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Metal fixation under soil pollution and wastewater reuse

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ABSTRACT

The relation of heavy metals fixation to their bioavailability, beet plant uptake, plant dry matter yield, and pollution indices was studied under the effect of an artificially polluted soil. The experiment was conducted in a greenhouse, using a randomized block statistical design, including 12 treatments (T1, T2, … T12), each one being composed of a mixture of heavy metal combinations of Zn, Mn, Cd, Co, Cu, Cr, Ni, and Pb, where each metal was participating in the treatment with 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 mg/kg, respectively. It was found that the results obtained underlined the importance of the elemental interaction's impact on heavy metal fixation and on metal bioavailability, their plant uptake, plant yields, and pollution indices.

Keywords: Metal fixation; Bioavailability; Soil pollution; Wastewater reuse; Plant uptake

1. Introduction

The reuse of treated wastewater, which being a marginal one, due to the fact that it contains heavy metals, pharmaceuticals and xenobiotics, can lead to soil pollution after long-term application. In the context of these effects of reuse, the following questions are raised in relation to the fate of heavy metals: (i) what is the fate of metals in the soil under pollution, (ii) which are the factors affecting the mobility and bioavailability of heavy metals, and (iii) to what extent the heavy metals are being fixed under pollution and how the fixed metals affect the metal bioavailability, plant growth and yield, and soil pollution level?

According to Marques et al. [1], the metals in soil are subjected to a physicochemical process known as "fixation", which is related to various "mechanisms" such as sorption, according to which a metal ion is absorbed on the soil particle surface being removed from aqueous solution to solid phase. This process in reality includes: adsorption, surface precipitation and finally "fixation" [2]. Sorption of heavy metals may be affected by a variety of factors, the most important being the pH, type of metal speciation, competition, and soil inorganic composition.

Obviously, the metal fixation capacity of soil is not infinite. Thus, with the increase of heavy metal concentration in soil, the fixation capacity may be saturated. In such a case, the metals cannot anymore be fixed, and they can be either taken up by plants or leached away from the soil profile to the underground water.

The bioavailability of metals is significantly affected by their fixation. However, according to Chlopecka [3], metals are proportionately more bioavailable in contaminated soils than when their concentration is at the background levels.

Sesquioxides seem to be the basic soil components contributing to metal fixation [4]. These workers point out that the amorphous crystalline forms of the metal hydrous oxides exert a major influence on heavy metal fixation due to their strong affinity for heavy metals.

Many soil components or properties influence metal fixation. Organic matter (OM), for example, is related to the availability of heavy metals in soil due to their binding on the soil components, and also to the weak functional groups, which dissociate under alkaline conditions [5,6].

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Fixed metals are chemically inactive, contrary to their exchangeable metal forms, which are very active from the chemical and bioavailability point of view [7]. The mobility of positively charged metals decreases in the negative binding sites, while it increases with the decrease of pH due to proton competition with metals [8]. The increase of the mobility or of the bioavailability of metals, which are bound to OM, is due to dissociation of this soil component under alkaline conditions [6]. Heavy metal mobility may also be improved by high pH due to the competition of cations by organic complexing for soil surfaces. This effect is due mostly to pH dependent dissolution/precipitation and oxidation/reduction of the hydrated oxides in soil [4].

The soil pH is indeed an important factor affecting significantly the availability and the concentration of metals in the soil solution. At low pH, the solution of metal increases, while at higher pH decreases. At pH = 5, the adsorptive capacity of soil increases and becomes higher than that of the OM [9]. In general, sorption of metal cations increases with the increase of pH [10]. A factor of soil, which is related to heavy metal adsorption, is the particle size. Thus, the fine-grained soils absorb more heavy metal than coarse-grained. Soil minerals absorb metal ions by both the ion exchange and specific adsorption [11].

Regarding the absorption of heavy metals by the clay minerals, this absorption takes place in the lattice structure (specific adsorption) of the mineral. The metals are being fixed into pore spaces of the mineral structure.

Fixation of metals may also occur by precipitation. Violante et al. [12] reported that precipitation of Fe, Al, and Mn hydroxides may efficiently fix adsorbed trace metals. Similarly, the extent of mobility of some metals and metalloids such as As and Sb are influenced by biogeochemical processes. Also, some metal oxides are most effective in sorbing the cationic forms of heavy metals among phyllosilicates indicating that the sorption is specific, i.e., it takes place between the lattice layers. This type of sorption is the dominant binding mechanism [10]. Similarly, complexation reactions may have the following effects on heavy metals:

- Metal ions are prevented from being precipitated.
- Complexing agents can act as carriers for trace elements in soil solution.
- Complexation may often reduce the toxicity of metals.

The fixation of a given metal is generally inversely proportional to its bioavailability suggesting that with the increase of fixation the bioavailability decreases. This is due to the fact that fixation is leading to immobilization and hence to the decrease of its availability and metal uptake. The bioavailable fraction of the metal is mobile, and therefore accessible to plants and to food chain.

Since mobile or bioavailable metals can be easily taken up by the plants, under certain conditions they can accumulate in the various plant parts including the edible ones. The level of soil pollution may have a significant impact on plant metal concentration. However, other factors as well can affect the plant metal accumulation such as the chemical kind of the pollutant, soil physical and chemical properties and especially the species of plants and their genotypes, i.e., the plant growth characteristics [13].

The bioavailability of heavy metals is controlled by the following processes: adsorption/desorption, chemical complexation with either inorganic or organic legands, redox reactions, as well as by biotic and abiotic factors [10]. The bioavailable fraction of metals includes all those soil components, comprising a deposit (store) of potentially mobile metals, which are constituents of clays, oxides of Fe, Al, Mn, OM, and soil solution representing the soluble fraction, which contributes to the transfer and circulation of metals in the soil profile [14].

Any factor affecting fixation of metals positively or negatively may have analogous effect on bioavailability. For example, ageing of metal oxides, which causes dehydration and recrystallization of precipitates with the time, reduces the mobility of metals associated with the above oxides and hence influences the bioavailability of these metals [15]. Similarly the oxidation state of metals, which is controlled by the redox reactions, affects the mobility and hence the bioavailability of metals. Also, abiotic and biotic factors may affect the mobilization and immobilization of metals, influencing the bioavailability. The low solubility product of most metal sulfides may be affected by even a moderate output of sulfide, and as result, it can remove the metals from solution due to the formation of insoluble compound, i.e., due to fixation, and thus, affect the bioavailability [10].

The presence of humic substances in the soil, due to the fact that they contain large number of complexing sites, they behave as natural “multiligand complexing systems” [16], obviously affecting metal bioavailability.

Violante et al. [12] reported that strong complexes with ions of metals may be formed with inorganic or organic ligands, which reverse their association with negatively charged sorbents, as the phyllosilicates. They form stable dissolved or dispersed complexes with cations, which are negatively charged, affecting metal fixation and bioavailability.

OM affects significantly the bioavailability of metals depending on: (i) the nature of OM, (ii) the microbiological degradability, (iii) salt content, (iv) pH, (v) redox potential, (vi) soil type, and (vii) metal concentration [17,18].

Considerable attention has been paid to the study of fixation and bioavailability of heavy metals in the soil. However, the aspect of the heavy metal fate under soil pollution merits more attention in relation to fixation, as this subject has not been studied sufficiently.

The purpose of the present work is to examine the relationship of metals fixation to their bioavailability under the effect of soil pollution created by enrichment of the soil with heavy metals, with cultivation of *Beta vulgaris* L. and irrigation with treated municipal wastewater. The ultimate aim is to study the fate of heavy metals under conditions of pollution, with the view to more effectively manage of the polluted soils.

2. Materials and methods

The fate of heavy metals in relation to their fixation and bioavailability in artificially polluted soil was studied in a greenhouse experiment, conducted in Elias Region, Greece, using a randomized block statistical design.

Twelve treatments (T1, T2, … T12) were applied; each one composed of a mixture of the following heavy metal combination Zn, Mn, Cd, Co, Cu, Cr, Ni, and Pb, applied in the form of a mixture of the corresponding chemical compound (Table 1).

The vegetable *Beta vulgaris* L. (beet) was used as a test plant, due to its popularity, fast growth (requires 3 months) and the fact that being a root vegetable, it gives the possibility for the study of the accumulation of heavy metals in the edible part of the plant grown within the soil in direct contact with the metals.

2.1. Experimental soil

The experimental soil was collected from the top layer (0–30 cm depth) of a non-cultivated agricultural area in Amaliada, Western Greece. The soil was analyzed, and its physical and chemical characteristics are presented in Table 2.

The soil was of light texture sandy loam (SL), with slightly acidic pH, low electrical conductivity (EC), medium in OM content, and lacking completely $CaCO_3$ due to its acid pH nature, and a volume weight (VW) compatible with its particle size composition (1.5 g/cm^3). The soil's metal concentration was very low, and it was insufficiently supplied with available exchangeable K and P (Table 2).

A quantity of soil (about 700 kg) was collected and transferred into the experimental site. It was sieved by means of a plastic 3 mm sieve. An amount of 10.5 kg of this soil with a moisture content of 5% was transferred into each plastic pot of rectangular shape with dimensions 50 × 20 × 16 cm (length × width × depth), corresponding to 10 kg of dry soil. The bottom of each pot was closed, to avoid loss of nutrients and heavy metals due to drainage and leaching.

After the preparation of the pots, six seeds of the test plant were sown in each pot. The irrigation, with treated wastewater, was applied regularly according to the soil field capacity (FC) as well as to percent wilting point (PWP). The FC of the light SL experimental soil was 18% and the PWP 8% [19]. The total volume of treated wastewater added to each pot during the period of plant growth was 47 L.

2.2. Chemical analyses

2.2.1. Soil analysis

The soil samples were air-dried, sieved through a 2-mm sieve and oven dried at 75°C for 48 h. The dry soil samples were analyzed by means of internationally accepted classical methods, as follows: soil mechanical analysis by the classical Bouyoucos [20] method, modified by Gee and Bauder [21] and Gee and Or [22]; OM by the wet digestion procedure of Walkley and Black [23]; pH measured on water extract of saturated paste using a standard glass pH electrode and the conductivity by means of a conductivity meter [24]. Available soil P was analyzed by Olsen [25], the exchangeable Ca^{2+}, Mg^{2+}, and K^+ by extraction with NH_4Ac, pH = 7.0 [26], and were measured by inductively coupled plasma–atomic emission spectroscopy (ICP–AES; Perkin Elmer, Optima 2100 DV, Shelton, USA). The micronutrients Zn, Fe, Mn, and Cu and the metals Cd, Pb, Co, Ni, and Cr were extracted with diethylenetriaminepentaacetic acid [27] and measured by ICP–AES [28].

2.2.2. Plant analysis

Each plant sample was placed in a plastic sieve and was flushed with low-pressure tap water till the complete removal of the soil particles, then the root was washed separately, followed by stem and leaf samples with deionized water; and with dilute solution of 0.005% HCl, also thoroughly washed by means of a special detergent (Alconox, 0.1%) and rewashed repeatedly (four times) with distilled

Table 1
Heavy metal treatment mixtures composed of various chemical metal compounds

Heavy metal per treatment		$ZnSO_4\cdot 7H_2O$	$MnSO_4\cdot H_2O$	$CuSO_4\cdot 5H_2O$	$Na_2Cr_2O_7\cdot 2H_2O$	$Co(NO_3)_2\cdot 6H_2O$	$Ni(NO_3)_2\cdot 6H_2O$	$Cd(NO_3)_2\cdot 4H_2O$	$Pb(NO_3)_2$
MWCC		287.44	168.998	249.685	298	291.04	290.8	308.482	331.21
AWHM		65.38	54.938	63.546	52.01	58.933	58.7	112.41	207.2
	mg/kg	g of chemical compound per pot (10 kg soil)							
T1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
T2	2	0.088	0.062	0.079	0.057	0.099	0.099	0.055	0.032
T3	4	0.176	0.123	0.157	0.115	0.198	0.198	0.110	0.064
T4	6	0.264	0.185	0.236	0.172	0.296	0.297	0.165	0.096
T5	8	0.352	0.246	0.314	0.229	0.395	0.396	0.220	0.128
T6	10	0.440	0.308	0.393	0.287	0.494	0.495	0.274	0.160
T7	12	0.528	0.369	0.472	0.344	0.593	0.594	0.329	0.192
T8	14	0.616	0.431	0.550	0.401	0.691	0.694	0.384	0.224
T9	16	0.703	0.492	0.629	0.458	0.790	0.793	0.439	0.256
T10	18	0.791	0.554	0.707	0.516	0.889	0.892	0.494	0.288
T11	20	0.879	0.615	0.786	0.573	0.988	0.991	0.549	0.320
T12	22	0.967	0.677	0.864	0.630	1.086	1.090	0.604	0.352

Note: MWCC = molecular weight of the chemical compounds and AWHM = Atomic weights of heavy metals.

Table 2
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	6.2	0.21	2.11	0.0	1.5
Cd, mg/kg	Co, mg/kg	Cr, mg/kg	Ni, mg/kg	Pb, mg/kg	N–NO_3, mg/kg	P, mg/kg	–
0.04	0.42	0.03	2.8	0.9	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	>2,000	20.09	2.3	33.6	88.6	0.3

Note: S – sand, C – Clay, Si – silt, EC – electrical conductivity, OM – organic matter, and VW – volume weight.

water. The plant samples were left to drain on filter paper and dried in a ventilation oven at 70°C. Then the plant samples were ground and were ready for chemical analysis. 1 g of the ground sample was dry mineralized in a muffle furnace at 500°C for 10–12 h, and the ash was dissolved in a 5-ml 6N HCl solution. The micronutrients Zn, Mn, and Cu and the heavy metals Cd, Co, Cr, Ni, and Pb were measured by ICP–AES [28].

2.2.3. Wastewater analysis

Treated municipal wastewater was processed by the method given by APHA [29] in "Standard Methods for the Examination of Water and Wastewater", and the microelements and heavy metals Zn, Mn, Cu, Cd, Co, Cr, Ni, and Pb were measured by atomic absorption with graphite oven, except of Zn, which was measured only by simple atomic absorption without the use of the Graphite oven.

2.3. Statistical analysis

The experimental data were statistically analyzed by using the statistical package SPSS running regression analysis and ANOVA.

3. Results and discussion

The relation of the heavy metal fixation to metal bioavailability, metal uptake by beets, whole plant dry matter yield, and soil pollution indices was examined in the present work in a soil polluted artificially by enrichment with variable mixtures of heavy metals, irrigated with treated municipal wastewater, and cultivated with garden beets (*Beta vulgaris* L.).

The percent metal fixation was calculated using an empirical mathematical relation based on the obtained experimental data, and specifically on the balance between: "the sum of the native and added bioavailable metals multiplied by the soil mass used per pot" minus the "sum of the metal plant uptake plus the residual metal left in the soil after harvesting multiplied by the soil mass per pot" the "difference" being expressed as a percent of the total bioavailable heavy metal. The relation used is given below:

$$F(\%) = \frac{\left[\left(\mathrm{M}a + Mn\right) * Qs) - \left(Mp * dm * 10^{-3} + Mr * Qs\right)\right] * 100}{\left(\mathrm{M}a + Mn\right) * Qs} \quad (1)$$

where F is the percent of soil metal fixation (%); Ma is the added bioavailable metal in mg/kg of soil; Mn is the native bioavailable metal in mg.kg of soil; Mp is the plant dry matter metal uptake in µg/g dm; Qs is the soil mass in kg/pot; and Mr is the soil residual metal in mg/kg.

3.1. Relation of fixation to soil bioavailable metals

The metal fixation, which occurs in the soil, is a complex chemical and physical process that is subject to various influences taking place in the soil substratum such as due to adsorption, precipitation, oxidation/reduction, clays minerals [10,11], OM, and its components fulvic and humic acids high in organic ligands, including microbial activity [14]. For example, microorganisms participate in the reduction of sulfates, which lead to metal precipitation in strongly reductive environment such as in wetlands [30]. Also, immobilization may retard fixation, as this process can inactivate the metals in oxygen free environments such as in the presence of high level of water in the soil [31]. Generally, the biological processes involved in a wet soil environment lead to the fixation of metalloids [30].

In a soil environment, which has been enriched with heavy metals, and is irrigated with treated municipal wastewater, the availability of the heavy metals is subjected to considerable influences by the various soil processes mentioned above, which finally lead to the metal fixation. Consequently, these processes are expected to affect the bioavailability negatively. The fact that the factors, which control these processes, contain organic and inorganic ligands, OM, humic and fulvic acids, root exudates, and the microbial metabolites [10], complicates further the fixation–metal bioavailability relations.

In order to get a deeper insight into the effect of metal fixation on the bioavailability of heavy metals in polluted soils, the regression analysis was employed, and the regression equations obtained expressed the metal bioavailability as a function of metal fixation. The heavy metal fixation was calculated on the basis of beet metal uptake that is according to the edible plant part dry matter yield, and also on the basis of the whole plant dry matter metal uptake.

Thus, the regression equations reported in Table 3 were found to be in accordance to the metal uptake, by the whole plant and beets (edible part), respectively. Therefore, in using the empirical formula for the calculation of the percent fixation, the dry matter of whole plant and the beet metal uptake, respectively, must be taken into account.

Table 3
Soils EDTA extractable or bioavailable metals as a function of their soil fixation

No	Relation	Regression equation	R	Significance	Type of relation
Beets					
1	Zn_{fb}–Zn_s	$Zn_s = 0.002 \times (Zn_{fb})^2 + 0.253 \times (Zn_{fb}) - 1.859$	0.407	0.004	S
2	Mn_{fb}–Mn_s	$Mn_s = -0.002 \times (Mn_{fb})^2 - 0.23 \times (Mn_{fb}) + 46.57$	0.932	0.000	A
3	Cu_{fb}–Cu_s	$Cu_s = -0.008 \times (Cu_{fb})^2 - 0.699 \times (Cu_{fb}) + 95.765$	0.599	0.000	A
4	Co_{fb}–Co_s	$Co_s = 0.001 \times (Co_{fb})^2 - 0.117 \times (Co_{fb}) + 2.542$	0.713	0.000	S
5	Ni_{fb}–Ni_s	$Ni_s = 0.001 \times (Ni_{fb})^2 + 0.022 \times (Ni_{fb}) + 1.382$	0.551	0.000	S
6	Pb_{fb}–Pb_s	$Pb_s = 0.052 \times (Pb_{fb}) + 3.452$	0.395	0.005	S
Whole plant					
1	Zn_{fwp}–Zn_s	$Zn_s = 0.084 \times (Zn_{fwp}) + 0.692$	0.418	0.004	S
2	Mn_{fwp}–Mn_s	$Mn_s = -0.396 \times (Mn_{fwp}) + 50.766$	0.932	0.000	A
3	Cu_{fwp}–Cu_s	$Cu_s = -0.008 \times (Cu_{fwp})^2 – 0.698 \times (Cu_{fwp}) + 95.747$	0.598	0.000	A
4	Ni_{fwp}–Ni_s	$Ni_s = 0.001 \times (Ni_{fwp})^2 + 0.021 \times (Ni_{fwp}) + 1.385$	0.552	0.000	S

Note: fb – metal fixation calculated on the basis of metal beet yield uptake; fwp – metal fixation calculated on the basis of the whole plant metal uptake; S – synergistic relation; and A – antagonistic.

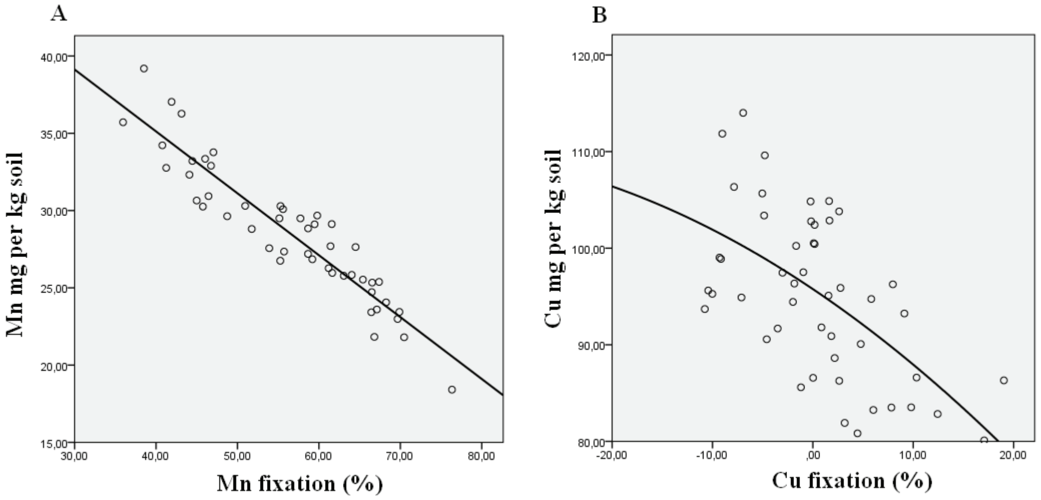

Fig. 1. Antagonistic relation of Mn and Cu fixation to their bioavailable soil level.

Careful study of Table 3 shows that generally, the metal fixation is related synergistically (positively), to the bioavailable soil metals, suggesting that an increase in the level of fixation increases the concentration of bioavailable soil metal. This synergistic relation is observed for the metals Zn, Co, and Pb whose calculation of their fixation was made on the basis of beet metal uptake. Similarly, the fixation of Zn and Ni was also synergistically related with their bioavailable soil level, calculated on the basis of the whole plant metal uptake. On the other hand, both Mn and Cu fixation was related antagonistically with their bioavailable soil level, irrespective of their calculation on the basis of the beet or whole plant metal uptake.

In spite of the fact that the metal fixation is generally expected to decrease the level of soil bioavailable metals as is the case of Mn and Cu (Fig. 1); in the case of Zn, Co, Ni, and Pb, the fixation of these metals was related synergistically, i.e., positively to their bioavailable soil level.

The explanation of this contradictory effect is given by the interactions of the above heavy metals with the bioavailable metals present in the soil, i.e., P, K, Mn, Cu, Cd, Co, Cr, Ni, and Pb. Applying the procedure of quantification [32,33], it was found that considerable quantities of the fixed metals, Zn, Co, Ni, and Pb including the Cd, were contributed to soil by their interactions with the above nine elements, and possibly with other unknown ones, as well as with the physical and chemical properties of soil. In fact, the amounts of these metals supplied to soil were as follows: Zn 3.42 mg/kg or 65.87%, Co 0.227 mg/kg or 49.14% Ni 4.46 or 76.1%, Pb 4.03 mg/kg or 70.1%, and Cd 4.47 mg/kg or 69.46% of the total bioavailable quantity of these metals in the soil. It is this contribution that offset the expected antagonistic (negative) relation of the metal fixation with the above five metals bioavailability. It must be mentioned that the fixation of heavy metals may occur in the soil till the saturation of its fixing capacity. Once this happens,

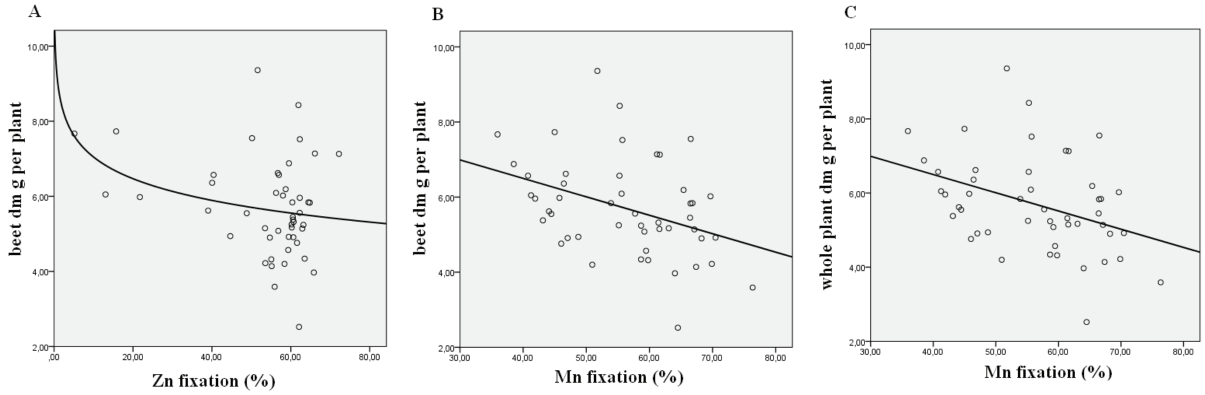

Fig. 2. The relation of Zn and Mn fixation to beet dry matter yield (A and B), and to dry matter yield of whole plant (C).

Table 4
Metal uptake by plants as a function of metal fixation in soils

No	Relation	Regression equation	R	Significance	Type of relation
Beets					
1	Zn_{fb}–Zn_b	$Zn_b = 0.123 \times (Zn_{fb}) + 24.35$	0.406	0.004	S
2	Mn_{fb}–Mn_b	$Mn_b = -2.066 \times (Mn_{fb}) + 203.161$	0.800	0.000	A
3	Ni_{fb}–Ni_b	$Ni_b = 0.001 \times (Ni_{fb})^2 + 0.015 \times (Ni_{fb}) + 2.45$	0.630	0.000	S
Whole plant					
1	Zn_{fwp}–Zn_{wp}	$Zn_w = 0.268 \times (Zn_{fwp}) + 47.204$	0.352	0.014	S
2	Mn_{fwp}–Mn_{wp}	$Mn_w = -7.524 \times (Mn_{fwp}) + 716.496$	0.579	0.000	A
4	Cd_{fwp}–Cd_{wp}	$Cd_w = 0.191 \times (Cd_{fwp}) + 12.372$	0.320	0.027	S
5	Ni_{fwp}–Ni_{wp}	$Ni_w = 0.003 \times (Ni_{fwp})^2 + 0.036 \times (Ni_{fwp}) + 3.308$	0.596	0.000	S

Note: fb – metal fixation calculated on the basis of metal removal by beet yield; fwp – metal fixation calculated on the basis of metal removal by the whole plant; wp – whole plant; S – synergistic relation; and A – antagonistic.

the excess bioavailable metals accumulate in the soil, where they are either taken up by the plants or they are leached toward the ground water if the soil is of light texture.

3.2. Relation of metal fixation to plant uptake

The relation between Zn and Ni percent fixation, and their uptake by beets, was found to be in agreement with the corresponding relation of fixation of these metals to their soil bioavailable level (Table 3) where it can be seen that the percent fixation of these two metals is synergistically related with their soil bioavailability. Thus, the Zn and Ni fixation was synergistically related to their beet uptake, and only Mn and Cu fixation was antagonistically (negatively) related to their uptake by the beets (Fig. 2), as well as to their soil bioavailable level (Fig. 1).

Similarly, the fixation of Zn, Ni, and Cd – of which the latter was contributed by its interactions with the other elements with 4.47 mg/kg or by 69.46% of its total bioavailable level – were synergistically (positively) related with their whole plant uptake (Table 4).

In spite of the logical expectation that the Zn and Mn fixation occurring in the investigated polluted soil would have increased the dry matter yields due to the inactivation of their high levels in this polluted soil, yet the yields decreased significantly (Fig. 2). The explanation is more or less similar to the previous cases.

Table 5
Accumulation of Mn and Zn in soils under the effect of metal fixation

Percent metal fixation	Accumulation in Mn and Zn in soils, mg/kg	
	Mn	Zn
15	–	1.85
20	–	2.40
25	–	3.22
30	–	3.85
35	36.07	4.54
40	34.17	5.06
45	32.17	5.48
50	30.07	5.79
55	27.87	6.00
60	25.57	6.13
65	23.17	6.14
70	20.67	6.05
75	18.07	–
80	15.37	–
85	12.57	–

In a polluted soil like the one under consideration, which has been used as a substratum for the growth of *Beta vulgaris* L., irrigated with treated wastewater, the elemental interactions seem to play an important role in offsetting the favorable role of fixation in plant growth. The high level of heavy metals due to the application of treatments, and the supply additional quantities of metals by the elemental interactions saturate the low fixing capacity of this SL soil, and therefore, considerable amounts of bioavailable heavy metals Zn and Mn accumulate in the soil, thus affecting negatively the dry matter yields (Fig. 2). In fact, only these two metals gave statistically significant results on dry matter yield, in spite of their fixation.

The above results were verified by means of the following regression equations between the metal fixation and metal bioavailable level in soil reported in Table 3, i.e.:

(a) $Zn_s = 0.002 \times (Zn_{fb})^2 + 0.253 \times (Zn_{fb}) - 1.859$ (R: 0.443, sign: 0.007)
(b) $Mn_s = -0.002 \times (Mn_{fb})^2 - 0.23 \times (Mn_{fb}) + 46.57$ (R: 0.933, sign: 0.000)

The solution of these two equations yielded the data reported in Table 5.

The data of the above Table 5 discloses that a considerable quantity of bioavailable Mn and Zn accumulated in the soil decreasing from 36.07 to 12.57 for the Mn and increasing from 1.85 to 6.05 mg/kg, for Zn respectively, attained with the increase of fixation from 35% to 85% for Mn and from 15% to 70%, for Zn (Table 5). It is seen that bioavailable Mn was decreased and Zn increased due to the antagonistic and synergistic relation of fixation of these metals to their respective bioavailable soil level.

Table 6
Pollution indices expressed as a function of metal fixation

Regression equation	R	Significance	Type of equation
$PLI = -0.001 \times (Zn_{fb})^2 + 0.164 \times (Zn_{fb}) - 0.91$	0.524	0.001	S
$PLI = 0.003 \times (Mn_{fb})^2 - 0.179 \times (Mn_{fb}) + 4.354$	0.815	0.000	S
Cu_{fb}–PLI		ns	
Cd_{fb}–PLI		ns	
$PLI = 0.011 \times (Co_{fb})^2 - 1.261 \times (Co_{fb}) + 27.303$	0.760	0.000	S
$PLI = 0.0002 \times (Cr_{fb})^2 + 0.018 \times (Cr_{fb}) + 0.534$	0.477	0.003	S
$PLI = 0.001 \times (Ni_{fb})^2 + 0.029 \times (Ni_{fb}) + 0.997$	0.586	0.000	S
$PLI = 0.0003 \times (Pb_{fb})2 + 0.039 \times (Pb_{fb}) + 1.993$	0.493	0.002	S
$EPI = -0.001 \times (Zn_{fb})^2 + 0.147 \times (Zn_{fb}) - 0.819$	0.524	0.001	S
$EPI = 0.003 \times (Mn_{fb})^2 - 0.160 \times (Mn_{fb}) + 3.897$	0.815	0.000	S
Cu_{fb}–EPI		ns	
Cd_{fb}–EPI		ns	
$EPI = 0.01 \times (Co_{fb})^2 - 1.131 \times (Co_{fb}) + 24.486$	0.761	0.000	S
$EPI = 0.0002 \times (Cr_{fb})^2 + 0.016 \times (Cr_{fb}) + 0.475$	0.478	0.003	S
$EPI = 0.001 \times (Ni_{fb})^2 + 0.026 \times (Ni_{fb}) + 0.891$	0.586	0.000	S
$EPI = 0.0003 \times (Pb_{fb})^2 + 0.035 \times (Pb_{fb}) + 1.785$	0.493	0.002	S
$HML = -0.018 \times (Zn_{fb})^2 + 1.873 \times (Zn_{fb}) + 101.478$	0.415	0.014	S
$HML = 0.037 \times (Mn_{fb})^2 - 3.012 \times (Mn_{fb}) + 194.884$	0.631	0.000	S
Cu_{fb}–HML		ns	
$HML = -0.006 \times (Cd_{fb})^2 + 0.128 \times (Cd_{fb}) + 154.832$	0.377	0.032	S-A
$HML = 0.078 \times (Co_{fb})^2 - 9.178 \times (Co_{fb}) + 312.46$	0.629	0.000	S
$HML = 0.003 \times (Cr_{fb})^2 + 0.084 \times (Cr_{fb}) + 114.678$	0.410	0.016	S
$HML = 0.508 \times (Ni_{fb}) + 122.708$	0.415	0.003	S
$HML = 0.292 \times (Pb_{fb}) + 134.438$	0.394	0.006	S
$TCF = 0.005 \times (Zn_{fb}) + 1.338$	0.348	0.015	S
$TCF = 0.0004 \times (Mn_{fb})^2 - 0.033 \times (Mn_{fb}) + 2.134$	0.630	0.000	S
Cu_{fb}–TCF		ns	
$TCF = -7 \times 10^{-5} \times (Cd_{fb})^2 + 0.001 \times (Cd_{fb}) + 1.695$	0.380	0.030	S-A
$TCF = 0.001 \times (Co_{fb})^2 - 0.101 \times (Co_{fb}) + 3.422$	0.628	0.000	S
$TCF = 3 \times 10^{-5} \times (Cr_{fb})^2 + 0.001 \times (Cr_{fb}) + 1.254$	0.410	0.016	S
$TCF = 0.006 \times (Ni_{fb}) + 1.343$	0.414	0.003	S
$TCF = 0.003 \times (Pb_{fb}) + 1.472$	0.392	0.006	S

Note: The subscript (fb) – metal fixation based on metal uptake by beets; PLI – pollution load index; EPI – elemental pollution index; HML – heavy metal load; TCF – total concentration factor; S – synergistic; A – Antagonistic; R – correlation coefficient; and sig. – level of significance ($P < 0.05$).

3.3. Metal fixation and soil pollution indices

So far the relation of metal fixation to: (i) bioavailable soil heavy level, (ii) their uptake by the beet and by the whole plant, and (iii) to whole plant dry matter, and beet metal level, has been examined. However, in order to have a more complete view of the role of metal fixation under soil pollution, the relation of metal fixation with the following soil pollution indices, i.e., pollution load index (PLI) [34], elemental pollution index (EPI) [35], heavy metal load (HML) [35], and total concentration factor (TCF) [35], has also been examined. The results obtained are summarized in Table 6.

Careful study of the equations reported in this table shows that the metal fixation was related positively with all the above pollution indices. This finding is opposite to what one could logically expect. As it has already been stated, the fixation is expected to inactivate the soil bioavailable heavy metals, and therefore, its relation to the bioavailable level ought to be antagonistic, i.e., negative.

The explanation of these contradictory results is given in the previous relations of metal fixation to soil available metals, to their uptake, and to dry matter plant yield. Synoptically, it can be said that the additional heavy metals contributed to soil due to elemental interactions, as well as to the high levels of the applied treatments, offset the inactivation effect of the role of fixation, thus adding considerable quantities of heavy metals to soil, which overcomes the soil's fixation capacity.

4. Conclusions

Based on the aforementioned, the following conclusions could be drawn:

The positive relation of metal fixation to: the soil bioavailable metal levels, their plant uptake, plant yields, and the soil pollution indices, is offset by the elemental interactions contribution in terms of heavy metals to soil.

These results suggested that the elemental interactions through their elemental contribution can modify significantly the impact of metal fixation on the metal–soil–plant relations, under conditions of soil pollution.

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