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Evaluation of interactions among sewage sludge bioavailable metals from WWTPs using DTPA agent

Evangelos Giannakopoulos[a], Ioannis K. Kalavrouziotis[a,*], Hlias Dimitrelos[b], Prodromos H. Koukoulakis[c], Soterios P. Varnavas[b], Frantzis Papadopoulos[c]

[a]*School of Science and Technology, Hellenic Open University, Tsamadou 13-15 & Saint Andrea, 262 22 Patras, Greece, email: ikalabro@eap.gr*
[b]*Department of Geology, University of Patras, 26504 Patras, Greece*
[c]*Hellenic Agricultural Organization 'Demetra' Soil Science Institute, Thermi, 57001, Thessaloniki, Greece*

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ABSTRACT

The diethylenetriaminepentaacetic acid extractable metal concentration, i.e., of Fe, Zn, Cu, Mn, Cd, Cr, Co, Ni, Pb, in the sewage sludge from six (6) municipal wastewater treatment plants (WWTPs) of Central Greece was evaluated. Also the elemental interactions occurring in the sludge and their quantitative contribution in heavy metals were studied according to the geographical grouping of the WWTPs in coastal and continental zone. Statistical analysis (ANOVA) between elemental interactions and quantification of their contribution in terms of heavy metals showed a significant difference in available metals between the above zones. Based on the quantification data of the interactions elemental contribution, it was found that more heavy metals were contributed to the sludge of the continental zone than to that of the coastal, due to the fact that more antagonistic interactions occurred in the coastal than in the continental zone.

Keywords: Sewage sludge; Biosolids; Metal interactions; Bioavailability; DTPA

1. Introduction

Sewage sludge is generated in an ever-increasing amount due to urbanization [1]. Sewage sludge is the solid material, often referred to as biosolid, which is generated as the by-product of municipal wastewater treatment [2]. The United States Environmental Protection Agency [3] defines biosolids as "the nutrient-rich organic materials resulting from the treatment of domestic sewage in a treatment facility. When treated and processed, these residuals can be recycled and applied as fertilizers to improve and maintain productive soils and stimulate plant growth".

Large quantities of domestic sludge are produced every year. For example, the estimated annual quantity of sludge produced by 13 member countries in EU during 1999 was 7.1×10^3 ton of dry matter [4]. The United States Environmental protection Agency estimated that in USA ~6.9×10^3 ton of biosolids were generated in 1998 [5]. Sixty percent of the biosolids generated were beneficially used either through land application, composting, or as a landfill cover. This number is expected to increase to 7.6×10^3 ton million Mg by 2005 and to 8.2×10^3 ton in 2010. Directive 91/271 of the European Union (EU) essentially prohibited the disposal of wastewater and sludge without treatment and permission. In USA, legislation on the disposal of sludge increased the regulations on land application of biosolids [6]. By implementing the EU Directive/1999/31, the amount of sludge produced to be buried by the process of the landfill should not exceed 35% of total waste generated in each EU country after 2016.

Sludge contains organic matter (OM), essential plant nutrients (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, and B), and variable amounts of various heavy metals (Cd, Cr, Pb, Hg, and Ni), rendering its application to soil problematic [4]. Therefore, it requires special management. The main concern about the

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* Corresponding author.

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biosolids is related to phytotoxicity, due to the accumulation of heavy metals in the soil [7] a fact that calls for special management of this soil input. Generally, the heavy metal accumulation in soil restricts its proper and effective functioning, because it may cause toxicity to plants, and affects unfavorably the food chain and human health. The heavy metals could be very toxic at high concentrations, and they may cause serious problems to the environment, plants, and human health [8].

Particularly important aspects that need to be investigated include long-term metal mobility and availability, plant trace metals uptake and toxicity, and leaching and colloidal mediated transport. Several methods have been proposed to assess metal availability in biosolid-treated soil. Chemical extractants such as chelating agents, dilute acids, and unbuffered salt solution are typically used to assess plant availability of trace metals. Ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid (DTPA) are the most commonly used chelating agents as a soil test for assessing plant availability of trace metals because of their greater extracting strength [9]. DTPA is the most popular chelating agent used because the concentration of trace metals extracted tends to correlate fairly well with metal uptake by plants [10–12]. Ure [13] found high correlation between DTPA extractable Cu, Zn, Ni, and Cd and their concentrations in lettuce and wheat. Additionally, Rappaport [14] found good correlation between DTPA extractable Zn and its concentrations in corn on 14 different Virginia soils. Mitchell et al. [15] reported significant correlations between DTPA extractable concentration and trace metals uptake by different plants in biosolids treated soils. Lake et al. [16] showed that Cd and Zn uptake by vegetables is proportional to DTPA extractable concentrations in soil. DTPA was also well correlated with Ni uptake by carrots roots and leaves [17] with Cu, Zn, Cd, and Ni uptake by winter wheat, carrots, and spinach [18] and with Cd, Cu, and Zn uptake by Swisschard, lettuce, tobacco, and peanut [19].

The subjective of this study is the statistical evaluation of the interactions among the metals that are found in the municipality sewage sludge (biosolid) and their bioavailability from the biosolid matrix to plants. For this aim, sewage sludge samples were obtained from six WWTPs in Central Greece (Fig. 1).

2. Materials and methods

In the present study, sewage sludges were collected from WWTPs, after mechanical dewatering of sludge and during last stage of conventional treatment process (conventionally treated), in plastic bags and brought to the field laboratory. The sewage sludge was air dried, finely powdered and sieved to 2 mm mesh size before use.

2.1. Reagents and solutions

Standard solutions were prepared by dilution of each pure element standards obtained from Merck (Darmstadt, Germany). The standard reference material of metals was used for the calibration and quality assurance for each metal. All aqueous solutions and dilutions were prepared with ultrapure water (Milli-Q, Millipore, Bedford, MA).

2.2. Physicochemical analyses

The determination of moisture (%), pH, OM, electrical conductivity (EC) (mS/cm), and $CaCO_3$ (%) content of the sludge was performed according to the Standard Methods for the Examination of Water and Wastewater of the American Public Health Association and AWWA [20]. The pH and EC were measured in 1:3 soil water suspension using a Crison

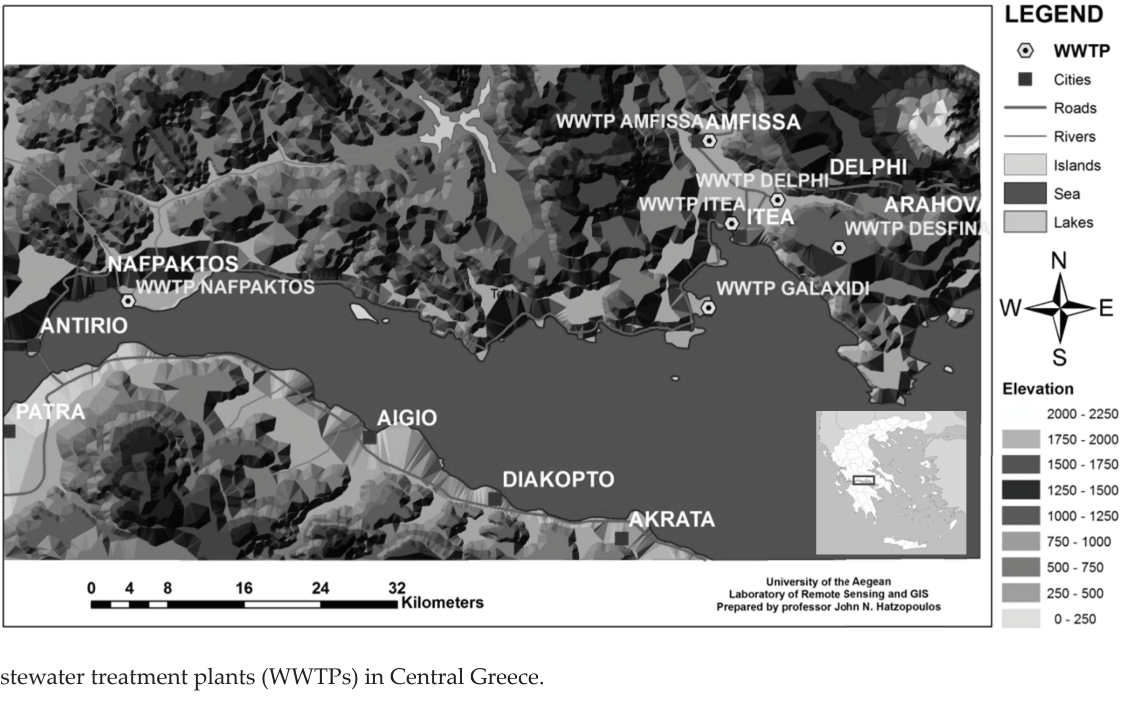

Fig. 1. Wastewater treatment plants (WWTPs) in Central Greece.

pH meter (Model GLP21) and Metrohm Conductivity meter (Model 712), respectively. OM is estimated by the method of Kalra and Maynard [21].

2.3. Single extraction of different metals from sludge

The methods used were laboratory analysis of metals by the extractive substance DTPA, which serve the equalization of conditions of availability of metals from soil to plants. According to Lindsay and Norvell, DTPA extractable fraction was obtained by mechanical shaking of sample 10 g dry sludge with 40 mL of 0.5 M DTPA, 0.01 M $CaCl_2$, 0.1 M TEA (triethanolamine) buffered at pH 7.3 for 2 h at room temperature [22]. The concentrations of metal ions were determined using Perkin Elmer Optima 7300 V inductively coupled plasma (ICP) optical emission spectrometers(PerkinElmer Inc., Waltham, MA, USA). The ICP spectrometer was calibrated to 0.1, 1, and 10 mg L^{-1}).

2.4. Statistical methodology

The statistical analysis was done by the statistical package SPSS (Ver. 17) and was based on the grouping of the results and the investigation of values variation of the metal concentration. The WWTPs were grouped into two zones depending on the altitude: (a) "coastal zone" for altitude <250 m and (b) "continental zone" for altitude >250 m. The "coastal zone" includes Nafpaktos, Galaxidi, Itea stations while the "continental zone" includes Amfissa, Delphi, and Desfina stations.

In all statistical models, the indicators of the level significance (Significance or Sig.) and variation chosen by *t*-test and *F*-test, respectively, while determined and the correlation coefficient (*R*) for each case. In addition, the percent elemental contribution (PEC, %) among metals and zones was calculated according to the methodology suggested by Koukoulakis et al. [23] and Kalavrouziotis et al. [24].

3. Results and discussion

3.1. Physicochemical properties of sewage sludge

Regarding the physicochemical properties of the sludge studied, the data obtained indicates that the mean values of the sludge physicochemical properties for all WWTPs of the two zones are found approximately the same, except for the EC values of Galaxidi and Delphi, WWTPs, which are quite high compared with other WWTPs (Table 1).

3.2. Concentration of metals

The heavy metal content of the sludge samples in the present work was extracted with DTPA, and therefore, the analytical results reported in Table 2 represent the

Table 1
Basic physicochemical properties of municipal sludge of WWTPs for coastal and continental zones

Physicochemical property	Coastal zone			Continental zone		
	Nafpaktos	Itea	Galaxidi	Amfissa	Delphi	Desfina
pH 1:3 soil water suspension	6.83	6.54	6.63	6.91	6.52	6.64
EC (mS/cm) 1:3 soil water suspension	4.569	4.899	9.637	4.882	14.151	5.954
$CaCO_3$ (%)	12.9	11.2	12.6	11.8	11.1	13.4
OM (%) Burning at 230°	22.6	22.6	24.8	23.6	25.8	26.4
Moisture (%)	85	86	92	85	95	85

Table 2
Mean (N = 4) DTPA extractable metal concentrations (mg/kg) of dried municipal sludge of WWTPs located in the coastal and continental zones

Metal (mg/kg)	Coastal zone				Continental zone			
	Nafpaktos	Itea	Galaxidi	Mean	Amphissa	Delphi	Desfina	Mean
Fe	211.7	234.1	314.4	253.4	334.8	427.5	356.8	373.0
Zn	123.6	313.1	219.6	238.8	194.4	246.1	276.0	226.4
Cu	73.7	109.4	12.5	65.2	26.0	11.4	48.6	28.67
Mn	209.0	181.7	12.2	134.3	12.8	32.9	72.5	39.40
Cd	0.261	0.565	0.345	0.390	0.246	0.587	0.441	0.43
Cr	0.124	0.08	0.06	0.088	0.088	0.059	0.361	0.169
Co	0.424	0.463	0.279	0.389	0.284	0.267	0.361	0.304
Ni	4.952	5.998	14.35	8.43	3.906	4.288	5.041	4.41
Pb	18.5	17.6	14.35	16.82	22.3	21.2	30.46	24.65

bioavailable forms of the metals, which are considered available to plants [2]. The concentrations of the metals investigated are generally lower than the maximum levels given by the EU Directive No 86 of July 1986 as it has been adopted by the Greek State. This difference may be probably due to the possible fact that the data reported by the EU directive refer to the total concentration of the metals in the sludge.

The variation of concentrations between the metals sampled from the different WWTPs is discussed below separately for each metal.

3.2.1. Iron (Fe)

Careful study of the data reported in Table 2 reveals that the concentrations of the metals vary between 211.7 mg/kg for Nafpaktos and 427.5 mg/kg for Delphi, while the mean concentration for all WWTPs was 313.2 mg/kg with a standard deviation 106.78 mg/kg. On the other hand, the mean values of Fe concentrations of WWTPs samples for the coastal and continental zones varied between 253.4 and 373.0 mg/kg, respectively. Based on the ANOVA, the difference in the Fe concentration between zones was statistically significant (Table 3). Also the mean level of Fe was found to be higher in continental zone than that of coastal. This may possibly be due to the high supply with Fe of the geologic formation of this zone through which the fresh consumed water is moving, in the continental zone.

3.2.2. Zinc (Zn)

The mean values of zinc concentration varied between 123.6 mg/kg for Nafpaktos and 313.1 mg/kg of Itea (Table 2), while the mean value of the WWTPs, irrespective of the zone, was 229.11 mg/kg, with a standard deviation equal to 65.68 mg/kg. Also the mean Zn concentrations of the coastal and continental zone were 238.8 and 236.4 mg/kg, respectively, the difference being according to t-test non-significant ($t = -1.372$ and Sig. 2-tailed 0.196).

3.2.3. Copper (Cu)

The mean Cu concentration of sludge samples varied between 26.0 mg/kg for Amphissa and 109.4 mg/kg for Itea, the mean value of all samples being 109.4 mg/kg, and the standard deviation 41.306, with a confidence limits 56 ± 88.24 mg/kg. Also, the mean concentration of Cu of coastal and continental zone was 65.2 m and 28.7 mg/kg, respectively (Table 2). As far the difference in Cu concentration between the samples of the WWTPs studied according to ANOVA (Table 3) was statistically significant, but between the coastal and continental zone it was non-significant based to t-test ($t = 2.086$ and Sig. 2-tailed 0.061).

3.2.4. Manganese (Mn)

The Mn mean concentration of sludge samples ranged between 12.2 mg/kg for Galaxidi and 209.0 mg/kg for Nafpaktos, the mean concentration of all samples being 86.9 mg/kg, with a standard deviation 83.6 mg/kg, and confidence limits 3.23 ± 170.504 mg/kg. Based on the ANOVA (Table 3), the Mn concentration between all the WWTPs differed statistically significantly, but not between the coastal and continental zones, according to t-test ($t = 2.761$ and Sig. 2-tailed 0.01), it was non-significant.

3.2.5. Cadmium (Cd)

Similarly, the mean values of Cd ranged from 0.246 mg/kg for Amphissa to 0.565 mg/kg for Itea, the mean value for all WWTPs being 0.407 mg/kg with standard deviation ±0.251 and confidence limits 0.155 ± 0.658 mg/kg. On the other hand, the mean values of Cd of costal and continental zones were 0.39 and 0.424 mg/kg, respectively. Also, according to ANOVA (Table 3), the difference between the WWTPs sludge Cd as well as between the coastal and continental zone did not differ significantly, the latter being based on the t-test ($t = -0.350$ and Sig. 2-tailed 0.733).

3.2.6. Chromium (Cr)

The mean concentration of Cr ranged between 0.059 mg/kg for Delphi and 0.124 mg/kg for Nafpakto (Table 2), while the mean value of all WWTPs was 0.083 mg/kg, the standard deviation being ±0.025 mg/kg and the confidence limits 0.059 ± 0.155 mg/kg. Also based on the ANOVA (Table 3), the concentration of Cr differed statistically significantly between the WWTPs, and the mean values of the coastal and continental zone were 0.88 and 0.071 mg/kg, respectively, the difference being non-significant according to t-test ($t = 1.399$ and Sig. 2-tailed 0.181).

3.2.7. Cobalt (Co)

As with the other metals and in the case of the Co, the mean concentration varied between 0.267 mg/kg for Delphi and 0.463 mg/kg for Itea (Table 2). Similarly, the mean value of all the WWTPs was 0.348 mg/kg with a standard deviation ±0.108 mg/kg and confidence limits 0.225 ± 0.443 mg/kg. The mean values of the coastal and continental zones were 0.39 and 0.29 mg/kg, respectively. Also, according to ANOVA (Table 3), the difference in the concentration of Co between all the WWTPs was statistically significant for both the two zones studied. But, the difference in Co concentration between the zones was statistically non-significant based on t-test ($t = 1.683$ and Sig. 2-tailed 0.124).

Table 3
ANOVA of metal concentration of sludge samples taken from wastewater processing plants of coastal and continental zones

Characteristics	Metals								
	Fe	Zn	Cu	Mn	Cd	Cr	Co	Ni	Pb
F-ratio	3.432	28.74	11.75	63.94	1.55	9.21	54.76	9.87	3.43
Significance	0.024	0.000	0.000	0.000	0.0225	0.000	0.003	0.000	0.024

3.2.8. Nickel (Ni)

In the case of the Ni, the mean concentration varied between 3.60 mg/kg for Galaxidi and 5.60 mg/kg for Itea (Table 2). Similarly, the mean value of all the WWTPs was 4.63 mg/kg with a standard deviation ±0.95 mg/kg and confidence limits 3.68 ± 5.58 mg/kg. The mean values of the coastal and continental zones were 4.85 and 4.41 mg/kg, respectively. Also, according to ANOVA (Table 3), the difference in the concentration of Co between all the WWTPs was statistically significant for both the two zones and for all the treatment plants, studied. But, the difference in Ni concentration between the zones was statistically non-significant based on *t*-test ($t = 0.815$ and Sig. 2-tailed 0.433).

3.2.9. Lead (Pd)

The mean concentration of Pb ranged between 3.6 mg/kg for Galaxidi and 5.6 mg/kg for Itea (Table 2), while the mean value of all WWTPs was 4.63 mg/kg, the standard deviation being 0.025 mg/kg with confidence limits of 3.68 ± 5.58 mg/kg. Also, based on the ANOVA (Table 3), the concentration of Pb differed statistically significantly between the WWTPs, while the mean values of the coastal and continental zone were 4.85 and 4.81 mg/kg, respectively, the difference being nonsignificant between the coastal and continental zones according to *t*-test ($t = 1.399$ and Sig. 2-tailed 0.181).

According to the above findings:

- The differences in the concentrations of the metals investigated between all the WWTPs were, according to ANOVA, statistically significant, reflecting different eating and food-consuming habits of the people of each area, as the wastewaters studied were sticky municipal.
- On the other hand, the difference in the concentrations of the metals between the two zones studied were statistically non-significant.

These conclusions suggest that the height of location of the WWTPs did have any significant effect on the heavy metal composition of the sludges investigated.

3.3. Effect of elemental interactions on sludge

It was found that considerable number of interactions are occurring in the sludge between heavy metals. The data of Table 4 disclose interesting information about the dynamics of the elemental interaction occurrence in the sludge. Thus, in total, 56 interactions occurred in coastal and 24 in continental zone. Furthermore, in the coastal zone, 16 synergistic interactions along with 12 partly synergistic (S–A) ones took place in the sludge of the WWTPs along with 13 antagonistic and 15 partly antagonistic (A–S) interactions. On the other hand, in the continental zone, the number of synergistic interactions that occurred were 13 and 6 partly synergistic (S–A), as well as 1 antagonistic and 4 partly antagonistic (A–S).

These results showed that the higher number of antagonistic interactions that occurred in the coastal zone were offset by the higher number of antagonistic interactions as it has been discussed previously. Therefore, less heavy metals were accumulated in the coastal zone than in the continental, where more heavy metals accumulated (Table 4). In Fig. 2, some synergistic interactions of coastal and continental zone are reported as an example.

It must be underlined that the relation between the interacting elements is a “cause to effect”, and consequently their interaction is described by regression models (equations) such those in Fig. 2, which can be linear, logarithmic, or quadratic form, depending on the level of significance of the corresponding model. The models of Fig. 2 are quadratic.

3.4. Elemental contribution of interactions

In order to evaluate the importance of the elemental interactions contribution, it is necessary to quantify their effect and express it in quantitative terms. Therefore, the experimental data that were obtained from this experiment were quantified following that reported procedure of Kalavrouziotis et al. [24] as modified by Koukoulakis et al. [23], where the theoretical aspect is synoptically analyzed and all steps involved were explained in detail. Thus, the calculated actual elemental contribution is given in Table 5.

Comparing the elemental contribution data of coastal and continental zones, the following was found:

- In the coastal zone, the elements Zn, Cu, Mn, Cd, Cr, Co, and Ni were contributed by the synergistic interaction, while the Fe and Pb were contributed negatively.
- In the continental zone, the positively contributed elements were Zn, Cu, Mn, Co, and Ni, and the negatively contributed elements were Fe, Cd, Cr and Pb, the last having contributed zero.
- The positively contributed elements in the continental zone supplied the sludge with much higher levels than the corresponding positively contributed in the coastal zone, for example, coastal zone: Zn 8.25%, Cu 12.8%, Mn 14,5%, and Ni 5.7%; continental zone: Zn 65%, Cu 36.8%, Mn 61.5%, and Ni 64%. Only Co was contributed in the coastal zone by 80.1% compared with 57% in the continental zone.

Summarizing the above results it can be concluded that the differences found in the elemental contribution to the

Table 4
Elemental interactions occurring in sludge samples originating from coastal and continental zones

Zone	Elemental interactions				
	Synergistic (S)	Antagonistic (A)	(S–A)	(A–S)	Total
Coastal	16	13	12	15	56
Continental	13	1	6	4	24
Total	29	14	18	19	80

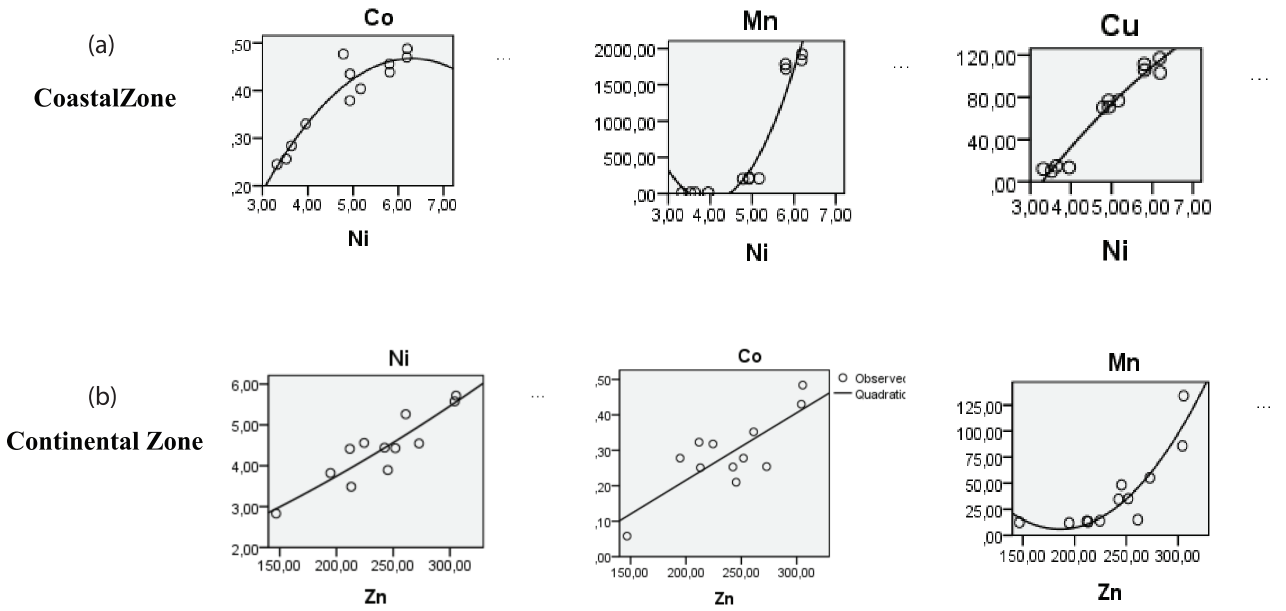

Fig. 2. Statistically significant synergistic interactions: (a) of Ni with Co, Mn, and Cu occurring in coastal zone and (b) of Zn with Ni, Co, and Mn occurring in continental zone (concentration [metal ions/dry sludge] at mg/Kg).

Table 5
Mean percent elemental contribution by the interactions occurring in sludge samples taken from wastewater processing plants located in coastal and continental zone

Metals	Percent elemental contribution, % (PEC)	
	Coastal zone	Continental zone
Fe	–28.5	–68
Zn	8.25	65
Cu	12.8	36.8
Mn	14.6	61.6
Cd	20.3	ns
Cr	25.5	ns
Co	80.1	57
Ni	5.7	64
Pb	–20.3	ns

Note: ns = non-significant.

sludge of the coastal and continental zone are explained on the basis of the fact that more antagonistic interactions took place in the coastal zone, as it has already been explained, and this is reflected clearly in the fact the more heavy elements were contributed to the continental zone sludge than to the coastal zone (Table 4).

4. Conclusions

A large number of elemental interactions are taking place in the sludge of the wastewater processing plants of the coastal and continental zones. Though more synergistic interactions occurred in the coastal zone, the higher number of antagonistic ones in this zone offset the positive effect of the synergistic interactions, thereby decreasing the elemental contribution significantly. Therefore, smaller quantities of heavy metals were contributed to the coastal zone. On the other hand, the smaller number of antagonistic interactions in the continental zone resulted in the contribution of much higher quantities of heavy metals in the sludge of this zone.

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References

[1] L.C. Morais, J. Dweck, E.M. Gonçalves, P.M. Büchler, An experimental study of sewage sludge incineration, Environ. Technol., 26 (2006) 1047–1051.
[2] D.C. Adriano, Trace Elements in Terrestrial Environments, Biogeochemistry, Bioavailability and Risks of Metals, Springer, NY, 2001.
[3] U.S. Environmental Protection Agency, 40 CFR Part 503 – Standards for the Use and Disposal of Sewage Sludge: Subpart B – Land Applications, 503.13 – Pollutant Limits, 58FR9387, Washington, D.C., 1993.
[4] G. Ntzala, I.K. Kalavrouziotis, P.H. Koukoulakis, A.H. Papadopoulos, Impact of sludge and wastewater on *Lactuca sativa* L. growth, Global NEST J., 17 (2015) 148–161.
[5] U.S. Environmental Protection Agency, Biosolids Generation, Use, and Disposal in the United States, EPA530-R-99–009, Municipal and Industrial Solid Waste Division Office of Solid Waste, Washington, D.C., 1999.
[6] U.S. Environmental Protection Agency, Process Design Manual for Land Application of Municipal Sludge, USEPA Rep. EPA-625/1–83–016, U.S. Government Printing Office, Washington, D.C., 1983.
[7] O. Stabnikova, W.K. Gog, H.B. Ding, J.H. Tay, J.Y. Wang, The use of sludge and horticultural waste to develop artificial soil for plant cultivation in Singapore, Biosour. Technol., 96 (2005) 1073–1080.
[8] WHO, Cadmium, Environmental Health Criteria, Vol. 134, Geneva World Health Organization, 1992, pp. 1–280.

[9] P.S. Hooda, D. McNulty, B.J. Alloway, M.N. Aitken, Plant availability of heavy metals in soils previously amended with heavy applications of sewage sludge, J. Food Agric., 73 (1997) 446–454.

[10] A.M. Bidwell, R.H. Dowdy, Cadmium and zinc availability to corn following termination of sewage sludge applications, J. Environ. Qual., 16 (1987) 438–442.

[11] L.E. Sommers, A.L. Page, T.J. Logan, J.A. Ryan, Optimum Use of Sewage Sludge on Agricultural Land, Western Regional Res. Pub. W-124, Colorado Agric. Exp. Stn, Fort Collins, 1991.

[12] P.S. Hooda, B.J. Alloway, The plant availability and DTPA extractability of trace metals in sludge-amended soils, Sci. Total Environ., 149 (1994) 39–51.

[13] A.M. Ure, Single extraction schemes for soil analysis and related applications, Sci. Total Environ., 178 (1996) 3–10.

[14] B.D. Rappaport, Availability and Distribution of Heavy Metals from Sewage Sludge in the Soil-plant Continuum, PhD Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, 1986.

[15] G.A. Mitchell, F.T. Bingham, A.L. Page, Yield and metal composition of lettuce and wheat grown on soils amended with sewage sludge enriched with cadmium, copper, nickel, and zinc, J. Environ. Qual., 7 (1978) 165–171.

[16] D.L. Lake, P.W.W. Kirk, J.N. Lester, Fractionation, characterization and speciation of heavy metals in sewage sludge and sludge-amended soils: a review, J. Environ. Qual., 13 (1984) 175–183.

[17] D.R. Sauerbeck, Plant element and soil properties governing uptake and availability of heavy metals derived from sewage sludge, Water Air Soil Pollut., 57 (1991) 227–237.

[18] P.S. Hooda, B.J. Alloway, The effect of liming on heavy metal concentrations in wheat, carrots, and spinach grown on previously sludge-applied soils, J. Agric. Sci., 127 (1996) 289–294.

[19] G.S. Miner, R. Guitierrez, L.D. King, Soil factors affecting plant concentrations of cadmium copper and zinc on sludge-amended soils, J. Environ. Qual., 26 (1997) 989–994.

[20] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 22nd ed., American Water Works Association, 2012.

[21] Y.P. Kalra, D.G. Maynard, Methods Manual for Forest Soil and Plant Analysis, Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta, Information Report NOR-X-319, 1991.

[22] W.L. Lindsay, W.A. Norvell, Development of DTPA soil test for Zinc, Manganese, and Copper, Soil Sci. Soc. Am. J., 42 (1978) 421–428.

[23] P.H. Koukoulakis, C. Chatzissavvidis, A. Papadopoulos, D. Pontikis, Interactions between leaf macronutrients-micronutrients and soil properties in pistachio (*Pistachio vera* L.) orchards, Acta Bot. Croat., 72 (2013) 295–310.

[24] I.K. Kalavrouziotis, P.H. Koukoulakis, A. Mehra, Quantification of elemental interaction effects on Brussels sprouts under treated municipal wastewater, Desalination, 254 (2010) 6–11.