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Copper and zinc fractionation in biosolid cultivated with *Pennisetum purpureum* in different periods

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Key words:
sequential extraction
sewage sludge
speciation of heavy metals

ABSTRACT

In order to reduce the effect of heavy metals on the biosolid, it is necessary to promote its phytoremediation. It is important to know the total content and chemical forms of these elements in the residue for analyzing its behavior and potential toxicity. Thus, the aim of this study was to evaluate the fractionation and behavior of Cu and Zn in biosolid cultivated with *Pennisetum purpureum* in different periods. The experiment was carried out using a randomized complete block design. The treatments, with five replicates, corresponded to *Pennisetum purpureum* cultivation in biosolid for 30, 60, 90, 120 and 150 days after planting. The total contents of Cu and Zn in the biosolid remained below the critical limits established by the CONAMA Resolution 357, and there was a reduction in these values with *Pennisetum purpureum* cultivation. Furthermore, the increment in the grass cultivation period caused intense reduction of Zn contents bound to organic matter, but there was an increase in soluble Zn and residual Zn. Additionally, there was an intense reduction in the content of Cu bound to sulfides. Therefore, for biosolid phytoremediation purposes, the grass should be cultivated for 150 days.

Palavras-chave:
especiação de metais pesados
extração sequencial
lodo de esgoto

Fracionamento de cobre e zinco em biossólido cultivado com *Pennisetum purpureum* em diferentes períodos

RESUMO

Para atenuar o efeito da presença de metais pesados no biossólido, faz-se necessária a sua fitorremediação, sendo fundamental o conhecimento das concentrações totais e das formas químicas destes elementos no resíduo para fins de análise do seu comportamento e potencial toxicidade. Assim, objetivou-se avaliar o comportamento e fracionamento do Cu e Zn em biossólido cultivado com *Pennisetum purpureum* em diferentes períodos. O experimento foi realizado utilizando-se delineamento em blocos casualizados. Os tratamentos, em cinco repetições, corresponderam ao cultivo de *Pennisetum purpureum* em biossólido por 30, 60, 90, 120 e 150 dias a partir do plantio. As concentrações totais de Cu e Zn no biossólido ficaram abaixo dos limites críticos da Resolução CONAMA 375, havendo redução destes valores com o cultivo de *Pennisetum purpureum*. Além disso, o incremento do período de cultivo da gramínea causou intensa redução da concentração de Zn ligado à matéria orgânica; por outro lado, houve aumento do Zn solúvel e residual. Já para o Cu houve intensa redução da concentração do elemento ligado a sulfetos. Assim, para fins de fitorremediação do biossólido, recomenda-se o plantio da gramínea por 150 dias.

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INTRODUCTION

Biosolids have been used in agriculture with good results (Nascimento et al., 2015). However, in tropical agricultural systems, the high humidity and temperature favor the intensification of organic matter degradation, causing the necessity of constant addition of this residue to the soil. This fact increases the risk of environmental contamination, because biosolids may contain high contents of heavy metals.

Procedures of sequential extraction are important and have been used to determine the total and available contents of metals associated with the exchangeable fraction up to those that are strongly bound to the residual fraction. This technique also allows more-detailed access to the distribution of these elements in the soil, their mobility, bioavailability and potential toxicity (Souza et al., 2012).

It is worth pointing out that the variation in the biosolid chemical composition is related to the origin, collection period and forms of treatment and stabilization, which leads to a wide multiplicity of results. Thus, studies on biosolids require more details, in order to establish safe information regarding its use, especially with respect to the necessity of practices of reduction in the contents of heavy metals, such as phytoremediation, to pose lower risk to the environment.

In this context, this study aimed to evaluate the different chemical forms of copper and zinc in biosolid, of the city of Montes Claros/MG, cultivated with *Pennisetum purpureum* in different periods.

MATERIAL AND METHODS

The experiment was carried out in a screened greenhouse, from September 2013 to March 2014, at the Institute of Agricultural Sciences of the Federal University of Minas Gerais (ICA/UFMG), campus of Montes Claros-MG, Brazil (16º 51’ 38” S; 44º 55’ 00” W; 652 m). According to Köppen’s classification, the predominant climate in the region is Aw - tropical savanna, with rainy summer and dry winter. The experimental units were built with dimensions of 1.0 x 1.0 x 0.5 m, coated with polyethylene to avoid slurry leaching, containing 0.5 m^3 of biosolid.

The biosolid was collected in the Sewage Treatment Station of Montes Claros, in September 2013. This city is known for being a regional center, because it has better infrastructure of health, commerce and service provision in general, besides having an industrial center. The produced sewage is processed as follows: the sewage is directed to the percolating biofilters, passing through the microbial decomposition process, in which organic matter is reduced by 90%. The liquid resulting from the process is centrifuged and, immediately after, transported to the thermal dryer, where it is subjected to temperatures of 350 °C for 30 min, being converted into granular material (pellets), showing the following contents and fractionation of Cu and Zn, and pH (Table 1).

The experiment was set in a randomized complete block design, with five replicates, and the treatments corresponded to 30, 60, 90, 120 and 150 days of cultivation from the planting of *Pennisetum purpureum* Schum. in biosolid. 20-cm-long cuttings were planted at depth of 10 cm, spaced by 20 x 20 cm, totaling 25 cuttings, with one bud each, per plot. This species was chosen for its high biomass production, promoted by the efficient fixation of atmospheric CO_2 (Flores et al., 2012).

Table 1. Chemical forms and contents of Cu and Zn, and pH in the biosolid before installing the experiment

Variable	Cu	Zn
	(mg kg^{-1})	
Exchangeable form	15.59 ± 2.93	19.43 ± 6.23
Soluble form	8.47 ± 0.69	11.39 ± 3.81
Form bound to organic matter	67.83 ± 1.75	168.33 ± 27.39
Form bound to carbonates	50.92 ± 6.24	230.00 ± 48.84
Form bound to sulfides	59.06 ± 12.58	96.37 ± 26.53
Residual form	53.77 ± 16.88	108.07 ± 39.63
Total	255.51 ± 0.41	633.59 ± 19.36
pH (1:2.5 in water)	6.67 ± 0.09	

Confidence interval of the mean by t-test at 0.05 probability level.
Sequential extraction: methodology of Sposito et al. (1982)

Irrigation depth was daily applied to maintain the substrate moisture close to field capacity (26.12% of volumetric moisture). The total water depth applied corresponded to 459 mm, which was estimated considering Elephant grass evapotranspiration of 0.85 (Lopes et al., 2003).

At the end of each cultivation period, plants were collected whole to determine total dry biomass and the biosolid was homogenized to collect the samples. Both plant and biosolid samples were dried in a forced-air oven at 65 ºC until constant weight.

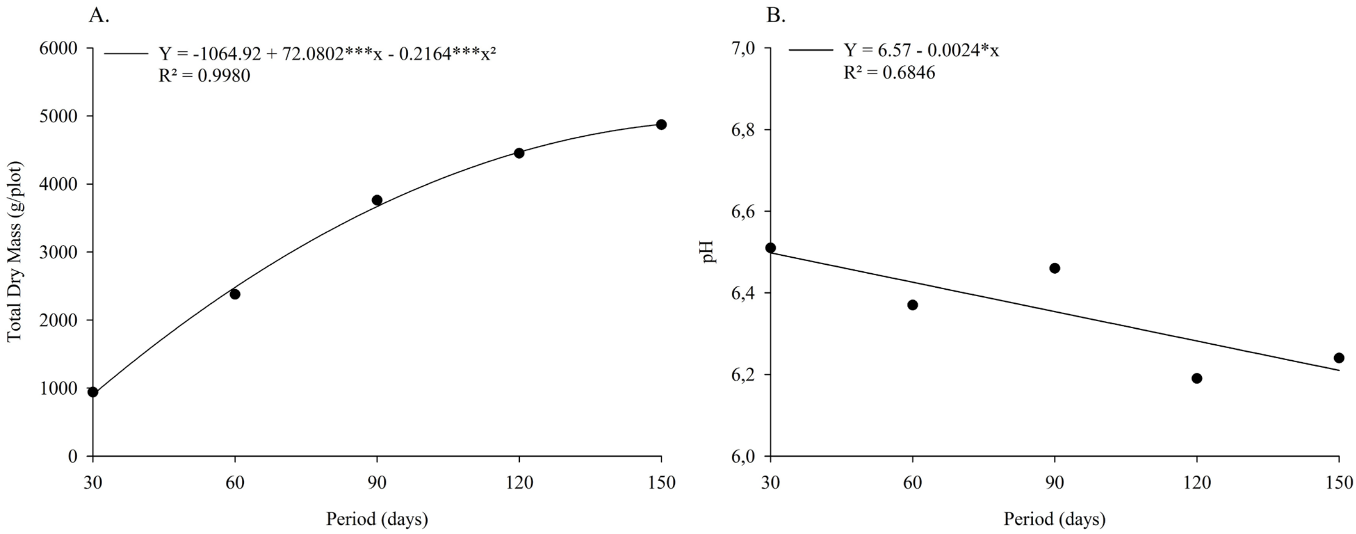
Biosolid pH was determined in water (1:2.5). Sequential extraction was performed according to the methodology of Sposito et al. (1982), which extracts metals from the exchangeable fraction, with 0.5 mol L^{-1} KNO_3; from the soluble fraction, with distilled water; from the organic matter fraction, with 0.5 mol L^{-1} NaOH; from the carbonate fraction, with EDTA (0.05 mol L^{-1}); from the sulfide fraction, with 4.0 mol L^{-1} HNO_3; and from the residual fraction, with the acids HF + HNO_3 (p.a.). In plant tissue, total extraction was performed according to the methodology of USEPA-3051 (EPA, 1994). Cu and Zn readings in the different samples were taken using an atomic absorption spectrophotometer (Varian, model AA 240).

The data were subjected to analysis of variance and related to the cultivation period through regression, testing the coefficients up to 0.1 probability level by t-test. For the variables not fitted to regression models, confidence intervals of the means were calculated at 0.10 probability level by t-test.

RESULTS AND DISCUSSION

Total dry mass increased with the increment in *P. purpureum* cultivation period (Figure 1A). Such increase evidences a good supply of the nutrients of the biosolid to the plant, without causing visual symptoms of toxicity. However, the highest growth rates occurred until 119 days, when total dry biomass production reached 90% of the maximum production.

Biosolid pH decreased linearly as the cultivation period increased (Figure 1B). The process of organic matter nitrification and presence of organic and inorganic acids released by microorganisms and roots may have contributed to the reduction in the pH values along the cultivation (Silva et



*Significant at 0.05 probability level by t-test

Figure 1. Total dry mass of the plant and biosolid pH as a function of the cultivation period

al., 2001). As reported by Caldeira Júnior et al. (2009), in soils fertilized with biosolid, there is a reduction of pH due to the presence of acid substances and to the processes of nitrification and oxidation of sulfides of this residue.

Nonetheless, although plants release acids into the rhizosphere, the buffering character of the organic matter prevents variations of pH. This characteristic is important because, under lower pH conditions, there might be greater availability of heavy metals to plants (Nachtigall et al., 2009). The results found for pH are satisfactory, because very alkaline biosolids require great caution in their application in the soils, since the soil-biosolid mixture must have at least pH 7.0, as established by the CONAMA Resolution 375 (Brasil, 2006).

Likewise, the total contents of Cu and Zn found in the biosolid used in the present study varied, respectively, from 158.15 to 172.16 mg kg^{-1} and from 407.24 to 587.50 mg kg^{-1}, respecting the critical limits established by the CONAMA Resolution 375 (Brasil, 2006), which are 1,500 mg kg^{-1} for Cu and 2,800 mg kg^{-1} for Zn.

The analysis of the exchangeable fraction evidenced the difference in the chemical behavior of Cu and Zn. The cultivation period contributed to the changes in the behavior of exchangeable Zn (Figure 2A), whose content increased until 97 days, possibly supplied by other chemical forms of the element. After this period, there was a decrease in the Zn content, which can be associated with the greater absorption by *P. purpureum*. Alvarenga (2015) highlights that the highest leaf biomass production of this species occurs at 139 days of cultivation in biosolid and Zhang et al. (2010) report that plants of the genus *Pennisetum* exhibit great capacity of Zn phytoextraction, which can partially justify its reduction in the exchangeable form.

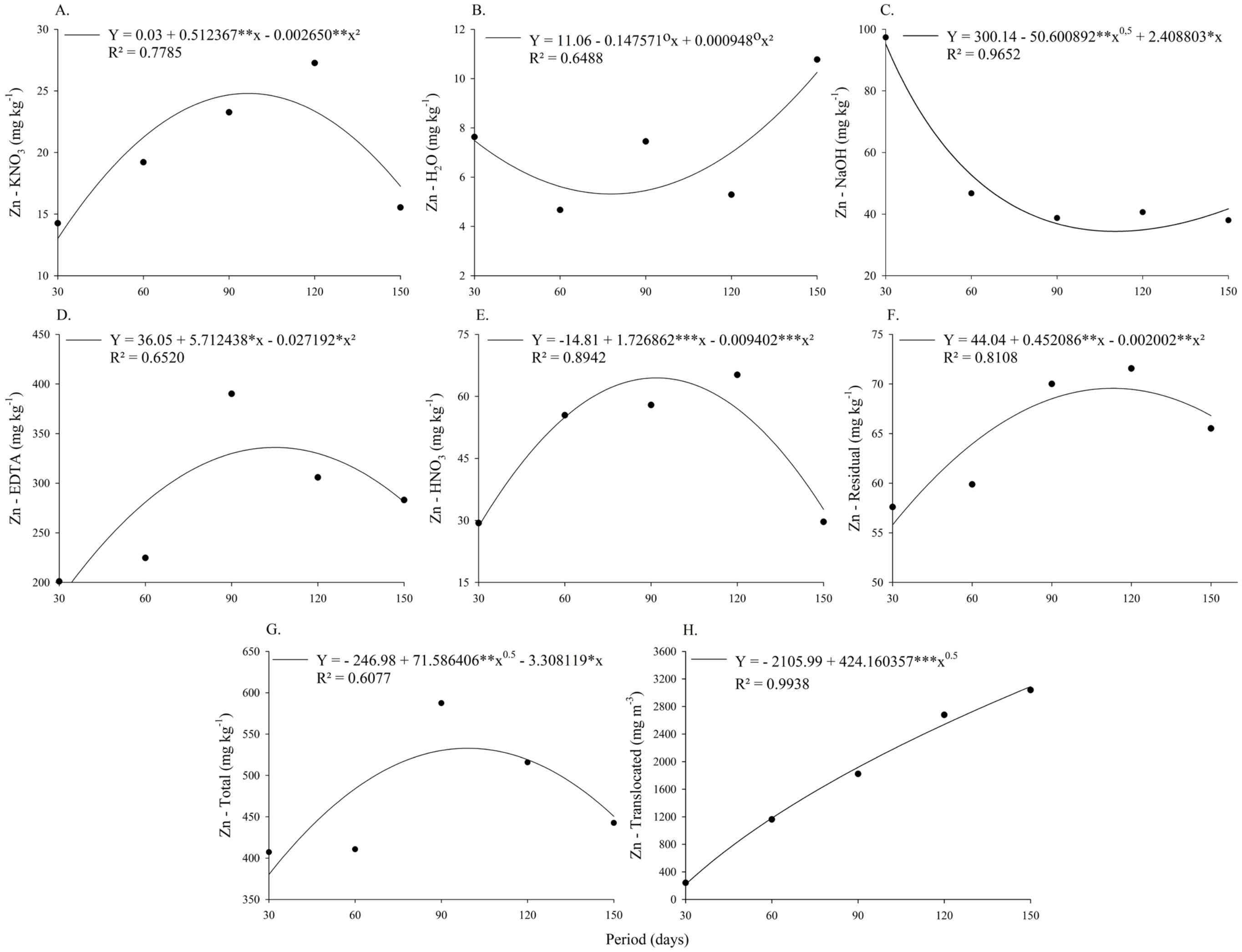
On the other hand, it was observed that the cultivation period did not influence the behavior of Cu in the exchangeable fraction, and its content remained constant along the experimental period (Figure 3A). Since Cu is an essential element to plants (Li et al., 2013), there must have been an adequate supply to the grass, because no symptoms of deficiency or phytotoxicity were observed. The results found for the biosolid are consistent with those of Chan et al. (2008), who report lower percentages of Cu for this fraction.

After 78 days from planting, there was an increment in the content of soluble Zn (Figure 2B), which became more available to plants, coinciding approximately with the period of reduction in the content of the exchangeable fraction, which presupposes that part of the soluble Zn may have been supplied by this fraction. At the end of the cultivation period, Zn content in the soluble form was about 10 mg kg^{-1}, coincident with the original content in the biosolid (Table 1). In general, the results in the literature show low percentages of heavy metals bound to the soluble fraction, in both composted and non-composted residues, being always in equilibrium with the exchangeable fraction (Amir et al., 2005; Zorpas et al., 2008).

For Cu soluble in water (Figure 3B), there was a slight reduction in the soluble contents with the planting of the grass, compared with its original condition (Table 1). Such fact mainly demonstrates the absorption of the nutrient by the plant, because the metals that are found in the soluble form, as free ions, soluble complexes with organic or inorganic anions, are easily absorbed by plants or leached. Nevertheless, as the cultivation period increased, the content of Cu in this form did not change much, reflecting that, although the plant absorbed part of the soluble element, with the reduction in the biosolid mass through decomposition, there was also an increase in its content, justifying the slight alteration of the content in this fraction with the cultivation.

The content of Zn bound to organic matter decreased along the grass growing period, reaching minimum value at 110 days of cultivation (Figure 2C). This fact can be attributed to organic matter decomposition and absorption of the nutrient by the plant, which showed intense growth rate along the entire cycle, besides the migration of the element to other chemical forms in the biosolid. As already mentioned, Zhang et al. (2010) report that plants of the genus *Pennisetum* have great capacity of Zn phytoextraction.

Based on the analysis of Cu bound to organic matter, its content remained constant (Figure 3C) along the entire cultivation period, with values lower than those originally



°, *, **, ***Significant at 0.10, 0.05, 0.01 and 0.001 probability levels by t-test, respectively

Figure 2. Zn contents in the fractions exchangeable (A), soluble (B), organic matter (C), carbonate (D), sulfide (E) and residual (F), total (G) and translocated from the biosolid to the plant (H), as a function of the cultivation period

found (Table 1). The process of organic matter decomposition along the cultivation period promoted the release of Cu in this form, for plant absorption or to compose other chemical forms, which explains the accentuated reduction of the element in this fraction.

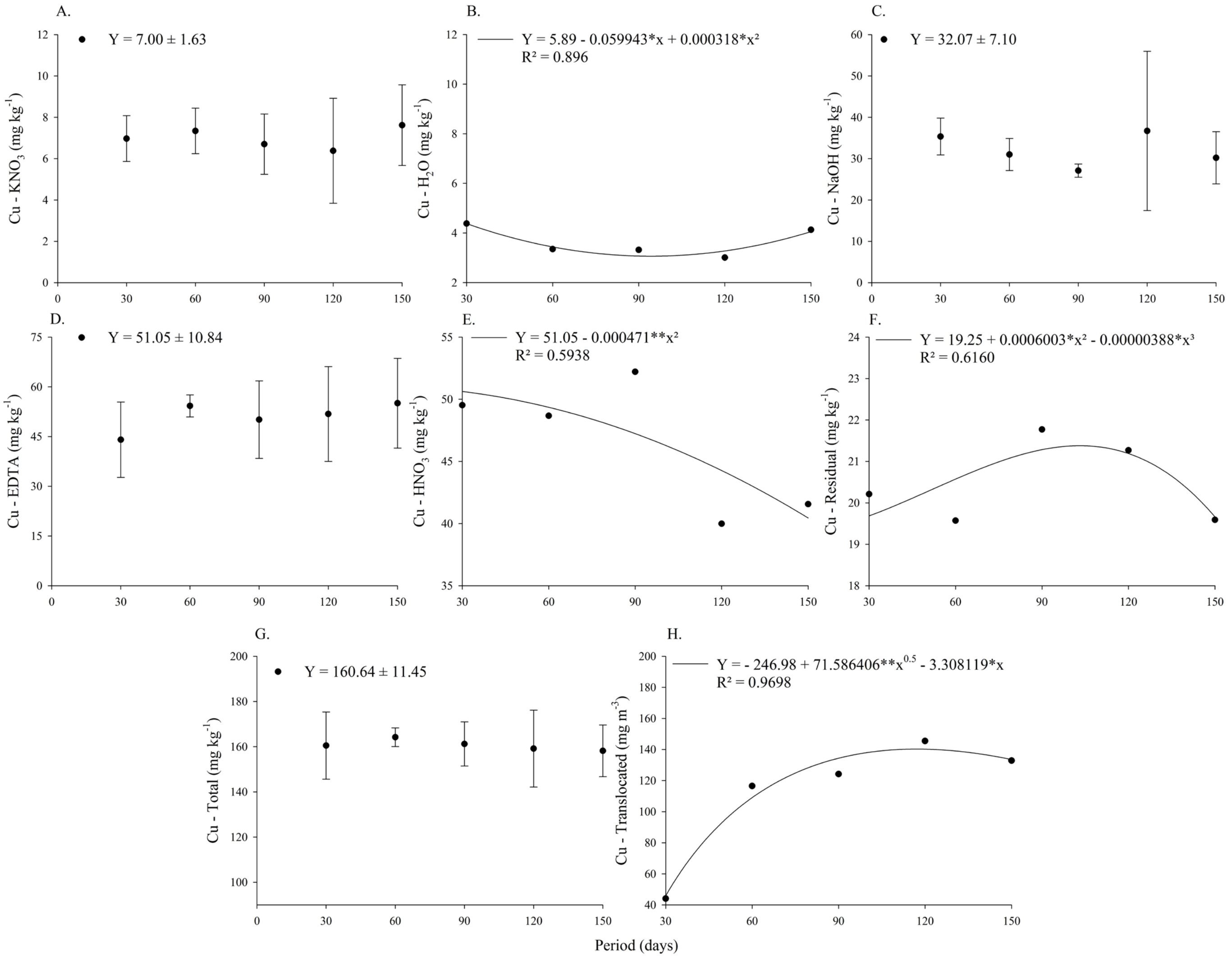
Among the studied fractions, the carbonate fraction showed the highest contents of Zn and Cu, with an increase in the content of Zn in this form, compared with the original content (Table 1) along the grass cultivation in the biosolid.

It should be highlighted that, since the municipality of Montes Claros is located in a karstic region (Sousa, 2013), there is a lot of carbonate dissolution in the water treated in the station and in the irrigation system used in the experiment. This fact is important, because it leads to lower risk of phytotoxicity or environmental contamination, since Zn and Cu are mostly precipitated with carbonates in the biosolid, making this fraction an important drain for Cu and Zn.

With the cultivation in the biosolid, the content of Cu bound to carbonates remained constant along the entire plant growth period (Figure 3D), which allows the interpretation that a small amount of the element in this form became available for absorption by plants. Oliveira et al. (2009) highlight the precipitation of Cu with carbonates as one way of maintaining the metal unavailable for reactions. The content of Zn bound to carbonates increased with the cultivation period until 105 days (Figure 2D), indicating that part of the element, which was found in other more labile chemical forms, such as water-soluble and exchangeable, changed to more stable ones, such as the carbonate form.

Regarding the sulfide fraction, it is observed that Cu and Zn showed different behaviors. For Zn bound to this fraction, there was a rapid increase in its content until 92 days (Figure 2E). After this period, there was a fast reduction, possibly associated with the change in the binding form of the element, its higher absorption by the plant and with the fact that sulfur is in constant transformation, involving oxidoreduction reactions promoted by microorganisms.

In the sulfide fraction, Cu showed reduction with the cultivation of the plant (Figure 3E). This fact can be attributed to the absorption of the element by the plant and to its mobilization to other less available chemical forms over the time of cultivation. It also explains its reduction with the grass cultivation, compared with the original content in the biosolid (Table 1). Ito et al. (2000) and Hullebusch et al. (2005) point out that, in biosolid, a large portion of Cu may be bound to sulfides.



*, **Significant at 0.05 and 0.01 probability level by t-test, respectively

Figure 3. Cu contents in the fractions exchangeable (A), soluble (B), organic matter (C), carbonate (D), sulfide (E) and residual (F), total (G) and translocated from the biosolid to the plant (H), as a function of the cultivation period

In the analysis of the residual fraction, compared with the original contents of Cu and Zn in the biosolid (Table 1), there was accentuated reduction in their contents in this chemical form with the grass cultivation. However, considering only the different growth periods, the cultivation in the biosolid led to increment in Cu and Zn contents, in the residual form, and the highest contents of the elements in this form occurred, respectively, at 103 and 114 days after planting, decreasing immediately thereafter (Figure 2F, 3F).

In this context, it is noted that, with the cultivation in the biosolid and decomposition of the organic matter, part of the Cu and Zn in the residual form became available to plants and to other chemical forms, occurring intensely after 100 days of cultivation. Nevertheless, the metals of this form are not much available to plants, under the normal conditions of nature, because they are chemically stable.

In a study on the fractionation of metals in biosolid, Alonso et al. (2006) observed that only 7% of the heavy metals were present in forms easily available to humans, while 90% were retained by the biosolid, with low risk of contamination. However, these authors point out that Zn was one of the elements that showed greater mobility and bioavailability in this residue.

Total Cu content in the biosolid remained constant along the *P. purpureum* cultivation period (Figure 3G). However, Zn content increased, reaching maximum value at 99 days of cultivation and, from this point on, it decreased reasonably until 150 days (Figure 2G).

Comparing the total contents of Cu and Zn in the biosolid, after *P. purpureum* cultivation, with their original contents in the biosolid (Table 1), it is noted that the final values remained around 63 and 71% of the initial ones, respectively for Cu and Zn at 150 days of cultivation. Such fact, naturally occurred due to the absorption of these elements by plants, made the biosolid even safer for agricultural use.

There was an increment in the quantities of Cu and Zn translocated from the biosolid to the plant with the increase in the cultivation period. Thus, at 117 days of *P. purpureum* cultivation, Cu translocation reached maximum value, of the order of 140.30 mg m^{-3} of biosolid (Figure 3H). This period of higher Cu accumulation in the plant is close to the period of highest leaf biomass production (Alvarenga, 2015), whose maximum value occurred at 139 days of cultivation. Regarding the quantity of Zn, the fit of the response curve over time was almost increasing linear, reaching maximum value of the element of 3,089 mg m^{-3} of biosolid, at 150 days of cultivation

(Figure 2H). Hence, it can be claimed that its translocation from the biosolid to the plant was intensified due to the nutritional requirement of the plant, increased quantity of the metal in the residue, greater availability of the element, high biomass production and organic matter decomposition, favored by the growth of the root system.

There was greater accumulation of Zn in the plant compared with Cu, which can be attributed to its higher content in the biosolid, besides a possible higher mobilization of Zn bound to organic matter (Figure 2C). Anyway, it was observed that the biosolid, despite the high total contents of these elements (Table 1), always promoted levels available in quantities that did not cause phytotoxicity in *P. purpureum*. These results corroborate with those of Liu et al. (2009), who found that this grass was able to develop in Cu-contaminated soils, tolerating a contamination of up to 1,500 mg kg^{-1} of Cu, without causing decrease in dry mass production.

Conclusions

1. The biosolid has total Cu and Zn contents below the critical limits established by the CONAMA Resolution 375/2006, with reductions in their contents along the *Pennisetum purpureum* cultivation for a period of 150 days.

2. The increase in *Pennisetum purpureum* cultivation period promotes a reduction in the content of Zn bound to organic matter. On the other hand, there is an increment in the contents of soluble and residual Zn, but with a small decrease of this latter at 150 days of cultivation.

3. For Cu, with the increase in *Pennisetum purpureum* cultivation period, there is a reduction in the content of the element bound to sulfides and a slight initial increment, with later reduction of the content of the metal in the residual form, at 150 days of cultivation.

4. The maximum quantity of Cu extracted from the biosolid by *Pennisetum purpureum* occurs at 117 days of cultivation, while for Zn it occurs at 150 days. Thus, for biosolid phytoremediation purposes, the grass should be cultivated for at least 150 days.

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