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Application of Sewage Sludge and Its Impact on Soil Characteristics, Including Morphological and Biochemical Properties of *Vigna radiata* Plant

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Abstract: This experiment was conducted in pots to study the effects of sewage sludge application on some morphological, physiological and accumulation characters of *Vigna radiata*. The experiment contained the following treatments: control (C) 0 gm sludge/30 kg soil, (T1) 300 gm sludge/30 kg soil, (T2) 600 gm sludge/30 kg soil and (T3) 900 gm sludge/30 kg soil. All sludge treatments showed a significant increase in all morphological, physiological and accumulation characters compared with control. (T2) treatment gave a significant increase as compared to other treatments in all morphological characters (height of the plant, leaf area, and total dry weight for shoots and roots). It increased by 34.1 cm, 33.1 cm^2, 29.8 gm and 3.3 gm, respectively, compared with the control. T2 treatment also gave significant values in all physiological characters (chlorophyll and protein content) as compared to other treatments and the control treatment, (2.60 µg/gm and 17.7%) respectively compared with the control. T3 treatment showed a higher accumulation of Cd and Pb in all plant parts, the root system showed greater susceptibility to bioaccumulation than the shoot system for both the heavy metals.

Key words: Sewage sludge, heavy metal, *Vigna radiata*.

Introduction

Sludge is a mixture of water and solids separated from various types of water as a result of natural or artificial processes (Eu, 200). Sludge has a lot of organic matter and is a rich source of nutrients, thus generating interest as cheap fertiliser. The value of municipal sewage sludge as a fertiliser was positively evaluated in a number of scientific studies (Evanylo, 1999). Agricultural use of municipal sewage sludge is legally regulated (Vaca et al., 2011) and is mainly based on the evaluation of the heavy metal content and its sanitary properties. Allowable amounts of sewage sludge that can be introduced into the soil according to the law do not constitute a direct threat to the environment. Note, however, that from the ecological point of view, components contained in the sewage sludge over many years can accumulate in the soil or be subject to loss by elution (Fao, 2014). Some plants have the ability to accumulate heavy elements without symptoms of toxicity, some of these elements such as copper, iron, nickel, and zinc are known to play physiological role in plants, while others such as cadmium, lead, cobalt and selenium are not known for their the physiological function (Wei and Liu, 2005). There are many ways to manifest the use of sludge in the asphalt industry, but it is economically expensive; one of the most common ways to add sludge to agricultural soils is that this method is easy to apply and low costs and has positive repercussions on the soil (Vaca et al., 2011). Soil supply

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with organic matter provide the plant with essential nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and others (He et al., 1996), also its effects on the physical and chemical properties of soil, such as the density of the surface and increases the porosity and the high capacity of the ion exchange to increase the amount of soil on water retention (Pinamonti and Zorzi, 2000), as well as increasing crop productivity and reduction of the use of chemical fertilizers and increase the beneficial organisms of the soil (Wei and Liu, 2005), and the equation of the content of organic matter decreases in the soil where the organic matter adsorption characteristic of the major and minor nutrients, making them available in soil around the roots (Vries, 2005). Dry and semi-arid ecosystems with nutrient-rich soils are ideal locations for sludge use because they reduce runoff, reduce soil erosion and improve production (Perez et al., 2005).

Mung bean (*Vigna radiata*) is one of the summer crops that belong to the Fabaceae family, a herbaceous or semi-existing plant as shown in Figure 1 that has a life cycle between 70 and 90 days, which is characterized by drought tolerance. It is cultivated in Iraq in most governorates, 92-88 thousand hectares per year (Kole, 2011).

Vigna radiata is classified according to Liddell and Scott (1980) as follows:

Figure 1: *Vigna radiata* plant (mung bean).

Kingdom: Plantae
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: Vigna Savi
Species: *Vigna radiata* (L.) R.Wilczek – mung bean

This research aims to know the feasibility of using sludge and the rates added to qualify poor soils and increase their fertility as well as their impact on the productivity of plants and to determine its ability to accumulate lead and cadmium from the soil.

Materials and Methods

The mung bean plant (*Vigna radiata*) was chosen for planting in pots, capacity is 30 kg soil per one pot. The experiment contained the following treatments: control (C) 0 gm sludge/30 kg soil, (T1) 300 gm sludge/30 kg soil which is equal to 30 ton per hectare, (T2) 600 gm sludge/30 kg soil (60 t/h) and (T3) 900 gm sludge/30 kg soil (90 t/h).

Chemical and Physical Analysis of Soil and Sludge

Soil and sludge samples were collected from all the treatments and placed in Nylon bags, the samples were dehydrated and tested and passed from a 2 mm diameter sieve and packaged in plastic containers. The chemical and physical properties of soil and sludge were determined by using the standard methods mentioned in the pH and electrical conductivity (E.C.) were measured in saturation extracts according to Demiralay (1991) and Di Martino (2003). Soil organic matter (O.M.) was determined using the Smith-Weldon method as described in by Rhoades (1996), heavy metals were measure according to Nelson and Sommers (1982) as shown in Table 1.

Table 1: The chemical composition of sewage sludge applied

Specification	*Sewage sludge*
pH	7.3
E.C.(dS/m)	3.65
O.M.* (gm/kgm)	364.28
Cd	2.2 (1.8-2.9)
Cu	350.33 (295-411)
Ni	9.22 (12.6-8.2)
Pb	65.55 (58-71)
Zn	1196.33 (1031-1255)

Note: *Organic matter.

Growth Measurements of Plant

At the end of the experiment, plant height, leaf area, plant dry weight, spike length, grains number in spike and total dry weight for shoots and roots were recorded.

Measurement of plant height and leaf area:

The height of the plant (cm) and the leaf area of the *Vigna radiata* leaf (cm^2) were measured using the following equation (Calculation has been performed by using the space and Science leaf method; Jackson, 1958):

Leaf area = leaf length * Maximum paper width *0.905

The plants were harvested and grains were weighed, plants were dehydrated and then placed in the oven at a temperature of 68 °C until proven weight and dry weight record for each treatment.

Chemical Analysis of Plant

Protein

Protein was estimated in wheat plants following the Fulen method (Senedcor, 1958) measuring the optical density at wavelength of 650 nm using the spectrophotometer.

Chlorophyll Content

Chlorophyll content in leaves was determined using the method given by Kemp (1960). Plant leaves were taken for each plant (the second sheet) of each treatment and placed in special bags until transferred to the laboratory, and then directly take 200 mg of each leaf and then crush the wet leaves using a ceramic mortar with 20 mL of acetone (80%) and separating the leachate from the remaining precipitate by centrifugation, adsorption was read at wavelength in the range of 663-645 nm using spectrophotometer. Following ratios were used to calculate the amount of chlorophyll type A&B:

$$\text{Chl.}a = (12.7\ (\text{D}\ 663) - 2.69(\text{D}\ 645)) \times \text{V}/(1000\times\text{W}).$$

$$\text{Chl.}b = (22.9(\text{D}\ 645) - 4.68(\text{D}\ 663)) \times \text{V}/(1000\times\text{W})$$

Accumulation of Heavy Metal in Plant

Cadmium and lead were estimated in mung bean plants (shoot, root system and grains) using the method given by Arnon (1990). The amount of elements was estimated within the plant parts that were digested as follows: The plants were dehydrated and ground using the ceramic mortar weight, 0.5 g of ground plant was put in special flasks for digestion. Then a mixture of perchloric acid $HCIO_3$ and concentrated nitric acid HNO_3 was added to make a certain volume. The samples were left for 24 hours. Then put in a water bath at a temperature of 100 m for an hour.

Obtained data were subjected to analysis of variance, and the means were compared using the least significant differences (LSD) test at the 0.05 level, as recommended by Makinny (1941).

Results and Discussion

Sewage Sludge on Electrical Conductivity, Reaction Degree and Content Soil of Organic Matter after Harvest

The addition of sludge resulted in a significant decrease in the soil reaction rate (pH). This may be due to the degradation of the organic activity of the substance by microorganisms and the production of acids and thus reduce the degree of soil interaction. It is clear from Table 2 that the addition of sewage waste resulted in a significant increase in the values of electrical conductivity at the high level of addition and may be due to the containment of these sludge on salt compared with control treatment (C).

The proportion of organic matter in the soil after the harvest increased with the level of addition of sludge and reached this increase to 22 gm/kg in T3.

In treatment 3, the increase in the level of sewage led to the increase in electrical conductivity, thereby reducing the activity of the organic microorganisms. This effect remains in the soil for a longer period of time. These results are consistent with what was found by Alkhafji et al. (2019).

Table 2: The chemical analysis of soil

	pH	*E.C.dS/m*	*O.M. (gm/kgm)*
C	7.6	4.5	11
T1	7.5	4.5	12
T2	7.5	4.7	18
T3	7.4	4.9	22
LSD 0.05	0.05	0.14	0.21

Effect of Sludge on Morphological and Chemical Characters of *Vigna radiata* Plant

The addition of sludge increased shoot, root and total length. This increase was 15.5% in T2 treatment. The differences between T2 and T3 treatments were significant (Tables 3 and 4), and in agreement with PiJuam (2010) and Alkhafaji and ELkheralla (2019).

The dry weight, protein content and amount of chlorophyll in the plant were also increased (16.9%, 9%, 22% and 17.7%) when treated with sludge in T2 as shown in Tables 5-7. The increase in wheat production and the improvement of growth indicators in the addition of sludge may be due to improved physical and chemical properties of soil, increasing nutrient readiness and thus increasing absorption and growth. Waste mineralisation produces a lot of waste

Table 3: Effect of sludge on length in *Vigna radiata*

	Treatment	*Shoot length (cm)*	*Root length (cm)*	*Total length (cm)*	*Increasing (%)*
Control	0	20.4a	9.1a	29.5	0%
Sludge	T1	20.5	9.0	29.5	0%
	T2	24.2	9.9	34.1	15.5%
	T3	20.2	8.9	29.1	-1.3%
LSD		0.799	0.221	0.964	

Table 4: Effect of sludge on leaf area of *Vigna radiata*

	Treatment	*Length of leaf (cm)*	*Width of leaf (cm)*	*Leaf area (cm^2)*	*Increasing (%)*
Sludge	T1	7.1	7.2	24.7	99.1%
	T2	7.1	7.1	33.1	166.9%
	T3	7.4	7.5	23.9	92.7%
LSD		0.139	0.128	3.586	

Table 5: Effect of sludge on dry weight of *Vigna radiata*

	Treatment	*Shoot dry weight (gm)*	*Root dry weight (gm)*	*Total dry weight (gm)*	*Increasing (%)*
Control	0	9.9	2.5	12.4	**0%**
Sludge	T1	22.1	2.6	24.7	**99.1%**
	T2	29.8	3.3	33.1	**166.9%**
	T3	21.4	2.5	23.9	**92.7%**
LSD		5.210	0.341	5.517	

Table 6: Effect of sludge on chlorophyll content in *Vigna radiata*

Fertiliser	*Treatment*	*Chl.a (mg/g)*	*Chl.b (mg/g)*	*Total chl. (mg/g)*	*Increasing (%)*
Control	0	1.47	0.66	2.13	**0%**
Sludge	T1	1.74	0.86	2.60	**22.0%**
	T2	1.86	0.74	2.60	**22.0%**
	T3	1.49	0.53	2.02	**-5.1%**
LSD		0.288	0.226	0.461	

Table 7: Effect of sludge on protein content in *Vigna radiata*

Fertiliser	*Treatment*	*Shoots (%)*	*Roots (%)*	*Grains (%)*	*Total protein*
Control	0	11.3	17.4	20.3	**12.3%**
Sludge	T1	12.3	19.2	21.7	**17.7%**
	T2	12.4	17.9	22.1	**17.5%**
	T3	11.6	17.8	21.3	**16.9%**
LSD		2.455	1.896	1.374	

nutrients essential for plant growth. Fertilisation due to the presence of metals in the soil has also played a significant role in increasing production, and supply plant with the elements necessary for its growth due to activity of nitrogen, phosphorus and potassium.

This is consistent with many studies confirming that the use of sludge increases the production of crops, especially under drought conditions where water and soil lack organic matter (Ali, 2008; He, 2000; Hussein, 2009).

Effect of Sludge on Cd and Pb Concentrations in *Vigna radiata* Plant

On adding 900 gm of sludge to 30 kg soil, the Pb concentration increases from 4.62 µg/gm in the control treatment to 5.20 µg/gm in T3 treatment, as shown in Table 8. This increase may be due to increase in the lead content in the waste, thus increasing the absorption. The greatest absorption of lead in found in plants of T3 treatment.

Table 8: Accumulation of lead in *Vigna radiata* (µg/gm)

	C	*T1*	*T2*	*T3*
Root system	4.62a	4.26b	5.02c	5.20ac
Shoot system	2.71b	2.43c	3.02a	3.28a
Grain	1.94c	1.86a	2.99b	2.74ab

Note. Values are presented as mean±SD of three replicates.

Corresponding letters in one column indicate that differences are not significant LSD according to the test. The accumulation of cadmium increased significantly in all treatments relative to the comparison treatment©. The highest absorption was when the treatment T3 reached a value of 1.15 µg/gm in the root part of the plant, shown in Table 9.

Table 9: Accumulation of cadmium in *Vigna radiata* (µg/gm)

	C	*T1*	*T2*	*T3*
Root system	0.95a	1.01a	1.13ab	1.15b
Shoot system	0.34b	0.40c	0.41c	0.49a
Grain	0.89c	0.86b	0.91a	0.98c

Note: Values are presented as mean±SD of three replicates.

Corresponding letters in each column indicate that the LSD differences are non-significant. The root system in plants shows a higher accumulation of cadmium and lead. So the plant *Vigna radiata* is an accumulator of heavy elements (McGrath, 2002). All concentrations were within permissible limits.

This corresponds to a study on element concentration cadmium in the parts of the radish plant, a group of cabbage, where it was found that the highest concentration was in the vegetative and not in the root while in tomato plant was the opposite of the same study (Eid, 2002).

Conclusions

1. The best productivity of the *Vigna radiata* plant was in the T2 (600g sludge/30kg soil).
2. According to this study, the *Vigna radiata* plant is accumulated for lead and cadmium.
3. The highest concentrations of the lead and cadmium elements were in the root part of the plant while their lowest concentrations were in grains.

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