

IMPACT OF SLUDGE AND WASTEWATER ON *LACTUCA SATIVA* L GROWTH AND ON SOIL POLLUTION

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Received: 14/02/2014

Accepted: 27/12/2014

Available online: 25/02/2015

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ABSTRACT

Two greenhouse pot experiments were conducted in Agrinio, Greece. The impact of treated municipal wastewater (TMWW) and sludge (i) on the growth of *Lactuca sativa* L. var Longifolia (lettuce) and (ii) on the extent of soil pollution with heavy metals was studied. Soil pollution was assessed by calculating the Pollution Load Index (PLI). Both of these experiments were conducted, using a randomized block design in four replications and seven treatments, respectively, as follows: (a) Experiment A: study of the effect of treated municipal wastewater (TMWW): [Control, 20%, 40%, 60%, 80%, 100%, (100%+30 t/ha Sludge)] and (b) Experiment B: Study of the effect of sludge (t/ha): 0, 6, 12, 18, 24, 30, (30+100%TMWW). The sludge affected significantly plant height and fresh and dry matter yield, as well as the dry matter N content of plants, while the TMWW affected significantly the dry matter yield and non-significantly the plant height. The pollution load index (PLI) was non-significant for both treatments (sludge and TMWW). According to PLI calibration scale, the soil was found to be slightly polluted with heavy metals under both treatments.

Keywords: Treated municipal wastewater, heavy metals, sludge, Pollution Load Index (PLI), *Lactuca sativa* L

1. Introduction

Sludge, a product of wastewater treatment, contains organic matter, 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, Ni) rendering its application to soil, problematic. Therefore, it requires special management. The main concern about the sludge is related to phytotoxicity, due to the accumulation of heavy metals in the soil (Stabnikova *et al.*, 2005), 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 (WHO, 1992). For example, long term exposure to Pb may lead to the deterioration of memory, causing reduced ability of understanding, behavioral disturbances, and learning difficulties (Jarup, 2003). Chronic poisoning after several years of exposure

to Cd may cause cadmium-induced nephropathy which is characterized by increased proteinuria, in particular 2-microglobulin (Friberg, 1948; Tsuchiya, 1967).

Long term use of sludge, may increase the soil levels of Zn, Cu, Ni, Cr, Cd, Pb, and Hg, but only Zn, Cu, Ni and Cd may increase in the dry matter of cereal grains, while Zn, Cu, Cr and Pb are increased in grains of wheat (Purves, 1977). Also application of sludge to grain sorghum increased the heavy metal content, but due to the short term effect, their levels were below the accepted critical concentrations (Akdeniz *et al.*, 2006). However, long term application must be managed accordingly to avoid toxic effects. Similarly, long term irrigation with TMWW may increase the heavy metal contents of soil, and therefore the assessment of soil metal accumulation is of vital importance.

Wastewater, and sludge have long been used as fertilizers and as irrigation water, especially as supplementary sources of N and other macro and micronutrients, also as amendment for the improvement of soil physical properties (Pereira *et al.*, 2011; Liu *et al.*, 2005; NRC, 1996; Metcalf and Eddy, 1991).

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 $7,136 \times 10^3$ t of dry matter. In the European Union (EU), almost all countries, have soils which are polluted with heavy metals (Ferguson, 1999), and protection of soils from heavy metal pollution has been one of the first priorities (EC, 2002). Also, in China nearly 1/5 of the total area (2.0×10^7 hm²) is contaminated with heavy metals (Zang *et al.*, 2012). Similarly, in India, large areas in the Patancheru region have been affected by the accumulation of heavy metals (Govil *et al.*, 2001).

It has been reported (Adriano 2001) that heavy metals rank very high among the chief contaminants of vegetables, as they appear to be more effectively accumulated in the edible plant parts. However, the sludge-borne metals have been reported as having lower phytotoxicity than the added metals, that is why phytotoxicity symptoms are very rarely observed even after application of large amounts of sludge (Adriano, 2001).

Reuse of treated municipal wastewater (TMWW) and sludge enriches soil with heavy metals, which affect crop yield and soil fertility (FAO, 2003). Thus, when high doses of sludge and TMWW are used, the accumulation of heavy metals, may exceed the critical concentration limits, and consequently may create toxic effects on plants.

In spite of the problems associated with the use of sludge as an alternative fertilizer or soil amendment material in agriculture, sludge is gaining popularity in many countries such as in the region of Near East (Egypt, Syria, Saudi Arabia, etc.) (FAO, 2003), China (Liu *et al.*, 2005), Iran (Kalavrouziotis *et al.*, 2013), and Brazil (Pereira *et al.*, 2011). It is used in agriculture such as for the production of field crops, (maize, sorghum, forage crops), and vegetables (Antonius *et al.*, 2010).

The purpose of the present study is to evaluate the effects of application of sludge and of the TMWW on lettuce (*Lactuca sativa* L. var, longifolia) growth and to assess the extent of soil pollution with heavy metals.

2. Materials and methods

Two greenhouse pot experiments were conducted, for the study of the effect of sludge and TMWW on lettuce plant growth and mineral content and on soil pollution, as follows:

Experiment A: Seven sludge treatment levels were applied, the 7th one being a combination of the highest sludge level plus 100% TMWW.

Experiment B: Seven TMWW treatment levels, were studied, the 1st one being the control (natural water) and the 7th one being a combination of 100% TMWW and the highest level of sludge (Table 1).

Table 1. Experimental treatment levels of sludge and TMWW

Serial number	Treatments	Treatment level	Dried sludge		fresh sludge	
			g/pot	kg/ha	g/pot	kg/ha
I-Experimental study of the effect of TMWW on the soil						
1	Control (C)	Natural fresh well water	–	–	–	–
2	TMWW ₁	20%TMWW+80%Control	–	–	–	–
3	TMWW ₂	40%TMWW+60%Control	–	–	–	–
4	TMWW ₃	60%TMWW+40%Control	–	–	–	–
5	TMWW ₄	80%TMWW+20%Control	–	–	–	–
6	TMWW ₅	100%TMWW+0%Control	–	–	–	–
II- Experimental study of the effect of sludge on the soil						
7	S ₁	–	22.6	600	41.8	1110
8	S ₂	–	45.2	1200	83.6	2220
9	S ₃	–	67.7	1800	125	3330
10	S ₄	–	90.3	2400	167	4440
11	S ₅	–	113	3000	199	5550
12	TMWW ₅ +S ₅	–	113+TMWW ₅	3000+TMWW ₅	199	5550+TMWW ₅

Both the sludge and the TMWW were derived from the wastewater processing plant (WPP) of Mesolongion, W.Greece. The sludge used was subjected to partial dehydration, aerobic biological stabilization, denitrification and dephosphorization.

The experiments were conducted in a non-heated greenhouse, and in pots with a total volume of 6 L, containing 10 kg of air dried soil, of known moisture content (12.5%, determined at 100 °C). Both experiments were conducted, using a completely randomized block design, including seven TMWW and sludge treatments, respectively, as shown in Table 1, both in four replications, with a total number of pots per experiment equal to 28.

In each pot, two young lettuce plants were transplanted, i.e. in total 112 plants. The vegetable lettuce or *Lactuca sativa* L. var longifolia was used as an experimental test plant. This plant was chosen because it is one of the main leafy vegetables consumed in Greece, and it grows very easily and produces biomass in a relatively short time under relatively cool conditions.

The sludge treatments were mixed with the soil according to Table 1, before being transferred into the pots. The soil was mixed thoroughly by means of a plastic rod, and it was transferred into the pot. Then, transplanting took place and the plants were irrigated with TMWW or fresh water, according to the experimental design.

The sludge used was watery containing 15% dry matter. The quantities of sludge shown in Table 1 were calculated on the basis of the above dry matter percent content. The transplanting was done on 15/11/2012 and the harvest on 20/03/2013. The growth period of lettuce lasted eighteen (18) weeks. Composite soil samples, i.e. each one being composed of a mixture of 3-4 subsamples were taken as follows:

1-Selection of the experimental soil: One composite sample was taken from three respective selected areas, based on the determination of the physical and chemical characteristics (pH, EC, mechanical analysis, and CaCO₃), which would be compatible with soil needs of the lettuce plants. A quantity of about 700 kg of the chosen soil, which satisfied the requirements of the experiment, was then taken to be used as the experimental soil for filling the pots.

2-From this experimental soil mass, a composite sample of about 1 kg was taken for the detailed determination of physical, chemical characteristics, and macro, micronutrients which were as follows: Mechanical analysis light sandy loam (SL), slightly acid pH 6.52, low salinity (0.829 mS cm⁻¹), moderate organic matter content (1.47%), rich in Olsen P (112.1 mg kg⁻¹), and adequately supplied with Zn (1.06 mg kg⁻¹), Mg(330 mg kg⁻¹), Mn (28.5 mg kg⁻¹) and Cu (2.53 mg kg⁻¹).

3-Pot soil sampling: Composite soil samples of 0.3 kg were taken from each pot during the middle plant growth period, and also at the completion of the experiment.

The first sampling of soil from the pots was conducted about three (3) months or twelve (12) weeks (17/02/2011) from transplanting, and the second eighteen (18) weeks after transplanting (20/03/2011), i.e. at the completion of the experiment.

Along with the soil sampling, whole plant samples were also taken from each treatment (pot) during both the mid-growth period and at harvesting. One of the two plants was separated into roots and above-ground section (head) and the other one was kept intact (whole plant).

Roots, heads, and whole plants of lettuce of both samplings, along with the soil samples, were chemically analyzed for macro, micronutrients and heavy metals.

2.1 Soil and Sludge Analysis

Chemical determinations were made on the soil samples as follows: soil mechanical analysis by the classical Bouyoucos method, pH measured on water extract of saturated paste using a standard pH meter, CaCO_3 by the method of Bernard, salinity of the saturated extract by a conductivity meter, available soil P by Olsen *et al.* (1954), ex-changeable K according to Lanyon and Heald, (1982), Ca & Mg according to Richards, (1954), micronutrients Fe, Zn, Mn & Cu were extracted with DTPA (Lindsay and Norvell, 1978), and similarly heavy metals Cd, Ni, Co, Pb, Cr were extracted with DTPA (Ure, 1995). Micronutrients and heavy metals were measured by inductively coupled plasma emission spectroscopy (ICP – ES) (Soltanpour *et al.*, 1998). The mean results of soil analysis are given in Table 2.

Table 2. Soil physical chemical characteristics, and macro, micronutrient and heavy metal content under the effect of sludge and TMWW, respectively

Soil Characteristic (n=56)	SLUDGE		TMWW ^a	
	Mean content	Standard deviation	Mean content	Standard deviation
pH	5.76	0.234	5.68	0.281
EC (mS cm^{-1})	0.52	0.626	1.22	0.744
OM (%)	1.56	0.222	1.52	0.235
Total N (%) ^b	0.12	0.016	0.116	0.019
P (mg kg^{-1})	99.6	15.2	99.7	16.1
K (mg kg^{-1})	108	26.4	118	28.5
Ca (mg kg^{-1})	197	19.9	199	20.4
Mg (mg kg^{-1})	30.3	13.5	33.4	15.0
Fe (mg kg^{-1})	25.1	2.73	23.5	2.73
Zn (mg kg^{-1})	2.30	0.931	2.11	1.38
Mn (mg kg^{-1})	41.4	8.54	39.9	8.31
Cu (mg kg^{-1})	2.32	0.480	2.27	0.504
Cr (mg kg^{-1})	0.042	0.012	0.038	0.015
Cd (mg kg^{-1})	0.061	0.017	0.058	0.015
Co (mg kg^{-1})	0.073	0.052	0.072	0.051
Ni (mg kg^{-1})	0.355	0.082	0.339	0.081
Pb (mg kg^{-1})	5.52	2.81	5.40	3.13

^a Treated municipal wastewater, ^b (n=28)

Furthermore, chemical analysis was made of dried sludge for the determination of extractable DTPA content of heavy metals and other nutrients (AOAC, 1996). The attained results are indicated in Table 3.

2.2 Plant Analysis

Root samples were washed by standard procedure, i.e. complete removal of soil particles by means of low-pressure tap water, followed by cleaning with dilute solution of 0.001% HCl and with 0.1%

Alcoonox solution (Kalavrouziotis *et al.*, 2008). Then, they were dried at 70°C and were ground. Leaf and head samples were also washed according to the above procedure (Kalavrouziotis *et al.*, 2013).

The plant samples were mineralized by heating 1.0 g in a muffle furnace at 500°C for 10-12 h, and the ash was dissolved in a solution 50:50 v/v H₂O/HCl, which was used for the measurement of micronutrients and heavy metals by ICP-ES (Soltanpour *et al.*, 1998). The results obtained are given in Table 4.

Table 3. Physical and chemical characteristics and DTPA extractable, macro, micronutrient and heavy metal content of sludge.,

pH	EC (mS cm ⁻¹)	P	K	Ca	Mg	Fe	Zn	Mn	Cu	Cr	Cd	Co	Ni	Pb
mg kg ⁻¹														
6.29	13.7	777.7	3333	460	309	221.5	171.8	43.6	22.70	0.32	0.36	0.61	3.7	322.3

Table 4. Mean lettuce head, roots and whole plant macro, micronutrient and heavy metal content (µg g⁻¹ dry matter)

Elements	HEADS				ROOTS				WHOLE PLANT			
	Sludge		TMWW		Sludge		TMWW		Sludge		TMWW	
	µg g ⁻¹	Stand	µg g ⁻¹	Stand	µg g ⁻¹	Stand	µg g ⁻¹	Stand	µg g ⁻¹	Stand	µg g ⁻¹	Stand
	dm	Dev	dm	Dev	dm	Dev	dm	Dev	dm	Dev	dm	Dev
N	1.810	0.402	1.464	0.329	0.739	0.140	0.618	0.127	1.273	0.240	1.041	0.200
P	0.367	0.054	0.325	0.048	0.230	0.057	0.226	0.073	0.298	0.046	0.275	0.045
K	2.192	0.373	2.034	0.388	0.542	0.166	0.629	0.173	1.367	0.231	1.331	0.221
Ca	0.615	0.323	0.642	0.329	0.469	0.190	0.433	0.147	0.542	0.244	0.538	0.227
Mg	0.230	0.063	0.236	0.072	0.170	0.047	0.145	0.030	0.199	0.049	0.190	0.044
Fe	135.8	146.7	122.6	127.0	2331.4	1051.4	2091.5	1128.0	1233.6	514.0	1107.0	555.60
Zn	35.81	6.215	34.99	4.725	23.06	9.13	17.69	80565	29.43	6.773	26.34	5.495
Mn	188.4	43.15	211.0	50.73	277.85	123.6	263.1	113.34	233.14	63.02	237.0	60.62
Cu	9.274	5.58	10.04	8.811	11.74	6.89	12.07	6.381	10.50	4.740	11.06	5.612
Cr	1.391	0.722	1.378	0.823	18.94	9.55	20.24	13.88	10.17	4.638	10.81	6.944
Cd	0.663	0.485	0.677	0.450	0.926	0.743	0.802	0.578	0.794	0.579	0.739	0.455
Co	0.731	0.925	0.759	0.842	6.606	4.97	7.331	7.489	3.669	2.708	4.045	3.956
Ni	1.594	0.680	1.604	0.590	8.682	3.94	8.143	4.522	5.137*	1.906	4.874-	2.202
Pb	-	-	-	-	-	-	18.33	-	-	-	-	-

2.3 Water and TMWW Analysis

The TMWW was processed and analyzed by the procedure given by AOAC (1996) and APHA (1995). Natural fresh well water was analyzed for Electrical. Conductivity (EC), pH, SAR, P, K, Ca, Na, Mg, Fe, Mn, B, Cl, Zn, Cu, As, Cr, Cd, Ni < Pb, Co) by the methods suggested by Richard (1954 and APHA, 1995). (Table 5)

According to Table 5, the inorganic synthesis of TMWW, with the exception of the Na, whose concentration is very high compared to the WHO standards given above, all the other components were within the acceptable limits. Similarly, the characteristics of the irrigation water are within the allowed critical levels.

2.4. Pollution Load Index (PLI)

Among the aims of this research work was the study of the sludge and wastewater impact, on soil pollution. The assessment of soil pollution level with heavy metals, was possible by means of a simple mathematical relationship, i.e. of the pollution load index (PLI), which is basically the geometric mean of "concentration factors" (CF) of various metals which are involved in the pollution of the soil. The CF is equal to the ratio of the content of heavy metal in the soil to its corresponding reference value, i.e.:

$$CF = M_s / M_r \quad (1)$$

Where: M_s is the concentration of metal in the soil (mg kg^{-1}) and M_r is the reference value of this metal (mg kg^{-1}) (Ntzala, 2011).

Table 5. Chemical and physical characteristics of the two water sources studied in the experiment

Chemical characteristics	Maximum allowable levels	Water source			
		TMWW (n=6)		Natural water (control)	
		Mean	Standard deviation	Mean	Standard deviation
Conductivity (ms cm^{-1})	0.70	1.269	0.173	0.244	0.228
pH	6.5*8.5 ^b	7.32	0.325	8.03	0.213
SAR	≤ 3	4.00	0.723	0.303	0.045
NH ₄ (mg l^{-1})	-	18.33	3.393	0.05	0.00
P (mg l^{-1})	-	0.90	1.098	0.015	0.0055
K (mg l^{-1})	-	11.67	4.082	0.95	0.0548
Ca (mg l^{-1})	0-400 ^b	69.65	5.512	43.53	1.502
Mg (mg l^{-1})	0-60 ^b	29.10	7.140	5.10	0.228
Fe (mg l^{-1})	5.0 ^b	0.09	0.102	0.02	0.0075
Na (mg l^{-1})	0-3.0	162.0	28.114	7.66	1.211
Mn (mg l^{-1})	0.20 ^a	0.082	0.0249	0.00068	0.0003
B (mg l^{-1})	<0.70	0.33	0.082	0.27	0.082
Cl (mg l^{-1})	0-350	274.17	118.84	12.00	4.648
Zn (mg l^{-1})	2.00 ^a	0.003	0.00	0.003	0.00
Cu (mg l^{-1})	0.20 ^a	0.0018	0.0012	0.0043	0.0028
As (mg l^{-1})	0.10 ^a	0.0031	0.0018	0.0012	0.0006
Cr (mg l^{-1})	0.10 ^a	0.001	0.0024	0.0003	0.00015
Cd (mg l^{-1})	0.01 ^a	0.00001	0.00	0.00001	0.00
Ni (mg l^{-1})	0.20 ^a	0.012	0.0097	0.0023	0.0014
Pb (mg l^{-1})	5.00 ^a	0.0002	0.00	0.0003	0.00
Co ^b (mg l^{-1})	0.05 ^a	-			

The concentration factor (CF) is an indicator of soil pollution by a single heavy metal, while the PLI shows the overall pollution of soil by more than one heavy metals, and it is equal to the geometric mean, i.e. to the the n-ith root of the product of n individual concentration factors (nCF), (Cabrera *et al.*, 1999) i.e.:

$$PLI = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \dots \times CF_n} \quad (2)$$

In order to calculate the concentration factor it was necessary to know the reference values of the metals, of a given region. In the present case of the prefecture of Aitolokarnania, where the experiment was conducted, the reference values of the metals were derived from soil samples taken from areas which are far away from pollution sources (industries, major highways, landfills, etc.) and as possibly evenly distributed in the area under consideration. Therefore, four compound soil samples were taken from 11 sites, scattered evenly all over the Aitolokarnanian Prefecture i.e.: 1. Avlaki 2. Paleros, 3. Vonitsa 4. Lepenou, 5. Melegros, 6. Lesini, 7. Arakynthos, 8. Kallithea, 9. Mandrini, 10. Elatou and 11. Trikorfo. These sites are located away from sources of pollution. In total 44 soil samples were taken for the determination of Cd, Co, Cr, Zn, Cu, Mn, Ni and Pb content.; The following mean reference values were obtained shown in Table 6.

Table 6. Heavy metals references values of the Aitoakarnanian Prefecture (Ntzala, 2011).

Metal	n	Minimum	Maximum	Mean	Standard deviation
Fe	44	2.67	76.66	14.53	12.7676
Cu	44	0.24	11.19	2.48	2.2851
Mn	44	1.37	61.93	11.39	14.2377
Zn	44	0.23	15.49	1.84	2.6023
Cd	44	0.013	1.315	0.10	0.1890
Co	44	0.023	0.459	0.11	0.1004
Cr	44	0.00	0.039	0.012	0.0086
Ni	44	0.066	2.594	0.90	0.6127
Pb	44	0.049	2.165	0.57	0.3937

2.5. Statistical Analysis

Experimental data was statistically processed by ANOVA, and regression analysis, using the statistical package of SPSS ver. 17.

3. Results and Discussion

The experimental results that have been obtained are given below and discussed accordingly as follows:

3.1 Effect of sludge and TMWW on plant height

It was shown that the plant height was significantly positively affected by the application of sludge (Table 7). A more detailed study of the effect of treatments on plant height, basically showed that the height was significantly increased under the effect of sludge at the fourth and fifth measurement. As the greenhouse was not heated, the actual effect of sludge on the height started much later than the transplanting date of lettuce, during which the temperature was low (10-13 °C). The actual effect of sludge started at the time the temperature of the greenhouse had been improved (>15 °C). The gradual increase of air temperature contributed to decomposition and mineralization of sludge organic matter, and consequently the release of nutrients N, P and other micro-nutrients, which favored the plants growth. As shown in Table7, the mean plant height increased significantly at the fourth measurement, and from a mean value of 15.86 cm, increased to 26.75cm at the fifth measurement.

Table 7. Descriptive statistics of the. effect of applied sludge treatments on the lettuce plant height in cms

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Firstheight	28	8.00	9.60	8.6929	.48605
Secondheight	28	8.40	16.90	12.4750	2.10777
thirdheight	28	10.00	17.90	13.5143	1.94626
fourthheight	28	10.70	23.00	15.8679	2.89022
fifthheight	28	13.40	62.50	26.7571	12.72671
Valid N (listwise)	28				

Analysis of variance (ANOVA) indicated that the effect of sludge on plant height was statistically significant at the 4th (F = 8.501 sign.0. 000) and 5th (F = 3.201 and sign. 0.025) measurement.

3.2 Effects of sludge on fresh and dry matter yields

In Figures 1A and 1B, fresh and dry matter yield was affected significantly under the effect of sludge. These results showed that the addition of sludge had positive effects on both dry and fresh matter yields.

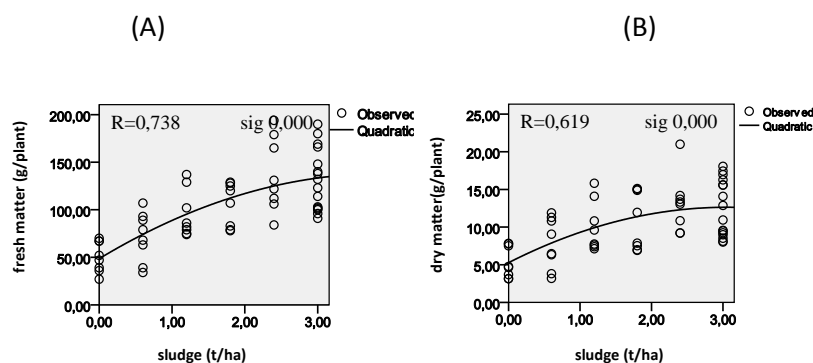


Figure 1. Effect of the applied sludge levels (t/ha) on the fresh (A) and dry (B) matter yield of lettuce heads (mean effect of both plant samplings (n=56))

Similarly, the effect of TMWW on both fresh and dry matter yields of lettuce head was statistically significant (Figure 2A and 2B).

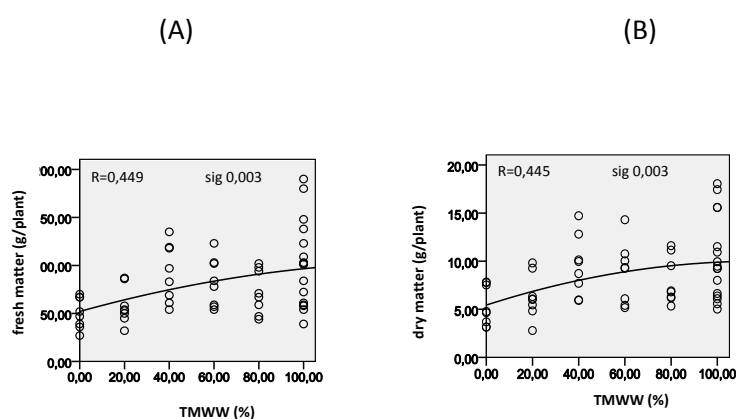


Figure 2. Effect of the applied TMWW levels on the fresh (A) and dry (B) matter yield of head (mean effect of both samplings) (n=56).

3.3 Effect of sludge and TMWW on physical and chemical characteristics of the soil

In the context of the present study, the effects of sludge and TMWW on some physical and chemical characteristics of the soil, such as pH, electrical conductivity (EC), organic matter, macro- and micro-nutrients, and heavy metals, were also studied.

Comparing the mean values of soil, pH under the effect of sludge and TMWW, respectively, it was found that the differences were statistically non-significant (Figure 3A) and under the TMWW (Figure 4A), suggesting that the effect of both sludge and TMWW on this parameter was similar, i.e. negative and quantitatively more or less equal.

However, the effect of TMWW on the soil EC was much stronger than that of sludge, increasing the EC from $0,40$ to $2,10 \text{ mS cm}^{-1}$ (Figure 4B) in comparison to that of sludge which increased EC from $0,45$ to $1,1 \text{ mS cm}^{-1}$ (figure 3B). These results are explained on the basis of the higher concentration of the soluble Na in the TMWW being 162 mg l^{-1} compared to the critical level of $0\text{--}3 \text{ Na mg l}^{-1}$ (Table 5). It is well known that Na ion is strong alkalizing factor, increasing the pH significantly (Richards, 1954).

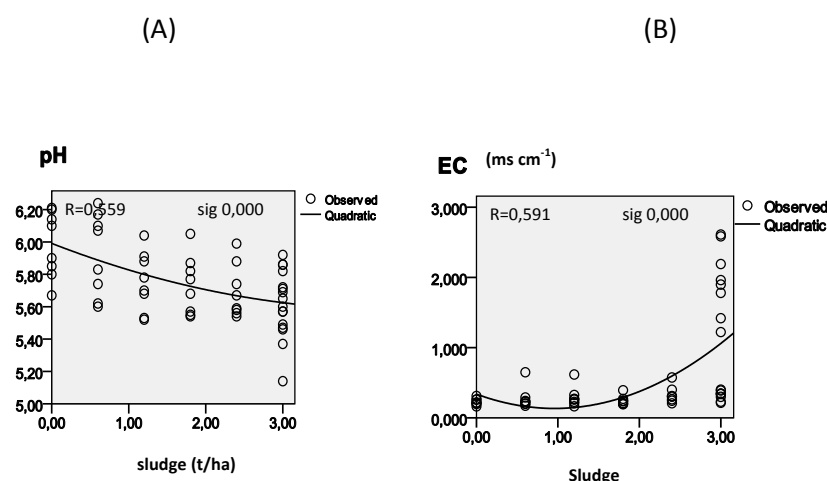


Figure 3: Effect of the applied sludge levels (t/ha) on pH and electrical conductivity (EC) of soil, during both samplings (n=48)

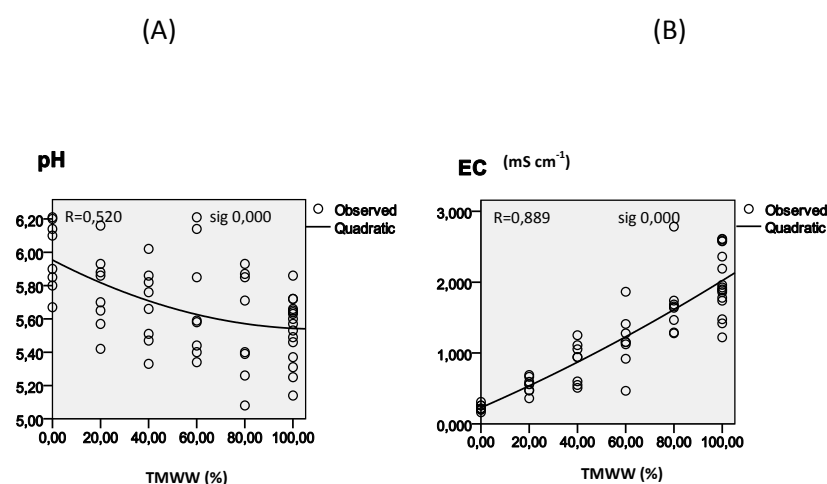


Figure 4. Effect of TMWW treatments on pH and electrical conductivity (EC) of soil, during both samplings n=58

3.4 Effect of sludge and TMWW on the nutrient and heavy metal content of heads, roots and whole plant dry matter

Application of sludge (S) increased linearly and statistically significantly the lettuce whole plant N, the head N and the root N content, as shown by the following regression analysis:

Plant organ	Regression equation	R	significance
Whole plant	$N\%(dm) = 0.013(S) + 1.045$	0.610	0.001
Heads	$N\%(dm) = 0.022(S) + 1.438$	0.590	0.001
Roots	$N\%(dm) = 0,005(S) + 0.652$	0.399	0.035

Contrary to sludge, the application of TMWW increased only the root N content of lettuce, the regression equation being quadratic, and statistically significant, i.e. : $N\%(dm) = 5.49E.005(TMWW)^2 - 0.006(TMWW) + 0.650$ (R 0.512, significance 0.023).

Excluding the N, the comparative study of the effect of sludge, and TMWW, on the macro, micronutrient and heavy metal head, root and whole plant content, respectively disclosed that neither of the above treatments affected significantly the mineral content of these lettuce plant parts As indicated by the analysis of t-test shown in Table 8,,the correlation coefficient between the compared

pairs is highly significant (0,999) and the t value non significant, suggesting that the difference between the treatments studied were statistically non significant and that both of these treatments had the same effect on the mineral composition of lettuce plant parts.

Table 8. Pair t-test analysis between the sludge and TMWW effect on the mineral content of lettuce head, root and whole plant dry matter

Pairs compared	Paired differences				Paired	t	df	Signif (two-tailed)
	Mean	Stand. Deviation	Standard error	95% confidence	95% Confid.			
					Upper			
Pair1 (h.SLUDGE-h.TMWW)	0.681	7.521	2.086	-3.863	5.226	0.327	12	0.749ns
Pair2 (r.SLUDGE-r.TMWW)	18.518	63.863	17.068	-18.355	55.392	1.085	13	0.298ns
Pair3 (wp.SLUDGE-wp.TMWW)	9.832	35.134	9.746	-11.402	31.065	1.009	12	0.333ns

The letters r, h, and wp in front of SLUDGE and TMWW stand for root, headsn and whole plants, respectively

3.5 The impact of sludge and TMWW on soil pollution

Among the main objectives of this work was to study and evaluate the level of soil pollution that may be caused by applying high levels of sludge and the long term reuse of TMWW and sludge. Despite of their favorable properties (high macro- and micro nutrients content, organic matter, and water), these two inputs also have a major drawback. They contain large amounts of heavy metals, (Stabnikova *et al.*, 2005; Akdeniz *et al.*, 2006) toxic compounds and various pharmaceutical substances and xenobiotics, which can easily enter into the food chain, eventually causing undesirable effects on the consumers' health (Fata-Kassinis *et al.*, 2010).

Long-term application of sludge or TMWW in agricultural soils at high rates can cause pollution due to the accumulation of heavy metals (Liu *et al.*, 2005; Zhang *et al.*, 2012. In this work, the experimental soil used, was a non polluted one. Therefore, sludge was applied at low and very high levels varying from 0 to 30 t/ha, in order to create a variable degree of soil contamination level. The objective was the evaluation of soil pollution level that would be caused by the addition of sludge, based on Pollution Load Index (PLI) (Tomlinson *et al.*, 1980).

The PLI was calculated for the various treatment levels of sludge and TMWW and the results obtained are given in Table 8.

The study of the data of this Table reveals the following: The values of the PLI corresponding to the treatment levels of the sludge and TMWW, were low at the first soil sampling, while they increased at the second sampling. These results are explained on the basis of the increase of temperature after the 1st soil sampling. Before this sampling, the air temperature was <15 °C. As a result soil chemical and biological processes (decomposition and mineralization of organic, organic metal complex formation) were less active, and the heavy metals were not released into the soil, consequently the PLI did not increase substantially. Therefore, plant growth and soil contamination were not differentiated.

The minimum and maximum PLI values attained at the 1st and 2nd soil sampling under both the treatments studied, ranged between 0,75 - 1,00 & 1,14-1,26 under sludge, and 0.61 to 1.09 & 1,33-1,55, under TMWW, respectively (Table 8). Previous work of Kalavrouziotis and Koukoulakis (2011) showed that the optimum PLI calculated under the effect of TMWW reuse varied from 1.99 to 2.50 and the cabbage dry matter losses related to these PLI values ranged from 1-11%, which were considered normal.

Table 9. Calculated Pollution Load Index under sludge and TMWW, respectively, at the 1st and 2nd soil sampling, on the basis of the soil analytical data

SLUDGE			TMWW		
Applied levels t/ha	PLI		Applied treatments %	PLI	
	1 st soil sampling	2 nd soil sampling		1 st soil sampling	2 nd soil sampling
Control(0)	0.75	1.14	Control(0)	0.61	1.42
6	0.91	1.20	20	0.77	1.43
12	0.81	1.26	40	0.98	1.38
18	0.87	1.20	60	0.93	1.52
24	1.00	1.22	80	0.98	1.33
30	0.90	1.23	100	1.04	1.37
30+100%TMWW	0.82	1.18	100+30t sludge	1.09	1.42

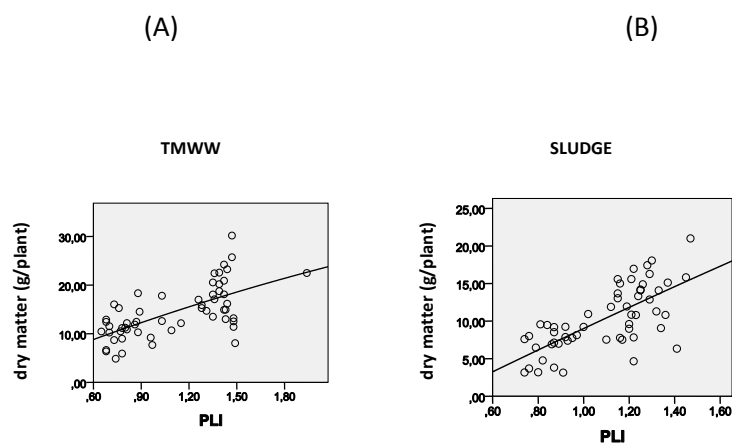
3.5.1 Relation between PLI and lettuce plant yield

In spite of the increase of the PLI values, it was found that this index was statistically significantly and curvilinearly related to lettuce yields, under the effect of sludge, and TMWW (Figure 5). This relationship is described by the following regression by equation for the sludge and the TMWW:

1- Under sludge: Dry matter = $-9.439(PLI)^2 + 37.438(PLI) - 11.883$ ($R^2 = 0.686$, $p < 0.000$):

2- Under TMWW: Dry matter = $-1,183(PLI)^2 + 13.322(PLI) + 1.242$, ($R^2 = 0.418$, $p < 0.000$)

Although it was expected that a decrease of lettuce dry matter yield would have occurred with the increase of PLI, this did not occur, because soil contamination by sludge and TMWW born metals, was very small (Table 8). Figure 5 shows that the value of PLI equal to 1.50 corresponds to the maximum lettuce plant dry matter yield under both the effect of sludge (15g/plant) and TMWW (24g/plant), respectively. These results suggest that the critical value of $PLI \leq 1$, set forth by Cambrera (1999) must be increased according to the capacity of the plant to resist the heavy metals.

**Figure 5.** Relationship between PLI and lettuce plant dry matter yield (in grams per plant) under the effect of sludge and TMWW

3.5.2 Relation between PLI and lettuce plant dry matter mineral composition

Since PLI is an indicator of the degree of soil contamination, it is expected to be directly related to the content of plant nutrients and heavy metals. Therefore, the relationship between PLI and mineral composition of lettuce plants, under the effect of sludge and TMWW, was investigated.

The data of Table 10 shows that the interaction of PLI with most micro-, macro-nutrients of lettuce head and roots, was mainly synergistic and statistically significant ($p < 0.000$), under the effect of sludge

and TMWW, respectively. This means that as the PLI increases, the nutrient and metal plant dry matter content also increases. Only between the PLI and Cd, Co and Ni, the relation is negative-antagonistic in lettuce heads, and roots, both under sludge, and TMWW, respectively (Table 10), suggesting that the increase of PLI reduces the dry matter content of the above three heavy metals in the plant.

These results are reflected in Figure 5 where the positive and statistically significant effect of PLI on dry matter yield is clearly shown. Since the PLI is positively and statistically significantly related to the macro and micronutrients, this relationship resulted in the increases of their concentration in the plants, and hence the plant growth was favourably affected.

Table 10. Relationship between PLI and lettuce root and head mineral composition, under the effect of sludge and TMWW

Relationship between PLI and metals	Under the effect of TMWW				Under the effect of sludge			
	Roots		Heads		Roots		Heads	
	sign	type	sign	type	sign	type	sign	type
PLI vs Ca	0.000	S	0.000	S	0.000	S	0.000	S
PLI vs Mg	0.000	S	0.000	S	0.000	S	0.020	S
PLI vs Fe	0.001	S	0.000	A	0.000	A	0.002	S
PLI vs Zn	ns	-	0.000	S	0.045	S	ns	-
PLI vs Mn	0.032	S	0.002	S	0.007	S	ns	-
PLI vs Cu	0.030	S	ns	-	ns	-	0.019	S
PLI vs Cr	ns	-	0.000	S	ns	-	ns	-
PLI vs Cd	0.000	A	0.000	A	0.000	A	0.000	A
PLI vs Co	0.000	A	0.000	A	0.004	A	0.000	A
PLI vs Ni	0.001	S	0.000	A	0.000	A	0.000	S
PLI vs Pb	-	-	-	-	-	-	0.000	S
PLI vs P	0.009	S	ns	-	0.000	S	ns	-
PLI vs K	0.007	S	ns	-	0.001	S	0.007	S
PLI vs Ca	0.000	S	0.000	S	0.000	S	0.000	S
PLI vs Mg	0.000	S	0.020	S	0.000	S	0.023	S

A=interaction competitive, S=synergistic, 0.000 = statistical significant 10^{-3}

4. Conclusions

The conclusions drawn from the aforementioned discussion are summarized as follows:

The increase of plant height was statistically significant under the effect of sludge, while it was non-significant under the TMWW.

Both sludge and TMWW affected positively and statistically significantly soil pH and negatively soil electrical conductivity (EC), the effect of TMWW being much higher.

Also, the addition of sludge increased the head, root and whole plant dry matter N content, while irrigation with TMWW increased significantly the total root N and P concentration.

The increase of PLI under the treatments studied, was considered low as shown by the positive relation of the PLI with plant dry matter production.

The relation between PLI and the plant macro and micronutrients of the head and root dry matter content was synergistic, and statistically significant, while between Cd, Co and Ni, plant content, was antagonistic, relationships, which affected favorably the plant growth. It was concluded that the applied sludge and TMWW treatments polluted the soil only slightly.

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