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Interactions between Cadmium, Lead, Cobalt, and Nickel in Broccoli, irrigated with treated municipal wastewater

I.K. Kalavrouziotis[1]*, P.H. Koukoulakis[2], G. Manouris[3], A.H. Papadopoulos[4]
[1] *Department of Environmental and Natural Resources Management, University of Ioannina, G.Seferi 2, GR-301 00, Agrinion-Greece – Email: ikalavru@cc.uoi.gr*
[2] *Assoc. Researcher, Soil Science Institute, NAGREF, Altladidos 8, GR-544 54, Thessaloniki*
[3] *Hellenic Ministry of Environment , Physical Planning and Public Works, General Directorate of Environment, Department of Environmental Planning ,147 Patission Str. GR-11251*
[4] *NAGREF, Soil Science Institute, of Thessaloniki, GR-570 01, Thermi, Thessaloniki, Greece*

Abstract: An experiment was conducted in a greenhouse, in Agrinion Greece, in order to study the effect of treated municipal wastewater on the interactions between heavy metals Cd, Pb, Co and Ni, in the various plant parts of Broccoli. The statistical design included two treatments, (a) Treated municipal wastewater (TMWW) and (b)-Ordinary irrigation water, denoted as "control", in six replications. Broccoli plants Brassica oleracea, var. Italica, was used as a test plant. The following were found: Many statistically significant interactions between heavy metals were taking place in the various parts of broccoli plants (roots, leaves and heads), under the effect of the treated municipal wastewater (TMWW), their distribution in the plant parts, being of special importance. The interaction CoxPb was found to be synergistic in the heads but at lower Co level. Similarly, the NixPb was synergistic in the leaves and antagonistic in he heads. Both of these interactions may be of importance, as they may concern the consumer, since the synergistic interactions contribute positively to the accumulation of heavy metals in the edible plant parts, while the antagonistic, negatively. However, the general effect of the treated municipal wastewater was not very conclusive. More work is necessary to elucidate the mechanism and the role of these interactions in plant growth and in the environment.

Keywords: heavy metals, roots, leaf, heads, interactions, broccoli

1. INTRODUCTION

Treated municipal wastewater (TMWW) is a source not only of water but also of essential nutrients (Jimenez-Cisneros, 1995), but also of heavy metals (Alloway, 1995).

In view of the irrigation water shortage faced in many countries, TMWW reuse constitutes an alternative source of irrigation water, which when used on a long term basis, may contribute to the accumulation of heavy metals cadmium (Cd), lead (Pb), nickel (Ni) and cobalt (Co), in the soil-plant system (Kalavrouziotis et al., 2008).

In recent years, there has been shown great concern about the accumulation of these metals in plant tissue, a problem that is directly related to human and animal health (Asano, 1998).

Many factors affect the accumulation of heavy metals in the plant tissues. Amongst them, the plant genotype is the most important (Woolhouse, 1983). Also, the content of the metals in the soil, as well as the chemical, physical and biological properties of the soil medium, may favour the increase of the metal concentration in the plant tissues (Alloway, 1995).

Generally, there is a positive correlation between soil metal content and plant tissue metal concentration (Kabata-Pendias and Pendias, 1995).

The reuse of TMWW usually enriches the soil with heavy metals and, therefore, enhances their accumulation in the soil-plant system (Kalavrouziotis et al., 2008). Under the influence of the TMWW reuse, several interactions are taking place between Cd, Pb, Ni, and Co in the plant tissues of Brassica oleracea var. Italica (Broccoli), which was used as a test plant in the present work. The synergistic interactions contribute positively to the accumulation of the metals, while the antagonistic, and may decrease the concentration of the interacting metals (Kalavrouziotis et al., 2008a). Therefore, the Cd, Pb, Co and Ni interactions can be of significant importance, especially from the health point of view, because, depending on their synergistic or antagonistic nature, they may either increase or decrease the concentration of one of the above mentioned metals or even of the essential elements, in the plant parts and especially in the edible ones, possibly posing health risk for the consumers.

The interactions can either be "two-way:, that is, the interacting elements can mutually affect each other's concentration, by increasing or decreasing the level of either of the elements involved, or of "one-way", by decreasing or increasing the level of only one of the interacting elements, the effect being non mutual (Kalavrouziotis et al., 2008a, 2009).

The interactions may also take place in the roots, leaves, or edible plant parts, i.e., in the broccoli heads, where they may contribute to the accumulation of the interacting heavy metals. Consequently, they may affect the distribution of the accumulation of these metals in the plant, a subject of prime concern, as it is related to the human health.

The extent of occurrence of heavy metal interactions, and especially their distribution in the plant parts is a matter that depends on the kind of plant, and the synergistic or antagonistic behaviour of the interacting heavy metals.

Thus, heavy metal interactions, which are characterized by a strong mutual or synergistic effect of either of the interacting metals on each other's concentration, may create health risk situations for the consumers, if they occur for example in the edible plant part. Under the effect of TMWW reuse, the heavy metal interactions may be intensified (Kalavrouziotis et al. 2008b), and thereby, they may further contribute to the accumulation of the interacting metals in the plant.

Among the TMWW heavy metals, lead (Pb) is found in relatively high levels in comparison to the concentration of the other heavy metals (Asano, 1988). The Pb soil content is usually positively correlated with Pb concentration in plant tissue (Karamanos et al., 1976: Korcak and Fanning 1985). Lead is accumulated mainly in the root (Kabata- Pendias and Pendias, 1995), and only a small fraction of this Pb is transported to the tops (3%).

Cadmium, a very toxic element, is usually found in TMWW in lower concentrations. This metal can easily accumulate in the plant and its various organs and its distribution in the plant tissues, is of extremely great concern (Alloway, 1995).

Cobalt accumulation in the plant takes place in a specific manner, and is related to the plant organ, and mainly to leaf. Thus, leafy vegetables may accumulate significant quantities of Co, for example cabbage Brassica oleracea var. capitata (Kloke, 1980).

Cobalt has been found to interact with many elements (Talukder and Sharma, 2007), and it is antagonistically related with Cd, as at high levels it suppresses its uptake. Moreover, it may synergistically be associated with Cd due to the biphasic mechanism involved in the CoxCd interrelationship (Werner, 1979). Similarly, Co is synergistically related to Ni, as it has been reported that toxic Co levels of 10-20 mg/kg dry matter, were associated with excess Ni (Anderson et al., 1973).

On the contrary, Co has been found to interact antagonistically with Fe, to the extent of causing Fe deficiency (Wallace and Abou-zamzam 1989). Nickel, it has been reported to be antagonistically related to the Fe leaf level, as low concentrations of Fe are found in the plant, in the presence of high Ni concentrations in the tissues (Foy et al., 1978).

Our understanding regarding the interactions between the heavy metals Cd, Pb, Ni, Co and their effect on the accumulation and distribution in the various plant parts of these metals is very limited. As this matter is related to the consumers health, the purpose of the present work is to study the role of these interactions on the accumulation of the heavy metals in Broccoli plant parts (roots, leaves and heads), under the effect of treated municipal wastewater, with the view to reuse TMWW for irrigation purposes of vegetables.

2. MATERIALS AND METHODS

A randomized block design experiment was conducted in a greenhouse, of the Department of Environmental Management and Natural Resources, Agrinion Greece, to study the comparative effects of the treated municipal wastewater (TMWW) and ordinary well irrigation water (Control), on the interactions of macro-, micronutrients, heavy metals, and physical and chemical properties of a soil cultivated with broccoli used as test plant.

The statistical design included two irrigation treatments (a) TMWW and (b) Control (ordinary well irrigation water) in six replications, with a total number of 2x6=12 experimental plots, of 2.5 m x 1.8 m=4.5 m^2 size.

The plots were separated by dikes of height: “0.1 m” Broccoli plants were transplanted in rows “0.8 m” apart from each other, while the distance between the plants in the row was “0.5 m”, respectively.

Transplanting was performed on December 11, 2005 and sampling of roots and leaves at eight weeks, and of the heads, at sixteen weeks after planting, i.e., April 20, 2006.

The treated wastewater was supplied by the Biological Treatment Plant of the Messolongion Municipality.

TMWW and the control were applied thirteen times during the plant growth period, at a rate of 30 L per application, the total volume of each treatment applied being 13x30=390 L per 4.5 m^2 or 867 mm/ha.

Ordinary well irrigation water, TMWW, and plant samples were analyzed, and the data obtained were processed statistically by ANOVA, t-test, and regression analysis.

2.1 Chemical analyses

2.1.1 Plant material

Each root sample was placed in a plastic sieve and was flushed with low pressure tap water, until the complete removal of the soil particles. Then, the parts of plants samples (root, leaf, and head) were washed with deionized water, followed by cleaning with a dilute solution of 0.005% hydrochloric acid (HCl), and then they were thoroughly washed, by means of a special detergent (alconox 0.1%), and rewashed repeatedly (four times) with distilled water, left to drain on a filter paper and dried in a ventilated oven at 700 ^oC. The dried samples were ground in a special hammer mill, and stored for chemical analysis. The plant samples were ashed in a muffle furnace at 500 ^oC for 10-12h. The inorganic ash was dissolved in a 50:50 (v/v) H_2O: HCI solution and this solution was analyzed for Pb, Cd, Co, and Ni, were by atomic absorption spectroscopy using an Variant, Model AA-IO (Sakata, 1987).

2.1.2 Water and TMWW analysis

Both ordinary well irrigation water (control) and the TMWW were analyzed before their application by methods suggested by AOAC (1996); APHA (1995). The pH was determined electrometrically by means of a precaliberated pH-meter while SAR was calculated according to Richards (1954).

Total nitrogen (N) was determined by the Kjeldhal method, i.e., by digesting a water and TMWW sample with sulfuric acid(H_2SO_4) to convert organic N to NH_3 , followed by distillation after alkalinization (AOAC,1996) and total N content was determined titrimetrically. Also, Clanions were determined in 100 ml of irrigation water or treated municipal wastewater, to which 1ml of indicator-acidifier was added, - composed of 250mg s-diphenylcarbazone, 4 ml HNO_3 and 30 mg of xylene cyanol FP in 100 ml alchohol . Then the solution was titrated with 0.0141N $Hg(NO_3)_2$ to definite purple end point (AOAC, 1996).

Calcium (Ca) and Magnesium (Mg) were measured by titration with ethyldiaminetetraacetic acid (Versenate) (Richard, 1954).

Potassium in water or in the wastewater was determined by pretreatment in a buffer solution, and it was determined by using atomic absorption spectrophotometer (AOAC, 1996).

Total P was determined by digesting an aliquot of the sample with persulfat, P was measured by direct colorimetric analysis by developing the phosphomolybdate complex, reduced to intensely blue color by means of ascorbic acid. The color intensity was measured colorimetrically (AOAC, 1996).

The well irrigation 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 nitric acid (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 were added and the mixture was reheated gently to dissolve the residue. The watch glass and the beaker 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.

The arsenic (As) content of the water or TMWW, was determined by evaporating 0,5 L of water or TMWW to dryness, and by adding a small quantity of Na_2CO_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 (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 nitric acid (HNO_3) up to $pH<2.0$. Curcumin (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. (Standard Methods for Examination of Water and Waste water, 1992).

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 flamephotometer. The water, and TMWW chemical analysis, is given in Table 1 (Kalavrouziotis et al., 2008a).

3. RESULTS AND DISCUSSION

Significant interactions occurred in Broccoli plants between the heavy metals Cd, Pb, Ni and Co under the effect of TMWW reuse for irrigation (Kalavrouziotis et al., 2008a, 2008c).

It was determined that most of these interactions were antagonistic, some of them were synergistic and a few were biphasic, i.e. they were both synergistic and antagonistic (Kalavrouziotis et al., 2009; Kabata-Pendias and Pendias, 1995).

On the other hand, these interactions were taking place in both roots and leaves such as the CdxPb, while others occurred in the leaves and heads (CoxPb and NixPb). Finally, some others were found to take place only in the leaves (CdxNi). These interactions are examined below, under the effect of both TMWW and the control, respectively, and relevant conclusions are drawn with respect to their impact on plant growth, health and the environment.

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

3.1 Cd x Pb

Both of these two heavy metals, involved in this particular interaction, were found as constituents of the TMWW, the Pb being less abundant (0.08-0.127 mg/l) than Cd (0.07-0.48 mg/l). They are toxic elements to humans as well as animals. Their concentration in the human diet is of great importance (Alloway, 1995). As far as the Pb is concerned, this metal is characterised as toxic element (Davies, 1995).

The CdxPb interaction, has been found to be mainly antagonistic, primarily in the leaves (Figure 1A), whereas in the roots it was biphasic (Figure 1B). Thus, it can be seen in this figure that, the root Pb concentration decreased under the effect of either treatment, with the corresponding increase of the dry matter Cd level up to 0.0075-0.008 μg/g (antagonistic effect). On the other hand, with the further increase of Cd, the Pb level increased significantly. Thus, the CdxPb interaction becomes synergistic (Figure 1B). Also, in this figure, it can be seen that with a small change in the Cd concentration i.e. by 0.005 μg/g, the root Pb level of the biphasic CdxPb interaction, decreased at the beginning and later on increased significantly.

Also, as shown in Figure 1A, the difference in the concentration of leaf Pb, under the effect of the TMWW and the control, increased significantly with the increase of Cd level above 0.05 μg/g dm, suggesting a relatively strong effect of TMWW on this interaction at the higher Cd levels, due probably to the TMWW higher Cd and Pb content, compared to that of the control. According to Kabata-Pendias and Pendias (1995), the Pb may probably stimulate the Cd plant uptake, indicating a possible synergistic interaction between these two heavy metals, though they postulate that this effect of Pb on Cd, may be a secondary one, related to trans membrane ion transport. This stimulating effect of Cd possibly takes place in the broccoli roots at the higher Cd levels, as shown in Figure 1B.

The CdxPb is a 'two-way' partially synergistic interaction, occurring in the broccoli, roots at the higher Cd levels (Figure 1B), suggesting that any increase of root Cd concentration may increase the Pb level, with possibly unfavorable effects on the plants and on the vegetation in general, which eventually may have environmental and health implications. However, it must be mentioned that contrary to the above CdxPb interaction, the PbxCd interrelationship is a synergistic interaction (Figure 2). The increase of Cd concentration, due to the effect of the increasing levels of Pb, shown in the above figure, may also have favorable environmental effects, as the Cd may act as a plant detoxifying agent, since it triggers the formation of phytochelatins (Grill et al., 1987) and therefore the Cd may influence favorably the plant growth and the environmental quality.

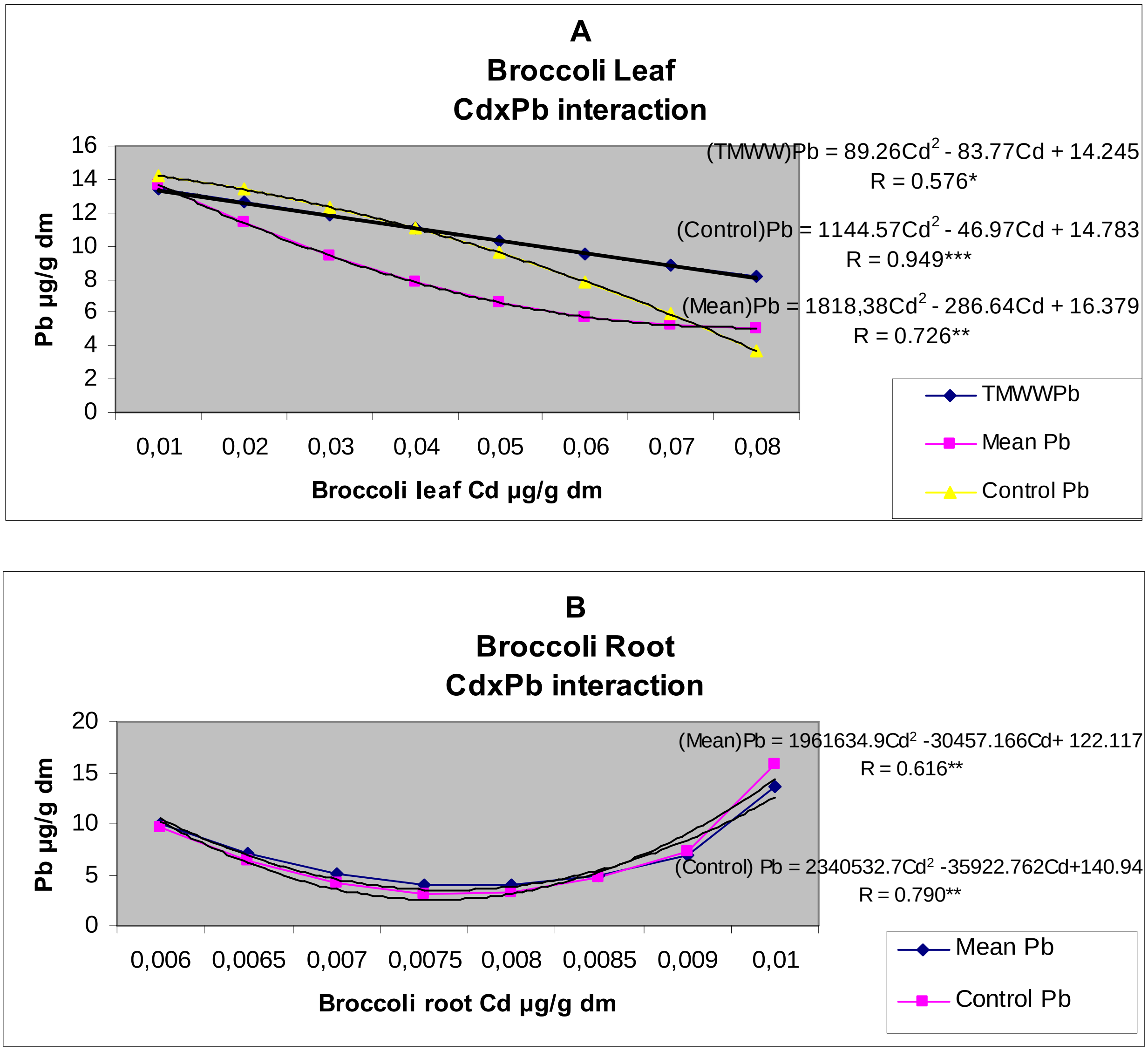


Figure 1: Interrelationships of Broccoli Cd and Pb in: A- Leaves and B- Roots under the effect of a-TMWW, b-control and c- mean treatment effect respectively
(*), (**), (***) statistically significant at P0.05, P0.01 and P0.001

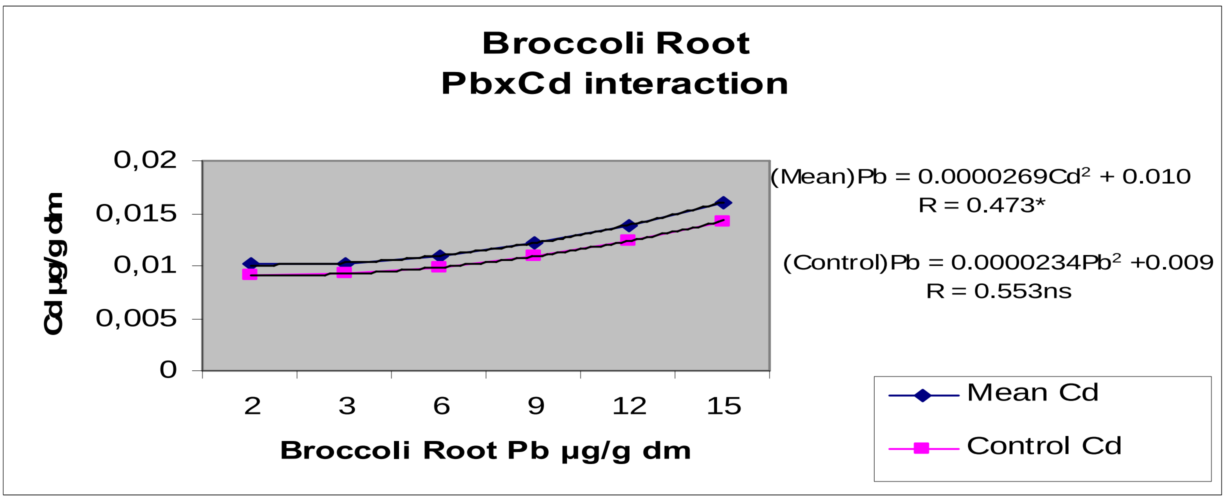


Figure 2: Interrelationships of Broccoli Root Pb and Cd content under the influence of: a-'control' and b- mean treatment effect respectively
(*), (**), (***) statistically significant at P0.05, P0.01 and P0.001

3.2 Ni x Pb

The NixPb interaction was found to be synergistic in the Broccoli leaf at the lower Ni levels (Figure 3A), and antagonistic in the heads under the TMWW (Figure 3B). Thus, it can generally be seen in these figures that a rise in the level of Ni increased the corresponding concentration of Pb in the leaf (Figure 3A) and it decreased in the head (Figure 3B).

However, in the case of leaf Pb content (Figure 3A), its concentration, under the effect of the control, increased with the increase of the Ni level up to 5μg/g and with further increase of Ni the Pb concentration started decreasing. Consequently, the otherwise synergistic NixPb interaction is changing into antagonistic at higher Ni levels (Figure 3A).

Conversely, the Pb concentration in the broccoli head, decreased under the effect of TMWW, with the increase of the Ni up to its level of 5μg/g, but with the further increase the Pb concentration started increasing, thus changing the otherwise antagonistic NixPb interaction into a synergistic one (Figure 3B).Thus these interaction were found to be biphasic.

The phenomenon of biphasic interactions has been observed in a number of cases (Kalavrouziotis et al., 2008a) and it is possibly related to the probable inability of the plant to function properly, when the accumulation of heavy metals in the plant tissues increases or it decreases, thus absorbing and accumulating lower or higher levels of the other interacting elements. Nevertheless, the mechanism involved is not well understood and more work is necessary for its elucidation.

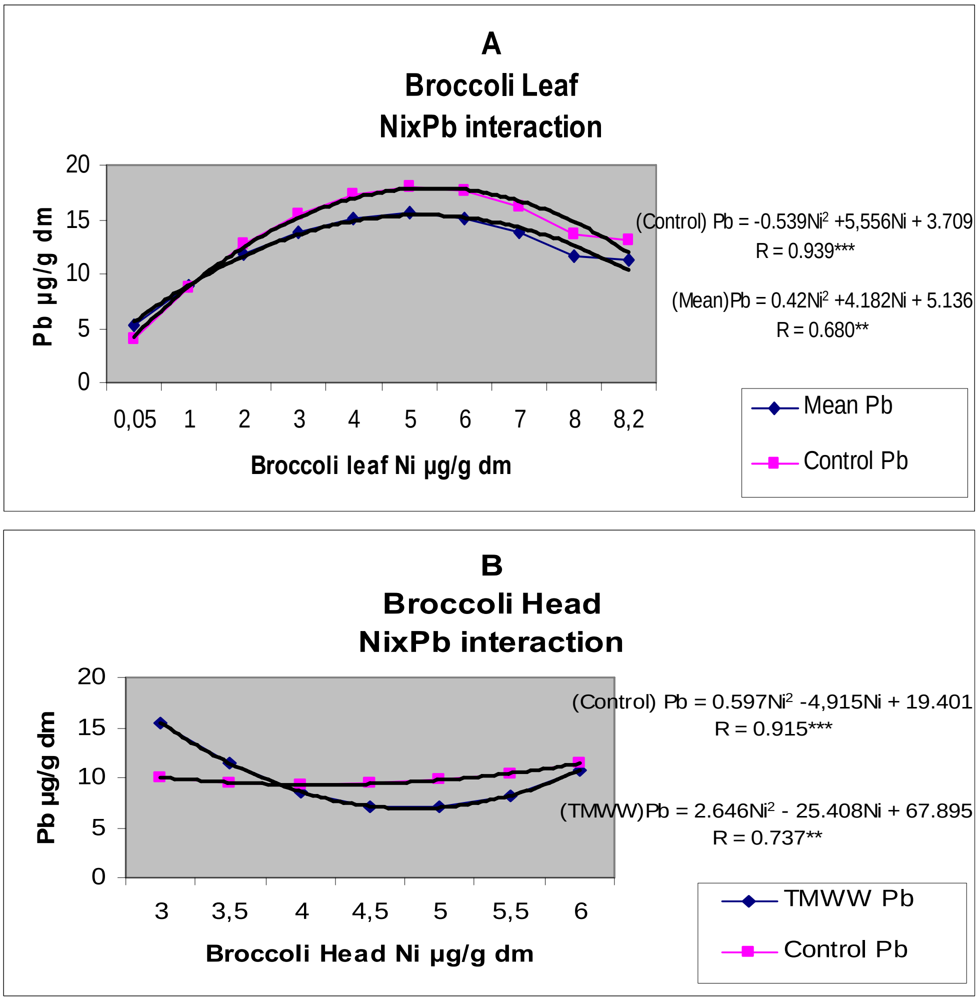


Figure 3: Interrelationships of Broccoli Ni and Pb in: A- leaf, and B-Head, under the influence of a-TMWW, b-control and c- mean treatment effect

(*), (**), (***) statistically significant at P0.05, P0.01 and P0.001 level of probability, respectively

The experimental evidence about the NixPb interaction is very scarce. The Ni has been reported to interact antagonistically only with Cu, Zn and Fe (Cataldo et al., 1978). On the other hand, it has been stated that Ni may act both antagonistically and synergistically on some heavy metals such as Cu, Zn and Mn (Kabata- Pendias and Pendias, 1995). So far no mention of the interaction of NixPb has been made in the literature.

This interaction must be studied further, as it is related to the human health, and to the environment. Any factor which will contribute to the decrease of the toxic Pb in the edible plant parts will be an important contribution to minimizing health risk.

In the case of Broccoli plant head, the antagonistic interaction of NixPb is of interest, as the Ni has been shown to be essential nutrient for some micro organism and animals (Alloway, 1995) and as reported by Brown (2007) Ni is now considered as an essential plant nutrient, in addition to the fact that by implication it may also play a role in the human metabolism, contrary to Pb, which is considered as a poisonous element (Davies, 1995).

The antagonistic effect of Ni on Pb found in Broccoli head is undoubtedly a very interesting result and must be studied further, since the available information about it is very limited.

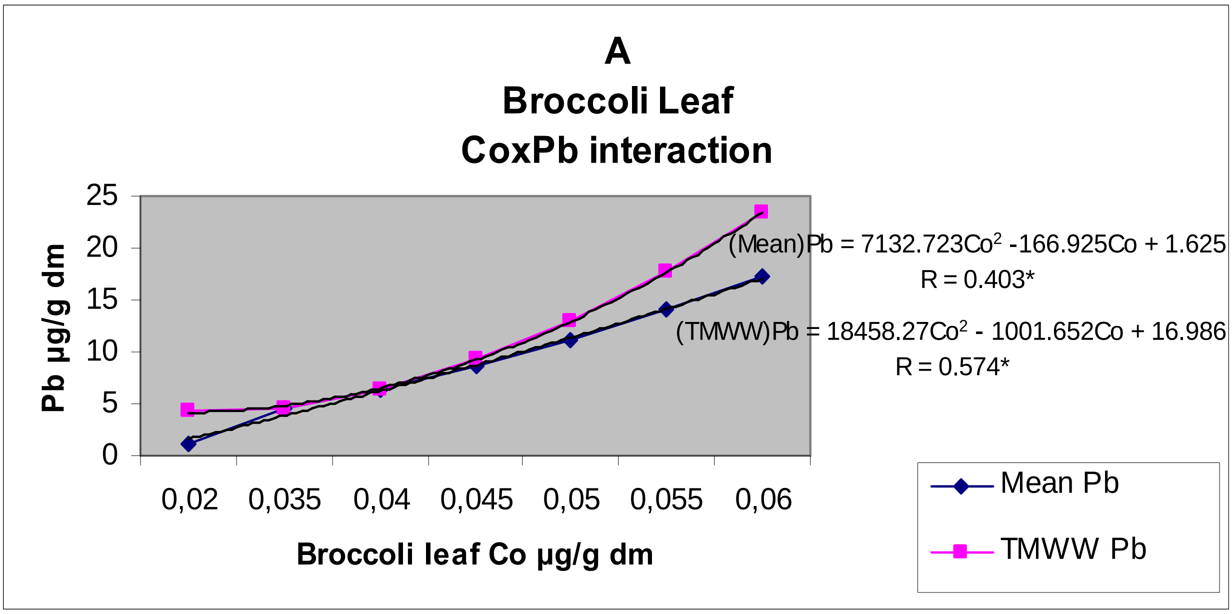
3.3 Co x Pb

Though Co is not an essential plant nutrient, its importance with respect to its biological function, stems from its essential role in biological N_2 fixation. Co is required by the Rhizobium bacteria, which forms symbiotic associations with legume plants (Smith and Paterson, 1995; Talukder and Sharma, 2007; Mengel and Kirkby, 2001).

The CoxPb interaction was found in this study to be synergistic in the Broccoli leaves (Figure 4A), while in the heads it is synergistic at low Co level (<0, 04-0,045 μg/g dm), whereas, at higher Co levels (>0,045 μg/g dm), it becomes antagonistic, because with the increase of the Co level, the Pb concentration decreases significantly (Figure 4B).

As shown in Figure 4A, the concentrations of Broccoli leaf Pb under the effect of TMWW, is much higher than that under the ‘mean’ treatment effect. This indicates the importance of TMWW in this interaction, the functioning of which, increases the Pb level in the leaves. Conversely, the TMWW reuse did not have a significant effect on the increase of the head dry matter Pb level, a situation which is of interest, as it concerns the edible Broccoli plant part (Figure 4B).

Even though the interactions of Co with other heavy metals and essential elements (Fe, Zn, Cd, Cu, Cr, Mg, S, Ni) have been studied considerably (Talukder and Sharma, 2007), no mention is made in the literature about the CoxPb interaction. The limited findings of the present work about this interaction, under the influence of TMWW, must be further examined by more detailed research, towards elucidating this very important interaction, especially under the effect of the TMWW reuse in vegetable crops, which is directly related to the consumer’s health. Though the environmental problems with Co are insignificant (Smith and Paterson, 1995) in comparison to problems caused by other toxic heavy metals, yet the synergistic interaction of Co with Pb in the Broccoli leaves and perhaps in other vegetables should be studied more carefully, as it is related to the accumulation of s toxic element (Pb) in the plant dry matter by the increase of the level of Co, an element which is necessary for the N_2 fixation of legumes.



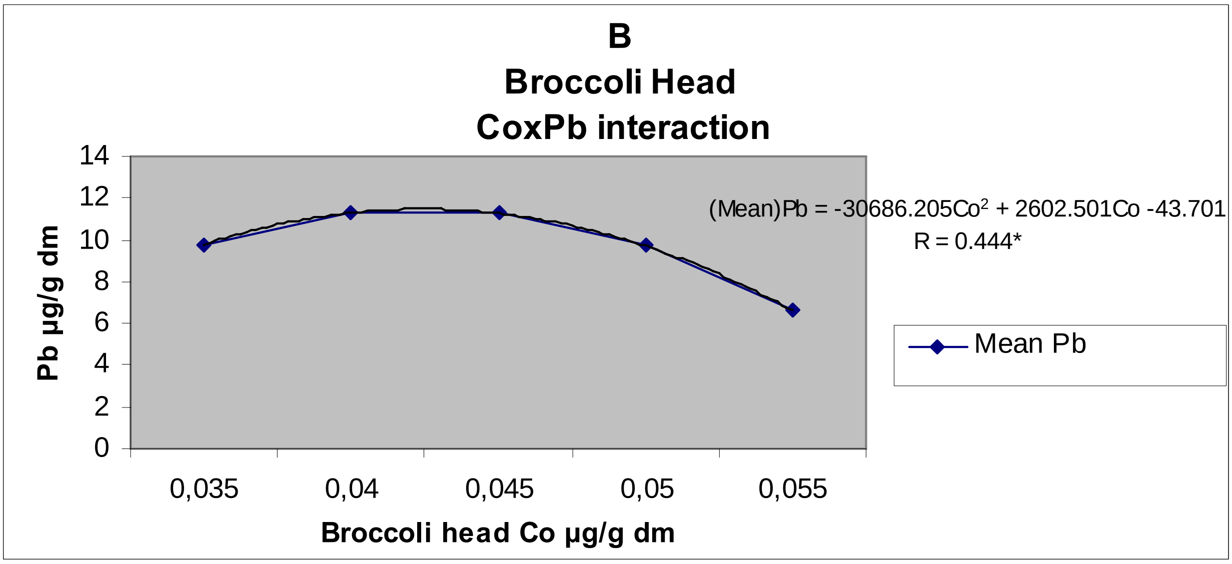


Figure 4: Interrelationships of Broccoli Co and Pb in : A- leaves, and B-Heads, under the influence of a-TMWW, b-control and c- mean treatment effect respectively
(*), (**), (***) statistically significant at P0.05, P0.01 and P0.001

3.4 Cd x Ni

According to the existing experimental evidence, this interaction has been reported as an antagonistic and/or synergistic (biphasic) (Kabata-Pendias and Pendias, 1995). Such biphasic interactions are very common and have been found in previous work of Kalavrouziotis et al. (2008c). In the present work, the interaction CdxNi was found to be antagonistic (Figure 5). It can be seen that with the increase of the Cd level, a significant decrease of the Ni concentration takes place, suggesting that the Cd antagonizes very strongly the Ni in Broccoli leaf. Furthermore, the difference in the concentration of Ni with the increasing levels of Cd, under the influence of both TMWW and the control, respectively, is statistically significant, showing that at the same increment of Ni, the control exerts much stronger negative effect on the decrease of Ni, than that of the TMWW (Figure 5).

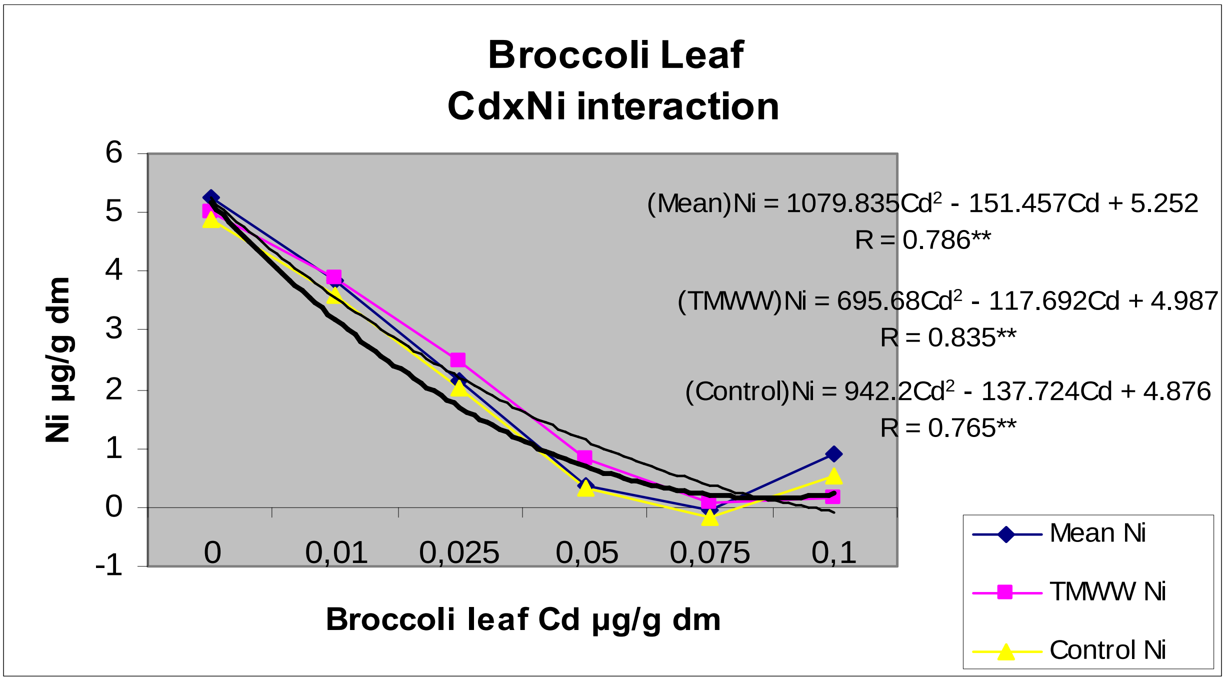


Figure 5: Interrelationships of Broccoli Leaf Cd and Ni under the influence of a-TMWW, b-control and c- mean treatment effect respectively
(*), (**), (***) statistically significant at P0.05, P0.01 and P0.001

4. CONCLUSIONS

Statistically significant interactions between heavy metals Cd, Pb, Ni and Co were found to take place in the various Broccoli plant parts, under the influence of the Treated Municipal Wastewater – a carrier of heavy metals – as follows:

In roots: CdxPb (two way synergistic), in leaves**:** CdxPb (antagonistic), NixPb (synergistic, CoxPb synergistic. CdxNi (antagonistic) and in Heads: NixPb (synergistic/antagonistic) and CoxPb synergistic/antagonistic.

The distribution of these interactions in the various plant parts and especially in the edible ones, such as of the CoxPb or NixPb, which include heavy toxic metals i.e. Co or the Pb, reflect health risk problems that merit special attention, as they constitute, apart from the microbiological load, the basic criteria for the decision making with respect to the reuse of TMWW for vegetable irrigation.

The general effect of the Treated Municipal Wastewater on the interactions studied was not very conclusive and though in some cases was definitely intensified. However, its effect on most of the interactions studied was not significant in comparison to those under the control. Nevertheless, more work is necessary in order to establish a sound and scientifically unquestionable basis for the safe and socially acceptable reuse of TMWW in the irrigation of broccoli and of vegetables, in general, aiming to health risk factors involved and making the TMWW reuse more safe for the consumer and the environment.

REFERENCES

Alloway, B.J, 1995. Cadmium. In Heavy Metals in Soil. Alloway, B.J, (ed) Blackie Academic Professional. An Imprint of Chapman and Hall. 2nd Edition, 3-4.

Anderson, A.J., Mayer, D.R., and Mayer, F.K, 1973. heavy metal toxicities: Levels of Nickel, Cobalt, and Chromium in the soil and Plants Associated with visual symptoms and variations in the growth of an oat crop. Aust.J.Agr.Res. 24:557.

AOAC.1996.Official methods of Analysis of AOAC International. 16th edition Publication International Suite AOAC 481 North Frederick Ave. Gauthersburg, Maryland 2027-2417 USA.

APHA, Standard Methods of Examination of Water and Wastewater, 1995, 19th Edition, American Public Health Association, Washington DC USA.

Asano, T, 1998. Waste-Water Reclamation and Reuse. Vol.10 Technomic Publishing CanCastev- Pennsylvania USA.

Brown, H.P., 2007.Nickel, In: Handbook of Plant Nutrition. BarkerA.V. and Pilbeam,D.J. (eds) CRC Taylor and Francis Grour, Boca Raton USA, 395-410.

Catalbo, D.A, Garland, T.P., and Wildung R.E., 1978. Nickel in Plants. Plant Physiol. 62(I):563 (II):566.

Davis, B.E., 1995. Lead. In: Heavy Metals in Soil. Alloway, B.J, (ed) Blackie Academic Professional. An Imprint of Chapman and Hall.

Foy, C.D., Chaney, R.L., and White, M.C., 1978. The physiology of Metal toxicity in plants. Annual.Plant.Physiolog. 29:511.

Grill, E., Winnacker, E.L, and Zenk, M.H., 1987. Phytochelatins, a class of heavy metals binding peptides from plants are functionally analogous to metalloproteins. Proc. Nat. Acad.Sci. 84:439-443 USA.

Jimenez-Cisneros, B., 1995. Wastewater reuse to increase soil productivity, J.Water.Sci.Technol. 32(12):173-180.

Kabata-Pendias A. 1989. Unpublished data. In Kabata-Pendias, A. and Pendias, H. 1995. Trace Elements in soils and plants, CRC Press. Boca Raton, USA. 2nd Edition.

Kabata-Pendias, A. and Pendias, H. 1995. Trace Elements in soils and plants CRC Press. Boca Raton USA. 2nd Edition.

Kalavrouziotis, I.K., P.Robolas, P.H. Koukoulakis, A.H. Papadopoulos, 2008. Effects of municipal reclaimed wastewater on the macro and micro elements status of soil and of *Brassica oleracea* var. Italica, and *Brassica oleracea* var. gemmifera. J. of Agricultural Water Management, Vol. 95, issue 4, 419-426.

Kalavrouziotis, I.K., P.H. Koukoulakis, P.Robolas, A.H. Papadopoulos, V. Pantazis 2008a, Interrelationships of heavy metals, macro and micro nutrients, and properties of a soil cultivated with *Brassica oleracea* var. Italica (Broccoli) under the effect of Treated Municipal Wastewater, Journal Water, Air and Soil Pollution, Vol 190, no 1-4, 309-321.

Kalavrouziotis, I.K., P.H. Koukoulakis, P.Robolas, A.H. Papadopoulos, V. Pantazis 2008 b. Macro and micronutrient interactions in soil under the effect of *Brassica oleracea* var. Italica, irrigated with Treated Municipal Wastewater,' International Journal 'Fresenius Environmental Bulletin 17(9a) :1271-1281.

Kalavrouziotis, I.K., P.H. Koukoulakis, Maria Sakellariou-Makrantonaki, Christos Papanikolaou 2009. 'Effect of Treated Municipal Wastewater on the essential Nutrients interactions in the plant of *Brassica oleracea* var. Italica' s. Desalination.242:297-312

Karamanos, R.E., Bettany, J.R., and Stewart, J.W.B. 1976.The uptake of native and applied lead by alfalfa and bromegrass from soil, Can.J.Soil Sci, 56:485-496.

Kloke, A., 1980. Effects of heavy metals on soil fertility, flora and frauna, as well as its importance for the nutrition of fodder chain. In : Proceedings of utilization of refuse and sewage in Agriculture, Zurich, 58-87.

Korcak, R.F., and Fanning, D.S., 1985. Availability of Applied Heavy Metals As A Function of Type of Soil Material and Metal Source ,Soil Science 140:23-34.

Mengel, K., and Kirkby, E.A., 2001. Principles of plant nutrition. Kluwer Academic Publication, Dordrecht.

Richards,L.E.,1954.Diagnosis and improvement of saline and sodic soils, Handbook No 60,USDA.

Sakata, M., 1987. Movement Neutralization of alkaline leachate at coal and disposal sites. Envir. Sci. and Technology 21:771-777.

Smith, K.A., and J.E. Paterson, 1995. Manganese and Cobalt. In: Alloway, B.J, (ed). Heavy Metals in Soil. Blackie Academic Professional. An Imprint of Chapman and Hall, London, 224-244.

Tuckendorf, A., and Rauser, W.E., 1990. Changs in Glutathione and Phytochelatins in roots of maize seedings exposed to Cadmium. Plant Sci.70:155-160.

Talukder,G., and Sharma, A, 2007. Cobalt. In : Handbook of plant nutrition, Barker, Av., and Pilbeam, D.J. (eds) CRC Taylor and Francis Group, Boca Raton, USA pp.449-514.

Wallace,A., and Abou-zamzam A.M., 1989. Low levels but excesses of five different trace elements, singly and in combination in bush beans growon in solution culture. Soil Sci. 147:439-441.

Werner, V., 1979. Effect of Ni, Cd, and Co on the uptake of Cu by intact barley (Hordeum distichon) roots, Zh. Planz. Physiol. 93:1-10.

Woolhouse, H.W., 1983. Toxicity and tolerance in response of plants to metals. In : Encyclopedia of plant Physiology, new series, Langer, O.L.et al., Editors, Vol 12c, 45-300 Springer- Verlag, Berlin.