ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
v.21, n.8, p.573-578, 2017
Campina Grande, PB, UAEA/UFCG – http://www.agriambi.com.br

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v21n8p573-578

Phytoremediation of chlorobenzenes in sewage sludge cultivated with *Pennisetum purpureum* at different times

Anarelly C. Alvarenga[1], Regynaldo A. Sampaio[1], Gevany P. Pinho[1], Paulo H. S. Cardoso[1], Izabelle de P. Sousa[1] & Mário H. C. Barbosa[1]

[1] Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias. Montes Claros, MG. E-mail: engagronoma@hotmail.com (Corresponding author); rsampaio@ufmg.br; gevanypp@yahoo.com.br; paulohenrique.sc@hotmail.com; izabelle-sousa@hotmail.com; marioh.ufmg@gmail.com

Key words:
sewage sludge
toxic organic substance
environmental pollution

ABSTRACT

One of the major limitations of sewage sludge agricultural use is the presence of chlorobenzenes (CBs), which are toxic to the environment. The use of techniques of sewage sludge phytoremediation can be an important alternative for the degradation of these substances in the waste. The aim of this study was to evaluate the effect of *Pennisetum purpureum* cultivation time on the reduction of the concentrations of 1,4-CB and 1,3,5-CB in sewage sludge. The experiment was conducted in a greenhouse, using a randomized block design. The treatments consisted of five periods of *Poaceae* cultivation in sewage sludge plots (30, 60, 90, 120 and 150 days) and a control (sludge plots not cultivated). At 150 days, the control, in the layer of 0-20 cm, showed higher concentrations of 1,3,5-CB, compared with the same layer of cultivated sludge. In the cultivated sludge, in the 10-20 cm layer, there was a decrease in the concentrations of 1,4-CB, while in the layer of 20-30 cm, both concentrations decreased. The observed reductions may be the result of mutualistic interactions between decomposer organisms and the roots of *P. purpureum*, favoring the decomposition process.

Palavras-chave:
biossólido
substância orgânica tóxica
poluição ambiental

Fitorremediação de clorobenzenos em lodo de esgoto cultivado com *Pennisetum purpureum* em diferentes períodos

RESUMO

Uma das grandes limitações do uso agrícola de alguns lodos de esgotos é a presença de clorobenzenos (CBs) que são tóxicos ao meio ambiente. O uso de técnicas de fitorremediação no lodo de esgoto pode ser uma importante alternativa para a degradação dessas substâncias no resíduo. Objetivou-se, neste trabalho, avaliar o tempo de cultivo de *Pennisetum purpureum* na redução das concentrações de 1,4-CB e 1,3,5-CB em lodo de esgoto. O experimento foi realizado em casa de vegetação, utilizando-se o delineamento em blocos casualizados. Os tratamentos corresponderam a cinco períodos do cultivo da *Poaceae* em parcelas de lodo de esgoto (30; 60; 90; 120 e 150 dias a partir do plantio de estacas) e uma testemunha (parcelas de lodo não cultivado). Aos 150 dias a testemunha, na camada de 0-20 cm, apresentou maiores concentrações de 1,3,5-CB, quando comparada à mesma camada do lodo cultivado. Já no lodo cultivado na camada de 10-20 cm, houve decréscimo das concentrações de 1,4-CB, enquanto, na camada de 20-30 cm, tanto as concentrações de 1,4-CB quanto de 1,3,5-CB diminuíram. As reduções observadas podem ser resultado das interações mutualísticas entre organismos decompositores e as raízes de *P. purpureum*, favorecendo o processo de remediação.

Ref. 168-2016 – Received 10 Oct, 2016 • Accepted 24 Feb, 2017 • Published 29 Jun, 2017

Introduction

Sewage sludge is a waste rich in organic matter and essential elements to plant nutrition. Its utilization in agricultural systems is of great economic and environmental relevance, because it promotes improvements in the physical-chemical and biological attributes of the soil, causing increments in the yield of crops.

Despite all benefits from the utilization of sewage sludge in agricultural systems, care must be taken, because this waste may have in its composition high concentrations of organic contaminants, such as chlorobenzenes (CBs). These compounds are generally found at high concentrations in sewage sludge, for being present in the composition of cleaning material, solvents, pharmaceutical products, graphic materials, pesticides, deodorants and air purifiers (Kamarei et al., 2010).

CBs constitute a class of aromatic halogenated chemical compounds of high hazard and persistence in the environment (Zhang et al., 2011). Because of the risks posed by CBs, it is necessary to conduct studies on the chemical behavior of these contaminants in bioremediation processes, to define the ideal conditions for their biodegradation (Adebusoye et al., 2007). Such fact can be extended to studies on sewage sludge, since the presence of the previously mentioned substance is inherent to this waste.

It becomes evident, therefore, the need to minimize the environmental impacts caused by the utilization of sewage sludge in agricultural systems, reducing as much as possible the concentrations of any contaminants present in the waste. Thus, this study aimed to evaluate the effect of *Pennisetum purpureum* Shum. cultivation, for different periods, on the concentrations of 1,4-CB and 1,3,5-CB in sewage sludge.

Material and Methods

The experiment was carried out in a greenhouse, at the experimental farm 'Professor Hamilton de Abreu Navarro', at the Institute of Agricultural Sciences ICA/UFMG, along five months. The study was conducted in a randomized block design, with six treatments, which corresponded to five evaluation periods (30, 60, 90, 120 and 150 days, from the planting of cuttings) during the cultivation of *P. purpureum* Shum. Grupo Merker, in plots of sewage sludge and one control (plots with sludge and without *P. purpureum*). The treatments had five replicates, totaling 30 experimental units. The units were made with the following dimensions: 1.0 m long x 1.0 m wide x 0.5 m high, containing 0.5 m^3 of sewage sludge.

The sewage sludge was collected in the Treatment Station of Montes Claros (ETE Vieira), in September 2013 and showed the following composition: organic matter = 42.5 dag kg^{-1}; pH in water = 6.2; P_2O_5 (total) = 25 g dm^{-3}; K_2O (total) = 2.9 mg dm^{-3}; Ca (total) = 75 g dm^{-3}; Mg (total) = 26 g dm^{-3}; S = 10.1 g dm^{-3}; Si (soluble) = 14.2 mg dm^{-3} (Tedesco et al., 1995). The initial concentrations of chlorobenzenes found in the sewage sludge, prior to the installation of the plots, were: 0.004 mg kg^{-1} of 1,4-CB and 0.023 mg kg^{-1} of 1,3,5-CB (Pinho et al., 2014).

Immediately after filling the plots with sewage sludge, cuttings of *P. purpureum* Shum. Grupo Merker, collected in the beds of forage species of the ICA/UFMG, were planted. The propagation material was cut in 20-cm-long setts, leaving only one bud in each cutting. Planting was performed at depth of 10 cm, spacing of 20 cm, totaling 25 plants per experimental unit.

Substrate moisture was daily monitored using a T.D.R. (Time Domain Reflectometry) device, to maintain the amount of water necessary for the development of the grass without slurry outflow. The temperature of the plots was daily measured before the irrigations.

Sewage sludge samplings, for the analyses of 1,4-CB and 1,3,5-CB concentrations, were made at the end of each treatment, at different depths: 0-10, 10-20, 20-30, 30-40 and > 40 cm (initially 40-50 cm). For the plot without grass cultivation, sampling was performed at 150 days from the beginning of the study. CBs were extracted using the solid-liquid extraction technique (Pinho et al., 2014). The present study monitored two contaminants: dichlorobenzene (1,4-CB), with retention time of 7.004 min and trichlorobenzene (1,3,5-CB), with retention time of 10.400 min.

The extracts were analyzed in gas chromatograph, Agilent Technologies (GC 7890 A) and the chromatograms were processed using the Data-analysis program.

The obtained data were subjected to analysis of variance and the means of the treatments of chlorobenzenes, present in sewage sludge cultivated for different periods, were compared with the mean of the treatment of uncultivated sewage sludge at 0.05 probability level by Dunnett's test. Means relative to the CBs concentrations in the sewage sludge cultivated with *P. purpureum* and collected at different times, were fitted to regression models, testing the coefficients up to 0.10 probability level by t-test.

Results and Discussion

The concentrations of 1,4-CB and 1,3,5-CB in the sewage sludge, prior to the experiment, were already below the acceptable critical concentrations according to the CONAMA Resolution nº 375 (BRASIL, 2006), which establishes limits of 0.39 mg kg^{-1} of 1,4-CB and 0.50 mg kg^{-1} of 1,3,5-CB, in sewage sludge. However, the remediation of these compounds, even at low concentrations, is relevant because CBs can accumulate in the soil through the successive applications of sewage sludge.

The low biodegradability of CBs causes great environmental concern, because these organic compounds are widely used in the production of dyes, pesticides, degreasers, lubricants, solvents and in electric transformers (Schroll et al., 2004), which causes great diffusion of this contaminant in the environment. The harmful effects of CBs have long duration, due to their high stability in the soils (Koe & Shen, 1997).

In the experimental plots, there was large variation of temperature during the first 2 months, following the patterns observed in the composting processes. During this period, there was a reduction in the volume of the plots, around 4% for uncultivated ones and 10% for the cultivated ones. Along this phase, there may be losses between 15 and 25% in the total mass of the compost, because of the high decomposition of the organic matter (Oleszczuk, 2007).

During the initial phase, there was a sharp decrease in the concentration of both CBs monitored (Table 1). At 30 days of

Table 1. Concentration of 1,4-CB and 1,3,5-CB in sewage sludge with and without *P. purpureum* cultivation in five evaluation periods (30, 60, 90, 120 and 150 days, from the planting of cuttings)

Period Depth (cm)	150 CSSNC	30 CSS	60 CSS	90 CSS	120 CSS	150 CSS	CV (%)
	mg kg^{-1}						
			1,4-Chlorobenzene				
0-10	0.0410 A	0.0650 A	0.0870 A	0.0250 A	0.0280 A	0.0840 A	137.19
10-20	0.0067 A	0.0910 B	0.0450 A	0.0330 A	0.0430 A	0.0240 A	62.75
20-30	0.0023 A	0.1150 B	0.0630 B	0.0310 A	0.0460 A	0.0340 A	74.22
30-40	0.0020 A	0.0740 B	0.0820 B	0.0560 B	0.0970 B	0.0340 A	56.40
> 40	0.0020 A	0.0391 B	0.0740 B	0.1300 B	0.0680 B	0.0230 A	37.02
			1,3,5-Chlorobenzene				
0-10	0.431 A	0.161 B	0.115 B	0.048 B	0.229 B	0.185 B	31.85
10-20	0.483 A	0.288 B	0.152 B	0.095 B	0.261 B	0.083 B	34.56
20-30	0.313 A	0.268 A	0.155 A	0.114 A	0.172 A	0.102 A	85.39
30-40	0.201 A	0.175 B	0.384 B	0.189 A	0.505 B	0.159 A	38.68
> 40	0.217 A	0.070 B	0.315 A	0.335 A	0.573 B	0.106 A	36.16

CSSNC - Control sewage sludge with no cultivation; CSS - Cultivation in sewage sludge; Means of the treatments referring to the CBs concentrations in sewage sludge cultivated for different periods, with the same letter of CBs concentrations in sewage sludge without cultivation, in the row, do not differ at 0.05 probability level by Dunnett's test

cultivation, the mean concentrations of 1,4-CB and 1,3,5-CB in sewage sludge were on the order of 19.21 and 8.37 times higher, respectively, than the concentrations obtained before the beginning of the test.

Possibly, the increase of temperature in the experimental units favored the activity of decomposer microorganisms, such as bacteria and fungi, which use carbon as energy source (Kulikowska & Klimiuk, 2010), causing loss of carbon, mainly, and increase in the concentrations of the contaminants. These data are consistent with those of Haroun et al. (2009), who observed that during the sewage sludge mineralization stage there is a substantial increment of contaminants.

The 1,4-CB concentrations, found in the sewage sludge cultivated for 30 days, were higher than the concentrations found in the sludge without cultivation in almost all evaluated layers, except 0-10 cm (Table 1). Along the cultivation period, other treatments differed from the control, namely: 60 days of cultivation in the layers from 20 to > 40 cm, and 90 and 120 days of cultivation in the layers from 30 to > 40 cm. The influence of cultivation period on CB concentration in the sewage sludge became evident, because, comparing the concentrations of the sludge cultivated for 150 days with those of the sludge without cultivation, there is no difference in any of the evaluated depths. CBs are semi-volatile compounds and the reduction in the concentration of this contaminant during the sewage sludge mineralization stage is directly related to the supply of aeration to the system. Every five days, the manual turning of sludge compost piles promotes better aeration during the process and, consequently, sharp reduction in the concentration of CBs (Cai et al., 2012). Based on the principle that sewage sludge turning is the main factor defining the biodegradation rate of the CBs, as previously mentioned, this explains the similarity of the 1,4-CB concentrations in the treatments with sludge not cultivated and sludge cultivated for 150 days, because there was no turning of the sewage sludge in any treatment along the entire experimental period.

The control, in the layers of 0-10 and 10-20 cm, exhibited higher 1,3,5-CB concentrations compared with the concentrations of the cultivated sewage sludge, in all experimental periods (Table 1). The lowest concentration of the contaminant in these layers of the cultivated sewage sludge may have been influenced by the *P. purpureum* cultivation, since these layers were explored by a larger volume of roots of the grass. Studies confirm the potential of using Elephant grass for phytoremediation of toxic compounds, due to its excellent capacity of development in contaminated environments and capacity to absorb and accumulate high concentrations of aromatic hydrocarbons, besides organochlorinated pesticides (Sojinu et al., 2012).

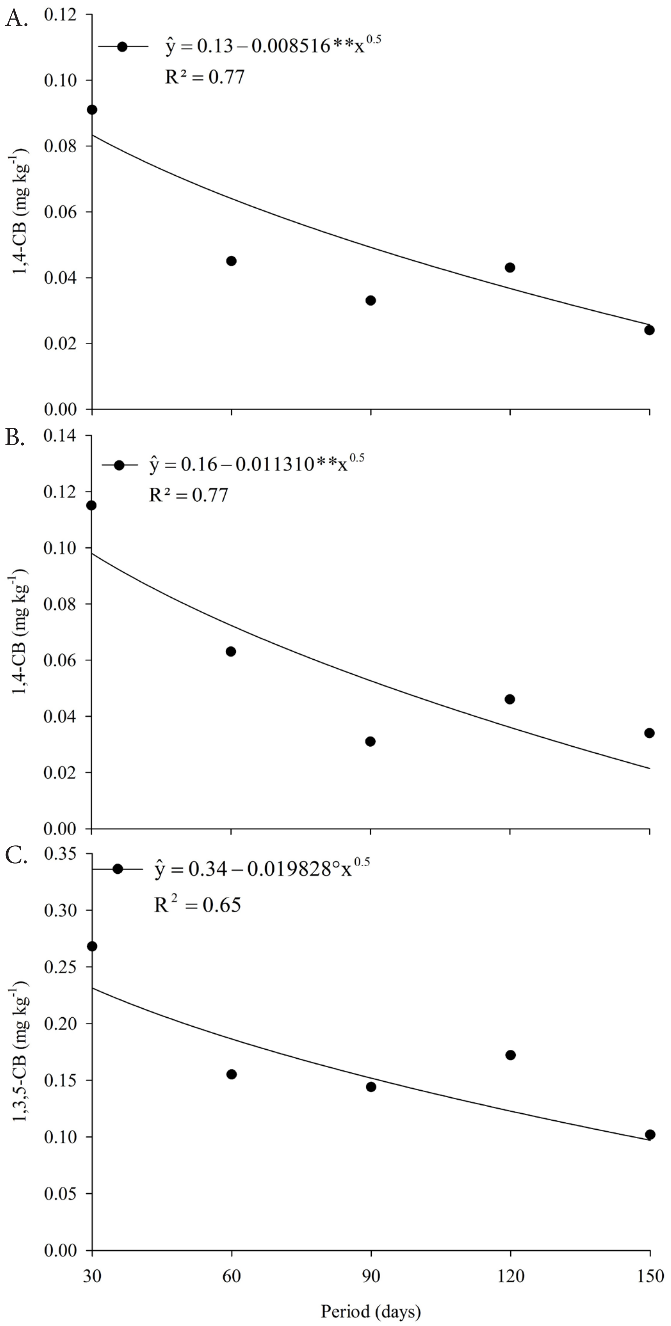
After 150 days of experiment, almost all treatments, except only the treatment of 1,3,5-CB with cultivation in sludge for 120 days, in the layers from 30 to > 40 cm, were below the acceptable critical concentrations established by the CONAMA Resolution No. 375 (BRASIL, 2006). Although the CBs concentrations were very low, in almost all treatments, there is always the necessity of a constant monitoring to avoid damages to the environment and risk to animal health, because in tropical systems the high temperatures and high humidity favor a rapid decomposition of the organic matter, requiring constant application of this waste in the soil.

The persistence of these two CBs can be related to their chemical structures, which are known to be recalcitrant. CBs are halogenated compounds, with aromatic nucleus, which promote greater stability in the compost and limit its chemical and microbial degradation (Häggblom et al., 2006).

The persistence of these contaminants in the sewage sludge can limit the agricultural use of the waste because some plant species, such as wheat and tomato, can absorb and translocate CBs to the shoots (Su & Liang, 2011). These chlorinated organic compounds are highly volatile and soluble in fat (Li et al., 2014), exhibiting high toxicity to humans, possibly entering the food chain and accumulating along it (Monferrán et al., 2005). The symptoms observed in living organisms exposed to the contaminants are agitation, tremors and muscle spasms; in the long term, they have carcinogenic, teratogenic and mutagenic effects, besides affecting the central nervous system (Moreira et al., 2012).

The concentrations of 1,4-CB, which initially were already low, in the layers of 0-10 and 30-40 cm were not influenced by the cultivation periods, remaining with means of 0.06 and 0.07 mg kg^{-1}, respectively, along the entire experimental period. Likewise, there was no variation in the concentrations of the contaminant 1,3,5-CB in the layers of 0-10, 10-20 and 30-40 cm in sewage sludge cultivated for different periods, remaining with means of 0.15, 0.18 and 0.28, respectively. Possibly, the high content of organic matter in the sewage sludge preserved the CBs from the decomposition, since they can be strongly adsorbed by this matrix, which limits their mineralization by decomposer microorganisms (Lee et al., 2009). One way of trying to reverse this phenomenon would be through the increase in the cultivation period, favoring the mineralization of the sewage sludge and, consequently, exposing the CBs to degradation by the microorganisms native to the sewage sludge.

Another technique to accelerate the decomposition of CBs would be the addition of isolates of microorganisms with scientific evidence of action in the degradation of these contaminants. According to Nishino et al. (1992), isolates of the bacteria *Pseudomonas putida* were able to mineralize approximately 54% of the CBs contained in an environment contaminated by these compounds, in the period of 7 days.



*, **Significant at 0.10 and 0.01 probability levels, respectively, by t-test

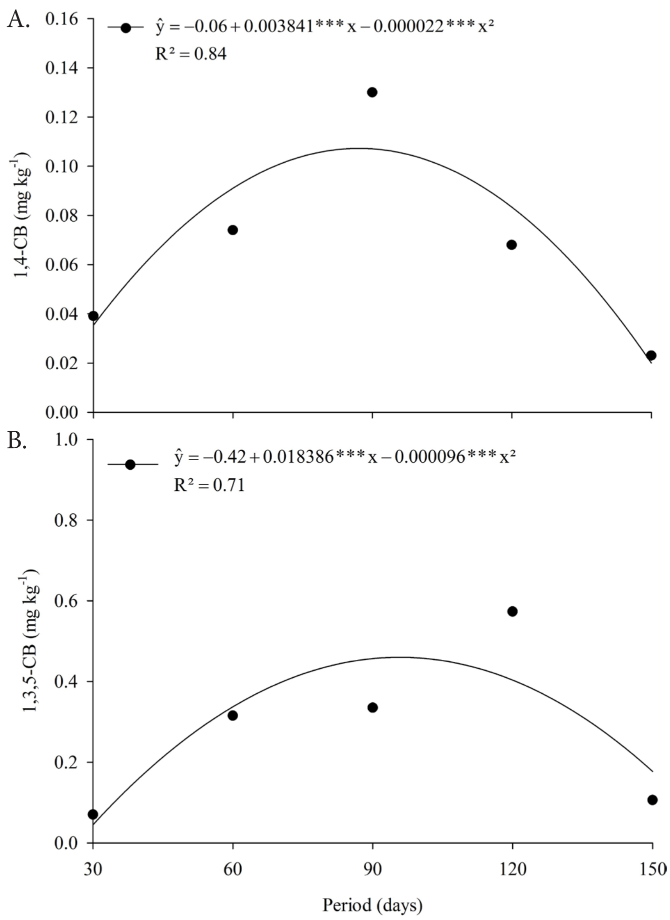
Figure 1. Concentrations of 1,4-CB (A and B) and 1,3,5-CB (C) in sewage sludge in the different periods of the experiment

Despite the great stability of CBs, some microorganisms found in the sewage sludge are able to degrade the contaminant (Guerin, 2008). In this case, the bacteria *Enterobacter* sp. SA-2 and *Pseudomonas* sp. SA-6 are able to use 1,3-chlorobenzene and 1,3,5-chlorobenzene as sources of carbon and energy (Adebusoye et al., 2007).

There were reductions in the contents of 1,4-CB in the layers from 10 to 30 cm (Figure 1A and B) and reductions in the contents of 1,3,5-CB in the layer of 20-30 cm (Figure 1C). In these cases, considering the cultivation period of 150 days, the minimum values observed for the 1,4-CB concentrations were 0.026 and 0.022 mg kg^{-1}, while for 1,3,5-CB the concentration was 0.097 mg kg^{-1}.

On the other hand, the grasses favor an adequate environment for the development of these decomposer bacteria. This plant-microorganism interaction can explain the reduction in the concentrations of CBs in the layers from 10 to 30 cm, because the roots were more concentrated at this depth. Some plant species can improve the chemical and physical conditions of the contaminated environments, promoting the increase in microbial activity (Ali et al., 2013). It is assumed, therefore, that these layers had a larger amount of bacteria responsible for the biodegradation of the contaminant.

At the depth > 40 cm, both evaluated CBs exhibited a very similar chemical behavior. In the period of up to 87 days for



***Significant at 0.1 probability level by t-test

Figure 2. Concentrations of 1,4-CB (A) and 1,3,5-CB (B) in sewage sludge in the different periods of the experiment

1,4-CB and up to 96 days for 1,3,5-CB, there was an increase in the concentrations of the contaminants and, immediately after, a decrease in their values in the sewage sludge (Figures 2A and B). These results can be explained by the mode of action of the decomposer microorganisms, which initially act on the organic matter of the sewage sludge, since it is a material of easy decomposition and high energetic value (Lerch et al., 1993).

Along the sewage sludge mineralization, however, there are losses of carbon, released in the form of CO_2, causing a decrease in the volume of the waste and, consequently, increase in the concentration of CBs. With the reduction of this process, the decomposers are believed to start acting on the organic pollutants, degrading these compounds. The minimum concentrations reached at the end of 150 days of *P. purpureum* cultivation, in the last layer, were 0.02 and 0.18 mg kg^{-1} of 1,4-CB and 1,3,5-CB, respectively.

Conclusions

1. *P. purpureum* cultivation in sewage sludge for 150 days promotes reductions in the concentrations of 1,3,5-CB in the layers with greater concentration of roots, compared with uncultivated sewage sludge, but it does not influence the concentration of 1,4-CB.

2. In general, the concentrations of 1,4-CB and 1,3,5-CB, in sewage sludge, decrease with the increase in the *P. purpureum* cultivation period; thus, cultivation for at least 150 days is advisable to maintain the levels of the contaminants within the safest limits for the agricultural use of the waste.

Acknowledgments

To the Minas Gerais Research Support Foundation (FAPEMIG) and National Council for Scientific and Technological Development (CNPq), for the financial support; to the Coordination for the Improvement of Higher Education Personnel (CAPES), for granting the scholarships and to the Minas Gerais Sanitation Company (COPASA), for providing the sewage sludge.

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