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Effects of Different Sewage Sludge Concentrations on the Soil Microorganism Sensibility and Metals Accumulation during *Helianthus annuus* L. Cultivation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GMBB and TLO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GMBB and EBB managed the analyses of the study. Authors EMB and PJS performed the statistical analysis. Authors GMBB and EBB managed the literature searches, analyses of the study performed and discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

The agricultural use of sewage sludge have been presented as an alternative both to reduce the volume of this environmental liability as to obtain a product to be used in agricultural soils as fertilizer. However, when the household sewage is also composed of water from the industrial area, the sludge is richer in potentially toxic elements. Hence, the present study aimed to evaluating the effects of different concentrations of sewage sludge on soil microbial activity and contents of Cd, Cu, Cr, Pb and Zn in soil, plant and seed. The experiment was conducted at the greenhouse during

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the crop season of 2012 to 2013, with evaluations of soil total organic carbon, microbial biomass carbon, metabolic quotient and heavy metals in soil, plant and seed. According to the results, application of 20 Mg ha^{-1} of sewage sludge promoted higher microbial activity, lower metabolic quotient and in increased production of dry plant matter. Resulted in 480.77 µg g^{-1} of microbial carbon and 9.75% of soil organic carbon. Heavy metal contents in seeds were 1.29, 14.26, 1.52 and 80.57 mg kg^{-1} of Cd, Cu, Pb and Zn, respectively, with the lead content above the maximum extent recommended in food by WHO (Word Health Organization).

Keywords: Sunflower; microbial activity; waste; residue.

1. INTRODUCTION

The sunflower (*Helianthus annuus L.*) is yearly crop native of North America [1]. It is one of the five oilseed crop producing edible vegetable oil in the world, It is also used as a source of protein for animal feed, grain pastry, honey production, crop rotation and cycling nutritional and production of biodiesel [2]. The area cultivated with sunflower in Brazil during the season 2012/2013, was approximately 2.37 millions of hectares [3]. With increased sunflower production also comes the increased land degradation by use of pesticides. One way to reduce this impact is the use of waste as a nutrient for plants in this context arises the potential for applying tons of solid waste generated by the treatment of industrial and domestic sewage in the world [4]. Sewage sludge has considerable percentage of organic matter and essential elements for plants and can replace, even partially, mineral fertilizers, playing an important role in agricultural production and the maintenance of soil fertility [4]. The sewage treatment is able to reduce river pollution and improve public health. However, several sewage treatment projects do not include the final destination of sludge produced and thus cancel in part the benefits of collection and treatment of wastewater [5].

Although the agricultural use of sewage sludge presents himself as one of the most attractive alternatives for final disposal of this waste, potentially toxic elements can limit your application, due to the possibility of soil contamination of aquatic systems and the atmosphere, increasing the risk of their transfer to the food chain as highlighted by [6]. The sludge from sewage treatment plants when destined for domestic sewage treatment, it is framed as waste Class II A or even B according to the Brazilian Association of Technical Standards NBR 10004 [7], allowing its use with increased safety in agriculture. This is possible since the waste is evaluated for agronomic value, and the usage limits are respected, determining the potential impacts of their application on soil properties, to meet technical and safety criteria for human and environmental health established for agricultural use of such material [8].

Interest in the production of organic compounds from these materials have been presented as an alternative both to reduce the volume of this environmental liability as to obtain a product to be used in agricultural soils [9]. However, when the household sewage are also composed of water from the industrial area, the sludge is richer in potentially toxic elements (PTE), including metals: cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb) and zinc (Zn),reference prevention values are respectively 1.3, 60, 75, 72, 300 mg kg^{-1}, according to the Brazilian National Environmental Council (CONAMA) Resolution No. 420 of 2009 [10]. Repeated application of sewage sludge on agricultural soils can raise the levels of organic and inorganic compounds in the soil, requiring periodic evaluations in order to avoid environmental contamination [11].

In the face of these problems, this work was developed with the objective of evaluating the effect of different concentrations of sewage sludge on soil microbial activity and Cd, Cu, Cr, Pb and Zn contents in soil, plant and seeds. The hypothesis of this study is that the application of sewage sludge on agricultural soils enhances soil microbial activity and the concentration of heavy metals in soil and food.

2. MATERIALS AND METHODS

The experiment was conducted at the greenhouse of the experimental area of the Federal Institute of Rio Grande do Sul in the crop season of 2012 to 2013. The soil is classified as dystrophic Red-Yellow Argisol (U.S. soil taxonomy). Soil and sludge chemical and physical properties were obtained according to [12] before the experiment soil properties in the following Table 1.

The sewage sludge used was obtained from Sewage Treatment Plant - SANEP (Pelotas Sanitation stand-alone service) after treatment by anaerobic fluidized bed reactor - RALF. The plant receives around 30% of the domestic sewage from the city of Pelotas/RS. It was collected in March 2013 in a dry day with ambient temperature of 20℃ and kept refrigerated. Its chemical and heavy metal properties were measured according to [12,13] (Table 1).

Table 1. Soil and sludge chemical and physical properties

Variable	Soil	Sludge
pH (H20)	5.6	5.21
clay (%)	16	-
organic matter (%)	1.1	-
N (mg dm^{-3})	-	39,960
P (mg dm^{-3})	6.5	1,560
K (mg dm^{-3})	68	1,920
Ca ($cmol_c$ dm^{-3})	1.5	22,120
Mg ($cmol_c$ dm^{-3})	0.7	1,180
Al ($cmol_c$ dm^{-3})	0.2	-
CEC ($cmol_c$ dm^{-3})	6.6	-
Cr (mg kg^{-1})	5.56	32.48
Zn (mg kg^{-1})	0.05	404.31
Cu (mg kg^{-1})	0.2	133.38
Pb (mg kg^{-1})	0.5	175.47
Fe (mg kg^{-1})	-	575.90

Chemical analysis of the sludge allowed to frame that waste as Class II A or even B according to the Brazilian Association of Technical Standards NBR 10004 [7], allowing its use with increased safety in agriculture. Current law requires that waste is disposed in special landfills denominated ARIP (Landfill of Hazardous Industrial Waste). The amounts used in this work, however, amounted to Cr additions below the maximum limits accepted by Resolution 375 of the National Environment Council Environment [10] for sludge to be applied to agricultural soils that are 1000 kg ha^{-1} Cr.

The experimental design was completely randomized with four treatments and a control, with four replications: T1 - control (no fertilizer, no sludge); T2 - NPK only; T3 - 4 Mg ha^{-1} of sludge; T4 - 12 Mg ha^{-1} of sludge; T5 - 20 Mg ha^{-1} of sludge. The experiment consisted of 20 plastic pots with 10 kg capacity, with drains and dishes. Each pot received a layer of about 3 cm of gravel. Soil and sewage sludge were air dried, homogenized and sieved (2 mm mesh size). The sludge concentration of T3 was determined to meet the N requirement of sunflower variety [13], assuming that 1/3 of N contained in the residue would be available for plants. The concentrations of T4 e T5 were 300 and 500% of the T3 concentration, respectively. For T2, the NPK fertilizers were urea, triple superphosphate and potassium chloride, and their concentrations were determined to meet the sunflower requirements according to SBCS/NRS [13], which were: N = 40 kg ha^{-1}; P_2O_5 = 180 kg ha^{-1}; and K_2O = 170 kg ha^{-1}. For pH adjustment, all pots received 3 t ha^{-1} of lime in the form of a mixture of $MgCO_3 + CaCO_3$ (2:1).

In each pot, about four sunflower seeds (Agrobel 369, 90% germination) were planted at a 2 cm depth. Fifteen days after germination, thinning was done leaving about two plants showing well-developed leaves in each pot. Soil moisture was kept near field capacity for a period of 90 days.

At 70 days after planting (DAP), four soil samples were collected from each pot at 0-10 cm depth and about 10 cm from the plants, homogenized and analyzed for soil total organic carbon (TOC), microbial biomass carbon (MBC), basal respiration (BR), and heavy metals. The TOC content was determined by the Walkley-Black method [9], and the MBC according to [14]. For MBC, soil microorganisms were killed by irradiation at 2450 Mhz for four minutes instead of fumigation with chloroform. The MBC was determined by the difference of C between the irradiated (Ci) and non-irradiated (Cni) soil sample, corrected by a correction factor (Kc) of 0.33 according to [14], as follows: Equation 1) MBC = (Ci - Cni)/Kc. The MBC to TOC ratios were also assessed.

Basal respiration rate (BR) is a measure of microbial activity that consists of quantifying the CO_2 released per hour from organic carbon decomposition by microbes. It was determined according to [14], where the quantity of CO_2 released from the soil is trapped in alkali during seven days of incubation and subsequently titrated against HCl. For the calculation of CO_2 efflux the equation 2) BR = (B - S) x M x 4 was used, where B is the volume of HCl to titrate the blank flask; S is the volume of HCl to titrate the remaining NaOH from the soil sample; M is the HCl concentration; and 4 (standard value) is the equivalent gram of respired carbon by soil microorganisms. The BR to MBC ratios, or metabolic quotients (qCO2), were derived.

When the plants reached the grain formation stage, after 86 days after planting, they were removed from the pot and proceeded then to the careful cleaning of the root system. Plants were harvested at the height of arms to separate air and roots. The shoots were dried at 60℃ to constant weight to determine the dry matter, and shoot the ground in a Wiley mill.

The shoots and sunflower seeds were analyzed for heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) according to [15] using atomic absorption spectrometry.

Data were subjected to analysis of variance and comparison of means by the Tukey method (0.05 probability), using the Statistix 8.0 software (Analytical Software Inc., Tallahassee, FL, USA).

3. RESULTS AND DISCUSSION

The highest microbial biomass C (MBC) was observed in the T5 treatment, which was 38% larger than in the control treatment (T1) and 45% larger than in the NPK only treatment (T2), the average for MBC was 357.8 μg g^{-1} (Table 2). The MBC increases were associated with organic matter increases from sludge applications, according [16] the application of the sewage sludge in soil have possibility of recycling valuable components such as organic matter, N and P. In contrast, Sullivan et al. [17] did not observe changes in MBC as an effect of the application of sewage sludge (between 2.5 and 30 Mg ha^{-1}) and suggested that, in this case, a rapid adaptation of soil microbes occurred. Other negative effects on biological soil properties, such as MBC, from applications of sludge contaminated with heavy metals are reported in the literature [18], and show the harmful impact of these chemicals on soil microbiology. These deleterious effects on soil microbial communities were not observed in this study.

The largest TOC contents were observed in T5 and were 59.02% larger than in T1 and 32.8% larger than in T2. These results correlate with the sludge applications and agree with the observed MBC trends. Similar results were obtained by [19], where increasing concentrations of anaerobic sludge from parboiled rice effluent treatment station resulted in larger TOC and MBC.

The MBC/TOC ratio, which indicates the percentage of TOC represented by the microbial carbon, decreased relative to the control in T1 and T2. For qCO_2, the largest values were obtained by T2 and the smallest values by control T1, T4 and T5 treatments. The qCO_2 has been used as a biological efficiency indicator of the soil, since as the microbial biomass becomes more efficient, more carbon is incorporated in the microbial biomass and less carbon is released as CO_2 due to respiration [14]. In general, sewage sludge application resulted in qCO_2 reduction compared to T2. It is recognized that stress factors (herbicides, toxic metals and pH) cause microbial inefficiency [14]. A potential effect of the application of sewage sludge in the soil is the inhibition of soil microorganisms with detriment of important functions such as mineralization and immobilization processes [20].

However, in this study the application of sludge increased microbial efficiency (smaller qCO_2), especially at large concentrations. This is confirmed by the larger MBC/TOC ratios.

The largest DM contents were observed in T4 and T5, witch was 41.55% larger than in T1 and 40.1% larger than in the NPK only treatment (T2). These results correlate with the sludge applications (Table 2). The sludge addition favored the development of plants resulting in increased amount of dry matter. Similar results to those in soils treated sewage sludge application was obtained by [21,22].

The higher plant growth was observed in treatments with sludge applications and by the NPK only treatment (T2) (Fig. 1). During the first developments phase of plants (0-20 days) T5

Table 2. Soil Microbial Biomass Carbon (MBC), Total Organic Carbon (TOC), ratio of MBC/TOC, metabolic quotient (qCO_2) and Dry Matter (DM) from the different treatments 70 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	MBC (μg g^{-1})	TOC %	MBC/TOC	qCO_2 (10^{-3})	DM(g)
T1-control	267.74±26.12d	4.50±1.59cd	1.97±0.87c	17.54±2.54c	2.36±0.34c
T2-NPK only	294.97±27.98c	6.55±1.68c	2.42±0.92bc	35.72±2.34a	2.87±0.45b
T3-sludge 4 Mg ha^{-1}	346.77±31.35b	7.90±0.87bc	3.43± 0.46b	27.63±3.05b	3.59±0.71a
T4-sludge 12 $Mgha^{-1}$	398.73±29.04b	8.05±0.97b	3.69±1.29ab	19.29±3.89c	4.16±0.54a
T5-sludge 20 $Mgha^{-1}$	480.77±16.67a	9.75±1.02a	5.13±1.40a	14.71±4.02c	4.38±0.86a

Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability < 0.05 treatment resulted in increased growth compared to other treatments. After 20 days, treatment with NPK represented a significant improvement in plant growth similar to the sludge treatments, did not differ to the applied sludge dosages.

In general, the treatments that received sewage sludge did not affect BR compared with treatments with NPK only (T2) and control (T1) (Table 2). There was increase in the production of CO_2 with increasing sewage sludge concentrations (Fig. 2), even though it was expected since MBC increased. Similar results were found by [23] when evaluating the effect of sewage sludge (0, 10, 20, 40, 80 and 160 Mg ha^{-1}) on the microbial activity of the soil, they did not found negative effect from the sludge applications.

The concentrations of Cd, Cu, Cr, Pb and Zn in soil were largest in T5 (Table 3). According to the Brazilian National Environmental Council (CONAMA) Resolution No. 420 of 2009 [10], reference prevention values for Cd, Cu, Cr, Pb and Zn are respectively 1.3, 60, 75, 72, 300 mg kg^{-1}. Therefore, the Cd in treatments with sludge and Pb in treatment T5 concentration was above the prevention limit. Larger heavy metal contents in soils with sludge applications were also observed by [9]. The metals are also present in the organic fraction of the soil, and are released to the soil solution upon the decomposition of organic matter. The soil solution provides a means for chemical reactions and transfer of metals between the soil and organisms [24]. According to the same authors, metals and non-metals are involved in a series of complex biological and chemical interactions, and the most important factors affecting their mobility are the pH and presence and concentration of inorganic and organic ligands, including humic and fulvic acids, exudates and nutrients. Furthermore, redox reactions are of great importance in the control of the mobility and toxicity of various elements such as Cr, Pb, Ni and Cu.

In parts of the plant the Cd, Cu, Pb and Zn average for treatment with sludge were 3.06, 20.12, 9.5 and 126.63 mg kg^{-1} respectively.

The contents were generally proportional to the sewage sludge additions, with largest values in T5 (Table 4). In parts of the plant the Cd, Cu, Pb and Zn average for treatment with sludge were 3.06, 20.12, 9.5 and 126.63 mg kg^{-1} respectively. The contents were generally proportional to the sewage sludge additions, with largest values in T5 (Table 4). Low levels of copper were detected in parts of the plant. This result can be attributed to the strong complexation that this element may suffer from organic matter and to the antagonism between Cu and Zn in soil solution [25]. Chromium was not detected in any of the treatments in parts of the plant (Table 4). This could be due to the soil adsorption and chelation capacity, making Cr not available for the plant [26]. This metal can be strongly complexed by humic acids, thus reducing its availability in the soil [25].

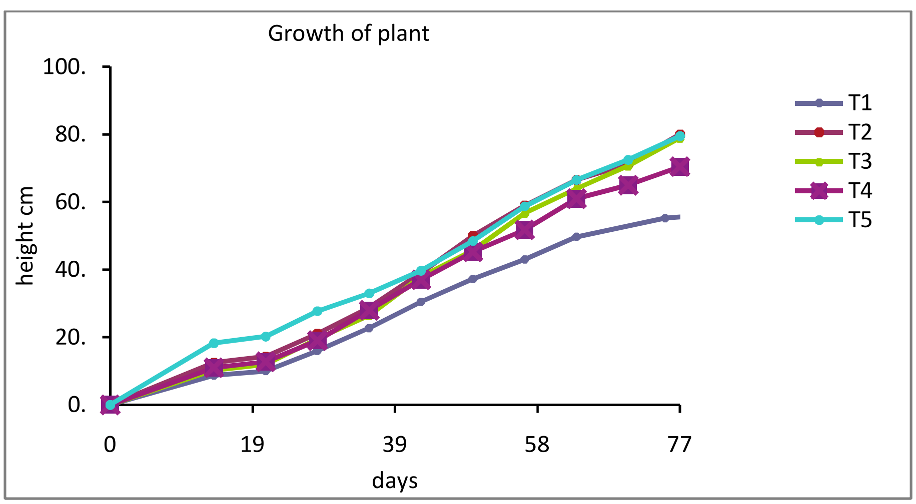

Fig. 1. Growth of plant from the different treatments 77 days during planting. T1- Control, T2- NPK only, T3- sludge 4 Mg ha^{-1}, T4- sludge 12 Mg ha^{-1} and T5- sludge 20 Mg ha^{-1}

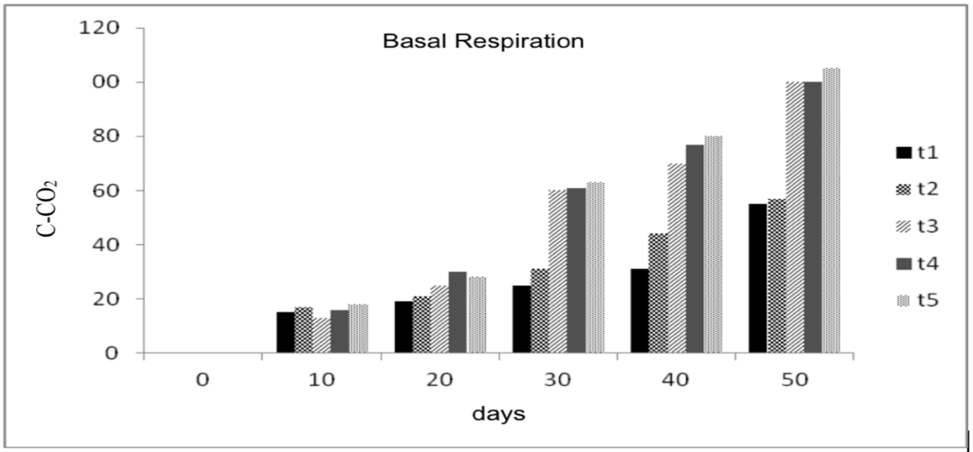

Fig. 2. Effect of sewage sludge in Argisol on the cumulative release carbon (CO_2) in the soil in 50 days. T1- Control, T2- NPK only, T3- sludge 4 Mg ha^{-1}, T4- sludge 12 Mg ha^{-1} and T5- sludge 20 Mg ha^{-1}

Table 3. Soil heavy metal (Cd, Cu, Cr, Pb and Zn) contents from the different treatments 70 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	Cd	Cu	Cr	Pb	Zn
			mg kg^{-1}		
T1-control	1.02±0.76c	6.32±0.15c	2.67±0.86c	0.45± 0.12e	1.45±0.34d
T2-NPK only	1.13±1.23c	7.35±0.23c	3.44±0.98c	2.13±0.56d	55.52±10.45c
T3-sludge 4 Mgha^{-1}	4.36±1.43b	11.60±1.13b	6.34±1.22b	35.21±8.34c	155.53±13.60b
T4-sludge 12Mgha^{-1}	5.02±1.75a	13.68±1.74a	8.24±1.45a	48.35±12.35b	182.32±18.90a
T5-sludge 20 Mgha^{-1}	6.40±1.05a	15.45±1.87a	9.46±1.04a	75.24± 9.21a	197.76±16.43a
DL	0.051	0.02	0.014	0.06	0.03
QL	0.192	0.13	0.04	0.08	0.12

DL: detection limit; QL: quantification limit. Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability < 0.05

Lead contents in parts of the plant increased with sludge additions, with T5 showing the higher values (Table 4). T1 and T2 had statistically significant smaller Pb contents than all sludge treatments. Zn contents in parts of the plant increased with sludge additions. In [27], Zn contents in the parts of the plant except the seeds also increased with the sewage sludge additions. This suggests that a fraction of Zn contained in the sewage sludge becomes available to and is absorbed by the plant. Another study reported increases in Zn levels in parts of corn plants grown in soil treated successively with sewage sludge [28]. However, factors such as stage of plant development, exposure time and the presence of other ions in solution interfere with the distribution of metals in plants. A similar result was found by [28], evaluating the effects of application of sewage sludge on soil with improved acidity, found increases in Zn concentration in the leaves and kernels of corn, showing that other sludge constituents can alter the chemical processes occurring in the soil in such extension that liming pass to provide little protection against the absorption of this metal by plants.

In seeds the Cd, Cu, Pb and Zn the average for treatment with sludge were 1.29, 14.26, 1.52 and 80.57 mg kg^{-1}, respectively. The contents were generally proportional to the sewage sludge additions, with largest values for Cu and Zn in T5. The results obtained for Cu and Zn in the seeds are similar to those obtained by [29] where the transfer of heavy metals were those with the highest transfer rates to the plant.

Chromium was not detected in any of the treatments in seeds (Table 5).

Lead contents in seeds were increased with sludge additions, with T5 showing the largest values (Table 4). These high Pb levels found in parts of the plant are considered harmful for human consumption, according to Ordinance No. 685 of 1998 of the Ministry of Health [30], which

Table 4. Plant heavy metal (Cd, Cu, Cr, Pb and Zn) contents from the different treatments 70 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	Cd	Cu	Cr	Pb	Zn
			mg kg^{-1}		
T1-control	0.59±0.19b	6.30±0.13d	Nd	0.35±0.21c	2.12±0.45e
T2-NPK only	1.03±0.42ab	9.50±1.50c	Nd	1.57±0.54c	32.61±9.34d
T3-sludge 4 Mgha^{-1}	2.87±1.55a	16.32±1.63b	Nd	8.42±1.23b	95.53±11.32c
T4-sludge 12Mgha^{-1}	2.93±1.31a	21.03±0.52a	Nd	9.57±0.84ab	120.52±20.35 b
T5-sludge 20 Mgha^{-1}	3.40±1.03a	23.02±1.72a	Nd	10.51±0.87a	154.85±15.87a
DL	0.08	0.04	0.02	0.10	0.06
QL	0.39	0.24	0.08	0.20	0.25

DL: detection limit; QL: quantification limit. Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability < 0.05. ND- Not detected

Table 5. Seed heavy metal (Cd, Cu, Cr, Pb and Zn) contents from the different treatments 70 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	Cd	Cu	Cr	Pb	Zn
			mg kg^{-1}		
T1-control	0.13±0.79a	1.10±0.12d	Nd	0.28±0.19	3.48±1.02d
T2-NPK only	0.43±0.28a	6.56±1.43c	Nd	1.08±0.94	27.15±7.14c
T3-sludge 4 Mgha^{-1}	1.26±0.69a	13.21±1.28b	Nd	1.29±0.59	62.80±9.80b
T4-sludge 12Mgha^{-1}	1.33±0.78a	12.33±0.73b	Nd	1.55±0.96	82.00±5.80a
T5-sludge 20 Mgha^{-1}	1.30±0.50a	17.24±1.35a	Nd	1.72±0.47	96.91±11.33a
DL	0.08	0.04	0.02	0.10	0.06
QL	0.39	0.24	0.08	0.20	0.25

DL: detection limit; QL: quantification limit. Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability < 0.05

recommend maximum allowed Pb values in food of 0.05 to 2.0 mg kg^{-1}, but there is no direct reference to vegetables. The Codex Alimentarius [31] recommends a maximum limit of 0.3 mg kg^{-1} of Pb in plants. The Pb contents found in seeds were also above this limit.

4. CONCLUSION

Sewage sludge applications, during the cultivation of sunflower, provided greater soil microbial activity, resulting in increased microbial biomass carbon, soil organic carbon decrease in the metabolic quotient and in increased production of dry plant matter. The contents of heavy metals Cd, Cu, Cr, Pb e Zn in soil were higher in treatment of sludge with higher dose. The concentrations of Cd and Pb in soil resulted in values above the limit of prevention. The lead content found in the seeds was above the maximum extent recommended by the Codex Alimentarius.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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