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The Effects of Sewage Sludge from Different Regions and Seasons on Plant Growth and Mineral Composition

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***Abstract*:** *Sewage sludge (sludge) samples were sampled montly from ten high sized wastewater treatment plants in the coastal regions of Antalya, Turkey. In a greenhouse experiment, seasonaly grouped sludges were applied soil and lettuce plant were growed. Plant dry matter and concentrations of total N, P, K, Ca, Mg and Na, Fe, Mn, Zn, Cu, Ni, Pb, Cd and of lettuce plant were determined, and sludge effects on plant were evaluated with regard to sampling region and the sampling seasons of sludge. In greenhouse experiment, sewage sludge applications were increased dry matter yield, plant nutrients and heavy metal contents of lettuce plants. Although total heavy metal contents of sludge samples were determined below the regulation limits, Zn, Pb, Cd and Cr contents of lettuce were exceeded food codex limits for these metals. Although sludge composition and characteristics were changed depending on the region and time, sludge of a certain region has not dominated for all parameters of lettuce plant and all type of sludge applications increased heavy metal concentrations in lettuce plant.*

Keywords: *Sludge, Heavy Metals, Seasonal Variations, Lettuce*

1. Introduction

Due to the population growth the amount of sludge produced as a result of wastewater treatment increases and millions of tons of residual sludges worldwide every year are generated. That brings the challenges to its proper management. The basic disposal methods for such large quantities of sludge are land application, landfilling, incineration, ocean dumping and lagooning [1]. The disposal of sludge in landfills or by incineration is a feasible option but both these strategies are expensive and may cause environmental problems. Compared to this, the utilization of sludge in agricultural lands is the best alternative for sludge disposal because it recycles both nutrients and organic matter.

The plant nutrient value of sludge has been evaluated by many investigators [2,3], and the nutrient composition is considered to be similar to other organic-waste based soil amendments that are routinely applied on cropland, such as animal manure. In addition to major plant nutrients, sludge also contains trace elements that are essential for plant growth. Soils which have been tilled for decades are often deficient in certain trace elements, such as zinc and copper [4]. Land applications of municipal sludge can help to remedy these trace metal deficiencies [5]. Sewage sludge contains all the elements essential for the growth of higher plants. Because nitrogen and phosphorus are the most abundant major plant nutrients in sludge [6], its agricultural use is almost exlusively as a supplemental source of nitrogen and/or phosphorus fertilizer. However, toxic chemicals in low concentrations are introduced into municipal wastewater and sewage sludge often contains potentially toxic elements, which cause soil contamination, phytotoxicity and undesirable residues in plant and animal products [7]. As a matter of fact, pollution problems may arise if toxic metals are mobilized into the soil solution and are either taken up by plants or transported in drainage waters. Risk for human health may then occur through consumption of such crops. In the long term, the use of sewage sludge can cause a significant accumulation of

Zn, Cu, Pb, Ni and Cd in the soil and [8]. Sewage sludge also contains human pathogens, although it can be treated to significantly reduce the number of pathogens present. Pathogens and toxic chemical pollutants may be introduced into sludge-amended soil.

Antalya region of Turkey have a high economic potential, familiar with tourism, agriculture and industrial activities for all season. Due to high variability of the tourism, industrial and and agricultural activities of Antalya, various characteristics and quantities of sludge are generated by wastewater treatment plants.

The objective of this paper is to provide information on the effects of soil applications of sewage sludges sampled from different regions and at different seasons on lettuce plant gwroth and to evaluate regional and seasonal differences of sludges on plant nutrient and heavy metal concentrations.

2. Material and methods

2.1. Sludge Sampling and Analysis

Samples were monthly collected from ten wastewater treatment plants located at coastal regions of Antalya, namely: Tekirova (1), Beldibi (2), Göynük (3), Çamyuva (4), Kemer (5), Antalya-Hurma (6), Serik (7), Belek (8), Side (9) and Alanya (10) during one year (Figure 1). All wastewater treatment plants receive domestic and a little amount of industrial effluents. Sludge samples were collected from belt filtered raw material before disinfection, lime stabilization or thermal microbial stabilization processes from the treatment plant.

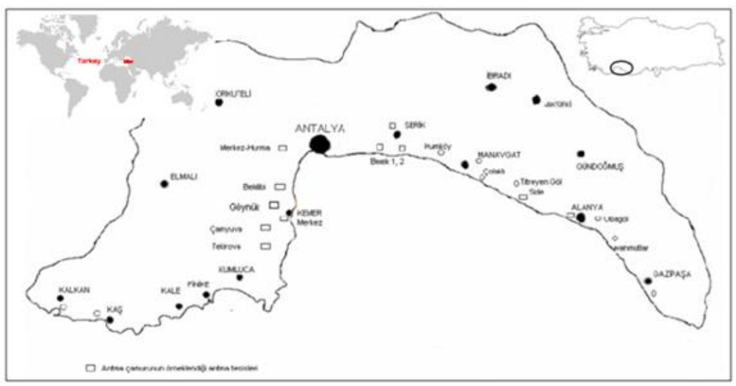

Fig. 1. Big and middle sized biological wastewater treatment plants located at the coastal regions of Antalya

The sludge samples were air-dried, mixed, ground and sieved through a 1- mm-mesh sieve for analysis. The final material was stored in plastic bottles at room temperatures until analysis. Three replicates of each dried sample were analyzed for several physiochemical analysis.

In sludge samples pH, total organic matter (OM), conductivity (EC) Kjeldahl nitrogen (N) were analysed by using standard methods [9]. In sludge samples available phosphorus [10], exchangeable Na, K, Ca and Mg [11], total and DTPA extractable Fe, Mn, Zn, Cu, Ni, Pb, Cd and Cr were analysed. For the determination of 'total' heavy metal concentrations, sludge samples were digested in aqua regia (1:3 HNO_3/HCl) and $HClO_4$ according to the international standard [12]. Bioavailable fractions (DTPA-extractable) of metals were extracted from soil with diethylenetriaminepentaacetic acid-$CaCl_2$-triethanolamine adjusted to pH 7,3 [13]. Total and bioavailable Zn, Cd, Ni and Pb concentrations of greenhouse soil samples were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 ^{0}C) material.

2.2. Greenhouse Experiment

The pot experiment was conducted on the greenhouse in calcareous soil with slightly alkaline reaction. The experimental soil belong to red mediterranean big soil group was taken from the Aksu, representative of the major vegetable growing area of Turkey Antalya. Soil was taken from 0-30 cm and then air dried and sieved from 2 mm for pot experiment. The analytical characteristics of the greenhouse soil are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [14].

Table I. The analytical characteristics of the experimental soil and seasonally grouped sludge samples

	Soil	Seasonaly grouped sludges				Metal limits[3] of soil (pH <6-7<)	Metal limits[3] o sludge
		Spring	Summer	Autumn	Winter		
Texture	Clayed loam						
Lime ($CaCO_3$), %	10,5						
pH	7,7	6,25	6,10	6,38	6,79		
E.C., dS m^{-1}	0,311	5,58	6,85	8,41	6,07		
Organic M., %	2,45	65	75	74	60		
Total N, %	0,124	5,45	6,35	5,87	4,64		
Available P, %	9,6	0,145	0,194	0,181	0,134		
Exchangeable K, mg kg^{-1}	266	2923	3558	3854	1811		
Exchangeable Ca, mg kg^{-1}	2156	5813	5991	5891	5163		
Exchangeable Mg, mg kg^{-1}	377	1381	1448	1475	1578		
Exchangeable Na, mg kg^{-1}	336	782	967	1789	702		
Fe, mg kg^{-1}	14554[1](0,64)[2]	13733 (835)	11533 (753)	12463 (744)	16878 (875)	-	-
Mn, mg kg^{-1}	420 (8,2)	356 (108)	286 (97)	263 (59)	460 (153)	-	-
Zn, mg kg^{-1}	66 (3,4)	812 (422)	1023 (523)	808 (525)	818 (310)	150-300	2500-4000
Cu, mg kg^{-1}	22 (1,3)	226 (15,10)	186 (12,21)	176 (19,32)	200 (9,42)	50-140	1000-1750
Ni, mg kg^{-1}	19 (0,73)	74 (6,26)	54 (5,74)	48 (4,87)	110 (11,5)	30-75	300-400
Pb, mg kg^{-1}	48 (2,14)	410 (8,82)	397 (9,49)	387 (8,98)	421 (8,73)	50-300	750-1200
Cd, mg kg^{-1}	* (*)	4,84 (0,26)	3,70 (0,27)	3,38 (0,27)	4,90 (0,20)	1-3	20-40
Cr, mg kg^{-1}	12 (0,18)	41 (0,80)	34 (0,77)	30 (0,40)	47 (0,42)	100	1200

[1]: total concentration, [2]: Values in pharenthesis are belong to DTPA-extractable concentration; *: below the detection limits (<0,001 mg kg^{-1}),
[3]: EU standarts (86/278/EEC [14].

Experiment was carried out in a plastic covered greenhouse under controlled climatic conditions. The sludge sampled monthly from each sampling area was seasonaly combined in equal amounts. Sludge samples representing each season were applied at the level of 36 g / pot as dry weight basis (approximately equal to 3 tonnes/da) and total pot weight was completed to 3 kg with experimental soil. Pot experiment was arranged in a completely randomized block design with four replicates.

Lettuce seeds (*Lactuca sativa var. Yedikule*) were germinated in peat-perlite substrate then seedlings of lettuce were transplanted as two plants per pot. Pots were maintained around field capacity by daily watering with distilled water. Sixty-five days after transplanting, in the maturity stage of lettuce, plants were harvested by cutting from soil surface, washed with pure water and then leaves were dried at 65 ºC in a air-forced oven for 48 h for determination of dry matter yields. In dried plant samples, total kjeldahl nitrogen [15] was determined. For the determination of total mineral and heavy metal concentrations, plant samples were ground and then digested in aqua regia (1:3 HNO_3/HCl) according to the international standard [12]. Total mineral and heavy metal concentrations in plant samples were analyzed using ICP-MS under optimized measurement conditions and values were adjusted for oven dried (12 h at 105 ^{0}C) material.

The effects of sludges on the dry matter and mineral contents of lettuce plants were evaluated at seasonal and regional level. Analysis of variance was used to evaluate the effects of different factors,. Statistical analyses were performed by using SPSS-16 for Windows program.

3. Results and discussion

3.1. The physicochemical properties and metal contents of soil and sludges

Experimental soil has a clayed loam texture, slightly alkaline reaction, high $CaCO_3$ content, low salinity, low N and available P, high levels of K, Ca, Mg and Na. The heavy metal contents of untreated experimental soil (control) are well within the accepted normal range of values. A comparison of metal contents of sewage sludge with that of untreated soil showed that the metals Zn, Cu, Ni, Pb, Cd and Cr were present in sewage sludge in greater concentrations than in the soil (Table 1).

Seasonaly grouped sludges from various watewater treatment plants located at the coastal regions of Antalya demonsrated significant differences in their physicochemical characteristics and metal contents. pH, EC, organic matter, total N, P, Mn, Cu, Ni and Cr contents; available P; exchangeable K, Na; DTPA-extractable Mn, Cu, Ni and Cr contents of sludge showed significant changes with regard to sampling seasons. The heavy metal concentrations of sewage sludge are below the pollutant levels indicated by the EU (86/278/CEE [14] for the agricultural use of sewage sludge (Table 1). According to soil pollution criteria, it is seen that all sludges could be used for agronomic purposes in soil amendments without pollution risks according to regulation limits.

EC values showed a big variation among sludges, ranged between 4.28-8.61 dS m^{-1}. All sludges had high EC values, which indicated a high salt content above the critical level 4 dS m^{-1} [16]. High EC values would likely be the soluble salts responsible for the high salinity. It has been reported that the sludge can cause soil salinity due to its high conductivity value, which leads to an increase in the availability of certain metals and thus cause toxic accumulation of heavy metals during long-term sludge application to the soil [17, 18]. Sludges showed a big exremity with regard to organic matter and total nitrogen content. However all sludges have a high rate of organic matter above 50 %. Total and available P rates were found generally higher in sludge for an organic matter source. Nitrogen and P have been reported as the most abundant essential plant nutrients in the treatment sludge for the development of higher plants [19]. As can be seen from the examination of Table 1, although the contents of organic matter, N and P in sludge are generally high, and high conductivity values is considered as limiting factors in agricultural application of sludge.

3.2. Plant growth, nutrient and metal contents of lettuce

Dry matter yield and concentrations of N, P, K, Ca, Mg, Na in the leaves of lettuce plant grown in sludge treatments are presented in Table 2. Plant nutrient content of lettuce plant was determined above the deficiency threshold level in control application. All sludge applications increased dry matter yield and concentrations of N, P, K, Ca, Mg, Na in lettuce plant as compared with control (no sludge) application. This shows that all sludges have a high nutrient content to support plant growth. Seasonal effects of sludge samples on lettuce plant were significantly found on P, K and Na contents. Previous studies showed that soil application fo sludge increased the amount of crop yield and plant nutrients content but also increased the heavy metal content [20, 21].

Total Fe, Mn, Zn, Cu, Ni, Pb, Cd and Cr concentrations of lettuce plant grown in sewage sludge treatments, and also background concentrations (Davis and Carlton-Smith, 1980) and phytotoxic metal limits and food codex limit values are presented in Table 3. Concentrations of metals (Fe, Mn, Pb, Cd and Cr) in the control application were determined in representative of background levels but Zn, Cu and Ni was determined below the background level. Sewage sludge treatments increased all heavy metal concentrations in lettuce plants as compared control. Specially, sludge applications to soil increased Cd and Cr contents of lettuce in extreme degree. This expected increase in metal content with sludge applications is attributed to the high level of total and available plant nutrients and heavy metal content of the sludge. It has been reported that soil sludge applications increased the concentrations of Cd, Cu, Ni, Zn in the lettuce plant and that the heavy metal accumulation is closely related to the sludge metal characteristics [24].

Table II. The effects of sludge applications on the dry matter yield and plant nutrient content (mg kg-1) in lettuce.

	Control	Seasonally grouped sludge applications				Significancy	Deficiency threshold level[1]
		Spring	Summer	Autum	Winter		
Dry matter, g pot^{-1}	8,96	10,60	10,92	10,76	10,77	ns	
N, %	3,50	4,32	4,26	4,17	4,03	ns	2,49
P, %	0,410	0,868	1,068	1,086	0,922	*	0,39
K, %	4,55	6,53	7,56	6,87	5,50	*	1,49
Ca, %	1,05	1,23	1,32	1,38	1,35	ns	0,80
Mg, %	0,36	0,58	0,63	0,61	0,62	ns	0,29
Na, mg kg^{-1}	370	491	559	655	633	*	-

*: P<0.05, **: P<0.01, ***: P<0.001. ns: no significancy ,. 1: [22].

Table III. The effects of sludge applications on the micronutrient and heavy metal concentrations (mg kg-1) in lettuce

	Control	Seasonally grouped sludge applications				Significancy	Background levels[1]	Pytotoxic threshold levels [2]	Food codex limits[3]
		Spring	Summer	Autum	Winter				
Fe, mg kg^{-1}	67	316	306	313	340	ns	100-500	<500	-
Mn, mg kg^{-1}	27	76	68	55	127	***	20-30	400-500	-
Zn, mg kg^{-1}	29	82	108	106	63	***	40	100-400	5
Cu, mg kg^{-1}	5,1	9,1	7,5	10,3	6,5	*	8	20-100	5
Ni, mg kg^{-1}	1,3	7,6	7,5	6,5	12,3	***	2	10-100	0,2
Pb, mg kg^{-1}	3	34	36	31	32	ns	3	30-300	0,2
Cd, mg kg^{-1}	<0,01	1,52	1,77	1,64	1,28	ns	< 0,5	5-30	0,1
Cr, mg kg^{-1}	0,1	6,3	6,5	3,7	4,0	***	-	5-30	0,05

*: P<0.05, **: P<0.01, ***: P<0.001. ns: no significancy ; [1]: [25], [2]: [23], [3]: Values were given for vegetables and equivalent foods as fresh matter basis [26].

Significant differences were found among the treatments. According to background and toxicity limits, metal status of leaves was generally ranged in normal and high levels. Seasonal effects of sludge samples on lettuce plant were significantly found on Fe, Mn, Zn, Cu, Ni and Cr contents. It was determined that the metal concentrations of lettuce plant were much higher than the background levels of Zn, Cu, Ni, Pb, Cd and Cr elements. Nevertheless, all metal concentrations were below the phytotoxic levels although the limit values reported for plant phytotoxicity were exceeded in terms of Zn, Ni and Pb contents in some sampling seasons. This is thought to be caused by lettuce plant being a high-level metal accumulating plant [27] and proportional accumulation of heavy metals in edible parts of vegetables and fruits were significantly higher in lettuce plants than other crops [15, 28].

It is considered that the content of N, P, K, Ca and Mg of lettuce plant is in the appropriate range for optimum development and therefore the sludge can be regarded as an organic fertilizer source in this respect. However, as can be understood from the examination of Table 3, the heavy metal content were significantly increased in lettuce compared to the content of the control application, especially for Zn, Ni, Cd and Cr. According to food codex of Turkish Food Legislation [26] and European Union Standards [25], metal limits for leafy vegetables have been reported as 0.2 and 0.3 for Pb and 0.1 and 0.05 mg / kg for Cd respectively.Table 3 clearly shows that Zn, Pb, Cd and Cr contents of lettuce plant exceeded the declared food codex values (as converted for fresh weigh basis), and in a sense it seems to be disadvantageous to consume the sludge amended lettuce vegetable as a food.

3.3. Effects of seasonal and regional sludge differences on lettuce plant

The effects of the soil applied sludges sampled at different seasons on the P, K, Na, Mn, Zn, Cu, Ni and Cr contents of the lettuce plants were found statistically significant. This could be due to the fact that the characteristics of the sludge applied to the soil on the plant nutrients and heavy metal contents of lettuce plant have changed according to the seasons. Leaf P, K, Zn concentration of lettuce plant was higher in summer and autumn seasons.

Data on the dry matter, nutrient and heavy metal contents of lettuce plant grown in the sludge applied soil are given in Table 4 and Table 5 with regard to the different regions of sludge. The effects of sludges collected from different sampling regions on total N, P, K, Mg, Na, Fe, Mn, Zn, Cu, Ni, Pb, Cd and Cr contents of lettuce plant were found statistically significant. These findings show the determinant effect of the sludge characteristics of the different regions on the criteria mentioned in the lettuce plant.

Soil sludge applications increased the amount of dry matter in the lettuce plant but the effect of different regions of sludge on the dry matter amount of lettuce plant was not found statistically important. All macro and micro plant nutrients and heavy metals with the exception of Ca in lettuce plant were increased by sludge applications. As can be seen, although the application of sludge of a certain region could increase the most of examined criteria in lettuce, some of other criteria could be influenced at low levels. Sludge of a certain region has not dominated on all parameters of lettuce plant. According to these findings, it can be concluded that there are no differences with regard to regions of sludge that has a well accepted criteria for agronomic purposes than others.

Table IV. The effects of different regions of sludges on the dry matter and some nutrient contents of lettuce

Sludge Sampling Regions	Dry matter, g/pot	N	P	K	Ca	Mg	Na, mg kg^{-1}
		%					
Control	8,96	3,50	0,410	4,55	1,05	0,36	370
1	10,62	4,09	0,806	6,19	1,22	0,69	1006
2	10,88	5,16	1,379	8,55	1,13	0,67	556
3	11,14	4,43	1,022	7,14	1,46	0,61	553
4	10,04	4,69	1,151	7,45	1,15	0,58	702
5	11,44	3,43	1,134	5,75	1,28	0,49	404
6	9,96	4,18	1,436	11,24	1,32	0,75	758
7	10,44	3,52	0,609	4,60	1,50	0,64	429
8	11,02	4,29	0,956	6,50	1,37	0,62	521
9	10,55	5,76	0,763	7,28	1,33	0,55	508
10	10,44	3,09	0,859	4,75	1,34	0,51	407
Significancy	ns	***	***	***	ns	*	***

*: P<0.05, **: P<0.01, ***: P<0.001; ns: no significancy

Table V. The effects of different regions of sludges on some nutrients and heavy metal contents of lettuce

Sampling Regions	Fe	Mn	Zn	Cu	Ni	Pb	Cd	Cr
	mg kg^{-1}							
Control	67	27	29	5,1	1,3	3	<0,001	0,1
1	362	105	88	7,5	5,4	33	1,56	3,9
2	242	32	104	14,9	2,5	33	1,49	3,2
3	357	92	49	4,0	10,2	11	0,88	5,2
4	286	59	91	7,9	7,9	28	1,32	5,7
5	208	74	120	10,1	10,1	49	2,05	4,4
6	342	54	109	14,7	8,3	39	1,48	5,6
7	242	150	79	9,1	10,8	47	3,14	4,2
8	428	86	97	4,2	11,6	19	0,48	6,8
9	270	49	114	9,6	6,5	25	1,84	6,4
10	319	34	89	6,9	5,7	48	1,55	7,1
Significancy	***	***	***	***	***	***	***	***

*: P<0.05, **: P<0.01, ***: P<0.001; ns: no significancy

4. Conclusion

Sludge composition was changed depending on the sampling region and time. Concentrations of heavy metals in sewage sludge have been determined below the maximum permissible limit values. However, the DTPA extractable fraction of heavy metals is quite high for Zn, Ni. Zn, Pb, Cd and Cr. All type of sludge applications to soil increased heavy metals in lettuce plant but sludge of a certain region or season has not dominated on all parameters of lettuce plant. Nevertheless, all metal concentrations were below the phytotoxic levels although the limit values reported for plant phytotoxicity were exceeded in terms of Zn, Ni and Pb contents in some sampling seasons.

Content of N, P, K, Ca and Mg of lettuce plant is in the appropriate range for optimum development and therefore the sludge can be regarded as an organic fertilizer source in this respect. However, the heavy metal content was significantly increased in lettuce compared to the content of the control application, especially for Zn, Ni, Cd and Cr. Heavy metal contents of lettuce plant exceeded the recommended food codex values for a healthy vegetable product by sludge applications in the greenhouse experiment. In plant nutrition and soil organic matter amendments, more than regulation limits, potential bioavailability of heavy metals in sludge for their harmfull effects should be considered.

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