Simulation of soil heavy metal pollution environmental stress on plant growth characteristics in the presence of wastewater

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Abstract

Soil pollution was simulated by means of artificial heavy metal enrichment, in the presence of treated wastewater, and its effect on the growth characteristics of *Beta vulgaris* L. (red beets) i.e. on the dry matter yield, and on beet quality expressed in terms of dry matter heavy metal, nitrogen and crude protein content, was investigated in a greenhouse pot experiment. Twelve treatment combinations of heavy metals mixtures composed of Zn, Mn, Cd, Co, Cu, Cr, Ni, and Pb, with each one metal participating in the mixture at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 mg kg^{-1} were applied, respectively. It was found that the increase of soil DTPA extractable heavy metal concentrations reflecting high soil pollution, decreased statistically significantly the beet dry matter (dm) yield, but increased the metal and the, the nitrogen concentrations, and consequently, contributed statistisically significantly to the increase of beet crude protein yield.

Keywords: *Beta vulgaris*, heavy metals, nitrogen protein, soil pollution.

1. Introduction

The application of treated wastewater for irrigation of vegetables has been shown to be a very appropriate recycling option for environmental and economic purposes (Mufeed *et al*., 2011). Furthermore, it is a common practice in many countries the reuse of municipal wastewater in urban and periurban ecosystems (Pedrero *et al*., 2010). The main purpose of the water reuse in agriculture is to provide a sufficient quantity of high quality water for irrigation and to ensure food safety (Pedrero *et al*., 2010). The wastewater is reused mainly for agricultural irrigation and recharge of aquifers. In addition, treated wastewater contains fractions of organic matter, suspended solids, nutrients and trace contaminants (Kalavrouziotis *et al*., 2013). It is noteworthy that the application of wastewater on agricultural land involves environmental hazards as the wastewater may contain a range of metals, which are toxic when the thresholds are exceeded. Several studies have indicated that long-term irrigation with wastewater can increase the contents of toxic metals in soils (Kalavrouziotis *et al*., 2013).

Plants have a natural ability to extract elements from soil (i.e. micro-nutrients, to roots, shoots and fruits by means of biological processes (Kalavrouziotis *et al*., 2008). In many cases, vegetables using wastewater irrigation have normal appearance, without visual symptoms of contamination and are therefore consumed by humans (Preeti *et al*., 2015). However, vegetables which are cultivated in areas with high heavy metal content may have adverse consequences on human health due to the high level of these metals in the edible plant parts (Kalavrouziotis *et al*., 2008). In addition, heavy metals tend to bioaccumulate in plants and animals, consequently, entering the food chain (Levei *et al*., 2009).

Proteins are macromolecules which are considered as being the major work factors of living cells. Apart from the important physiological role of proteins in plants, they also constitute a major source of food for humans and animal. Among the plants that are popular in human beings, vegetables are important parts of the human diet because they constitute rich sources of carbohydrates, proteins, vitamins, minerals whilst also having significant antioxidative effects (Vicente *et al*., 2009). The food quality and crop growth are affected by heavy metal accumulation in soils (Fergusson, 1990). The extent of accumulation of heavy metals in toxic levels depends on the plant and heavy metal species (Alloway, 2013). The consumption of vegetables which are heavy metal contaminated may set a risk to human health and this is one of the most important aspects of food quality deterioration (Guerra *et al.,* 2011). During the last decades, the emission of heavy metals into the environment has increased considerably, due to rapid industrialization, urbanization and excessive usage of agricultural improvers such as fertilizers, wastewater, and biosolids. According to Shahid *et al.* (2014), heavy metals may affect the structure of plant proteins and consequently the quality of these compounds.

While the formation, accumulation and mechanisms of establishing tolerance to heavy metal pollutants have been studied by physiologists, nutritionists and other scientists, the quantitative effects of heavy metals on plant proteins under the presence of heavy metals and their effects on these vital compounds of plants have not been studied adequately. As the heavy metals are toxic and at higher levels pose health risk, it is necessary to study their quantitative changes of their presence in the plant. Therefore, the purpose of the present work is to study the effect of heavy metals were added to soil via wastewater reuse and artificial enrichment on the quality and quantity characteristics of *Beta vulgaris* L. proteins, studied in relation to beet yield, and nitrogen concentration.

2. Materials and methods

A greenhouse experiment was conducted in an agricultural area of Amaliada located in Western Greece, in order to study the heavy metal bioavailability effect on proteins of *Beta vulgaris* L. plants grown in an artificially polluted soil using a randomized block statistical design. Treated municipal wastewater was applied for irrigation of plants. The wastewater was used basically as a source of irrigation water. It may however contain heavy metals which can in the long run accumulate in the soil increasing their load in addition to those heavy metals added via enrichment. All these metals are reflected in soil analysis which was made at the completion of the experiment. The above procedure was necessitated by the fact that the wastewater due to each relatively low heavy metal content, can increase the soil pollution level after long time irrigation of crops. As the level of soil pollution due to reuse cannot be increased in the short time of the experiment, to the extent of causing problems to the plant, it was considered necessary to enrich the soil with various levels of heavy metal mixtures in order to create various levels of heavy metal load in the soil so that their impact can be more clear on the plants. The study of the quantitative effect of wastewater on plant growth was not included with the aims of the present work, as the wastewater was applied at the same level to all plants.

2.1. Experimental soil

The experimental soil was collected from the top layer (0-30 cm depth) of a non cultivated agricultural area of Amaliada and was analyzed by the methods which refer to paragraph 2.3 of the present work. The physicochemical characteristics of soil are presented in Table 1.

Approximately 600 kg of soil was collected and sieved by means of a plastic 2 mm sieve. Twelve treatments i.e. (T1-T12) were applied to soil, composed of a heavy metal mixtures (Zn, Mn, Cd, Co, Cu, Cr, Ni and Pb), where each one of these metals was participating in each of the twelve mixtures with 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 mg kg^{-1}, respectively. The chemical compounds used as carriers of the above metals were $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Na_2Cr_2O_7 \cdot 2H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, and $Pb(NO_3)_2$, respectively. The application of each metal

mixture treatment to each pot was made by preparing a solution of high concentrations which was then diluted to the above metal concentration per treatment, and added to the soil. The bottom of each pot was closed, to avoid loss of nutrients and heavy metals due to drainage and leaching.

Table 1. Physicochemical characteristics of the experimental soil

Sand		56		OM	%	2.11
Clay	%	12		EC	mS cm^{-1}	0.21
Silt		32		pH		6.2
Cd	Co	Cr	Ni	Pb	N-NO$_3$	P
			mg kg^{-1}			
0.04	0.42	0.03	2.8	0.9	25	6
K	Mg	Ca	Fe	Zn	Mn	Cu
			mg kg^{-1}			
75	260	>2000	20.09	2.3	33.6	88.6

EC-electrical conductivity, OM-organic matter

2.2. Wastewater collection and analysis

Treated municipal wastewater, was collected from the municipal wastewater treatment plant of Amaliada. For the determination of the heavy metals, the treated municipal wastewater was processed by the method given by APHA (2012) and the microelements and heavy metals Zn, Mn, Cu, Cd, Co, Cr, Ni, Pb were measured by atomic absorption with graphite oven, except of Zn which was measured only by simple atomic absorption spectrophotometer without the use of the graphite oven.

2.3. Soil analysis methods

The soil samples were air-dried, sieved through a 2 mm sieve and oven dried at 75 °C for 48 hrs. The dry soil samples were analyzed by the methods, as follows: soil mechanical analysis by the classical Bouyoucos method as modified by Gee and Or (2002); organic matter by the wet digestion procedure method of Walkley and Black (1934); pH measured on water extract of saturated paste, using a standard glass pH electrode and the conductivity by means of a conductivity meter. Available soil P was analyzed by Olsen *et al.* (1954), the exchangeable Ca^{2+}, Mg^{2+}, and K^+ by extraction with NH_4Ac, at pH = 7.0 (Lanyon *et al.,* 1982) and were determined by inductively coupled plasma atomic emission spectroscopy (ISP-OES, Perkin Elmer, Optima 2100 DV, Shelton, USA). The soil extractable fraction of Zn, Fe, Mn, Cu, Cd, Pb, Co, Ni, and Cr, which is considered available to plants, were extracted with diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell, 1978) and measured by ICP-AES.

2.4. Experimental plants

After the soil preparation of the pots, six seeds of the test plant *Beta vulgaris* L. (beet) were sown in each pot followed by light irrigation with treated wastewater. The plants were regularly irrigated during the 3.5 month growth period. A total volume of 47 L treated wastewater, added to each pot during the growth plant period.

2.5. Plant tissue analysismethods

The plant samples were flushed with tap water until the complete removal of the soil particles. Subsequently, the plant parts, root, beet (the edible plant part) and leaf samples, were washed separately with deionized water; with dilute solution of 0.005 % HCl, and also thoroughly washed by means of a special detergent (Alconox, 0.1%) and rewashed repeatedly four times, with distilled water. The samples of plant parts were left to drain on filter papers, dried in a ventilation oven at 70 °C, and then they were weighted for the determination of dry matter, and grounded. In turn, a dry matter sample of 1 g was mineralized by dry ashing in a muffle furnace at 500 °C for 10-12 h; then the ash was dissolved in a 5 ml 6 N HCl solution. The Zn, Mn, Cu, Cd, Co, Cr, Ni, and Pb were measured by ICP-AES. Determination of total Nitrogen was realized by the Kjeldahl method and the Nitrogen Protein was estimated by multiplying the total Nitrogen by 6.25 (AOAC, 1990).

Table 2. Mean value of DTPA extractable soil heavy metal concentrations per treatment, determined at the finish of beet harvesting period

Treatments	Heavy metals in soil (mg kg^{-1})							
	Zn		Mn		Cu		Cd	
	mean	SD	mean	SD	mean	SD	mean	SD
T1	1.28	0.10	32.35	2.50	85.51	5.83	0.09	0.03
T2	2.05	0.08	31.78	1.96	92.01	4.29	1.15	0.09
T3	2.49	0.28	29.08	2.88	89.03	5.24	2.23	0.15
T4	3.01	0.20	30.97	2.77	92.51	7.73	3.23	0.39
T5	3.72	0.11	36.57	2.24	90.17	4.67	4.16	0.55
T6	4.44	0.40	26.34	3.23	91.68	6.73	5.56	0.64
T7	4.83	0.91	28.16	2.36	89.83	7.99	6.18	1.06
T8	5.95	0.22	26.88	2.82	101.46	4.48	8.19	0.41
T9	6.60	0.50	26.56	2.39	100.34	4.47	9.81	0.39
T10	7.88	0.79	25.44	3.25	102.57	7.60	10.76	0.84
T11	9.85	0.69	25.38	2.68	103.39	5.54	11.82	0.79
T12	10.18	0.88	23.72	3.93	102.25	11.57	14.07	1.97
Treatments	Co		Cr		Ni		Pb	
	mean	SD	mean	SD	mean	SD	mean	SD
T1	0.13	0.00	0.02	0.02	1.65	0.15	1.16	0.3
T2	0.19	0.01	0.03	0.02	2.36	0.18	1.67	0.17
T3	0.23	0.01	0.02	0.01	2.75	0.18	2.45	0.25
T4	0.32	0.03	0.04	0.02	3.53	0.37	3.15	0.08
T5	0.46	0.05	0.05	0.03	4.86	0.43	3.94	0.19
T6	0.44	0.03	0.04	0.02	5.42	0.56	5.39	0.66
T7	0.46	0.06	0.07	0.04	5.86	0.81	5.99	0.72
T8	0.52	0.05	0.10	0.01	6.82	0.17	6.88	0.37
T9	0.65	0.07	0.09	0.01	8.09	0.49	8.18	0.39
T10	0.62	0.10	0.10	0.04	8.62	0.86	8.85	0.93
T11	0.73	0.04	0.18	0.05	9.28	0.46	9.97	0.81
T12	0.80	0.16	0.22	0.06	11.15	0.46	11.38	1.36

SD: standard deviation (N = 48 and each value is mean of 4 samples)

Table 3. Dry matter yield of *Beta vulgaris* of the above ground plant part, beet, root and whole plant produced per treatment

Treatments	Plant dry matter (g)							
	above ground		beet		root		whole plant	
	mean	SD	mean	SD	mean	SD	mean	SD
T1	1.89	0.44	6.86	0.97	0.27	0.02	9.02	1.38
T2	2.44	0.52	5.87	0.75	0.35	0.09	8.65	1.09
T3	2.23	0.55	6.83	1.74	0.31	0.07	9.37	1.59
T4	2.46	0.29	5.77	1.56	0.38	0.11	8.62	1.56
T5	2.22	0.25	5.78	0.85	0.31	0.06	8.31	0.76
T6	2.43	0.49	5.13	0.62	0.29	0.06	7.85	0.85
T7	3.03	0.43	7.20	0.96	0.45	0.17	10.68	1.14
T8	2.02	0.57	5.36	0.18	0.34	0.15	7.71	0.59
T9	2.15	0.59	4.75	0.61	0.23	0.05	7.13	0.48
T10	2.14	0.79	5.32	0.85	0.29	0.09	7.75	1.59
T11	2.32	0.52	5.90	1.19	0.48	0.19	8.70	1.70
T12	2.21	0.45	3.62	0.78	0.35	0.06	6.18	0.89

SD: standard deviation (N = 48 and each value is mean of 4 samples)

Table 4. Heavy metals concentrations of beets dry matter ($\mu g\ g^{-1}$)

Treatments	metals in beet dry matter ($\mu g\ g^{-1}$)							
	Zn		Mn		Cu		Cd	
	mean	SD	mean	SD	mean	SD	mean	SD
T1	25.84	2.48	118.15	10.38	28.64	2.84	1.10	0.62
T2	27.97	2.35	93.97	13.19	28.38	1.93	0.90	0.29
T3	26.70	2.20	85.20	15.31	26.78	1.48	1.27	0.61
T4	28.99	3.51	103.36	24.55	28.89	4.27	2.02	0.47
T5	29.73	1.39	131.45	9.31	26.67	1.60	2.80	1.51
T6	34.95	3.02	87.59	16.72	28.22	2.67	4.85	2.46
T7	32.34	3.61	98.49	12.60	24.87	2.75	4.49	2.96
T8	32.12	2.86	80.38	10.57	27.20	3.48	3.06	1.01
T9	33.89	3.40	67.46	12.21	25.44	2.90	1.98	0.85
T10	32.44	4.92	60.91	16.04	22.71	2.89	3.95	1.41
T11	37.14	2.73	64.99	4.92	25.40	2.64	4.43	2.93
T12	29.71	2.34	50.85	6.90	24.36	2.37	3.49	2.71
Treatments	Co		Cr		Ni		Pb	
	mean	SD	mean	SD	mean	SD	mean	SD
T1					2.63	0.21		
T2					3.21	0.31		
T3	<0.35				3.48	0.27		
T4					4.94	1.00		
T5	0.55	0.13			7.46	0.74		
T6	0.36	0.03			6.59	0.37		
T7	0.52	0.06	<0.35		7.51	0.80	<2.10	
T8	0.42	0.07			6.42	0.50		
T9	0.58	0.17			6.80	0.43		
T10	0.50	0.09			6.68	1.35		
T11	0.69	0.06			7.98	0.40		
T12	0.80	0.28			8.68	2.00		

SD: standard deviation (N = 48 and each value is mean of 4 samples), ND: not detected

2.6. Statistical analysis

The experimental data were statistically analyzed by using the SPSS (21) program, running regression analysis and ANOVA.

3. Results and discussion

The relationship of heavy metals to beet quality of the edible plant part was studied in relation to the effect of heavy metals on beet yield, N, and crude protein content of beet dry matter.

3.1. Effect of soil heavy metal levels to plant beet yield

The applied treatments increased significantly the extractable heavy metal concentration in soil (Zn, Mn, Cu, Cd, Ni, Pb) (Table 2). Due to the above increase of available metal concentrations in soil the dry matter yield of beets and of the whole plant decreased significantly while the root and above ground parts dry matter yield was decreased statistically not significant (Table 3).

In Figure 1, the regression between Zn and beet dry matter yield as well Cd and beet dry matter yield, was found linearly negative and statistically significant. This figure emphasizes the toxic effect of the high available soil heavy metal levels on plant growth. These findings are in agreement with the results of Singh and Agrawal (2010), who found that *Beta vulgaris* grown in wastewaters containing a range of heavy metals reduced the plant yield. They related the higher bioavailability of heavy metals in wastewater irrigated sites to a reduction in the nutrient availability to plants, hence reducing the biomass.
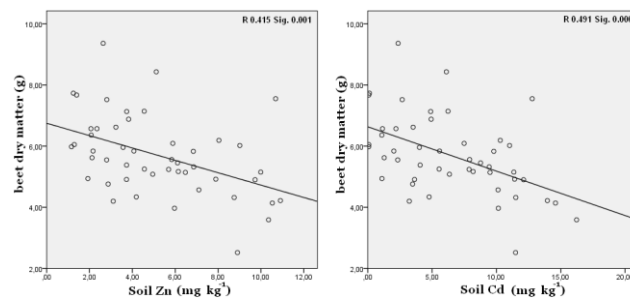

Figure 1. The effect of soil metals Zn and Cd on beet dry matter yield, under the influence of the applied heavy metal mixtures and wastewater reuse

3.2. Effect of soil heavy metal composition on beets

The concentrations of heavy metals in beet dry matter are reported in Table 4. Based on these data, it is shown that the soil heavy metals are transferred from soil to *Beta vulgaris* L. in variable quantities. Factors which influence the transfer of metals from soil and their accumulation to plant are: (i) the soil type (ii) the metal concentration in the soil, (iii) the bioavailability of metals, (iv) the metal chemical forms, and (v) the differences in uptake potential

and growth rates of plant species (Tinker, 1981; Wang *et al.,* 2012).

The relation of the heavy metal concentration in soil with that in beet tissue was found positive for the metals Zn, Mn, Cd, Co, and Ni where concentrations in the plant increased with the corresponding increasing soil concentrations, reflecting elevated soil pollution (Figure 2) and unfavorable effect of the beet quality. These results are important in the sense that the uptake of the heavy metals by the plants under soil pollution, and the possible harmful effects on consumer's health.
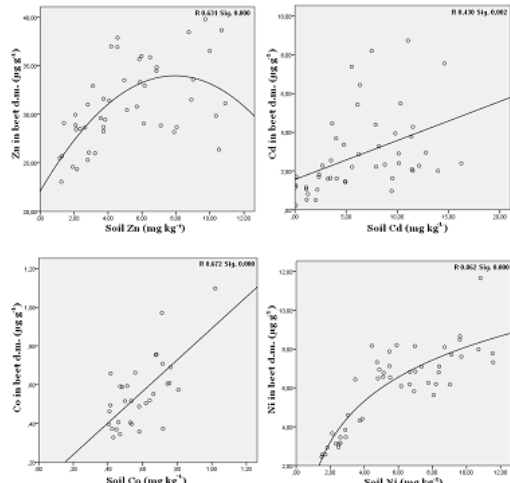
Figure 2. Relation between soil Zn, Cd, Co and Ni to their concentration in beet dry matter, under the effect of the applied metal mixtures treatments and wastewater reuse

3.3. Effect of metals on beet N concentration and crude protein yield

The mean beet dry matter nitrogen concentration was positively affected by the applied treatments as shown in Figure 3. Consequently, the beet percent crude protein also increased with increasing heavy metal concentrations calculated by multiplying total N by the factor 6.25 (AOAC, 1990). The results are given in Table 5.

As shown in Figure 3, an increase of the applied levels of heavy metal concentrations in soil increased the percentage nitrogen content in the beet, respectively. Also, all the applied heavy metals, with the exception of Cu, increased the beet crude protein content as shown in Table 6 and Figure 4 which is presents for example the interaction of Zn, Cd, Ni and Co. According to Shah and Dubey (1997), heavy metal stress has been shown to induce a variety of proteins, resulting in an overall increase in protein content. Singh and Agrawal (2007) also showed that the protein content of *Beta vulgaris* plants increased when grown in sludge amended soils with high concentrations of heavy metals. Metals in soils such as Fe, Mn, Zn, Cu, and Ni are referred to as essential micronutrients for normal plant growth, and in tiny quantities have a pivotal role in the structure of enzymes and proteins (Zengin and Munzuroglu, 2005). However, the metals concentration is an important factor in the growing process of plants, and the existence in excess can

lead to the reduction and inhibition of plant growth (Emamverdian *et al.,* 2015).
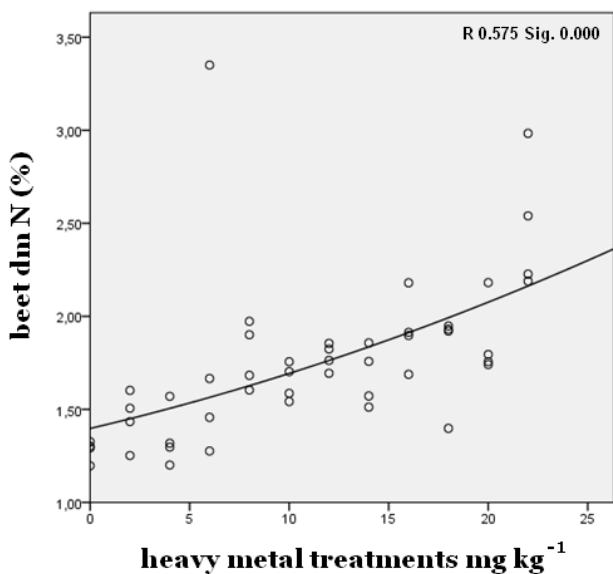

Figure 3. Effect of the metal treatments on the beet dry matter N contents

Table 5. Nitrogen protein expressed as percentages (%) of the dry matter

Treatments	Nitrogen protein expressed as percentages (%) of the dry matter	
	mean	SD
T1	8.00	0.36
T2	9.05	0.92
T3	8.42	0.98
T4	12.11	5.97
T5	11.19	1.09
T6	10.29	0.62
T7	11.15	0.44
T8	10.47	1,00
T9	12.00	1.26
T10	11.25	1.67
T11	11.68	1.31
T12	15.53	2.30

The examination of the data of Table 6 reveals that almost all the heavy metals studied, either by the increase of their concentration in the soil or within the *Beta vulgris* L. plants, favorably affected the beet nitrogen content, therefore increasing the percent beet crude protein. The results in Figure 4 clearly show that the increase of DTPA extractable level of soil Zn, Cd, Ni and Co, increases respectively the beet crude protein content and this increase is statistically significant (Table 6). The above findings are in line with the fact that Zn is an essential micronutrient taking part in the formation of proteins, known as zinc fingers that bind to DNA and RNA and contributes to their regulation and stabilization (Fergusson, 1990).
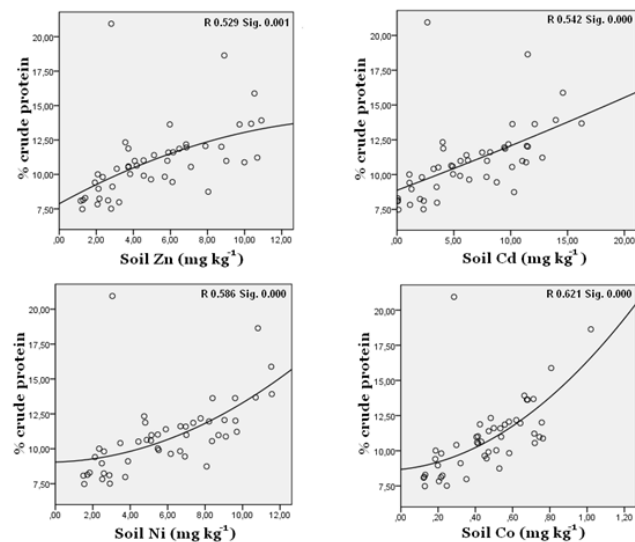
Figure 4. The effect of DTPA extractable soil Zn, Cd, Ni and Co in mg kg^{-1}, on beet percent crude protein under the effect of heavy metal mixture treatments and wastewater reuse

Table 6. Regression equations expressing the beet crude protein yield (CP) as a function of the soil heavy metals under the effect of the metals treatments studied and the wastewater reuse

Regression equation	R	significance	Type of regression
$CP = -0.002(Zn_s)^2 + 0.739(Zn_s) + 7.87$	0.529	0.000	S
$CP = -6.422\ln(Mn_s) + 32.389$	0.372	0.009	A
$CP = 6.337(Co_s)^2 + 1.334(Co_s) + 8.675$	0.621	0.000	S
$CP = 0.001(Cd_s)^2 + 0.307(Cd_s) + 8.837$	0.542	0.000	S
$CP = 6.337(Co_s)^2 + 1.334(Co_s) + 8.765$	0.621	0.000	S
$CP = -15.948(Cr_s)^2 + 22.585(Cr_s) + 9.29$	0.465	0.004	S
$CP = 0.039(Ni_s)^2 + 0.037(Ni_s) + 9.051$	0.686	0.000	S
$CP = 0.002(Pb_s)^2 + 0.419(Pb_s) + 8.449$	0.548	0.000	S

s: soil heavy metal, Type of regression: A = antagonistic and S = synergistic

3.4. Relation of heavy metal soil pollution to the beet quality

The present work examines the beet quality in terms of dry matter heavy metal, N, and crude protein percent. Examining the relation between the soil heavy metal pollution level, determined quantitatively by means of pollution indices such as Pollution Load Index (PLI), Elemental Pollution Index (EPI), Heavy metal Load (HML) and Total Concentration Factor (TCF) (Kalavrouziotis *et al.,* 2012), it is interesting to underline that, based on Figure 5, the beet dry matter crude protein content increases with the quantitative increase of soil heavy metal pollution. This result is in line with the effect of increasing

individual heavy metal levels on beet N and on crude protein concentration as depicted by the Figures 3 and 4.
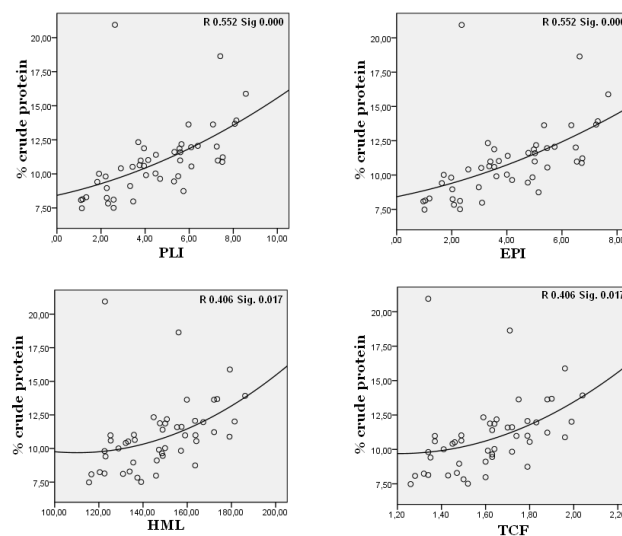

Figure 5. Relation of soil Heavy metal pollution indices to beet dry matter crude protein content under the heavy metal mixture treatments and wastewater

4. Conclusions

Based the above mentioned, the following conclusions could be drawn:

The applied soil heavy metals treatments under the effect of the wastewater decreased the beet dry matter yield, due to the metal toxic effect. They, on the other hand, affected positively and statistically significantly the beet quality parameters, such as the total N concentration, the heavy metal content, and beet crude protein yield.

Also, the heavy metal soil pollution level, quantitatively determined by the use of the pollution indices, increased significantly the total beet N concentration and consequently the percent crude protein concentration of beets. These results showed that while the high concentration of soil heavy metals may have negative impact on beet biomass yield, but it can improve the beet protein quality parameter significantly.

It is concluded that more extensive research be conducted for the study of the heavy metal effect of the polluted soils on other quality parameters of beets, such as sugars and amino acids content.

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