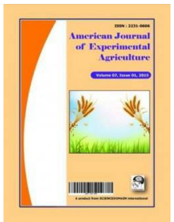

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Effects of Different Sewage Sludge Concentrations on Soil and Cultivated *Raphanus sativus* L

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Authors' contributions

This work was carried out in collaboration between all authors. Author GMBB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RMK, CH and EMB reviewed the experimental design and all drafts of the manuscript. Authors RMK and CH managed the analyses of the study. Authors GMBB and RCOM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Sewage sludge is a residue rich in organic matter and nutrients important for plant growth and soil fertility, but it may contain in its composition heavy metals that can result in toxicity to the plant, soil and humans when used as fertilizer. The objective was to assess microbial activity and heavy metal residues in soil and radish tubers grown with different concentrations of sewage sludge. Carbon microbial biomass, total organic carbon, soil basal respiration and zinc, copper, chromium and lead levels in soil and radish tubers were analyzed. According to the results, application of 30 Mg ha^{-1} of

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sewage sludge promoted higher microbial activity and lower metabolic quotient, and resulted in 490 tili^{-1} of microbial carbon and 11.12% of soil organic carbon. Heavy metal contents in radish tubers were 266.15, 2.82 and 15.42 mg kg^{-1} of zinc, chromium and lead, respectively, with the lead content found in the samples were above the maximum extent permitted recommended by the Codex Alimentarius.

Keywords: Radish; microbial carbon; heavy metal; basal respiration.

1. INTRODUCTION

The sewage sludge is the solid residue from the urban sewage treatment. It is rich in organic matter and used in agricultural as source of nutrients for plants [1]. Furthermore, the agricultural use has been recommended for providing agronomical benefits [2] such as greater soil water retention capacity, porosity (aeration of the roots) and aggregate stability, greater resistance to erosion, and possibly controls soil pathogens [3]. It also provides reduction of potential acidity and improved physical, chemical and microbiological soil conditions [4].

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 and to obtain a product to be used in agricultural soils [5,6]. 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: zinc, copper, chromium and lead [7,8]. 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 [3]. The accumulation of toxic metals in soil treated with sewage sludge is of concern due to its potential phytotoxic effects on plants, animals and humans, and its effect on soil microbial activity [1,2,3]. The use of sewage sludge in agriculture is restricted to specific provisions of the National Environmental Council [6], in order to avoid PTE transfer to the food chain.

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 Zn, Cu, Cr and Pb contents in soil and radish tubers. 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 greenhouses of the experimental area of the Federal Institute of Rio Grande do Sul in the period from March to April 2013. The soil used in the experiment was classified as dystrophic Red-Yellow Argisol. Soil and sludge chemical and physical properties were obtained according to [9], and heavy metal contents were measured according to [10] using before the experiment soil properties were: pH (H_20) = 5.6; clay = 16%; organic matter = 1.1%; P = 6.5 mg dm^{-3}; K = 68 mg dm^{-3}; Ca = 1.5 cmol$_c$ dm^{-3}; Mg = 0.7 cmol$_c$ dm^{-3}; Al = 0.2 cmol$_c$ dm^{-3}; cation exchange capacity = 6.6 cmol$_c$ dm^{-3}; Cr = 5.56 mg kg^{-1}; Zn = 0.05 mg kg^{-1}; Cu = 0.2 mg kg^{-1}; and Pb = 0.5 mg kg^{-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: pH (H_2O) = 5.21; N = 39.96 g kg^{-1}; organic C = 348.47 g kg^{-1}; P = 1.56 g kg^{-1}; K = 1.92 g kg^{-1}; Ca = 22.12 g kg^{-1}; Mg = 1.18 g kg^{-1}; Fe = 575.90 g kg^{-1}; Cu = 133.38 mg kg^{-1}; Zn = 404.31 mg kg^{-1}; Cr = 32.48 mg kg^{-1}; and Pb = 175.47 mg kg^{-1}.

The experimental design was completely randomized with five treatments and a control, with four replications: T1 - control (no fertilizer, no sludge); T2 - NPK only; T3 - 6 Mg ha^{-1} of sludge; T4 - 12 Mg ha^{-1} of sludge; T5 - 18 Mg ha^{-1} of sludge; T6 - 30 Mg ha^{-1} of sludge. The experiment consisted of 24 plastic pots with 5 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 radish, according to

SBCS/NRS [11]. The concentrations of T4, T5 and T6 were 200, 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 radish requirements according to SBCS/NRS [11], 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 2.7 t ha^{-1} of lime in the form of a mixture of $MgCO_3 + CaCO_3$ (2:1).

In each pot, about five radish seeds (Saxa (220), Isla brand, lot 22133, 90% germination, 99.5% purity) were planted at a 1 cm depth. Fifteen days after germination, thinning was done leaving about three plants showing well-developed leaves in each pot. Soil moisture was kept near field capacity for a period of 40 days.

At 30 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. At 35 DAP the radish plants were harvested and analyzed for heavy metals in the tuber.

The TOC content was determined by the Walkley-Black method [9], and the MBC according to [12]. 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 (C_i) and non-irradiated (C_{ni}) soil sample, corrected by a correction factor (K_c) of 0.33 according to [12], as follows: $MBC = (C_i - C_{ni})/K_c$. 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 [5], 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 formula $BR = (B - S) \times M \times 4$ 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 (qCO_2), were derived.

The radish tubers were analyzed for heavy metals (Cr, Cu, Pb and Zn) according to [13] 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

Average MBC was 408.31ion $^{-1}$, largest MBC was observed in the T6 treatment, which was 26% larger than in the control treatment (T1) and 30% larger than in the NPK only treatment (T2) (Table 1). The MBC increases were associated with organic matter increases from sludge applications. In contrast, Sullivan et al. [14] 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 [15], 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 T4 and T6, and were 22.5% larger than in T1 and 19% larger than in T2. These results correlate with the sludge applications and agree with the observed MBC trends. Similar results were obtained by [16], where increasing concentrations of anaerobic sludge from a parboiled rice effluent treatment station resulted in larger TOC and MBC.

In general, the treatments that received sewage sludge did not affect BR compared with treatments with mineral fertilizers (T2) and control (T1) (Table 1). There was no increase in the production of CO_2 with increasing sewage sludge concentrations, even though it was expected since MBC increased. Similar results were found by [17] 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 found no negative effect from the sludge applications.

The MBC/TOC ratio, which indicates the percentage of TOC represented by the microbial carbon, decreased relative to the control in T2, T3 and T4, whereas larger sludge additions did not affect it. For qCO_2, the largest values were obtained by T2 and the smallest values by T5 and T6 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 [12]. In general, sewage sludge application resulted in qCO_2 reduction compared to T1 or T2. It is recognized that stress factors (herbicides, toxic metals and pH) cause microbial inefficiency [12]. 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 [18]. 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 concentrations of Zn, Cu, Cr and Pb in soil were largest in T6 (Table 2). According to the Brazilian National Environmental Council (CONAMA) Resolution No. 420 of 2009 [6], reference limit values for Zn, Cu, Cr and Pb are respectively 300, 60, 75, 72 mg kg^{-1}. Therefore, only the Pb concentration in T6 was above the limit. Larger heavy metal contents in soils with sludge applications were also observed by [19]. 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 [20]. 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 radish tubers, the Zn contents were generally proportional to the sewage sludge additions, with largest values in T5 and T6 (Table 3). In [21], Zn contents in the parts of the plant except the grain 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 [2]. 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.

Copper was only detected in radish tubers in T6 treatment, showing that Cu was absorbed by the plant only when the largest amount of sludge was applied. This lack of response from Cu from sludge additions 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 [8].

Table 1. Soil microbial biomass carbon (MBC), total organic carbon (TOC), basal respiration rate (BR), ratio of MBC/TOC and metabolic quotient (qCO_2) from the different treatments 30 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	MBC µg g^{-1}	TOC %	BR µg CO_2 g^{-1} h^{-1}	MBC/TOC	qCO_2 x 10^{-3}
T1- control	364.72c±24,14	8.68c±0,82	0.394a±0,23	0.42ab±0,08	10.8b±1,13
T2- NPK only	346.75c±18,32	9.09bc±0,93	0.511a±0,35	0.38bc±0,05	14.74a±0,98
T3- sludge 6 Mg ha^{-1}	383.04bc±37,03	10.63abc±0,76	0.240a±0,21	0.35c±0,03	9.16b±0,97
T4- sludge 12 Mg ha^{-1}	406.03abc±45,3	11.36a±0,56	0.335a±0,13	0.36c±0,05	8.25bc±1,23
T5- sludge 18 Mg ha^{-1}	460.55ab±38,90	10.91ab±0,74	0.332a±0,09	0.42ab±0,04	7.21c±1,05
T6- sludge 30 Mg ha^{-1}	489.01a±13,54	11.18a±0,33	0.543a±0,15	0.44a±0,03	6.26c±1,11

Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability of 0.05

Table 2. Soil heavy metal (Zn, Cu, Cr and Pb) contents from the different treatments 30 days after planting. Average values followed by the standard deviation from the four replicates

Treatments	Zn	Cu	Cr	Pb
		mg kg^{-1}		
T1- control	1.15e±2,13	0.20c±1,15	5.567c±3,50	0.50e ±1,54
T2- NPK fertilization	65.32d±24,21	0.35c±0,36	5.454c±2,13	2.53e±5,43
T3- sludge 6 Mg ha^{-1}	255.533bc±28,13	12.560b±7,20	8.345c±1,12	35.321d±7, 132
T4- sludge 12 Mg ha^{-1}	287.542b±35,07	15.658b±3,85	10.324b±0,95	48.435c±8, 12
T5- sludge 18 Mg ha^{-1}	293.676b±18,11	17.435b±5,08	11.436b±1,32	65.234b±11 ,10
T6- sludge 30 Mg ha^{-1}	338.157a±14,03	25.432a±2,19	18.120a±2,51	93.67a±7,09
DL	0.03	0.02	0.014	0.06
QL	0.12	0.13	0.04	0.08

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 of 0.05

Table 3. Heavy metal (Zn, Cu, Cr and Pb) contents in radish tubers from the different treatments. Average values followed by the standard deviation from the four replicates

Treatments	Zn	Cu	Cr	Pb
		mg kg^{-1}		
T1- control	0.18e±.18e	Nd	Nd	0.354c±.354
T2- NPK fertilization	32.610d±2.610	Nd	Nd	1.567c±.567
T3- sludge 6 Mg ha^{-1}	115.533c±15.53	Nd	Nd	11.424ab±1.42
T4- sludge 12 Mg ha^{-1}	180.523b±80.52	Nd	Nd	11.572ab±1.57
T5- sludge 18 Mg ha^{-1}	254.850a±54.85	Nd	Nd	12.511ab±2.51
T6- sludge 30 Mg ha^{-1}	266.151a±66.15	2.822 1age	Nd	15.420a±5.42
DL	0.03	0.02	0.014	0.06
QL	0.12	0.13	0.04	0.08

DL: Detection limit; QL: Quantification limit; Nd: Not detected. Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability of 0.05

Chromium was not detected in any of the treatments in radish tubers (Table 2). This could be due to the soil adsorption and chelation capacity, making Cr not available for the plant [5]. This metal can be strongly complexed by humic acids, thus reducing its availability in the soil [8].

Lead contents in radish tubers increased with sludge additions, with T6 treatment showing the largest values (Table 3). T1 and T2 had statistically significant smaller Pb contents than all sludge treatments. These high Pb levels found in radish tubers are considered harmful for human consumption, according to Ordinance No. 685 of 1998 of the Ministry of Health [22], which 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 [23] recommends a maximum limit of 0.3 mg kg^{-1} of Pb in plants. The Pb contents found in radish tubers were also above this limit.

4. CONCLUSION

Sewage sludge applications during the cultive of radishes provided greater soil microbial activity, resulting in increased microbial biomass carbon, soil organic carbon and decrease the metabolic quotient. The contents of metals in soil increased directly with the increase of sludge doses applied to the soil, resulting in transfer of zinc metals, chromium and lead for tubers of radishes grown with sewage sludge. The lead content found in the samples were above the maximum extent permitted recommended by the Codex Alimentarius. The use of sewage sludge in the cultivation of radishes results in improvement in the microbiological soil quality, but results in possible toxicity by heavy metal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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