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Application of Industrial Treatment Plant Sludge and Heavy Metal Accumulation in Lettuce Plant (*Lactuca sativa*)

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Industrial treatment plant sludge application in agriculture was investigated. The effects of sludge at different loadings (0, 25, 50, 75 and 100 % sludge mixtures) and barnyard manure application as control set in lettuce plant, number of leaves, weight and heavy metal accumulation (Cd, Cr, Ni, Zn, Cu) in lettuce leaves were evaluated. The experiment was conducted using a completely randomized design with four replicates in Tekirdag province of Trakya region, Turkey in 2004. Sludge generated from organized industry region that is mostly composed of textile industries is applied to pots. The least significant difference (LSD) method is used to test the significance of sludge loadings. In general, 0-25-50-75-100 % of sludge treatments resulted in increase the number of leaves and weight in lettuce due to high inorganic nutrient (N and P) content of sludge loadings. In addition, heavy metal concentrations were measured in plant leaves and compared with the acceptable limits given for lettuce plant. The results indicated that application of sludge did not significantly increase nickel, cadmium and chromium contents of lettuce plant while zinc and copper content had significant ($p < 0.05$) effect. Although, copper content was between limits, zinc content was higher than the limit values. The results from pot experiments demonstrated that sludge used in the study can not be used as a partial substitute of chemical fertilizers. Accordingly, application doses of sludge below 25 % (20, 15, 10, 5 % sludge ratio) has been prepared and further experiments should be conducted to evaluate the heavy metal intake rates and accumulation in the plants.

Key Words: Sludge, Heavy metals, Lettuce, Land application.

INTRODUCTION

Sludge, the solid portion which remains after wastewater treatment, must be disposed properly[1-4]. In recent years, the production of sludge has increased sharply in Turkey due to the demand for quality water and the imposition of strict environmental laws. The accumulation of such biowastes poses a growing environmental problem. The disposal of sludge products in landfills or by incineration are feasible options currently practiced in many parts of the world, but both these strategies are expensive and can cause environmental problems[5]. Due to its high nutrient and organic matter contents, sludge has been applied worldwide in increasing amounts to agricultural lands during the past several decades[1,6]. The application of sludge could reduce pollution problem and soil productivity is favoured and the need for synthetic fertilizers is reduced[5,7].

Wheat[8], pepper[9], winter barley[10], iceberg lettuce[4] and cabbage species[11] grown in the process of applying various sludge ratios into soils were positively influenced from the increasing sludge from the point of productivity criteria.

The evaluation of sludge toxicity by chemical and biological testing is therefore very important when deciding on the suitability of sludge for land application, as well as for obtaining its optimum application rate[5]. Due to their persistence and potential toxicity, they may pose a risk to ecosystems[7,12-14]. However, the main problems of an excessive application of sludge are related to the plant toxicity due to the accumulation of heavy metals in soils[15]. Previous studies indicated that excessive amounts of heavy metals (*e.g.* lead, cadmium, zinc, mercury) can accumulate in the plant tissue and human health can be negatively influenced with food chain[11,16-18]. Therefore, use of industrial wastewater treatment plant sludge in agricultural has not been a common practice due to heavy metals and their possible negative environmental and health impacts.

Lettuce plant can accumulate heavy metals in its leaves. Thus, it can have negative effects on food chain. The objectives of the study are to investigate the applicability of organized industrial region wastewater treatment plant sludge in agriculture and to examine the effect of various sludge loadings on the number of leaves, weight and heavy metal concentrations (Ni, Cd, Cr, Cu and Zn) of lettuce plants. Finally, the optimum ratio of sludge is evaluated.

EXPERIMENTAL

The sludge belongs to the largest wastewater treatment plant of the Trakya Region and is accumulated at a rate of 4 to 5 tons per day. The sludge used in this experiment was obtained from Cerkezkoy Organized Industrial Region. The organize industry region composed of mainly textile and metal industries. 85 % Of the wastewater in the treatment plant is fed by the textile industry and the remaining comes from the other sectors located. Disposal of the sludge is an exponentially growing problem from day to day.

The dewatered sludge used in experimental studies obtained from drying beds of the treatment plant. Initially, the heavy metal concentrations of dewatered sludge were determined. The sludge samples were dried in open air ground and sieved through a 2 mm sieve. The part passing the sieve was put into bags and stored for the analysis and the experiements.

The soil samples were taken from 0 to 20 cm depth in Tekirdag Agriculture Faculty experiement field, placed into pots and labelled. They were brought to the laboratory. The soil samples dried in open air ground and sieved through a 2 mm sieve. Sieved soils were stored into glass jars for the analyses.

The soil characteristics determined were: clay loam; $CaCO_3$ content was 8.5 %; total salt was 0.052 %; available P was 1.83 kg da^{-1}; pH, 7.69 and organic matter, 1.27 %. Particle size distribution was determined using the hydrometer method[19]. Soil pH was determined in a 1:2.5 soil water suspension[20]. Organic matter was analyzed calorimetrically using the modified Walkley Black method[21] calcium carbonate was measured with a calcimeter. Available P was determined by the molybdenum blue method in a sodium bicarbonate extract[22].

Extractions were taken for total heavy metal analyses in soil and sludge. For extraction purposes, EPA-600/4-82-055, Method 3050 was employed. Accordingly, 10 g of oven dried (105ºC) soil was weighed. 50 mL 1:1 HNO_3 was added and shaken. Then the mixture was heated at 95ºC for 10 to 15 min without boiling (reflux) and left to cooling. The sample was heated before boiling and vapourized until there remained 25 mL and cooled. The reheating of the sample started adding 10 mL of distilled water and 15 mL of 30 % H_2O_2 (5 mL at each time) until the foams reduced and ended. The cooled sample was filtered and centrifuged. The centrifuged sample was completed to 500 mL.

In the extracted soil and sludge sample, Cd, Cr, Ni, Zn and Cu were determined by UNICAM 929 Atomic Absorption Spectrophotometer with the respective wavelengths: Zn; 213.9 nm, Cu; 324.8 nm, Pb; 217.0 nm, Ni; 232.0 nm, Cd; 228.9 nm, Cr; 357.9 nm.

Results are presented in Tables 1 and 2, separately. Land application limits set by Turkish Regulations (Soil Pollution Control Regulations, SPCR) issued by Ministry of Environment and Forestry are also given in tables. The concentrations of heavy metals in the sludge and soil used for plant growth are lower than those of allowable limits (Tables 2 and 3).

The reason for choosing lettuce (*Lactuca sativa*) as the experimental material is its shorter maturation for harvest and resistance to cold climates and availability for under cover growing. It is consumed uncooked and it can accumulate heavy metals in its leaves.

TABLE-1
COMPARISON OF HEAVY METALS CONTENT OF THE SOIL TESTED IN THE EXPERIMENT WITH THE ANNEX I – LIMIT VALUES OF SOIL CONTAMINANTS REFERRED TO IN THE SOIL POLLUTION CONTROL REGULATIONS

Heavy metals	pH < = 6 ($mg\ kg^{-1}$) Furnace dry soil	pH > 6 ($mg\ kg^{-1}$) Furnace dry soil	Soil sample ($mg\ kg^{-1}$) Furnace dry soil
Pb	50**	300**	32.7
Cd	1**	3**	–
Cr	100**	100**	73.4
Cu*	50**	140**	14.0
Ni*	30**	75**	63.4
Zn*	150**	300**	44.8
Hg	1**	1.5**	–

*If the pH value is greater than 7, the Ministry may increase the limit values to 50 %. **In the areas where fodder plants are grown and where it is proven by scientific studies that the same is not threatening environment and human health, such limit values may be exceeded.

TABLE-2
COMPARISON OF HEAVY METALS CONTENT OF THE SLUDGE WITH THE SOIL POLLUTION CONTROL REGULATIONS

Heavy metal	SPCR[a] ANNEX-II[b]	Sludge sample ($mgkg^{-1}$)
Pb	1200	10.00
Cd	40	1.20
Cr	1200	66.80
Cu	**1750**	**315.20**
Ni	400	29.20
Zn	**4000**	**756.00**
Hg	25	–

[a]SPCR: Soil pollution control regulations; [b]ANNEX-II: The maximum allowable heavy metal contents in the sludge to be used in agriculture (in terms of $mg\ kg^{-1}$ dry sludge).

In the experiment conducted as a completely randomized design with 4 replicates, soil sieved, sand and barnyard manure in 1:1:1 mixture rate was used. These ratios are:

A = 0% S (sludge) + 100% M (manure),
B = 25% S + 75% M
C = 50% S + 50% M
D = 75% S + 25% M
E = 100% S + 0 % M

TABLE-3
INFLUENCE OF THE SLUDGE DOSES ON THE WEIGHTS OF THE LETTUCE PLANTS

Application rate	Range	Mean	LSD 5 %
Weight (g pot^{-1})			
100 % Manure (Control)	95.0-133.3	113.3	
25 % Sludge + 75 % Manure	138.3-173.3	154.1	
50 % Sludge + 50 % Manure	120.0-156.7	132.9	
75 % Sludge + 25 % Manure	128.3-166.7	142.5	
100 % Sludge	100.0-175.0	143.7	
LSD Value			n.s.[a]
Number of leaves (pot^{-1})			
100 % Manure (Control)	20.7-22.7	21.7	c
25 % Sludge + 75 % Manure	22.3-31.0	26.5	a
50 % Sludge + 50 % Manure	23.0-26.7	24.4	abc
75 % Sludge + 25 % Manure	22.3-24.7	23.4	bc
100 % Sludge	20.7-28.3	25.4	ab
LSD Value			3.17

[a]n.s. = Difference between the averages is not statistically significant.

TABLE-4
INFLUENCE OF THE INCREASING SLUDGE DOSES ON THE Zn AND Cu CONTENT OF THE LETTUCE PLANTS

Application rate	Range	Mean	LSD 5 %
Zn (mg kg^{-1})			
100 % Manure (Control)	43.9-53.3	49.4	c
25 % Sludge + 75 % Manure	97.3-113.4	105.7	b
50 % Sludge + 50 % Manure	135.9-152.1	143.9	a
75 % Sludge + 25 % Manure	120.9-165.6	141.6	a
100 % Sludge	125.9-159.5	139.9	a
LSD Value			19.7
Cu (mg kg^{-1})			
100 % Manure (Control)	1.8-3.1	2.4	c
25 % Sludge + 75 % Manure	2.9-4.6	3.9	b
50 % Sludge + 50 % Manure	3.5-6.3	5.4	a
75 % Sludge + 25 % Manure	3.8-5.5	4.7	ab
100 % Sludge	2.0-4.9	3.5	bc
LSD Value			1.43

The seeds were planted into multipods and germinated. Lettuce plants (2-3 leaves) were transplanted into each pot. The harvested plants were brought to the laboratory and the rest of the plants in pots were collected from pots. Fresh weight and number of leaves were recorded and then heavy metal contents were analyzed.

Fresh plant samples were subjected to a series of processes before analysis started. Soils were completely removed from plant parts. To keep chemical and biological changes as small as possible, the plant samples were dried right after the harvest. The plant samples were placed into the drying container in separate paper bags and dried in an oven at 60-70ºC within 24-48 h. The plant samples were ground, bottled and labeled for the further analyses.

The mineral substances in the plant samples can generally be determined after degradation of the organic substances. 0.5 to 2.0 g plant samples were weighed and placed in the porcelain ash container. Sulfuric acid and ethyl alcohol burning method was chosen[23].

In the extracted plant samples, Cd, Cr, Ni, Zn and Cu analyses were determined by UNICAM 929 Atomic Absorption Spectrophotometer.

Statistical analysis was performed by the analysis of variance procedure[24]. MSTAT computer program and LSD (Least Significant Differences) method were employed.

RESULTS AND DISCUSSION

Number of leaves and weight of plants

Table-3 shows the changes in the number of leaves and weight of plants with sludge applications. Sludge application did not significantly increase the lettuce weights in comparison to control set. The highest mean value (154.1 g pot^{-1}) was obtained for the 25 % sludge ratio. For the 50, 75 and 100 % sludge treatments, mean values were almost the same and they were 132.9, 142.5, 143.7 g pot^{-1}, respectively. However, this insignificant result does not mean that the sludge application has negative impacts on plant growth.

Regarding the number of leaves, the highest mean value was obtained for 25 % sludge ratio (26.5 leaves pot^{-1}) that was almost equal to that of the 100 % sludge application (25.40 leaves pot^{-1}). Mean values of 50, 75 and 100 % applications were almost the same, 24.40, 23.40 and 25.40 leaves pot^{-1}, respectively. The smallest leaves numbers was obtained as 21.65 leaves pot^{-1} at control set. LSD value was calculated as 3.17 for the probability level 0.05. The LSD test results show significant statistical interaction between control set and each sludge treatments. Although, for the treatments of 50, 75 and 100 % sludge ratio, mean values were almost the same, when the mean values of 50, 75 and 100 % sludge ratios were compared with that of the control, significant differences were found. Thus, the number of leaves were increased at 50, 75 and 100 % sludge ratio when compared to the control. The numbers of leaves was the maximum (26.50 leaves pot^{-1}) at 25 % sludge ratio.

Heavy metal concentrations

Heavy metal uptake by lettuce plants was determined in the leaves. Metal concentrations were measured for five treatments (control, 25, 50, 75 and 100 % sludge ratio) and results were given in Table-4. Cadmium, nickel and chromium were not measured in the leaves even at high sludge loadings. Zinc and copper were accumulated in leaves with high rates at sludge treatments. The highest mean values were obtained at 50, 75 an 100 % sludge ratio for zinc, as 143.940, 141.627 and 139.84 mg kg^{-1}, respectively. The lowest zinc content was obtained as 49.383 mgkg^{-1} for control treatment. The content of zinc was also increased at 25 % sludge ratio when compared to control set. LSD value was calculated as 19.799 for the probability level 0.05. The LSD test result shows significant statistical interaction between the control and each sludge treatments. Although, for the treatments of 50, 75 and 100 % sludge ratio, mean values were almost the same, when the mean values of 50, 75 and 100 % sludge ratios were compared with that of the control, significant differences were obtained. Thus, the contents of zinc were increased at 50, 75 and 100 % sludge ratios when compared to control. Similarly, the mean value of 25 % sludge ratio was increased when compared to control.

Copper was also accumulated in leaves with the sludge treatments. The highest mean value for copper was obtained at 50 % sludge ratio (5.375 mgkg^{-1}). The lowest copper content was obtained as 2.357 mg kg^{-1} at control set. The copper content was increased at sludge treatments when compared with control set. LSD value was calculated as 1.4387 for the probability level 0.05. The LSD test result shows significant statistical interaction between the control and each sludge treatments. The contents of copper were decreased at 75 % sludge ratio when compared to 50 % sludge ratio. Similarly, the copper content was decreased at 100 % sludge ratio when compared to 75 % sludge ratio. The LSD test shows significant increase copper at sludge treatments.

In Turkey, the application of sludge to vegetable and fruit production has been limited by Ministry of the Environment regulations. After the analysis of sludge and soil, permission must be obtained from the relevant officials[25]. As far as the agricultural potential and the cost of sludge disposal systems in Turkey are concerned, application of sludge in agricultural field is the most appropriate solution[3]. Thus, the industrial wastewater treatment plant sludge used in this study was suitable for agricultural purposes in terms of the investigated heavy metal contents[4].

Sludge treatments increased the weights of lettuce plants, it was not statistically significant. Due to high organic nutrient (N and P) content of sludge loadings, 0-25-50-75 and 100 % of sludge treatments increased the number of leaves and weights of lettuce in comparison to control set[8-11,26].

Since specific standards for lettuce have not been included yet in Codex Standards except cadmium (0.2 mg kg^{-1}), the maximum limits for other metals are adopted from recent literatures. In this study, sludge treatments did not significantly increase the contents of nickel, cadmium and chromium in lettuce plants[4]. This can be explained by the high pH value of sludge and soil and strong absorption of heavy metals through soil.

Lettuce leaves accumulate metals[4]. The results indicated that zinc and copper were increased in leaves at sludge treatments when compared to the control set[8,11,16,27,28]. Zinc and copper are essential micronutrients for plants, however, can cause toxicity in plant when maximum limits exceeded. The maximum limits and edible limit values zinc and copper were taken from the Mills and Jones[29] (20-250 mg kg^{-1} zinc, 5-20 $mgkg^{-1}$ copper) and Bergman[30] (30-80 mg kg^{-1} zinc, 7-15 mg kg^{-1} copper), respectively.

At sludge treatment ratios, zinc and copper contents in lettuces were found lower than the maximum limit value cited in the literature[29]. However, when zinc and copper were compared with the edible limits, zinc was higher and copper was lower than the that of limit values[30].

In order to determine optimum sludge ratios, not only the numbers of leaves and weights, but also zinc and copper contents in lettuce plants were evaluated. All sludge treatments increased the number of leaves and plant weight as compared to control set. Although, copper content in lettuces was lower than maximum limit values, zinc was higher than limit values at all sludge treatments even at 25 % sludge ratios.

In 25 % sludge application ratio (20 ton da^{-1} sludge), positive results were obtained for other parameters except zinc content. Accordingly, The ratios below 25 % (20 ton da^{-1}), *i.e.* (20, 15, 10, 5 % *etc.*) should be prepared and further experiments need to be conducted to evaluate the intake rates of the heavy metals by plants and their level of accumulation in the plants. The toxic effects of the heavy metals to human body through vegetables, fruits or such other agricultural products should also be noted. Whether Cerkezköy Organized Industrial Region waste water treatment plant sludge imposes heavy metal risks or not, it is concluded that it is available for agricultural use.

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