Heavy metal transfer to *Beta vulgaris* L., under soil pollution and wastewater reuse

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Abstract
The transfer of heavy metals under soil pollution wastewater reuse was studied in a Greenhouse experiment using a randomized block design, including 6 treatments of heavy metals. mixtures composed of Zn, Mn, Cd, Co, Cu, Cr, Ni, and Pb, where each metal was taking part in the mixture with 0, 10, 20, 30, 40, 50 mg/kg respectively, in four replications. The *Beta vulgaris* L (beet) was used as a test plant. It was found that the DTPA extractable soil metals, the soil pollution level as assessed by the pollution indices, and the pH, played an important role in determining the TF of the various metals studied, and hence, the extent of heavy metal transfer from soil to plant.

Keywords
Transfer factor; heavy metals; soil pollution; wastewater reuse

INTRODUCTION

With the rapid economic development, water shortage has become an important factor for the people's lives and the social development. The use of municipal wastewater for the irrigation of vegetables is a common practice in urban and periurban ecosystems of many countries (Chang et al. 2013). The application of treated wastewater for agricultural purposes has been evaluated as the most convenient recycling option for environmental and economic reasons. The treated wastewater contains plant nutrients and organic matter, but also contains variable levels of heavy metals. Plants have a natural ability to extract elements from soil and to translocate them between roots, shoot, and fruits. Studies have shown that long-term irrigation of wastewater considerably increased the contents of toxic heavy metals in soils (Singh et al. 2004). The transfer of metals from soil to plant root may be evaluated by means of the transfer factor (TF) (Cui et al. 2004), which under wastewater reuse may change considerably due to the variation of heavy metal pollution load caused by the heavy metal inputs entering the soil via wastewater long term reuse.
The purpose of the present work is to study and assess the transfer of heavy metals from a heavy metal enriched polluted soil to *Beta vulgaris* under the effect of wastewater reuse.

MATERIALS AND METHODS

The experimental soil was collected from the top layer (0 - 30 cm depth) from a non cultivated agricultural area. The soil was a light textured sandy loam (SL), suitable for the growth of root crop, as is the beet. It was slightly acid, with low electrical conductivity, medium in organic matter content, and of course lacking completely $CaCO_3$, with a volume weight compatible with its particle size composition (1.48 g/cm^3). Its heavy metal composition was very low. The soil's physical and chemical characteristics are reported in Table 1.

Table 1. Physical and chemical characteristics of the experimental soil

S %	**C** %	**Si** %	**pH**	**EC** mS/cm	**OM** %	**$CaCO_3$** %	**VW** g/cm^3
56	12	32	6.17	0.206	2.11	0.00	1.48
Cd mg/kg	**Co** mg/kg	**Cr** mg/kg	**Ni** mg/kg	**Pb** mg/kg	**$N\text{-}NO_3$** mg/kg	**P** mg/kg	**-**
0.042	0.424	0.026	2.777	0.856	25	6	-
K mg/kg	**Mg** mg/kg	**Ca** mg/kg	**Fe** mg/kg	**Zn** mg/kg	**Mn** mg/kg	**Cu** mg/kg	**B** mg/kg
75	260	>2000	20.09	2.32	33.58	88.55	0.25

S-sand, C=Clay, Si=silt, EC=electrical conductivity, OM-organic matter, VW- volume weight

Preparation of the pots

A quantity of soil was collected and transferred into the experimental site. It was sieved by means of a plastic 3 mm sieve. 10.5 kg of this soil with a moisture content 5% was transferred in each plastic pot of rectangular shape with dimensions 50x20x16cm (length x width x depth), corresponding to 10 kg of dry soil. Each treatment (T1, T2, ….T6) was composed of a mixture of heavy metals i.e Zn, Mn, Cd, Cu, Co, Cr, Ni and Pb. in the form of chemical compounds given in Table 2, the concentration of each metal being 0, 10, 20, 30, 40 and 50 mg per kg soil for the six treatments, respectively. The bottom of the pots was closed, so that there was no loss of metals due to leaching. Each of the above mixtures was replicated 4 times being applied in each of the four pots, respectively. After the preparation of 24 pots according to the above procedure, the sowing took place; the test plant used was the garden beet (*Beta vulgaris* L*)*. The plants were irrigated with treated municipal wastewater (TMWW).

Table 2. Chemical compounds used for the preparation of the experimental treatments.

$ZnSO_4 \cdot 7H_2O$	$MnSO_4 \cdot H_2O$	$CuSO_4 \cdot 5H_2O$	$Na_2Cr_2O_7 \cdot 2H_2O$
$Co(NO_3)_2 \cdot 6H_2O$	$Ni(NO_3)_2 \cdot 6H_2O$	$Cd(NO_3)_2 \cdot 4H_2O$	$Pb(NO_3)_2$

Chemical Analyses

Soil analysis

The soil samples were air-dried and sieved through a 2mm sieve. Soil samples were dried at 70^oC under ventilation for 48 hrs and they were analyzed by means of internationally-accepted classical methods, as follows: soil mechanical analysis by Bouyoucos method. Organic matter by the wet digestion procedure of Walkley and Black and calculated for the total organic carbon, $CaCO_3$ by the method of Bernard, pH and conductivity by pH-meter and a conductivity apparatus. Available soil P was analyzed by Olsen method. The exchangeable Ca^{2+}, Mg^{2+}, and K^+ by extraction with NH_4Ac, pH= 7.0, Ca and Mg by inductively coupled plasma atomic emission spectroscopy (ICP-AES), also K by flame photometer. Zn, Fe, Mn, Cu and heavy metals Cd, Pb, Co, Ni, Cr were extracted with DTPA (Soltanpour et al., 1998) method and measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The mean values of the heavy metals concentration determined in soil samples are given in Table 3.

Table 3. Mean concentration of soil heavy metals determined at the period of the beet harvesting

Treatments	Heavy metals in soil (mg kg^{-1})															
	Zn		Mn		Cu		Cd		Co		Cr		Ni		Pb	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
T1	4.058	0.851	82.157	9.105	95.924	11.851	0.932	0.502	0.700	0.246	0.038	0.009	2.696	0.618	2.087	0.570
T2	10.013	1.532	72.412	12.889	14.765	16.245	10.221	1.943	2.983	0.499	0.104	0.058	11.095	2.303	8.267	1.696
T3	17.515	3.496	61.299	11.142	31.034	17.698	20.507	3.386	4.803	0.896	0.205	0.090	18.984	3.735	15.991	2.951
T4	20.719	2.369	55.921	3.448	22.747	12.533	23.433	2.388	7.480	0.555	0.406	0.018	21.844	2.611	18.437	2.645
T5	30.680	6.876	69.692	15.361	39.222	26.461	37.925	6.653	15.527	3.923	1.325	0.381	32.858	6.163	28.994	4.202
T6	39.734	6.575	84.397	17.303	52.162	21.196	47.082	7.444	22.409	2.421	3.079	0.571	39.130	5.109	39.889	6.276

SD=standard deviation

Plant tissue analysis

Plant samples, were washed using standard procedure, dried at 70 ^{o}C and ground. 1.0 g was mineralized at 500 ^{o}C for 10–12 h and the ash was dissolved in a 5 ml 6N HCl solution. The micronutrients Fe, Mn, Cu, and Zn and the heavy metals Cd, Co, Cr, Ni, and Pb were measured by ICP-ES. The results obtained are reported in Table 4.

Table 4. Heavy metal concentration of the above ground plant part, beets and root dry matter of *Beta vulgaris* (µg/g).

Plant Analysis																
above ground																
Treatments	Zn		Mn		Cu		Cd		Co		Cr		Ni		Pb	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
T1	112.8	4.52	895.21	122.86	138.03	29.98	1.81	0.30	0.48	0.08	1.05	0.46	12.20	2.10	0.54	0.35
T2	131.71	14.56	611.91	36.43	87.16	4.41	106.25	11.77	3.73	0.71	2.31	0.54	57.11	7.18	1.91	0.65
T3	125.71	21.27	395.61	27.18	73.37	15.75	100.34	33.74	5.64	1.53	1.91	0.50	70.03	17.12	1.61	0.46
T4	94.55	7.24	412.76	66.90	69.45	12.46	63.18	14.34	7.42	2.59	2.09	0.28	69.55	10.99	1.79	0.56
T5	90.20	8.37	423.26	69.42	76.80	28.09	81.26	22.78	19.48	8.15	4.25	2.95	77.48	9.35	2.29	1.01
T6	102.32	13.95	498.38	78.85	90.67	8.64	109.49	38.92	42.38	20.76	7.40	5.12	96.77	27.91	3.59	1.26
beet																
Treatments	Zn		Mn		Cu		Cd		Co		Cr		Ni		Pb	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
T1	28.83	1.40	131.54	38.13	42.32	10.69	0.11	0.05	0.19	0.01	1.15	1.04	5.36	1.22	0.22	0.14
T2	45.75	3.66	126.62	22.33	45.7	7.53	14.00	1.86	1.37	0.33	0.99	0.14	16.94	3.22	0.45	0.21
T3	47.97	7.24	116.82	37.59	50.24	10.78	21.12	10.60	2.44	0.94	1.41	0.56	19.54	3.05	0.63	0.28
T4	68.33	37.88	145.62	39.55	83.13	23.16	30.85	5.08	4.54	1.95	2.85	2.21	29.19	4.43	1.45	0.94
T5	69.87		152.32		82.01		39.09		10.40		2.65		34.62		1.89	
T6																
root																
Treatments	Zn		Mn		Cu		Cd		Co		Cr		Ni		Pb	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
T1	63.01	8.61	580.22	134.51	354.01	78.83	0.70	0.16	8.04	1.89	22.35	7.01	69.05	6.83	6.30	3.80
T2	63.04	19.09	1025.21	306.27	562.23	252.80	46.80	15.57	21.95	4.99	43.06	10.70	96.07	30.86	18.01	4.37
T3	76.20	6.44	851.13	213.31	661.28	159.25	55.50	12.01	22.08	5.33	36.27	13.30	119.68	12.40	17.35	4.56
T4	89.33	11.16	740.16	216.95	630.37	40.51	75.74	23.61	25.72	5.56	60.39	42.13	140.30	28.44	14.80	3.35
T5	105.45	21.62	649.97	92.58	564.05	172.04	93.24	33.74	34.41	15.32	50.06	17.07	131.92	34.19	11.33	2.41
T6	102.35	22.73	689.69	110.81	401.04	121.25	115.37	20.86	62.80	20.51	45.31	16.89	146.58	35.12	16.89	5.89

TMWWs analysis

The TMWW was processed by the procedure given by APHA (1995) in "standard methods for the examination of water and wastewater" and the microelements and heavy metals were measured by means of ICP-ES (Soltanpour et al., 1998). The chemical properties of the wastewater are shown in Table 5.

Table 5. The chemical and physical properties of the wastewater used for irrigation of plants

Cd	**Cr**	**Mn**	**Ni**	**Pb**	**Cu**	**Zn**	**Co**
mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00015	0.00057	0.0094	0.0037	0.0032	0.002	0.06	0.00396
pH 25^{o}C	**EC** µmhos/cm	**COD** mg/l(O_2)	**BOD_5** mg/l(O_2)	**Precipitated solids** (ml/l)	**Suspended solids** mg/l	**N Kjeldhal** mg/l	**Total P** mg/l
7.2	642	25	11	<0.1	2	6.8	0.7

RESULTS AND DISCUSSION

The transfer of heavy metals from polluted soil, enriched with a mixture of metals, to *Beta vulgaris* L. roots was assessed by means of the Transfer Factor (TF,) in order to evaluate the plant capability to absorb the heavy metals under soil pollution. Transfer Factor is defined as the ratio of plant dry matter metal concentration (Mpc) to the concentration of the same metal in soil (Msc) (Cui et al. 2004).

$$\mathrm{TF} = \frac{\mathrm{Mpc}}{\mathrm{Msc}}$$

The variations in TF values for different heavy metals depend on the bioavailability of metals, their concentration in the soil, their chemical forms, the soil type, the differences in plant uptake potential and growth rates (Tinker, 1981; Wang et al., 2012).

Transfer Factor relation to soil heavy metal concentration

The relation between Transfer factor (TF) and DTPA extractable soil metals was found according to regression model to be antagonistic, i.e. with the increase of soil metal concentration the TF decreases, respectively (Figure 1). Thus, the highest TF value corresponds to the lowest soil metal concentration, the regression equations of this relationship could possibly be used for the calculation of TF based only on the data of soil metal analysis or of the plant analysis data.

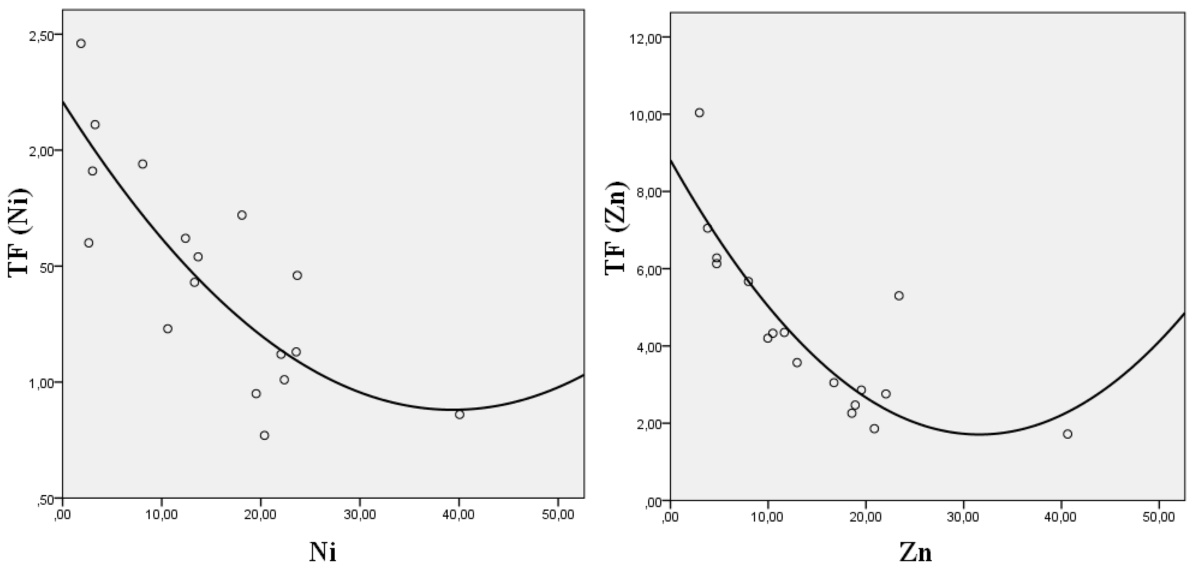


Figure 1: Relation of DTPA soil extractable Ni and Zn with the respective transfer factor (TF).

Relation between Transfer Factor and pollution indices

The pollution indices used as a tool for the evaluation of soil pollution level. The following soil pollution indices have been studied in the present work:

1st- Pollution Load Index (PLI), (Tomlinson et al., 1980)

$\mathrm{PLI} = \sqrt[n]{cf_1 \text{ x } cf_2 \text{ x ... x } cf_n}$

Concentration Factor (cf) of each metal was calculated as the ratio of the concentration of soil's heavy metal divided by the reference metal concentration obtained from uncontaminated soil. In the present study, the concentration of the soil metals under the applied treatments was higher than their corresponding reference values of the studied region, i.e. of Elias Prefecture, being as follows: Zn: 2.00, Mn: 80, Cu: 5.6, Cd: 0.052, Co: 0.47, Cr: 0.014, Ni: 0.75 and Pb: 1.8 mg/kg soil (Papaioannou et. al. 2015).

2nd- Elemental Pollution Index (EPI), (Kalavrouziotis et al., 2012)

$$EPI = \sqrt[n]{M_1 x\ M_2 x \ldots x\ M_n}$$

where M_1, M_2, $M_3 \ldots M_n$ are the concentrations of soil heavy metals involved in the pollution in mg/kg soil.

In table 6 and 7 the PLI and EPI values are reported. The study shows that both indices increase with the increase of heavy metals application to soil.

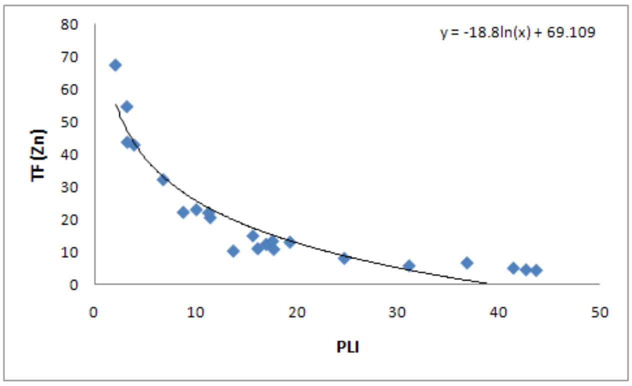
Table 6: Pollution Load Index values attained under the effect of applied heavy metal treatments

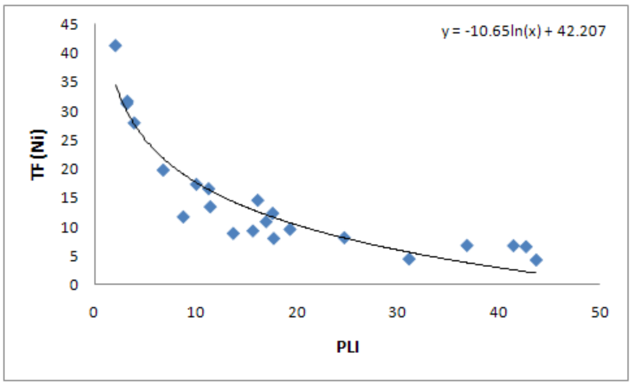
Treatments mg/kg soil	**Replications**			
	I	**II**	**III**	**IV**
0	3.95	3.29	3.25	2.10
10	10.08	11.42	6.82	8.71
20	16.94	13.80	15.65	11.29
30	17.72	19.35	17.60	16.15
40	31.08	36.81	24.69	24.11
50	42.66	43.66	41.41	31.01

Table 7: Elemental Pollution Index values attained under the applied heavy metal treatments

Treatments mg/kg soil	**Replications**			
	I	**II**	**III**	**IV**
0	3.54	2.95	2.92	1.88
10	9.04	10.24	6.11	7.81
20	15.18	12.37	14.03	10.12
30	15.88	17.34	15.77	14.48
40	27.86	32.99	22.13	21.61
50	38.23	39.13	37.12	27.79

The relationship between the pollution indices and TF of heavy metals from soil to plants showed that it is generally antagonistic (negative) (Figure 2 and 3) and Table 8. This relation could be explained on the basis of the reduction of metal plant concentrations due to the toxic effect of the increasing heavy metals concentration in soil due to the applied metal mixtures treatments.





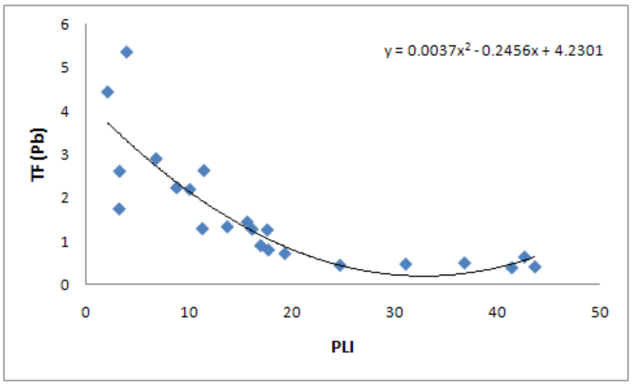


Figure 2. Relationship between PLI and TF (Zn, Ni, Pb) under the effect of the experimental treatments.

The antagonistic relation of TF to the heavy metal concentration decrease is also shown in table 8 which reveals that the TF of the metals Zn, Mn, Cu, Cd, Co, Cr, Ni, and Pb, decreases with the increase of the soil pollution as reflected by the applied treatments. This conclusion seems to be important in relation to the uptake of the essential elements by the plants under soil pollution. That is, the uptake of these nutrients must be expected to decrease with the gradual increase of the soil pollution level, as supported by the decrease of TF(Cr) and TF(Zn) with the increase of pollution indices EPI (Kalavrouziotis et al. 2012) and PLI by the decrease of TF(Ni), TF(Zn), & and TF(Pb) (Cambrera et al. 1999). This means that less heavy metal was taken up by the plants due to the toxic effect by the increasing concentration of heavy metals in the soil.

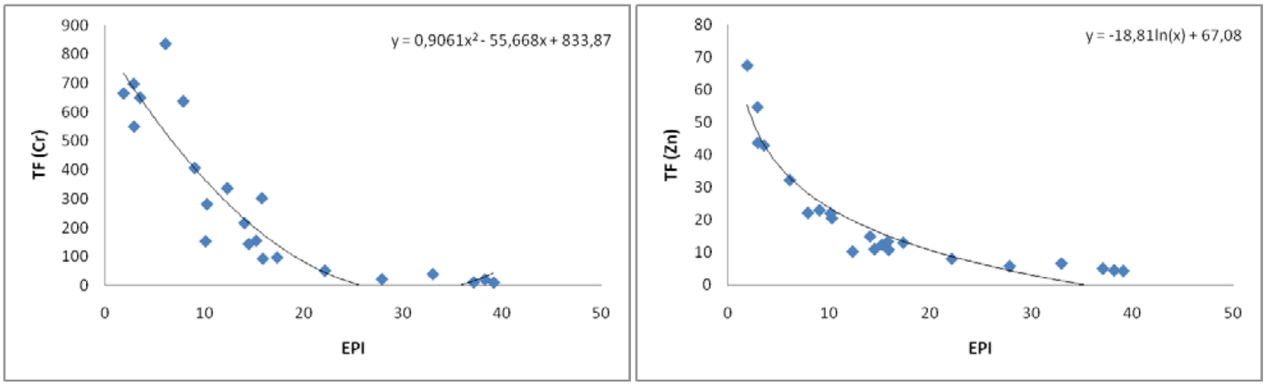


Figure 3. Relationship of pollution index EPI (Elemental Pollution Index) and TF (Zn and Cr) under the effect of the experimental treatments.

Table 8. The TF mean values of the heavy metals studied for the “above ground” and “whole” plant part of *Beta vulgaris*.

TF_{HM}		Treatments					
		T1	**T2**	**T3**	**T4**	**T5**	**T6**
Above ground plant	TF_{Zn}	28.99	13.48	7.63	4.60	2.99	2.38
	TF_{Mn}	11.04	8.60	6.68	7.43	6.07	5.38
	TF_{Cu}	1.45	0.77	0.58	0.57	0.56	0.57
	TF_{Cd}	2.89	10.82	5.09	2.72	2.15	2.21
	TF_{Co}	0.80	1.28	1.25	0.98	1.26	1.82
	TF_{Cr}	29.18	26.32	12.90	5.17	3.31	2.17
	TF_{Ni}	4.65	5.23	3.85	3.24	2.33	2.36
	TF_{Pb}	0.31	0.24	0.11	0.10	0.08	0.08

Whole plant	TF_{Zn}	17.41	8.20	5.01	4.05	3.10	2.39
	TF_{Mn}	6.57	8.29	7.48	7.78	7.17	6.42
	TF_{Cu}	1.85	2.07	2.07	2.13	2.00	1.56
	TF_{Cd}	1.32	5.63	2.94	2.44	2.16	2.25
	TF_{Co}	4.57	3.09	2.13	1.68	1.58	2.24
	TF_{Cr}	213.41	180.05	71.99	53.31	17.25	7.80
	TF_{Ni}	11.02	5.21	3.82	3.73	2.88	2.97
	TF_{Pb}	1.18	0.83	0.41	0.34	0.21	0.24

Transfer Factor and plant dry matter

In order to establish a more complete picture of the TF on the plant growth the relation of some of the main heavy metals TF with the beet and whole plant dry matter yields, was examined. Based on figure 4, the following conclusions are drawn: In the case of beets its seen that the maximum dry matter yield is attained at a value of TF(Zn) 10, of while in the case of TF(Cd) the maximum dry matter yield is attained as expected at the minimum value 0.25, obviously due to Cd's toxic effect the dry matter decreases to its minimum at the value of TF(Cd) 1.00. On the other hand in the case of the whole plant dry matter the maximum yield was attained at the value of TF(Co) 7 and TF(Pb) 1.8. These results suggested that the beet plant was more susceptible to Cd and more resistant to Co and Pb accumulation.

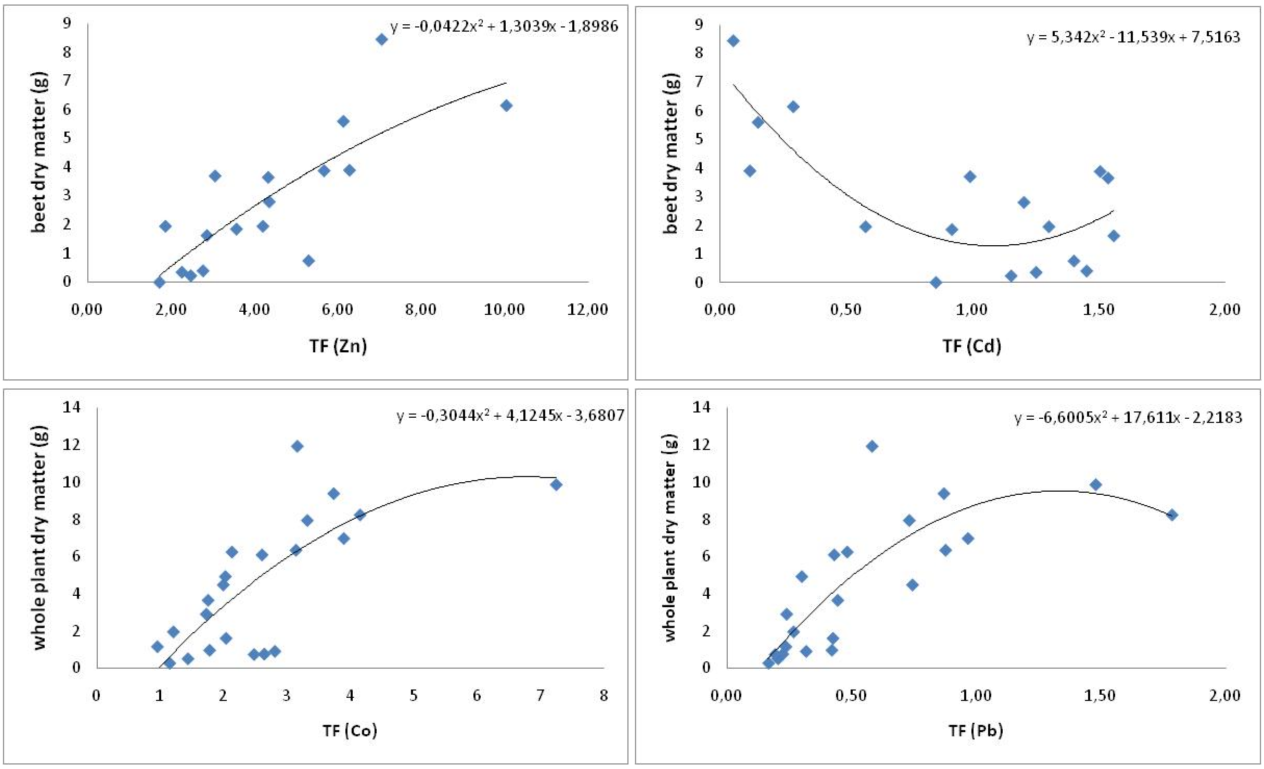


Figure 4. Relationship of TF(Zn), TF(Cd), TF(Co), TF(Pb) to dry matter of beet and whole plant, respectively.

Transfer Factor and pH

The heavy metals accumulations in the soil depend on different factors, the soil pH being an important parameter. Transfer factor is also affected variably by the soil pH, possibly depending on the kind of metal. Figure 5 shows that the TF(Zn) and TF(Cr) are affected positively by the increase of pH. Conversely, the TF(Cd) and oTF(Co) are influenced negatively.

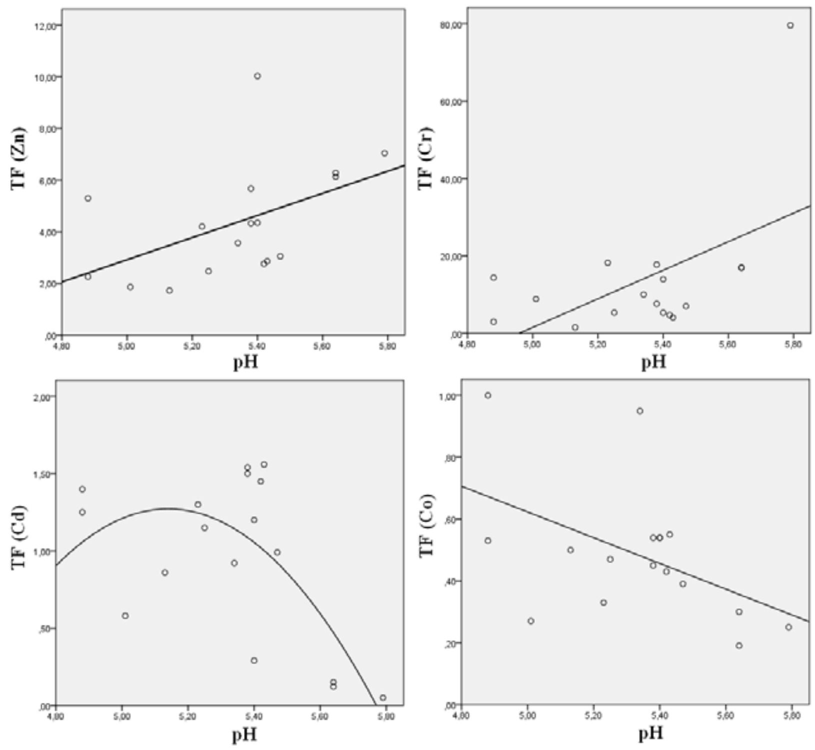


Figure 5. The effect of pH on Zn-TF, Co-TF, Cd-TF and Cr-TF in soil cultivated with beets under the influence of soil enrichment with heavy metals and wastewater reuse.

CONCLUSIONS

Based on the above findings, the following conclusions may be drawn:
The transfer factor (TF) of heavy metals under soil pollution and wastewater reuse was found to be related negatively with the DTPA extractable soil metals, whose concentration increase decreased the TF of the metals studied.
The applied treatments of the heavy metals mixture decreased statistically significantly the TF. Similarly, the increase of soil pollution level, as assessed by the pollution indices, decreased the TF which was generally negatively related to the increase of the pollution indices studied i.e Pollution load index, and Elemental Pollution Index. However, as to the relation of TF with pH it was found that the latter affected variably the values of the TF i.e. positively and negatively, possibly depending on the degree of solubility and bioavailability of the metals. Thus, the pH increase affected positively the TF(Zn) and TF(Cr), and negatively the TF(Cd) and TF(Co).

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