

Short Research Article

Influence of sewage sludge application on cultivate *Raphanus sativus* L.

ABSTRACT

Sewage sludge is a residue rich in organic matter and nutrients important for plant growth and soil fertility, but may contain in its composition various pollutants which can result in toxicity to the plant and soil. When used as fertilizer may result in heavy metal residues in the soil and therefore residue in foods. In this context, it developed an experiment in the period March-April 2013, to assess microbial activity and residues heavy metals in soil and in tubers of radishes grown under different applications of sewage sludge. Were analyzed carbon microbial biomass, total organic carbon, basal respiration in the soil and zinc levels, copper, chromium and lead in soil and in tubers of radish. According to the results, application 300 g/v sewage sludge in cultivation of radishes provided higher microbial activity, 490 $\mu\text{g g}^{-1}$ of microbial carbon, 11.12% organic carbon total soil and lower metabolic quotient. Regarding the contents of metals, resulted in contamination of tubers with 266.15; 2.82 and 15.42 mg kg^{-1} zinc, chromium and lead, respectively, and the lead content resulted above the limits allowed in food.

Keywords: radishes, microbial carbon, metals, basal respiration

1. INTRODUCTION

The sewage sludge is the solid residue from the urban sewage treatment, which in wealth in organic matter is used in agricultural as source of nutrients for plants [1]. Furthermore, the agricultural use has been recommended by providing agronomical benefits such as reduction of potential acidity and also provides improved physical, chemical and microbiological soil conditions [2, 4, 8]. Several works has been evidence the cultivation with sewage sludge also provides greater soil water retention capacity, porosity (aeration of the roots) and aggregate stability, greater resistance to erosion, usable for subsequent cultures residual effect and possibly induce soil suppressiveness to pathogens [3].

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 [5]. However, when the household sewage are also composed of water from the industrial area, the sludge is richer in potentially toxic elements (PTE), as the metals zinc, copper, chromium and lead. Repeated application of sewage sludge on agricultural cultivation 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 worrying due to phytotoxic effects on plants, into the food chain with phytotoxic effects on animals and humans and changes in 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 soil microbial activity and Zn, Cr, Cu e Pb content in soil and on radishes tubers during cultivation in the presence of different doses of sewage sludge. The hypothesis of this study is that the application of sewage sludge on agricultural farming enhances soil microbial activity, but may result in transfer of metals for soil and food.

2. MATERIAL AND METHODS

43 The experiment was conducted in experimental area of the Federal Institute of Rio Grande
 44 do Sul in the period from March to April 2013. The soil used in the experiment was classified
 45 as dystrophic Red-Yellow Argisol (PVAd). The chemical-physical characterization of soil was
 46 carried out according to the methodology described in Tedesco et al. [9] and presented as
 47 follows: pH (H₂O) = 5.6; clay = 16%; organic matter = 1.1%; P = 6.5 mg dm⁻³; K = 68 mg dm⁻³;
 48 ³; Ca = 1.5 cmol_c dm⁻³; Mg = 0.7 cmol_c dm⁻³; Al = 0,2 cmol_c dm⁻³; CTC cmol_c = 2.6 dm⁻³,
 49 being carried out before starting the experiment. The analysis of Cr, Zn, Cu e Pb (Cr = 5.56
 50 mg kg⁻¹, Zn = 0.05 mg kg⁻¹, Cu = 0.2 mg kg⁻¹ and Pb = 0.5 mg kg⁻¹) in soil were performed
 51 according to the method described by Hortellani et al. [10], and the determination by atomic
 52 absorption spectrometry.

53 The anaerobic sludge used was obtained from Sewage Treatment Plant - SANEP (Pelotas
 54 Sanitation stand-alone service) after treatment by anaerobic fluidized bed reactor - RALF,
 55 which receives around 30% of domestic sewage of the city of Pelotas/RS. The same was
 56 collected in March 2013 in dry day with ambient temperature of 20°C and kept refrigerated.
 57 Its physicochemical characterization was performed as methods described in Tedesco et al.
 58 [9] and presented as follows: pH (H₂O) = 5.21; N = 39.96 g kg⁻¹; organic C = 348.47 g kg⁻¹; P
 59 = 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 =
 60 133.38 mg kg⁻¹; Zn = 404.31 mg kg⁻¹; Cr = 32.48 mg kg⁻¹; and Pb = 175.466 mg kg⁻¹. The
 61 analysis of toxic metals in the sludge followed the same procedures used for analysis of
 62 metals in the soil according to the method described by Hortellani et al. [10], and the
 63 determination by atomic absorption spectrometry.

64 The treatments were six with four replications: T1 - control; T2 - NPK fertilization; T3 - 6 Mg
 65 ha⁻¹; T4 - 12 Mg ha⁻¹; T5 - 18 Mg ha⁻¹; T6 - 30 Mg ha⁻¹.

66 The experimental design was completely randomized with 24 plastic pots with 5 kg capacity,
 67 with holes in the base and dishes. In each pot was applied a layer of about 3 cm gravel.
 68 Samples of soil and sewage sludge alone were dried, homogenized and passed through a
 69 sieve of 2 mm mesh size. Seeds were planted with a depth of 1cm. In each experimental
 70 unit (pots) was planted about 5 seeds type Saxa (220), Isla brand, lot 22133, 90%
 71 germination, purity 99.5%. The incorporated amount of sludge in the treatment 3 was
 72 obtained after analysis of N content of this material, being determined the amount needed to
 73 meet the requirement of N radish culture, according to the SBCS / NRS [11]. The remaining
 74 doses in treatments 4, 5 and 6 were respectively 200, 300 and 500% of the dose applied in
 75 the treatment 3. These doses were equivalent to 10, 20, 30 and 50 mg.h⁻¹ respectively.

76 For the treatment T2, the NPK fertilizer levels were determined according to the soil analysis
 77 and fertilization according to the manual and liming SBCS/NRS [11] for radish culture. Were
 78 applied to the soil 40 kg N ha⁻¹, 180 kg ha⁻¹ of P₂O₅ and K₂O 170 kg ha⁻¹ as a source of
 79 urea NPK is used, triple superphosphate and potassium chloride, respectively. For pH
 80 adjustment, the treatments received 2.7 t ha⁻¹ of lime in the form of a mixture of MgCO₃ +
 81 CaCO₃ (2: 1).

82 Fifteen days after germination, thinning was done to leaving about three plants showing well
 83 developed leaves in each pot. The humidity was maintained near field capacity for a period
 84 of 40 days.

85 At 30 DAP were removed with the aid of a small shovel plastic, four soil samples, 10 cm
 86 from the plant, the depth of 0 to 10 cm from each experimental unit for chemical analysis
 87 (Total Organic Carbon- TOC) microbial activity of the soil (microbial biomass carbon-BMC
 88 and basal respiration-BR) and metals in soil. At 35 DAP was the radish harvest made for
 89 analysis of metals in the tuber.

90 The TOC content was determined by the Walkley-Black [9]. Carbon in the microbial biomass
 91 (MBC) [12]. According to this method, soil microorganisms are killed by irradiation at 2450
 92 Mhz for four minutes instead of fumigation with chloroform. The MBC was determined by the
 93 difference between the irradiated and non-irradiated soil sample after K₂SO₄ extraction and
 94 C determination by dichromate oxidation and titration with ferrous ammonium sulphate. The
 95 value for MBC was calculated by the following formula: $MBC = (C_i - C_{ni})/K_c$, where C_i and C_{ni}
 96 are the C content of the irradiated and non-irradiated samples, respectively; and K_c is the
 97 correction factor with a value of 0.33 [12]. The results were expressed in µg CO₂ g⁻¹ soil.
 98 The results were expressed as µg.g⁻¹ soil. The relationship CBM / COT was obtained by the
 99 ratio of microbial biomass carbon and total organic carbon soil.

100 Basal respiration (BR), which consists in measuring microbial activity derived from organic
 101 carbon decomposition and the quantification of CO₂ released, was determined according to
 102 the method described by Bohm et al. [5]. The quantity of CO₂ released from soil of each

treatment was trapped in alkali during seven days of incubation and subsequently titrated against HCl. For the calculation of CO₂ 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 results were expressed in mg C-CO₂ 100 g⁻¹. The rate of respiration per unit of biomass and metabolic quotient (qCO₂) was obtained by the ratio between basal respiration rate which is measured in CO₂ production resulting from the metabolic activity of the soil and microbial biomass [5].

For analysis of metals in the soil with sewage sludge applications, the samples were dried at 60 °C for 48 hours and sieved. The fraction <63µm was used for the chemical analysis. About 2g were weighed, and each pellet was added 4 mL of aqua regia (3:1 HCl: HNO₃), 4 mL of Milli-Q water and 1 mL of HClO₄, heating for 30 min at 90°C Water Bath [10]. The resulting solution was filtered and transferred to the volumetric flask of 25 mL, and the volume was made up with Milli-Q water. The extracts were subjected to analysis by atomic absorption spectrometry.

The tubers of the radishes were subjected to analysis of Cr, Cu, Pb and Zn, according to the methodology described by Abreu et al [13]. It proceeded to the application of this method by dividing the sample into masses of 1.0 g with 5 mL of HNO₃ and 1 mL of HClO₄. These tubers were taken to the block and the digester heated to 210°C. The extracts were subjected to analysis by atomic absorption spectrometry.

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

3. RESULTS AND DISCUSSION

The MBC presented average levels 408.31µg g⁻¹, and the T6 treatment showed the highest levels, 26% above the levels provided by the control treatment T1 and 30% higher of the levels obtained for the treatment with mineral fertilizer T2 (Table 1). Thus, the MBC increases were associated with organic matter that occurred due to the increase of sludge doses. In contrast, Sullivan et al. [14] did not observe changes in the microbial biomass carbon as an effect of the application of sewage sludge (doses between 2.5 and 30 Mg ha⁻¹) and suggested that, in this case, a rapid adaptation of soil microbes. Negative effects on biological soil properties, such as MBC, depending on the use of sludge doses contaminated with heavy metals are reported in the literature Kizilkaya & Bayrakli [15], and show the harmful impact of these chemicals on soil microbiology. These effects communities deleterious were not observed in this study.

Table 1. Contents of microbial biomass carbon (MBC), total organic carbon (TOC), basal respiration rate (BR), ratio of MBC/ TOC and metabolic quotient after 25 days of culture with the application of sewage sludge.

Treatments	MBC µg g ⁻¹	TOC %	BR µg Co ₂ g ⁻¹ h ⁻¹	MBC/TOC	qCO ₂ x10 ⁻³
T1- control	364.72c	8.68c	0.394a	0.42ab	10.8b
T2- NPK fertilization	346.75c	9.09bc	0.511a	0.38bc	14.74a
T3- sludge 6 Mg ha ⁻¹	383.04bc	10.63abc	0.240a	0.35c	9.16b
T4- sludge 12 Mg ha ⁻¹	406.03abc	11.36a	0.335a	0.36c	8.25bc
T5- sludge 18 Mg ha ⁻¹	460.55ab	10.91ab	0.332a	0.42ab	7.21c
T6- sludge 30 Mg ha ⁻¹	489.01a	11.18a	0.543a	0.44a	6.26c
Mean	408.31	10.31	0.39	0.39	9.72

CV	9.84	5.75	9.11	8.93	9.49
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Means followed by the same letter in the same column are not statistically different according to the Tukey test at a probability < 0.05 .

As for the TOC the largest contents obtained by T4 and T6 treatments, being 22.5% higher than the levels obtained by the control treatment T1 and 19% higher than treatment with mineral fertilizer T2. Probably this response is a function of sludge levels added to the treatments, agreeing with the result obtained for MBC. Similar results were obtained by Vieira et al. [16]. The authors found that an increase of anaerobic sludge applied dose of parboiled rice effluent treatment station, resulting in higher values of TOC and MBC.

In general, the treatments that received sewage sludge did not affect the basal soil respiration compared with treatments with mineral fertilizers (T2) and untreated sludge (T1) (Table 1). With increasing sewage sludge doses, there was no increase in the production of CO_2 , although it was expected that there would be due to higher levels of MBC. Similar results were found by Cardoso and Strong Neto [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 and found that there was no negative effect with the addition of the sludge.

To the ratio MBC/TOC which indicates the percentage of total organic carbon represented by the microbial carbon, the larger sludge dosages do not represent this relationship decreases, indicating no negative effect on the sludge population of soil microorganisms. As for the metabolic quotient (qCO_2), the greatest results were obtained by T2, with mineral fertilizer. The lowest values were obtained by T5 and T6 treatments, indicating the lowest level of CO_2 . The qCO_2 has been used as a biological indicator balance of the soil, since as microbial biomass becomes more efficient, less carbon is released as CO_2 for breathing and a higher proportion of carbon is incorporated in the microbial biomass [12]. In general, sewage sludge application resulted in qCO_2 reduction compared to the control treatment with T1 or T2 mineral fertilizer. It is recognized that stress factors (herbicides, toxic metals and pH) cause microbial inefficiency. A potential effect of the application of sewage sludge in the soil is the stimulant to the inhibition of soil microorganisms with important functions such as the mineralization processes and immobilization of nutrients [18].

The metals Zn, Cu, Cr and Pb in soil with sludge applications were higher in treatments with higher doses of sludge applied, and 338.16; 25.43; 18.12 and 93.67 mg kg^{-1} respectively (Table 2). According to the resolution in CONAMA 420 [6] reference values for prevention Zn, Cu, Cr and Pb are respectively 300, 60, 75, 72 mg.kg^{-1} . Therefore, only the Pb levels are above the limit within the prevention and research limit to the highest dose tested sludge in this study. Higher metal contents in soils with sludge applications were also observed by Araújo and Nascimento [19]. The metals are also present in the organic fraction of the soil, however the availability of metals occur as a result of decomposition of organic matter. The aqueous phase provides a mobile means of chemical reactions as transfer of metals through the soil for organisms [20]. According to these authors, metals and non-metals are involved in a series of complex biological and chemical interactions. The most important factors affecting their mobility are the pH, the absorbent nature, 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.

Table 2. Contents of metals Zn, Cu, Cr and Pb in soil with sewage sludge.

Treatments	Zn	Cu	Cr	Pb
	----- mg Kg^{-1} -----			
T1- control	1.15e	0.20c	5.567c	0.50e
T2- NPK fertilization	65.32d	0.35c	5.454c	2.53e

T3- sludge 6 Mg ha ⁻¹	255.533bc	12.560bc	8.345c	35.321d
T4- sludge 12 Mg ha ⁻¹	287.542b	15.658b	10.324b	48.435c
T5- sludge 18 Mg ha ⁻¹	293.676b	17.435b	11.436b	65.234b
T6- sludge 30 Mg ha ⁻¹	338.157a	25.432a	18.120 ^a	93.67a
DL	0.03	0.02	0.014	0.06
QL	0.12	0.13	0.04	0.08
Mean	206.396	11.939	9.874	40.948
CV	6.75	3.52	2.81	4.74

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

The Zn content in plant tuber were generally proportional to the applied doses of sewage sludge. The largest zinc levels were obtained by T6 (266.151 mg.kg⁻¹) and the treatments T1 and T2 mineral fertilizer not significantly different (Table 3). For Nogueira et al. [21] Zn contents in the parts of the plant except the grain increased with the applied sewage sludge doses. This suggests that a fraction of Zn contained in the sewage sludge becomes available to the plant, so that the increase in dose of the residue causes an increase in content available in the soil and its absorption by the plant. Other authors 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, presence of other ions in solution and species of elements interfere with the distribution of metals in plants.

The Cu levels in plant tuber were detected only in the T6 treatment, showing that metal has been absorbed by the plant applied only when a higher dosage sludge. This lack of response from copper levels the addition of increasing doses of sewage sludge can also be attributed to strong complexation that element suffers from organic matter and the antagonism that occurs between the Cu and Zn in soil solution [8].

Chromium-Cr in plant tuber was not detected in any of the treatments (Table 2). This could be due to soil adsorption capacity with chelated training, not being fully available for the plant [5]. This metal can be strongly complexed by humic acids, thus reducing its availability in the soil.

For lead metal- Pb in plant tuber, higher levels in treated sludge were detected, the T3 treatment showed the highest levels 15.42 mg kg⁻¹ with a significant difference from the control treatment T1 and T2 mineral fertilizer (Table 3). These high Pb levels found in samples of sludge with radishes may exhibit toxic if used for human consumption, as in Brazil, according to Ordinance No. 685 of 1998 of the Ministry of Health [22] ceilings tolerance for lead in food is 0.05 to 2.0 mg kg⁻¹, but there is no direct reference to vegetables. The Codex Alimentarius [23] recommend a maximum limit of 0.3 mg kg⁻¹ of lead in plants. Compared to these limits for vegetables, the lead content found in samples tested were above the limits set for human consumption.

Table 3. Contents of metals Zn, Cu, Cr and Pb in radish tuber with application of sewage sludge.

Treatments	Zn	Cu	Cr	Pb
	-----mg Kg ⁻¹ -----			
T1- control	0.18e	Nd	Nd	0.354c

T2- NPK fertilization	32.610d	Nd	Nd	1.567c
T3- sludge 6 Mg ha ⁻¹	115.533c	Nd	Nd	11.424ab
T4- sludge 12 Mg ha ⁻¹	180.523b	Nd	Nd	11.572ab
T5- sludge 18 Mg ha ⁻¹	254.850a	Nd	Nd	12.511ab
T6- sludge 30 Mg ha ⁻¹	266.151a	2.822	Nd	15.420a
DL	0.03	0.02	0.014	0.06
QL	0.12	0.13	0.04	0.08
Mean	141.619	2.822	-	8.692
CV	10.12	3.22	-	6.74

DL: detection limit; QL: quantification limit; CV: coefficient of variation. 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.

4. CONCLUSION

Sewage sludge applications during the cultivate 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.

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