Effect of Sewage Sludge on Heavy Metals Accumulation in Soil and Wheat, Mung Bean and Quinoa Crops

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Abstract. This study was conducted to study the effects of sewage sludge application to agricultural soil on the heavy metal accumulation in soil and plant and on availability of certain nutrients for plant together with the productivity of wheat, mung bean, and quinoa. The experiment were included four treatments i.e., (Control; inorganic fertilizer according to Ministry of Agriculture-Iraq.; sludge (the amount of sludge containing MoAR recommendation) of N without any addition of mineral N; and twice the amount of sludge without any addition of mineral N) with four replications in the feed crop studies station of the Ministry of Agriculture - Haditha agriculture division located in the village of Sakran (Hasweh Al-Jazeera) of Haditha district 260 km west of Baghdad during the seasons 2017-2018.2019. The applied sludge was described and soil was analyzed prior to cultivation. Upon harvesting, the heavy metals (Cd, Pb, Ni and Cr) were estimated in soil and plant in addition to certain nutrients (Total N, mineral N, available P, macronutrients) and organic matter. About heavy metal in soil and plant, significant buildup of the heavy metal in soil and plant was noticed by increasing the addition of sludge as compared to the control. Significant increase in organic matter and total N of soil in sludge-fertilized treatment was noticed as compared to the control. Moreover, a significant increase in mineral N, available P, Cu, Mn and Zn was also noticed by increasing the addition of sludge. There was significant increasing in plant productivity for various crops during the seasons by increasing the addition of sludge as compared to the control.

1. Introduction:

The daily quantities produced from sewage waste, which is the final product deposited from the treatment of sewage waste, are estimated in thousands of cubic meters, which are only used in a limited way in the field of land reclamation. Note that its quantities are constantly increasing worldwide due to the increase in population and the expansion of sewage treatment plants. The application of organic waste or compost on soils used for crop production is of great importance due to the nutritional input and low cost[1]. Organic waste, such as sewage sludge and sewage sludge compost, can improve the availability of nutrients thanks to the low molecular weight aliphatic compounds that interact strongly with the soil minerals [2]; moreover, it increases the soil's cation exchange capacity (CEC)[3]. The factors that affect the bio-availability of elements in soil are waste source, pH, organic matter content and chemistry of the elements[1]. Contamination of agricultural soils and crops by heavy metals have considered one of a serious environmental problem due to their non-biodegradable nature and long biological half-life and also, their potential accumulation in different body parts [4]. Some heavy metals such as zinc (Zn), cadmium (Cd), lead (Pb) and copper (Cu) are mostly found as contaminants in vegetables[5]. Many researchers note a high and increasing need for soil fertilization due to a significant anthropogenic load [6];[7]. Analysis of these literature sources has shown that in a number of countries, including Russia, there is a positive experience of using SS as a fertilizer in agriculture[8];[9]. On this basis, we made an assumption about the possibility of using the SS of treatment facilities as an organ mineral fertilizer on gray forest soil in the cultivation of carrots. To confirm this hypothesis, a number of studies were conducted, the results of which are presented below. Analysis of
numerous experiments has shown that the use of fertilizers in science-based technological modes does not pose a danger to nature, but violation of the norms and technological requirements for the use of fertilizers leads to unfavorable environmental consequences. One of these consequences is the abnormal use of sewage sludge a source of heavy metals entering the soil, ground water, and plants. Therefore, the use of sewage sludge is environmentally safe with systematic monitoring and comprehensive assessment of possible negative consequences[10]. Cadmium is one of the most dangerous of these elements to humans when it enters the human food chain [11]. In addition to the danger of heavy metals in waste, there is another danger which is that this substance contains a large amount of nitrates, which in turn are washed into Ground water pose significant risks to human health[12]. Since there are few studies on residues in Iraq, we have found it necessary and useful to study the effect of this substance on crop productivity and the accumulation of heavy elements in soil and plants.

2. Materials and Methods

2.1. Soil description

A composite sample was taken to specify the following nutrient characteristics of soil: available P was determined according to Olsen method [13]. Total N was estimated by Kjeldahl's method [14]. Organic matter was estimated by wet oxidation [2], and Available K was extracted by ammonium acetates at ratio 1:5 (concentration 1 mole) by using flame photometer.

2.2. Sludge characteristics

Table 1 presents some characteristics for the average of three replicates used in the experiment, where sludge is mostly municipal from Baghdad city; it received secondary treatment and air dried under the sun for 3 months. Sample acidity was measured by pH meter of water/sludge suspension at 1:5 ratios. Electrical conductivity was also estimated in the same mixture using an EC meter. The samples were digested through wet method [15]. Total N and total P were estimated using auto analyzer (Skalar). Total K estimated using a Flame Photometer. Inorganic N estimated using KCl of the extract (1:10) and amounts were assessed using Auto analyzer Skalar,[16]( Isaac R. ,and J. D. Kerber, 1971). Sludge was digested by HClO4 for micronutrients determination and digested with Aqua regia to determine heavy metal by using Atomic Absorption Spectrophotometer (Varian model) (Ministry of Science and Technology Labs.). The sludge was bacterially analyzed (wet sample and dry sample) and total E. Coli and Salmonella bacteria were counted by proper gradual dilution and spread in plates Kucey (1983) containing dispersion agent (SS agar, EMB, nutrient agar). The plates were incubated at ± 28 Cú for three days. Table 1 shows some characteristics of the study soil.

| Organic matter | Total N | Total P | Available P | Available K |
|---------------|---------|---------|-------------|-------------|
| %            | mg Kg^-1 | %       | mg Kg^-1   | %          |
| 0.9          | 0.05    | 7.46    | 4.45       | 390         |

The prevailing soil was a silt loam texture with 428 g Kg^-1 sand, 528 gKg^-1 silt and 44 g Kg^-1 clay. The soil was classified as typic turifluvents. Soil bulk density 1.31 Mg m^-3, with a porosity of 55%, the available water within the limits of 0.14, the soil pH=8.7, it is considered from the non-saline soils as the average electrical conductivity of the saturated soil paste extraction = 0.6 dSm^-1, and the content of both available phosphorous and total nitrogen is low and poor in organic matter (0.5 mg kg^-1).

Table 2. Total content of trace elements and heavy metals in the applied sludge

| Sludge source | Zn  | Mo | Mn | Fe  | Cu  | B   | Pb  | Ni  | Cr  | Cd  | Moisture |
|---------------|-----|----|----|-----|-----|-----|-----|-----|-----|-----|----------|
| Moisture      | mg Kg^-1 | %       |     |     |     |     |     |     |     |     |          |
| Rustumiya Plant | 1015 | 28 | 155 | 2350 | 238 | 110 | 72.0 | 77.4 | 850 | 2.25 | 6.5      |
| Allowable     | 3000 | 30 | -   | -   | 1000 | -   | 800 | 200 | 1000 | 20  |          |
limits

Arab Standards and Metrology Authority[17]

Through the previous table, it is noticed that the concentrations of heavy metals are within the permissible limits.
The sludge were analyzed bacteriologically (wet sample and dry sample), where a total number of bacteria were counted, and E. coli and salmonella bacteria were counted also by performing dilutions to the appropriate dilution by means of gradual dilution and spreading in dishes containing differentiated environments (nutrient agar, EMB, SS). Agar plates were incubated at 28 ± 2 °C for 3 days (Table 3).
The layout of the experiment was Complete Randomized Block Design (CRBD) with four replications. The experiment contained four treatments as follow:
1. Control (no fertilizers and sewage sludge added)
2. Mineral fertilized addition (as Iraqi MoAR's) recommendations.
3. Sewage sludge addition (as crop N requirements and N content of sludge in conformity with Iraqi standard, Ministry of Ecology)
4. Sewage sludge (twice crop N requirements).Means was compared by using LSD0.05.

2.3. Cultivation
The seeds of the wheat crop Ibaa 99 for the winter season 2018-2019 were sown on 3/12/2018, then the quinoa crop was planted on 1/2/2018, and the mung bean was planted on 12/23/2018 at the fodder crop research station in the Sakran region – Haditha District, the plots dimensions were (2 x 2m) and the irrigation method used was sprinkler irrigation, the plants were watered according to crop evapotranspiration (ETc) depend on evaporation from pan class-A;
Sewage sludge was applied before planting as mixing with upper 15 cm at average of 15 tons ha⁻¹ for the treatment of waste T2 and its twice for the fourth treatment T4 (30 tons ha⁻¹), after analyzing both soil and sewage sludge from the Rustumiya waste water treatment plant. Mineral N and P were added just to treatment as per MoAR's recommendations (195 Kg urea ha⁻¹(46%) and 195 Kg P2O5 ha⁻¹ for wheat, 180 Kg urea ha⁻¹ and 75 Kg superphosphate ha⁻¹ Quinoa [18], 43 Kg urea ha⁻¹ and 120 Kg ha⁻¹ superphosphate for mung bean using the equation (2).
When estimating the amount of sewage sludge to be added to the soil, it is necessary to know and determine the amount of organic nitrogen in the sludge, which can be calculated according to the equation 1:

\[
\text{Applied N(ton.ha}^{-1}\) = \frac{\text{Available N in sewage sludge(Kg ton}^{-1}\))}{\text{N plant needs (Kg ton}^{-1}\) – N content in soil(ton.ha}^{-1}\)} \ldots \ldots (1)
\]

The amount of mineral nitrogen released and released from the remaining organic nitrogen in the soil during the years preceding must be calculated, so that this amount is added to the amount of available nitrogen for plants with sewage sludge, and that is from the data of Table (3).
To estimate sludge amount it is essential to know and assess organic N amount in sludge computed according the Equation 2.

\[
\text{ON} = \text{T.N} – \text{IN} \ldots (2)
\]

Where:
ON = organic nitrogen
T.N = Total nitrogen
IN = Mineral nitrogen (NO₃⁻, NH₄⁺)
Shows that the numbers and types of germs in Rustumiya sludge are very low and are within the permissible limits.

Table 3. The amount of mineral nitrogen released and released from the remaining organic nitrogen in the soil.

| Years of adding residues to the soil | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 |
|-------------------------------------|-----|-----|-----|-----|-----|-----|-----|
|                                     |     |     |     |     |     |     |     |
| 1                                   | 1.10| 0.60| 0.64| 0.77| 0.86| 1.00| 0.45|
| 2                                   | 1.10| 0.35| 0.64| 0.73| 0.82| 0.95| 0.41|
| 3                                   | 1.00| 0.30| 0.60| 0.68| 0.77| 0.91| 0.41|

Table 4. Some chemical and physical properties of used sewage sludge.

| Moisture % | Mineral N mgKg⁻¹ | P K₂O % | T.N % | O.M (1:5) | EC dSm⁻¹ | pH | Bulk density Megm⁻¹ |
|------------|------------------|--------|-------|-----------|---------|----|---------------------|
| 6.5        | 8.49             | 132    | 1.35  | 3.70      | 40.5    | 3.38 | 0.37                | 0.86 |

Table 5 presents some characteristics for the average of three replicates used in the experiment, where sludge is mostly municipal from Baghdad city; it received secondary treatment and air dried under the sun for 3 months. Sample acidity was measured by pH meter of water/sludge suspension at 1:5 ratios. Electronic conductivity was also estimated in the same mixture using EC meter. The samples were digested through wet method and total N and total P, total K was also estimated according to standard method[19].

3. Results and Discussion

3.1. Wheat production

Table 5 shows the yield of grains wheat yield, the results showed there was a significant difference at the level of \( p \leq 0.05 \), it was noticed from the same table also, the waste fertilization treatment was superior over the control. T4 treatment was superior over T1, T2, and T3 by 47.7, 33, and 30.2%, respectively during 2017-2018. The same trend in productivity is observed within a year of 2018-2019, but at a higher rate where T4 treatment which was superior over the treatments, T1, T2, and T3 by 57, 27, and 0.06%, respectively, the same table also shown there was no significant difference between T4 and T3. Sludge is rich in N and organic matter, providing more nutrients to plants and promoting plant growth. This agrees with [20] showed that clarifies the role of sludge in increasing available P. Sludge addition may noticeably rise soil content from N and available P. Recently the application of sewage sludge to agriculture soils could be considered as an alternative to urea fertilization due to their high contents in organic matter and essential nutrients such[21].

Table 5. Average of wheat yield (tons ha⁻¹)

| Treatments | 2017-2018 | 2018-2019 |
|------------|-----------|-----------|
| T1         | 2.475     | 2.69      |
| T2         | 3.150     | 4.50      |
| T3         | 3.300     | 5.82      |
| T4         | 4.725     | 6.20      |
| Mean       | 3.124     | 4.803     |
| LSD5%      | 0.376     | 0.912     |

It was observed that the productivity decreased during the year 2017-2018 compared to the year 2018-2019. The reason for lower productivity between the treatments is attributed to various factors such as: The most important of these are the significant drop in temperatures,
the occurrence of freezing, the decrease in precipitation rates, and the sudden and significant rise in temperatures during the different ripening stages.

Table 6 shows the productivity of the mung bean plant from dry weight matter (straw) and grains yield. The results showed that there was an increase in productivity of mung bean grain, when moving from mineral to sewage sludge fertilization, and then twice of the sewage sludge fertilization compared to control treatment. A significant difference was found, at \( p \leq 5\% \), between the mean yield of treatment T4, which is twice the sewage sludge fertilization and the control treatment, the productivity of treatment twice sewage sludge fertilization, was superior by 5.01, 9.82 for straw and 4.83, 2.86 (tons ha\(^{-1}\)) for grain during 2017-2018 and 2018-2019 respectively. This corresponds to a study of [22] who indicated that adding sludge at moderate rates has a positive effect on plant ion uptake and phosphorus availability in the soil.

Table 6. Mung bean mean yield of productivity (ton ha\(^{-1}\))

| Treatments | 2017-2018 | 2018-2019 |
|------------|-----------|-----------|
|            | Straw     | Grain     | Straw     | Grain     |
| T1         | 3.433     | 2.167     | 2.85      | 2.81      |
| T2         | 6.425     | 4.967     | 11.47     | 4.54      |
| T3         | 6.867     | 4.358     | 11.9      | 4.86      |
| T4         | 8.442     | 7.000     | 12.67     | 5.67      |
| Mean       | 6.29      | 4.62      | 9.72      | 4.47      |
| LSD5%      | 1.242     | 0.673     | 0.190     | 0.645     |

Table 7 shows the productivity of the quinoa plant from the shoots and grains, as the results of the statistical analysis showed that there was a significant difference at the level of 5% between the average productivity of each of the treatments T2, T3 and T4 compared with the comparison treatment, while the differences between the rest of the treatments were apparent differences, whether for seeds or shoots. Where the fourth treatment (twice the sewage sludge) superior over its productivity of seeds and shoots over the rest of the treatments. This is may be due to the availability of phytonutrients from nitrogen and phosphorous elements in the sludge in a way available to the plant at a concentration was used in this experiment, as well the improving of soil aggregation resulted from role of the microorganisms mycelium played as a cemented agent materials binding the soil particles resulted the forming of good aggregation which increase soil aeration and gas exchange easily.

Table 7. Results of statistical analysis of mean quinoa productivity

| Treatments | 2017-2018 | 2018-2019 |
|------------|-----------|-----------|
|            | Seeds     | Total vegetative | Seeds | Total vegetative |
| T1         | 4.46      | 9.19       | 3.9   | 16.48             |
| T2         | 5.43      | 10.71      | 4.62  | 18.18             |
| T3         | 5.46      | 12.57      | 6.19  | 21.79             |
| T4         | 6.43      | 15.13      | 6.13  | 20.58             |
| Mean       | 5.45      | 11.89      | 5.21  | 19.21             |
| LSD5%      | 1.152     | 5.062      | 1.98  | 6.65              |

3.2. Effect of sewage sludge on the accumulation of some heavy metals in the plant:

Tables [9];[10]; [11]; [12];[13] show the concentration of heavy metals in the vegetative and straw parts of wheat, mung bean and quinoa plants.

Table 8. Cadmium, Cr, Ni, and Pb concentration of wheat, ibaa99 (mgKg\(^{-1}\))

| Treatments | Vegetative part | Pb | Ni | Cr | Cd |
|------------|----------------|----|----|----|----|
|            | Fresh | 2.69 | 1.26 | 1.07 | 0.26 |
| T2         | Fresh | 2.44 | 0.12 | 0.68 | 0.10 |
|            | Fresh | 2.81 | 1.28 | 1.28 | 0.29 |
| T3         | Fresh | 2.56 | 0.2  | 0.86 | 0.18 |
|            | Fresh | 2.86 | 1.29 | 1.45 | 0.34 |
| T3         | Straw | 2.76 | 0.25 | 0.88 | 0.13 |
The results of the statistical analysis showed an increase in the concentration of heavy metals studied in the vegetable tissues of grain and straw for the fertilizer treatments, whether with metallic fertilizer or sewage sludge, compared to the control treatment, and the concentration of those heavy metals in straw was higher than in grains except for cadmium, which was higher in grain than in straw. However, the concentration of all mineral elements was within the limits of the natural content for their concentration in plant tissues according to national and international standards[14]. The results of the statistical analysis showed:

There was no significant difference between the mean cadmium concentration in all treatments, including the control, whether in straw or grains. It has shown there was an apparent increase in the grain content of chromium in the treatment when the metallic fertilization was used. Also, noticed there was a significant increase in the mean grain concentration of chromium in the two treatments T3 and T4 compared to the control treatment.

There was a significant difference at the level of 5% between the average concentration of nickel in straw for the two treatments T3 and T4, compared to comparison, while the differences between the remaining treatments, whether in straw or grains, were apparent, and the nickel concentration in straw and grain was superior in the treatment of twice application of sewage sludge treatment over the rest of the treatments, including the control treatment.

There was a significant difference at the level of 5% between the average concentration of lead in the straws of the two treatments T3 and T4 sewage sludge and the twice application compared to the treatment of mineral fertilization T2 and comparison. The lead concentration in grains also increased with the increase in the addition of sewage sludge.

| Treatments | Vegetative part | Pb  | Ni  | Cr  | Cd  |
|------------|----------------|-----|-----|-----|-----|
| Fresh      | 3.20           | 1.55| 2.24| 0.50|
| Straw      | 2.85           | 0.33| 1.30| 0.22|
| Fresh      | 0.822          | 0.244| 0.993| 0.164|
| Straw      | 1.077          | 0.117| 0.792| 0.090|

**Table 9. Cadmium, Cr, Ni, and Pb concentration of Mung bean (mgKg⁻¹)**

| Treatments | Vegetative part | Pb  | Ni  | Cr  | Cd  |
|------------|----------------|-----|-----|-----|-----|
| Fresh      | 2.72           | 12.7| 1.37| 0.24|
| Straw      | 2.35           | 1.875| 0.87| 0.06|
| Fresh      | 2.11           | 15  | 1.6 | 0.18|
| Straw      | 2.3            | 2.525| 1.18| 0.032|
| Fresh      | 2.46           | 11.4| 1.35| 0.18|
| Straw      | 2.45           | 2.075| 0.95| 0.042|
| Fresh      | 2.41           | 13.2| 2.79| 0.2 |
| Straw      | 2.42           | 2.125| 0.77| 0.055|
| Fresh      | 0.75           | 2.24| 1.96| 0.07|
| Straw      | 0.52           | 0.78| 0.58| 0.05|

**Table 10. Cadmium, Cr, Ni, and Pb concentration of Quinoa (mgKg⁻¹)**
Table 11. Results of the statistical analysis of the average concentration of heavy elements in the mung bean shoot.

| Treatments | Vegetative part | Pb  | Ni  | Cr  | Cd  |
|------------|-----------------|-----|-----|-----|-----|
|            | Fresh           | 0.115 | 1.58 | 3.15 | 4.93 |
| T1         | Straw           | 0.14  | 0.28 | 0.62 | 1.29 |
| T2         | Fresh           | 0.113 | 1.80 | 5.56 | 5.36 |
| T2         | Straw           | 0.103 | 0.35 | 1.16 | 1.53 |
| T3         | Fresh           | 0.128 | 1.71 | 3.30 | 6.16 |
| T3         | Straw           | 0.115 | 0.53 | 1.27 | 1.41 |
| T4         | Fresh           | 0.123 | 1.93 | 3.55 | 6.33 |
| T4         | Straw           | 0.128 | 0.80 | 1.31 | 1.43 |
| LSD5%      | Fresh           | 0.029 | 0.529 | 2.656 | 2.058 |
| LSD5%      | Straw           | 0.02  | 0.374 | 0.497 | 0.279 |

Table 12. Results of the statistical analysis of the average concentration of heavy elements in the wheat (ibaa99) shoot.

| Treatments | Vegetative part | Pb  | Ni  | Cr  | Cd  |
|------------|-----------------|-----|-----|-----|-----|
|            | Fresh           | 3.48 | 5.22 | 1.33 | 0.04 |
| T1         | Straw           | 0.447 | 1.48 | 1.68 | 0.043 |
| T2         | Fresh           | 3.04 | 5.58 | 0.685 | 0.02 |
| T2         | Straw           | 0.671 | 1.34 | 5.5 | 0.025 |
| T3         | Fresh           | 0.94 | 4.4 | 1.55 | 0.55 |
| T3         | Straw           | 0.61 | 1.67 | 1.2 | 0.55 |
| T4         | Fresh           | 3.06 | 5.65 | 2.78 | 0.032 |
| T4         | Straw           | 0.398 | 1.79 | 1.71 | 0.033 |
| LSD5%      | Fresh           | 3.01 | 2.47 | 2.83 | 0.016 |
| LSD5%      | Straw           | 0.43 | 0.45 | 0.0125 | 0.05 |

Table 12 shows the concentration of heavy elements in the mung bean: The following data are noted through the results:

There was a significant difference at the level of 5% between the average cadmium concentration in a treatment of twice application of the sewage T4 (0.6) mg kg⁻¹ with the rest of the treatments, including control treatment.

There was a significant difference at the level of 5% between the mean concentration of chromium in treatment T4 (0.63) mg kg⁻¹ with the rest of the treatments, including comparison.

Nickel and lead increased with increasing the addition of sewage sludge, and the observed differences were apparent.

In general, the concentrations of those heavy metals were increasing in the plant tissues of the mung bean crop, with the addition of mineral fertilizers and sludge's and twice application sewage sludge, but their concentration remained within the limits of the natural content of their concentration in plant tissues, according to national and international standards.

Ensure that the sewage sludge is not added to soils grown with edible crops.

Ensure that the sewage sludge to be used in agriculture is dry air (humidity is not more than 6%) and has been produced for a period of not less than 6 months.

Because the sewage sludge is poor in nitrogen content, it is preferable to fertilize the soil with nitrogen fertilizers with the addition of sewage sludge.

Chu and Wong [23] showed that there is accumulation of cadmium in soil and plants with increased addition of residues, as Rabi [24] showed that there was a significant increase in the element of chromium in the roots of the plant quinoa with increased addition of residues, and also reported a significant increase of the element nickel. In the quinoa plant, with increased
addition of residues. Schiptsova [25] found that in the carrot tops, the zinc concentration ranged from 9.75-14.45 mgKg⁻¹; these indicators in comparison with the control are 1.50-2.22 times higher. The copper content was in the range of 1.54-2.48 mg/kg, which is 1.03-1.66 times higher than the control. The lead concentration in the carrot tops varied within 0.153-0.245 mgKg⁻¹, which exceeded the control indices by 1.56-2.5 times.

The cadmium content in the tops was 0.01-0.017 mgKg⁻¹, which was 2.43 times higher than the control. The lower content of heavy metals in root crops and the higher content in leaves is explained by the fact that organic ligands increase the level of bioavailability, that is, they translate them into a more mobile form. Thus, in plant organisms, heavy metals (trace elements) are mainly involved in redox reactions occurring in mitochondria and chloroplasts, heavy metals (trace elements) from root crops mainly migrate to the leaves.

4. Conclusions

The addition of sewage contributed to increasing the availability of some elements such as nitrogen and phosphorous, and in increasing the soil's organic matter content, which reflected positively on the productivity of the cultivated crops.

The use of sewage sludge led to a convergence in production with the treatment of mineral fertilizers, which means that the addition of chemical fertilizers can be dispensed with.

The accumulation of heavy metals in the soil increased with the addition of sewage sludge compared to the control treatment.

Cadmium build-up occurred in the soil as it was transported to the plant tissue.

The accumulation of heavy metals in the soil was within the limits of the natural content of their concentration in the soil.

It was found that the concentration heavy metals in the plant tissues of the cultivated crops, within the permissible limits and far from the toxicity limits.

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