0.04	15.3	22.2	4.76	10.01	20.2
Standard rate transition	-	-	-	-	-	-	-
Dehkheir (Second harvest)	0.6	0.3	13.3	4.4	0.84	11.2	9.9
Standard rate transition	-	-	-	-	-	-	-

Table-7. Means of heavy metals accumulation in foliar parts of Lucerne at two harvests and three different areas (ppm).

Different harvests	Areas	Ni	Pb	Mn	Cr	Cd	Zn	Fe
First harvest of foliar parts	Dehkheir	5.583	12.700	58.867	10.133	8.850	93.900	299.917
Second harvest of foliar parts		4.417	20.750	42.983	3.350	1.633	74.017	131.167
First harvest of foliar parts	Talebabad	2.55	9.45	63.00	5.383	4.500	75.567	324.417
Second harvest of foliar parts		4.167	14.35	53.017	5.067	1.833	85.583	219.367
First harvest of foliar parts	Salehabad	5.567	10.683	4.117	2.717	2.650	77.938	284.683
Second harvest of foliar parts		6.100	7.7000	38.800	3.350	1.000	74.767	141.250

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