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HEAVY METAL INTERRELATIONSHIPS IN SOIL IN THE PRESENCE OF TREATED WASTE WATER

I.K. KALAVROUZIOTIS[1,*]
P.H. KOUKOULAKIS[2]
A.H. PAPADOPOULOS[3]

[1]Department of Environmental and Natural Resources Management, University of Ioannina, G.Seferi 2, Agrinion-Greece
[2]Assoc. Researcher, Soil Science Institute NAGREF, Altladidos 8, GR-544 54, Thessaloniki, Greece
[3]NAGREF, Soil Science Institute, of Thessaloniki GR-570 01,Thermi, Thessaloniki, Greece

*to whom all correspondence should be addressed:
e-mail: ikalavru@cc.uoi.gr

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ABSTRACT

An experiment was conducted in a greenhouse located in Agrinion, Greece, in order to study the effect of the treated municipal wastewater (TMWW) on the soil heavy metal interactions, in comparison to ordinary well irrigation water, denoted as control. The ultimate aim was the establishment of sound scientific basis for the TMWW reuse in the irrigation of vegetable production. A randomized block design was used including TMWW and the control, in six replications, the vegetable of *Brassica oleracea* var. Gemmifera (Brussels sprouts) being used as a test crop.

The following were found:

Forty interactions took place in the soil between heavy metals Pb, Ni, Co, Cd, Cu and Zn. Of these, 11 were significant under both the TMWW and the Control, respectively. Also, under only TMWW 15 interactions or 37.5% were statistically significant, while under the Control 21 or 52.5%. It was also shown that the effect of TMWW on most of the statistically significant interactions between heavy metals, did not differ significantly from that of the control, suggesting that from this point of view, the TMWW could be used for reuse in vegetable irrigation, provided that health risk related to microbiological factor is taken into account.

KEYWORDS: Heavy metal, interaction, treated wastewater, reuse.

1. INTRODUCTION

The reuse of the treated municipal wastewater (TMWW) is a multifactor problem, related to social, spatial, economic and environmental criteria, each of these including a number of subcriteria. The present paper aims at an ecologically sound reuse of the TMWW, and therefore it will focus on its effects on soil heavy metals, as well as on their interactions, which by themselves, constitute an environmental quality factor, related to the decision making with respect to the TMWW reuse for crop irrigation.

As the environment involves the soil and the higher plant life, the TMWW reuse is directly associated with environmental quality, because by necessity, it is in an immediate contact with the soil-plant system, and consequently it may have variable effects on it (Chang *et al.,* 1998). Therefore, the detailed study of the TMWW impact on the chemical, physical and biological properties of soil as well on its macro, microelements and especially heavy metal content and their interactions, need special attention. As the TMWW is a carrier of the above elements, its long term use may result in heavy metal accumulation in the soil. Furthermore, various interactions, especially among heavy metals, may take place, which enhance the previously mentioned accumulation, not only in the soil, but also in the plant tissues as well, depending on the extent of their synergism and antagonism, creating a potential risk factor for the consumer's health (Alloway, 1995).

Preliminary studies with respect to the effects of TMWW on *Brassica oleracea* var. Italica (Broccoli), had shown that the reuse of TMWW increased the heavy metal content in the soil (Kalavrouziotis *et al.,* 2008).

Also, it was found the TMWW could act as a factor that intensified some of the interactions between heavy metals as well as macro and microelements in the soil and plants (Kalavrouziotis *et al.,* 2008a; 2008b). These interactions, could increase or decrease the level of the interacting heavy metals, depending on whether they were synergistic or antagonistic. Also, they could take place not only in the soil but in the plant, and especially in its various organs i.e. roots, leaves, and heads or sprouts, thereby, contributing to the spatial distribution of the heavy metals in the plant , an issue of great importance, as it is related to human health (Kalavrouziotis *et al.,* 2008a; Alloway, 1995).

In our previous work it was found that under the effect of the TMWW, the heavy metal interactions in the soil may increase or decrease statistically significantly the level of each other, depending on their synergistic or antagonistic nature. The relevant information in the literature concerning the interactions of the heavy metals under the application of TMWW, not to mention their effect on plant growth, is very scarce. Traditionally, the heavy metal impact on plant growth is examined basically in terms of total bioavailable metal level or concentration in the soil. Obviously, this level originates from various sources such as weathering of local minerals, organic matter breaking down, application of sludge and wastewater, as well as from the heavy metal interactions. Consequently it is expected that the interrelationships between heavy metals may have a variable impact on the environmental quality, and on plant growth.

With the term “heavy metal” it is referred to those metals which have a density > 6 g cm^{-3} (Phipps, 1981). The heavy metals are also known as “trace metals” (Alloway, 1995; Kabata-Pendias and Pendias, 1995).

The treated municipal wastewater is basically a carrier of plant nutrients (N,P,S, etc) and generally has low levels of heavy metals (Pb, Cd, Ni, Co, Cr, etc). According to experiments conducted by Sheikh *et al.* (1998), long term environmental impacts of reclaimed wastewater reuse for the irrigation of crops, appeared to be minimal. Though generally domestic wastewaters do not contain high levels of heavy metals, occasionally however, they may contain high levels of these metals owing to non-point illegal connections to the sewage system, and metal pipes erosion.

Experimental results obtained by Kalavrouziotis *et al.* (2008), using TMWW for irrigation of *Brassica oleracea* var. Italica (Broccoli), resulted in the accumulation of Cd, Pb, and Zn in both soil and plants, especially in the heads i.e. the edible plant part. Though the TMWW was of domestic origin, the presence of heavy metals was attributed to probable non-point pollution sources, illegally connected to the sewage system.

An important aspect of TMWW reuse, which has not been given due attention, is the study of the interactions between essential nutrients and heavy metals under the effect of the TMWW, which by itself is a carrier of such elements. Similarly the effect of these interactions on the nutrient and heavy metal levels in the soil and in the plants, have not been investigated in detail so far

The relevant information on the effect of TMWW on the heavy metal interactions in the soil is very limited, and therefore more work is necessary to shed light on this aspect of TMWW reuse, which may be an important factor in decision making with respect to applying the TMWW as an alternative water source for vegetable irrigation.

In view of the fact that the TMWW reuse may add to the soil significant quantities of the above essential nutrients and heavy metals, the aim of the present work is to study the impact of TMWW on the heavy metal interactions in the soil, which had previously been cultivated with *Brassica oleracea* var. Gemmifera (Brussels Sprouts), the ultimate goal being to establish the necessary scientific basis for the reuse of TMWW in the irrigation of Brussels sprouts, and possibly of other vegetables.

2. MATERIALS AND METHODS

An experiment was conducted in a greenhouse using a Statistical Design of Randomized Blocks, located at the University of Ioannina, Department of Environmental Management and Natural Resources, Agrinion Greece.

The general purpose of the experiment was to study the effects of the Treated Municipal Wastewater (TMWW) on soil properties, macro- and micronutrients, and heavy metal content, as well as on the inorganic composition of Brussels sprouts plant parts (roots, leaves and sprouts), with a view to examine the possibility off the TMWW reuse, for the irrigation of vegetables.

The statistical Design used, included two sources of irrigation water, (a) TMWW and (b) control, (ordinary well irrigation water), while the vegetable *Brassica oleracea* var. Gemmifera (Brussels Sprout) was used as a test plant.

The experiment was conducted in six replications, with a total of 2 x 6 = 12 experimental plots of 2.5 x 1.8 m^2 size. The plots were separated by dikes of 10 cm height. Brussels sprouts plants were transplanted in rows, the distance between them being 0.8 m, while between the plants in the row 0.5 m.

Transplanting was made on December 11, 2005, and harvesting of the sprouts 16 weeks after transplanting, i.e. April 20, 2005. The Treated Municipal Wastewater used, was supplied by the Biological Treatment Plant of Messolongion, located 3 kms away from the town, adjacent to the river “Koukos”.

The TMWW and the “control” were applied at the 50-60% depletion of the available soil water, which was found by regular soil sampling and determination of moisture by drying the samples at 105^oC.

In total, the TMWW and Control, were applied nine times during the growth period at a rate of 30 L per 4.5 m^2 at each irrigation, or 867 mm per ha. The application of the TMWW was done by means of a special hand operated sprinkler.

Composite soil samples from 0-30 cm depth were taken before the application of the TMWW and control, during the following periods: a) shortly before the commencement of the experiment, b) 8 weeks after transplanting and c) 16 weeks after transplanting (just before harvesting). The samples were prepared by being air dried, ground, sieved, and stored for chemical analysis.

The soil analytical data obtained with respect to the heavy metals, was statistically processed by means of regression analysis, ANOVA and t-test, in order to study the heavy metal interactions and their impact on the environment (Kalavrouziotis *et al.*, 2008a).

2.1 Chemical analyses

2.1.1 Soil analysis

Soil analysis was done as follows: For the determination of available P 2.5 g of ground soil sample was extracted in 100 ml of 0.5 M $NaHCO_3$ after shaking steadily for 30 min. The P in solution was determined by developing the blue phosphomolybdate complex and its intensity was measured colorimetrically (Olsen *et al.*, 1954). Available K was determined by extracting 2.5 g of soil in 50 mL of 1 M MNH_4Ac, pH 7.0 (Lanyon and Heald,1982), and the K was measured by flame photometry. The soil micronutrients Mn, Zn, Cu and Fe, and the heavy metals Pb, Ni, Co and Cd, were extracted by using 20 g of soil in 40 ml of 0.005 M DTPA solution, and measured by means of Atomic Absorption Spectrophotometer, using a Variant AAS type (AA-IO) (Sakata, 1987). The EC, $CaCO_3$ organic matter (OM) and pH were determined by the methods suggested by Page *et al.* (1982).

2.1.2 Irrigation water and TMWW analysis

The well irrigation water and the TMWW were analyzed before their application by methods suggested by AOAC (1996) as follows: pH was determined electrometrically by means of a commercial pH-meter, while SAR was calculated (Richards, 1954).

Total N was found by the Kjeldhal method i.e. by digesting a water and TMWW sample with H_2SO_4 to convert organic N to NH_3, followed by distillation after alkanization and the total N being determined titrimetrically. Cl^- anions were determined in 100 ml of irrigation water or treated municipal wastewater, to which 1 mL of indicator-acidified was added-composed of 250 mg s-diphenylcarbazone, 4 ml HNO_3 and 30 mg of xylene cyanol FP in 100 ml alcohol. Then the solution was titrated with 0.0141 N Hg $(NO_3)_2$ to definite purple end point.

Ca and Mg were measured by titration with ethyldiaminetetraacetic acid (Versenate) (Richard 1954). Ammonium acetate and dispersed organic matter were removed from the sample prior to titration with versenate. Evaporation of an aliquot of the water or TMWW to dryness followed by treatment with aqua regia (3 parts of conc. HCl and 1 part of conc. Nitric acid). The residue was dissolved in a quantity of water equal to the original volume of the aliquot, taken for treatment. First Ca was determined by placing a 5 mL aliquot and by diluting it with distilled water to approximately 25 ml. Then 5 drops of the 4 N NaOH were added, and approximately 50 mL of ammonium purpurate indicator composed of 0.5 g of ammonium purpurate in 100 g of powered potassium sulfate. The solution was then titrated with versenate 0.01 N using a 10 ml microburet, the end of the titration being determined by the color change from orange to lavender or purple. In turn, (Ca+Mg) was determined in an aliquot of 5 mL, which was pipeted into a 125 Erlenmeyer flask. It was diluted to approximately 25 mLl and 0.5 mL (10 drops) of the ammonium chloride-ammonium hydroxide buffer solution was added, and also 3-4 drops of Eriochrom black T indicator, composed of 0.5 g of Eriochrom T indicator (F241), 4.5 g of hydroxylamine hydrochloride in 100 ml of 95 % alcohol. and the solution was titrated with versinate. The calculations were made by means of the following relation: Ca or (Ca+Mg) in meq L^{-1} = (mL of versinate solution used x Normality of versinate x 1000) mL^{-1} of aliquot used. The K in water or in the wastewater was determined by pretreatment in a buffer solution, and it was determined by using atomic absorption spectrophotometer.

Total P was determined by digesting an aliquot of the sample with persulfate. The P was measured by direct colorimetric analysis by developing the antimony phosphomolybdate complex, being reduced to intensely blue color by means of ascorbic acid. The color intensity was measured colorimetrically. The water Fe, Mn, Zn, Cu and Cr contents were determined by filtering a known volume of the sample through 0.45 μm membrane filter. The membrane with the residue was then transferred into a 250 ml beaker, and 3 ml of HNO_3 were added. They were covered with watch glass and heated gently to dissolve the membrane. The heat was then increased to evaporate the solution to dryness. After cooling, 3 ml of HNO_3 were added until digestion was complete. Then 2 ml of HCl (1+1) were added and reheated gently to dissolve the residue. The watch glass and the baker were washed with H_2O and the solution was filtered. The filtrate was diluted to concentration within the range of the instrument. Then the metals in solutions were determined by means of an Atomic Absorption Spectrophotometer by setting the instrument the following wave lengths: Fe=248.34 nm, Mn=6274.5 nm, Zn=213.9 nm, Cu=324.7 nm, and Cr=357.9 nm.

Arsenic (As) was determined by evaporating 0.5 l to dryness, and by adding a small quantity of $NaCO_{3.}$ The filter with the residue was then washed thoroughly with hot water. The alkaline filtrate was diluted to definite volume, and it was used for the determination of the As. The following AOAC (1996).

Soluble B was determined in the irrigation water without pretreatment, and in the treated wastewater by pretreatment of the sample, i.e. by filtering through 0.4 - 0.45 μm filter and by acidification of the filtrate with HNO_3 up to pH < 2.0. Curcumine (Eastman No 1179) was used for the development of the red color and its intensity was measured by means of a spectrophotometer at 540 nm, with minimum light path of 1 cm (APHA,1995).

Sodium (Na) in the wastewater was determined by pretreatment following the same procedure mentioned above for the B, and the concentration of Na in the filtrate was measured by means of flame photometer. The relevant analytical data are given in Table 1.

Table 1. Chemical characteristics of the two water sources used i.e. "Control" and "TMWW"

Chemical characteristics	Applied Treatments			
	Control (n=9)		TMWW (n=9)	
	Mean	Standard deviation	Mean	Standard deviation
Conductivity µS cm^{-1} (25^{o} C)	261.67	30.688	1305.22	242.975
pH	8.38	0.280	7.56	0.554
SAR[a]	0.38	0.052	4.29	0.247
N (mg l^{-1})	0.63	0.302	11.71	3.440
P (mg l^{-1})	0.05	0.042	0.64	0.328
K (mg l^{-1})	0.88	0.164	16.14	4.903
Ca (mg l^{-1})	49.03	6.106	90.74	10.760
Mg (mg l^{-1})	4.2	0.299	21.63	3.606
Fe (µg l^{-1})	35.56	16.667	102.89	80.901
Na (mg l^{-1})	10.22	1.394	175.56	16.576
Mn (µg l^{-1})	4.11	2.147	84.54	62.954
B (mg l^{-1})	0.67	0.1	1.18	0.303
Cl (mg l^{-1})	14.96	2.351	290.36	94.984
Zn (µg l^{-1})	6.22	8.318	109.76	58.061
Cu (µg l^{-1})	1.78	0.338	2.73	0.612
As (µg l^{-1})	0.19	0.078	0.62	0.354
Cr (µg l^{-1})	1.23	0.042	1.25	0.032

(a) Sodium adsorption Ratio (SAR). an index of the sodium hazard to which the soil be subjected due to the use of a given irrigation water including the wastewater

The chemical and physical characteristics of the treated wastewater shown in Table 1, used in this experiment, are all within the range of the recommended maximum concentration, with the exception of Fe content, which is higher (WHO, 2006; Ayres and Westcot, 1989).

3. RESULTS AND DISCUSSION

The present work is dealing exclusively with the interactions of heavy metals which occured in a soil that had previously been cultivated with *Brassica oleracea* var. Gemmifera (Brussels sprouts), and irrigated with treated municipal wastewater (TMWW).

3.1 The heavy metal interactions in the soil

The regression coefficients of forty regression equations representing equal number of respective interactions between Cd, Pb, Ni, Cu, Zn, and Co in the soil, are given in Table 2, respectively. The study of this table discloses that eleven interactions out of the forty, are statistically significant under both the effect of TMWW, the control, and mean treatments (average effect of TMWW and control), respectively. These results, show the consistency and the stability of these interactions, which are all synergistic, i.e. by increasing the level of the one interacting element increases the level of the other (Marschner, 2002). It can also be seen in the same table, that a number of other interactions are statistically significant only under the effect of the control, or under only the TMWW, or under the mean effect, while others are significant under the control and the mean or under TMWW and mean or finally under control or the TMWW, respectively. Similarly, a large number of interactions are non significant, under either of the above treatments, studied (Table 2).

Some of the afore mentioned interactions are" two-way" i.e. the increase of the level of the one interacting element results in the increase of the other, and vice versa, while others are "one-way" i.e. only the increase or decrease of one of the interacting elements increases or decreases the level of the other one (Kalavrouziotis *et al.,* 2008a).

Table 2. Coefficients of regression equations of heavy metal interactions, taking place in the soil under the influence of control, TMWW and Mean treatment effect, respectively cultivated with *Brassica oleracea* var. Gemmifera (Brussels sprouts)

No	Interaction[1]	Control	TMWW	Mean Treatment
1	PbxZn	0.385ns	0.284ns	0.307ns
2	ZnxPb	0.548*	0.171ns	0.411*
3	PbxCu	0.656**	0.879**	0.678**
4	CuxPb	0.470*	0.914***	0.578**
5	PbxCd	0.463*	0.382ns	0.347*
6	CdxPb	0.117ns	0.311ns	0.293ns
7	PbxNi	0.366ns	0.723**	0.333*
8	NixPb	0.626**	0.838***	0.583**
9	PbxCo	0.597*	0.465*	0.529**
10	CoxPb	0.731**	0.539*	0.546**
11	CdxZn	0.917***	0.893***	0.890***
12	ZnxCd	0.890***	0.887***	0.889**
13	CdxCu	0.047ns	0.288ns	0.255ns
14	CuxCd	0.171ns	0.256ns	0.207ns
15	CdxPb	0.117ns	0.331ns	0.293ns
16	PbxCd	0.463*	0.382ns	0.347*
17	CdxNi	0.028ns	0.043ns	0.021ns
18	NixCd	0.182ns	0.249ns	0.181ns
19	CdxCo	0.168ns	0.168ns	0.148ns
20	CoxCd	0.483*	0.254ns	0.326ns
21	NixZn	0.134ns	0.113ns	0.110ns
22	ZnxNi	0.375ns	0.099ns	0.194ns
23	NixCu	0.720**	0.746**	0.703**
24	CuxNi	0.303ns	0.698**	0.416*
25	NixPb	0.626**	0.838***	0.583**
26	PbxNi	0.366ns	0.723**	0.333*
27	NixCd	0.182ns	0.249ns	0.181ns
28	CdxNi	0.028ns	0.043ns	0.021ns
29	NixCo	0.498*	0.148ns	0.259ns
30	CoxNi	0.543*	0.433ns	0.213ns
31	CoxZn	0.380ns	0.256ns	0.284ns
32	ZnxCo	0.817**	0.173ns	0.563**
33	CoxCu	0.215ns	0.525*	0.211ns
34	CuxCo	0.259ns	0.363ns	0.189ns
35	CoxPb	0.731**	0.539*	0.546**
36	PbxCo	0.597*	0.465*	0.529**
37	CoxCd	0.483*	0.254ns	0.326*
38	CdxCo	0.168ns	0.168ns	0.148ns
39	CoxNi	0.543*	0.433ns	0.213ns
40	NixCo	0.498*	0.148ns	0.259ns

[1] In the regression equation, always the first element of any one of the above interactions represents the independent variable (X) and the second the dependent (Y). The interactions with bold letters are statistically significant and "two-way". (*), (**), (***) statistically significant at $p_{0.05}$, $p_{0.01}$, $p_{0.001}$ level of significance, respectively. ns: non significant.

The following representative heavy metal interactions will be examined bellow.

3.1.1 PbxCu

An interaction of special environmental importance. While Pb is neither an essential or a beneficial element, but basically a toxic metal, it interferes with the availability of an essential micronutrient, i.e. the Cu, by acting on it synergistically, thus increasing its level in the soil (Figure 1A). These two elements, are interrelated between themselves, in a “two-way” interaction (Figure 1B) where the increase of the Cu level results in the increase of the Pb concentration in the soil. The environmental significance of this interaction is based on the fact that the presence of either one of these two heavy metals in the soil, further enhances mutually the concentration of the other, which at very high levels in the soil, may affect adversely plant growth and the agroecological environment.

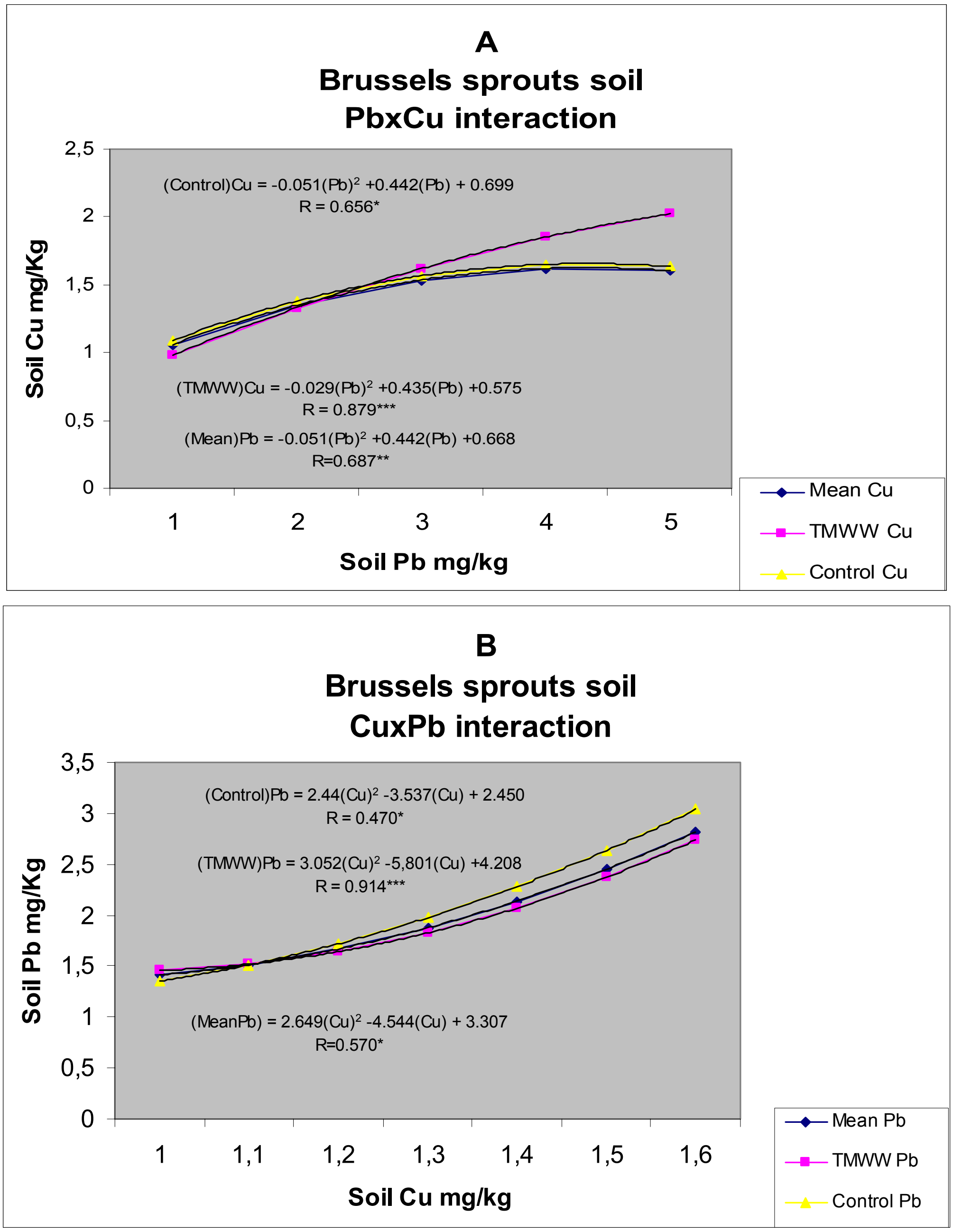

Figure 1. Interrelationships of soil DTPA extractable Pb and Cu under the effect of: a) TMWW b) Control and c) Mean treatment, respectively

Closer examination of Figures 1A and 1B discloses that though there is a trend of an increasing effect of TMWW on the level of Cu with the increase of Pb concentration (Figure 1A), and a similar effect of the control on the level of the Pb, with the increase of Cu concentration (Figure 1B), the difference, however, in the concentration of either of these elements, is statistically non-significant, neither under the TMWW or the control,respectively. These results, show therefore that both of the treatments studied, affect more or less similarly the bioavailable level of either Cu or Pb in the soil during their respective interactions, and consequently their effect on the interactions PbxCu or CuxPb, is similar.

Unfortunately, our knowledge about these interactions is very poor, and more work is necessary to throw more light on this interrelationship, which may be intensified under the TMWW effect, since the later will eventually be used as a alternative source of the irrigation water.

The interactions under consideration, must be studied in connection to the pH changes brought about by the TMWW (Kalavrouziotis *et al.,* 2008b) as well as in relation to organic compounds contained in the TMWW. Both Cu and Pb are complexed by the organic matter (Stevenson and Cole, 1999) and their solubility is very highly affected by the pH changes (Kabata-Pendias and Pendias, 1995).

The Pb is least mobile heavy metal in the soil, and the characteristic localization near the surface of most soils is primarily related to the surface accumulation of organic matter. Thus according to the above workers the greatest Pb accumulation is found in organically rich layers of soil. This is in agreement with the statement by Hughes *et al*., (1980), according to which steadily increasing amounts of Pb in surface soils of various terrestrial systems have been reported.

The proximity of both Pb and Cu accumulation, possibly favors the synergistic interaction between these two heavy metals, found in the present work.

3.1.2 CoxPb

This interaction was found to be "two-way" synergistic (Figures 2A and 2B). Similar results were obtained in another work with *Brassica oleracea* var. Italica(broccoli), it was found that the above interaction was "two way"synergistic in the the plants of this vegetable, irrespective of the plant parts (mean TMWW effect) (Kalavrouziotis *et al.,* 2009).

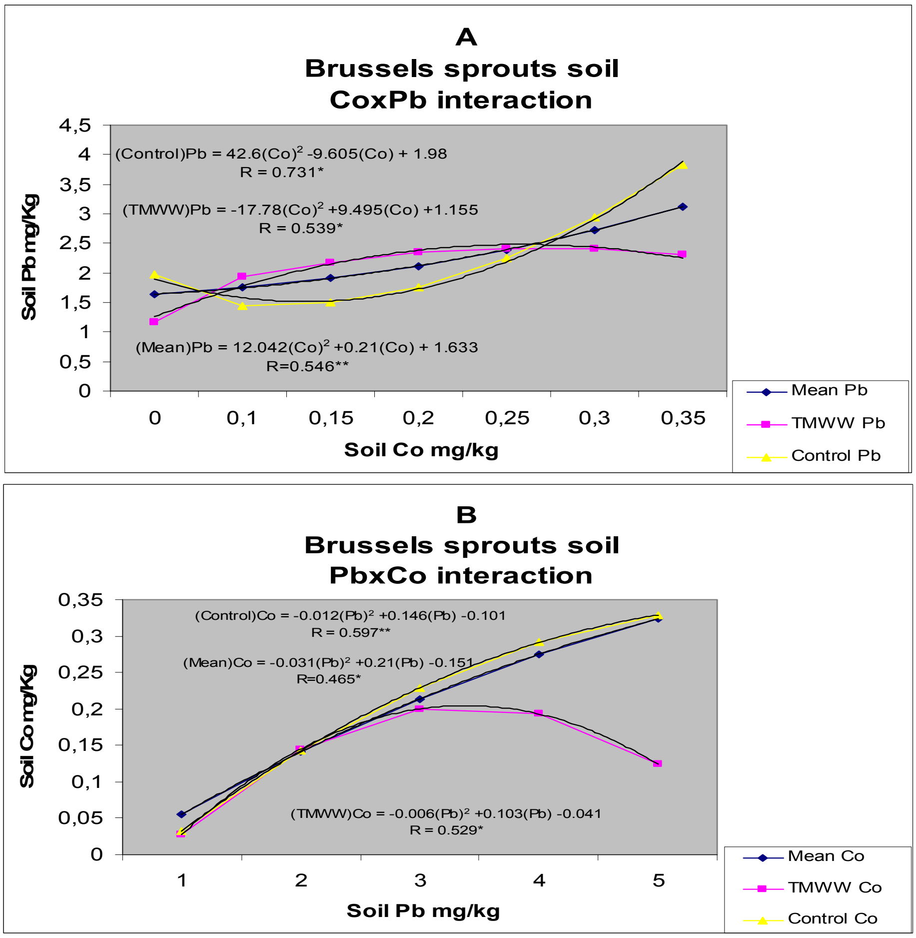

Figure 2. Interrelationships of soil DTPA extractable Co and Pb under the effect of: a) TMWW b) Control and c) Mean treatment, respectively

The findings of the present work in relation to the CoxPb interaction taking place in the soil which had been cultivated with *Brassica oleracea* var. Gemmifera (Brussels sprouts), are in agreement with the above reported results related to broccoli, thus reflecting the consistency of the occurrence of the above interaction.

Figures 2A and 2B however, show that both the level of the Pb and Co, respectively, decreases under the effect of TMWW with the corresponding increase of Co at evels > 0.25 μg g^{-1} (Figure 2A), and similarly of Pb, at levels >3.0 μg g^{-1} (Figure 2B), respectively.

These findings suggest that under the TMWW, the interactions CoxPb or PbxCo become antagonistic at the higher levels of the Co and Pb concentration in the soil, as reported for Broccoli plants (Kalavrouziotis *et al.,* 2009)

3.1.3 CdxZn

According to Kabata-Pendias and Pendias (1995), the CdxZn interaction has been reported as both antagonistic and synergistic. The synergism was found in rice by Kitagishi and Vamane (1981) who explained the occurrence of this interaction in terms of Zn competition for Cd sites, which results in an increase of Cd solubility and Cd translocation from roots to shoots. Wallace *et al.* (1980) also reported that in the presence of high Zn level. Cd accumulated in roots, thus suggesting the synergistic nature of this interaction. But Lagerwerff and Biersdorf (1971) had earlier reported that the CdxZn interaction was found to be antagonistic during its uptake-transport process.In fact they found that increasing concentration of Zn suppressed Cd uptake at low Cd concentration. It appears that the Cd/Zn ratio is the governing factor of the CdxZn synergism or antagonism (Kabata-Pendias and Pendias, 1995).

In the present work, the CdxZn interaction in the soil was synergistic and “two-way” (Figures 3A and 3B). As shown in Figure 3B, the Cd concentration increases very sharply with the increase of the Zn level, irrespective of the treatment effect. According to Alloway (1995), from the geochemical point of view, Cd is very closely related to Zn, as both elements have similar ionic structures and electronegativities. Also, they show the same tendency with respect to their downward movement within the soil profile. This seem to be in line with the reported statement that Zn exhibits greater inhibitory effect than all the other metals on Cd absorption on the solid phase of soil, thereby indirectly contributing to the latter’s availability (Kabata-Pendias and Pendias, 1995).

The above interaction was also found to be antagonistic, and concomitantly occurring in the “root-leaf” pathway of Brussels sprouts plants, under the effect of TMWW (Kalavrouziotis and Koukoulakis 2009), thus agreeing with the findings of Lagerwerff and Biersdorf (1971).

These seemingly controversial results signify the complexity of the interactions and the necessity of more scrupulous and detailed work that must still be done to fully explain them.

The geochemical similarities between Cd and Zn, may possibly explain their synergistic interrelationship in the soil, reported in this work. However, environmentally, the CdxZn interaction is very important. Thus, the increase of Cd in the soil by 1 mg kg^{-1} under the TMWW (Figure 2A), corresponds to an increase of Zn equal to 0.79 mg kg^{-1}, while for each increase of soil Zn by 1 mg kg^{-1}, the Cd increases on the average by 0.71 mg kg^{-1} (Figure 3A). The increase, especially of Cd, is high enough to potentially cause problems to plants and humans, and shoud be taken into consideration when reusing TMWW for the irrigation of crops.

The Cd is a heavy metal which when present in the food even in suboptimal levels, may cause chronic accumulation in kidneys and dysfunction, if its concentration increases in the kidney cortex above to 200 mg kg^{-1} of fresh weight (Fassett,1980).

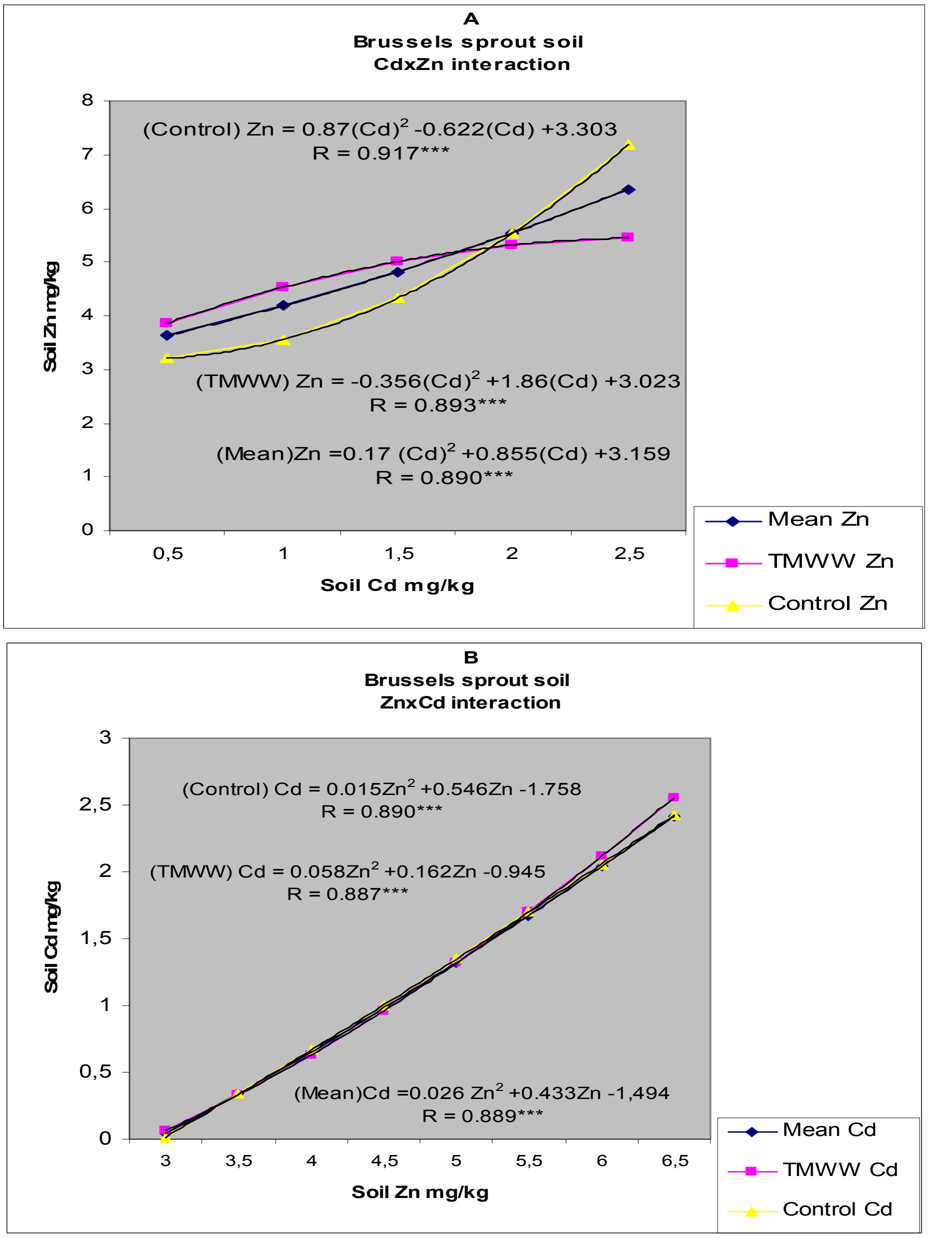

Figure 3. Interrelationships of soil DTPA extractable Cd and Zn under the effect of: a) TMWW b) Control and c) Mean treatment respectively

3.1.4 NixCu

This interaction was found to be in the soil synergistic, as the increase of the Ni level is followed by a corresponding increase of Cu, irrespective of the treatment effect (Figure 4).Conversely, according to our unpublished data, the NixCu interaction has been found in Brussels sprouts leaves antagonistic under the Control, and "antagonistic-synergistic" under TMWW, the latter contributing negatively to the plant, basically being antagonistic.

Unfortunately, the available bibliographic information about this interaction is very limited. According to Cataldo *et al.* (1978) the absorption of Ni by soybean roots and its translocation to roots and shoots was inhibited by the presence of Cu, suggesting that these two elements may be interacting antagonistically. Nevertheless, the results of the present work, showed that there is a great need need for more work based on specially designed experiments for the elucidation of the afore mentioned interaction.

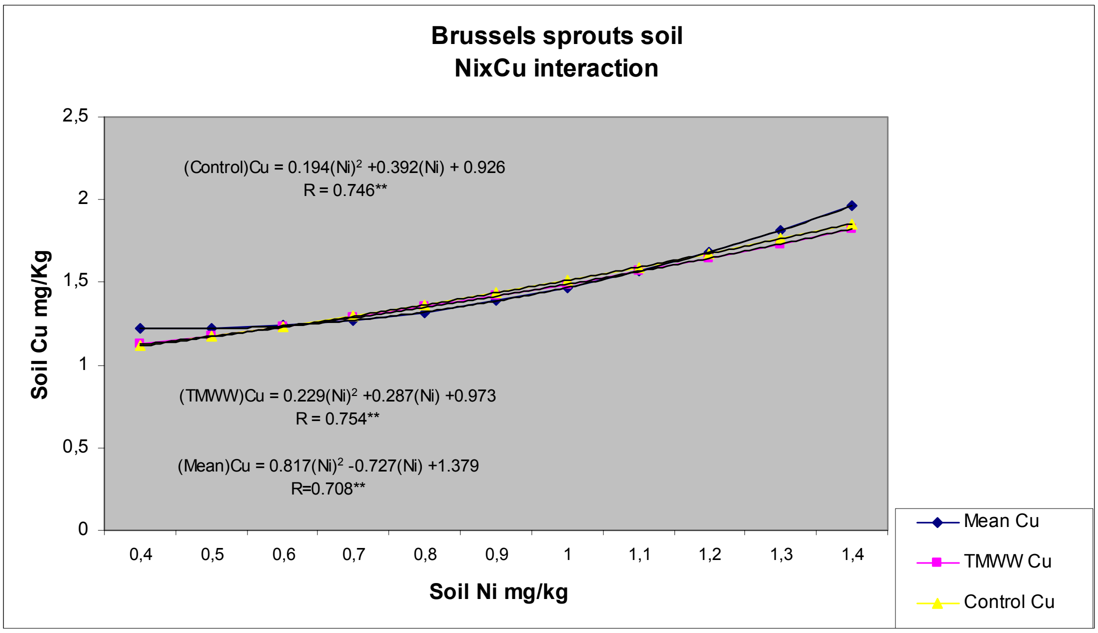

Figure 4. Interrelationships of soil DTPA extractable Ni and Cu under the effect of: a) TMWW b) Control and c) Mean treatment respectively

The Ni and Cu, apart from being characterized as heavy metals, they are also essential plant nutrients. In fact the Ni has recently been included in the essential elements (Brown, 2007). Therefore, the interaction NixCu is directly related to plant growth and development and their possible synergism in the soil, may mutually increase their concentration, thus favoring plant growth, and eventually, the environment.

3.1.5 NixPb

Results related to the above interaction, reported in previous work (Kalavrouziotis *et al.,* 2008b) showed that this interaction under both the effect of TMWW and Control, was found to be “two-way” and synergistic, in a soil cultivated with *Brassica oleracea* var. Italica (broccoli). Also, in another unpublished work, the NixPb interaction was shown to be synergistic in broccoli leaves under the TMWW and control, respectively, but antagonistic in the heads under the TMWW,where the Pb decreased, with the increase of Ni up to 5 μg g^{-1}, and it increased at an Ni concentration> 5 μg g^{-1}.

Similarly, in the present work this interaction (NixPb) was also found to be “one-way” synergistic in the soil, which was cultivated with *Brassica oleracea* var. gemmifera *(*Brussels sprout*)* (Figure 5).

Thus, the results of the present work seem to confirm the consistency of the synergism of the NixPb interaction in the soil, in the leaves, as well as n the edible plant part, but at higher than 5 μg g^{-1} level of Ni.

As shown in Figure 5, the differences observed in the concentration of Pb with the increasing levels of Ni, under the effect of the control, TMWW and Mean treatment, respectively, are statistically non significant according to t-test. This means that both the TMWW and Control affect the NixPb interaction more or less similarly.

As far as the relation of this interaction to the environment, it is of interest to note that the increase of Pb as a result of the synergistic effect of Ni, may be of concern, if the Pb level in the soil increases to toxic levels, as result of high levels of Ni that may possibly occur in the soil due to possible pollution with Ni.

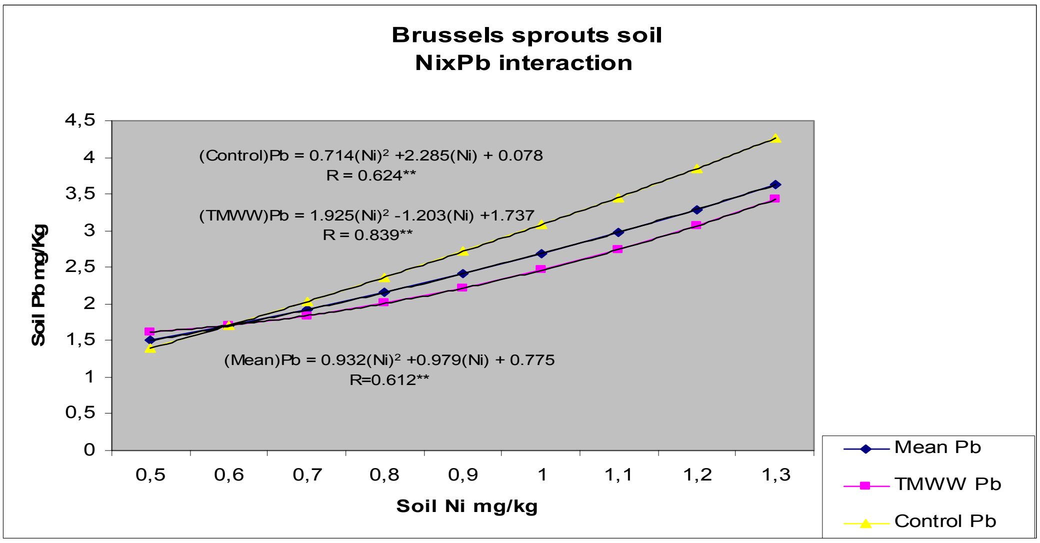

Figure 5. Interrelationships of soil DTPA extractable Ni and Pb under the effect of: a) TMWW b) Control and c) Mean treatment respectively

4. CONCLUSIONS

Of the 40 soil heavy metal interactions taking place between Pb, Ni, Co, Cu, Cd and Zn, under the respective effect of TMWW and Control, only 36 were statistically significant, being distributed on the basis of the applied treatments, as follows: a) under TMWW: 15 or 37.5 %, and b) under the Control 21 or 52.5 %.

Thus, most of the statistically significant interactions took place under the effect of the control, and less under the TMWW. Also, 11 of these interactions or 27.5% were found to be concomitantly occurring under TMWW and Control, respectively. This, indicated the consistency and stability of the occurrence of these interactions.

The general conclusion was that on the average, the effect of TMWW on the above interactions did not differ significantly from that of the control. Therefore, from this point of view, the TMWW could be used for vegetable irrigation, provided that the necessary measures are taken for minimizing the health risk involved, probably by improving the treatment of the wastewater in the Biological Treatment Plant.

Most of the heavy metal interactions in the soil, were found to be “two-way”, synergistic. This synergism, however, when it refers to heavy metals, it may be a cause of serious concern from the environmental and health point of view, since It may increase the level of toxic elements, such as for example of Cd and Pb.

More detailed experimental work is necessary to further elucidate the relations of heavy metals with the environment, and health, under the effect of the TMWW reuse.

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