Heavy metal and fertility in a Tropical Oxisol amended with sewage sludge under *Eucalyptus* plantation

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**Abstract.** The objectives of our study were to evaluate i) the soil fertility and fractionation of Ba, Cd, Cu, and Zn in the topsoil layer (0-0.20 m depth); and ii) production and concentration and accumulation of Ba, Cd, Cu, and Zn in the components of *Eucalyptus* trees at 36 months after sewage sludge (SS) application, with or without mineral P fertilizer, compared to mineral fertilization. Application of SS (at N criteria) with P increased soil organic matter and heavy metal concentrations, which were mostly bound to the oxidic and organic matter fractions. SS provided *Eucalyptus* production and heavy metal concentrations and accumulation in the trunk, branches, and leaves similar to mineral fertilization for high wood production. The application of SS (at N criteria) supplied with P increased soil heavy metal, fertility, and *Eucalyptus* production, without risk of environmental contamination.

**Keywords:** Biosolid, Forest management, Organic fertilizer, Potentially toxic elements, Sequential extraction.

1. Introduction

The presence of heavy metals in sewage sludge (SS) can prevent or restrict its use in agriculture and forestry. However, the potential for using SS in *Eucalyptus* plantations is great [2] since the products of these operations are not intended for human or animal consumption. After its application, SS interacts with soil particles and properties. During this process, heavy metals can bind to different soil components, such as organic matter, clay minerals, and/or remain in soil solution, changing their availability. Thus, sequential extraction methods can assist in the interpretation of the behavior of heavy metals in soil [8].

In *Eucalyptus* plantations, an increase in heavy metal concentrations and soil mobility have been observed but without toxic effects [1]. There may be a residual effect of heavy metals of up to 17 years after SS application [10]. In this context, *Eucalyptus* components have a high capacity to accumulate elements, which presents potential phytoremediation of soil in contaminated areas, with a significant advantage when cultivated for wood production since heavy metal return to the soil is limited [13].
We hypothesized that when following recommended plantation management criteria, SS application (i) does not contaminate soils with heavy metals; (ii) increases soil fertility and Eucalyptus wood production; (iii) and that there is a greater accumulation of metals in the wood in comparison to canopy. Our objectives were to evaluate heavy metal concentrations and fractionation in the topsoil layer (0-0.20 m depth); heavy metal accumulation and concentration in different Eucalyptus components at 36 months after SS application with or without mineral P fertilizer in comparison to mineral fertilizer application for high wood production.

2. Materials and methods

2.1. Experimental area and materials characterization

The experiment was installed in February 2015 in a commercial Eucalyptus plantation (Boa Esperança do Sul, State of São Paulo; 21°57’ S and 48°31’ W), on a soil classified as Typic Hapludox [18]. Eucalyptus production represents the only land use during the last 40 years and SS was never applied before the experiment. The climate is classified as Cwa, i.e., humid subtropical zone with hot summer and dry winter [4]. Class B SS was obtained from the Jundiaí wastewater treatment plant [7]. The detailed soil and SS physical-chemical attributes are presented in Abreu-Junior et al. (2020) [3].

2.2. Field procedures

Minimum tillage started in January 2015, which consisted of subsoiling to 0.40 m depth between the lines of the previous rotation over the entire area. After tillage, lime was uniformly broadcasted on the soil surface at 1.8 Mg ha⁻¹ to supply Ca and Mg. Eucalyptus seedlings were manually planted in February 2015. The genetic material used was a hybrid, produced via vegetative clonal propagation, from the cross between Eucalyptus grandis and Eucalyptus urophylla (E. urophylla, clone SP5727), spaced 3.00 x 2.25 m apart, totaling 1,481 trees per hectare.

The experiment was installed in a randomized complete block design with six treatments and four replications, totaling 24 experimental plots, each consisting of 100 plants (10 x 10 plants; 675 m²), with the 36 central trees corresponding to the data collection area (243 m²). Treatments consisted of the following: 1. Control, without fertilization (C); 2. Mineral fertilization for high wood production, as recommended by Suzano S.A. Company (MF); 3. 14.5 Mg ha⁻¹ of SS + 22 kg ha⁻¹ of P (S1P1); 4. 29 Mg ha⁻¹ of SS (S2); 5. 29 Mg ha⁻¹ of SS + 17.5 kg ha⁻¹ of P (S2P2); and 6. 43.5 Mg ha⁻¹ of SS (S3).

Triple superphosphate was applied to the planting pit next to the seedling. Seven months after planting, SS was applied in a continuous 0.6 m wide band, without incorporation into the soil, 0.2 m aside the planting rows. Sludge doses were calculated according to N criteria, following CONAMA Resolution No. 375 [6], to provide 192 kg ha⁻¹ of N, as recommended by the Suzano S.A. Company. Thus, the doses of 14.5, 29.0, and 43.5 Mg ha⁻¹ of SS, on a dry weight basis, were equivalent to 50, 100, and 150% of the recommended N. Doses of 22 and 17.5 kg ha⁻¹ of P were applied to provide 83 and 66% of mineral P recommendation (26 kg ha⁻¹), respectively. All treatments, except C, were supplemented with mineral K (165 kg ha⁻¹ K₂O) and B (6.5 kg ha⁻¹ B). Detailed nutrient application is presented in Abreu-Junior et al. (2020) [3].

2.3. Soil sampling, preparation, and analysis

Soil samples were collected in September 2018, 36 months after SS application (42 months after planting), at the depth of 0-0.20 m, considering superficial SS application. Each soil sample consisted of four subsamples collected per plot in the planting rows and interrow, after carefully removing fallen organic material. In the laboratory, samples were air-dried and sieved at 0.5 mm prior to analysis.

2.3.1. Soil fertility. Soil fertility analyses were performed according to Raij et al. (2001) [17]. Soil pH was measured in a soil/solution mixture of 1:2.5 1 mol L⁻¹ CaCl₂. Soil organic matter (SOM) concentration was estimated by the Walkley–Black method. Available soil P (P-resin) was extracted by an ion-exchange resin procedure and determined by the colorimetric method. Cation exchange capacity (CEC) was equal to Ca²⁺ + Mg²⁺ + K⁺ + (H+Al).
2.3.2. Soil heavy metal. The levels of heavy metals were extracted using the 3051A method [20], with concentrated nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) in a closed microwave system. Element (Ba, Cd, Cu, and Zn) determinations were performed by mass spectrometry with inductively coupled plasma (ICP-MS).

2.3.3. Soil sequential extraction. Soil fractionation was conducted as described by Colzato et al. (2018) [8]. The fractions evaluated were: F1 - soluble and/or exchangeable fraction (extracted with CaCl₂); F2 - carbonated fraction (extracted with NaOAC at pH 5); F3 - organic matter fraction (extracted with NaOCl at pH 8.5); F4 - oxides fraction (extracted with ammonium oxalate and oxalic acid at pH 3.0); F5 - residual fraction, determined by the 3050B method [19]. The elementary (Ba, Cd, Cu, and Zn) determinations were performed by optical emission spectrometry with inductively coupled plasma (ICP-OES).

2.4. Wood production assessment and plant analysis
Circumference at breast height (CBH) of all trees in the inner plot was measured at 42 months after planting. These data were used to estimate the volume of wood and biomass of wood, branches, and leaves according to methods presented by Abreu-Junior et al. (2020) [3]. The CBH data were also used to select one tree per plot for a non-destructive sampling of trunk, branches, and leaves to assess heavy metal concentrations.

Plant samples were washed with distilled water and then dried in a forced air oven at 60 °C to a constant weight. Then, the samples were ground in a Willey knife mill and digested with concentrated nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) in a closed microwave system. Element (Ba, Cd, Cu, and Zn) determinations were performed by mass spectrometry with inductively coupled plasma (ICP-MS).

2.5. Statistic analysis
Statistics were conducted using the software R [16]. Differences among treatments were compared using a one-way analysis of variance by the F test on each measured variable and, when a significant effect was observed, multiple comparisons were assessed by a Duncan’s post hoc test (p < 0.05).

3. Results
Soil pH, P-resin, and CEC did not differ among treatments, ranging from 4.7 to 5.4, 3.1 to 8.1 mg dm⁻³, and 26 to 34 mmol dm⁻³, respectively (Table 1). After 36 months of SS application, soil organic matter (SOM) was 27% higher in the S2P2 treatment (10.0 g dm⁻³), relative to other treatments. Wood volume production and wood, branch, and foliar biomass production increased after 36 months of SS application (Table 1). Wood volume production was higher in the S2 (203 m³ ha⁻¹), S2P2 (210 m³ ha⁻¹), and S3 (206 m³ ha⁻¹) treatments than the control (188 m³ ha⁻¹), but similar to mineral fertilization (199 m³ ha⁻¹). In general, wood, branches, and foliar biomass production were higher in the S2P2 treatment than the control and S2, but similar to mineral fertilization.

Table 1. Topsoil fertility (0-0.20 m depth) and *Eucalyptus* production after 36 months of sewage sludge application.

| Treat. | pH | SOM g dm⁻³ | P-resin mg dm⁻³ | CEC mmol dm⁻³ | Volume m³ ha⁻¹ | Trunk Mg ha⁻¹ | Branches Mg ha⁻¹ | Foliar Mg ha⁻¹ |
|--------|----|------------|----------------|---------------|----------------|---------------|-----------------|----------------|
| C      | 4.8 a | 8.0 b | 3.1 a | 26.7 a | 188 b | 69.8 c | 4.7 c | 7.6 c |
| MF     | 5.4 a | 8.1 b | 6.4 a | 26.7 a | 199 ab | 74.4 ab | 5.0 bc | 8.2 ab |
| S1P1   | 4.7 a | 7.8 b | 4.1 a | 26.0 a | 200 ab | 73.4 bc | 5.0 ab | 8.2 ab |
| S2     | 5.1 a | 7.8 b | 4.2 a | 29.0 a | 203 a | 73.3 bc | 5.0 bc | 8.2 b |
| S2P2   | 4.9 a | 10.0 a | 5.3 a | 34.5 a | 210 a | 78.4 a | 5.3 a | 8.7 a |
| S3     | 5.4 a | 7.6 b | 8.1 a | 34.3 a | 206 a | 76.5 ab | 5.2 ab | 8.5 ab |
Soil concentration of heavy metals increased with SS application, but there was no difference for Cu among the treatments, ranging from 2.81 to 4.27 mg kg\(^{-1}\) (Table 2). Soil Ba concentration was 43% higher in the S3 (4.65 mg kg\(^{-1}\)) treatment in relation to C, S1P1, and S2 (3.25 mg kg\(^{-1}\)), but similar to MF and S2P2 (4.02 mg kg\(^{-1}\)). The highest Cd concentration was observed in the S3 treatment (0.016 mg kg\(^{-1}\)), but similar to S2P2 (0.014 mg kg\(^{-1}\)) and higher than the control, MF, S1P1, and S2 (0.009 mg kg\(^{-1}\)). Soil Zn concentration was higher in the S3 (5.64 mg kg\(^{-1}\)) treatment than the C, S1P1, and S2 (3.43 mg kg\(^{-1}\)), but similar to MF and S2P2 (4.88 mg kg\(^{-1}\)).

### Table 2. Heavy metals concentration in the 0-0.20 m soil depth cultivated with *Eucalyptus* after 36 months of sewage sludge application obtained by the means of acid digestion and sequential extraction.

| Treat. | Acid Digestion | Exchangeable | Carbonated | Organic Matter | Oxides | Residual |
|--------|----------------|--------------|------------|----------------|--------|----------|
| C      | 3.13 c         | 1.93 a       | 0.85 a     | 1.19 b         | 20.7 a | 1.26 a   |
| MF     | 4.13 ab        | 2.77 a       | 0.99 a     | 1.60 b         | 20.3 a | 1.49 a   |
| S1P1   | 3.36bc         | 2.23 a       | 0.94 a     | 1.15 b         | 20.0 a | 1.25 a   |
| S2     | 3.26bc         | 2.10 a       | 0.96 a     | 1.27 b         | 20.0 a | 1.63 a   |
| S2P2   | 3.91 abc       | 2.75 a       | 1.10 a     | 1.92 ab        | 19.6 a | 1.37 a   |
| S3     | 4.65 a         | 2.75 a       | 1.12 a     | 2.52 a         | 20.4 a | 1.56 a   |
| CV (%) | 16.0           | 21.4         | 13.1       | 32.4           | 2.4   | 29.5     |

| Treat. | Acid Digestion | Exchangeable | Carbonated | Organic Matter | Oxides | Residual |
|--------|----------------|--------------|------------|----------------|--------|----------|
| C      | 0.009 bc       | 0.025 a      | < 0.01     | 0.039 a        | 0.340 a| < 0.01   |
| MF     | 0.010 bc       | 0.020 a      | < 0.01     | 0.031 a        | 0.303 a| 0.050    |
| S1P1   | 0.006 c        | 0.036 a      | < 0.01     | 0.023 a        | 0.303 a| < 0.01   |
| S2     | 0.010 bc       | 0.019 a      | < 0.01     | 0.040 a        | 0.325 a| < 0.01   |
| S2P2   | 0.014 ab       | 0.017 a      | < 0.01     | 0.038 a        | 0.323 a| < 0.01   |
| S3     | 0.016 a        | 0.026 a      | < 0.01     | 0.036 a        | 0.294 a| < 0.01   |
| CV (%) | 33.1           | 56.5         | -          | 48.5           | 11.4  | -        |

| Treat. | Acid Digestion | Exchangeable | Carbonated | Organic Matter | Oxides | Residual |
|--------|----------------|--------------|------------|----------------|--------|----------|
| C      | 2.81 a         | 0.84 a       | 0.74 a     | 3.56 c         | 0.48 a | 3.43 a   |
| MF     | 3.43 a         | 0.81 a       | 0.55 a     | 4.46 abc       | 0.72 a | 3.11 a   |
| S1P1   | 2.66 a         | 0.84 a       | 0.55 a     | 3.90 bc        | 0.49 a | 3.80 a   |
| S2     | 3.01 a         | 0.92 a       | 0.58 a     | 3.72 a         | 0.54 a | 4.05 a   |
| S2P2   | 4.27 a         | 1.02 a       | 0.65 a     | 5.31 a         | 1.00 a | 2.98 a   |
| S3     | 3.95 a         | 1.02 a       | 0.53 a     | 5.51 a         | 0.98 a | 3.15 a   |
| CV (%) | 27.9           | 15.4         | 25.4       | 21.1           | 49.8  | 25.2     |

| Treat. | Acid Digestion | Exchangeable | Carbonated | Organic Matter | Oxides | Residual |
|--------|----------------|--------------|------------|----------------|--------|----------|
| C      | 3.34 c         | 0.98 a       | 0.25 a     | 4.18 bc        | 1.91 b | 3.61 a   |
| MF     | 4.90 ab        | 1.14 a       | 0.52 a     | 5.33 ab        | 2.01 b | 2.92 a   |
| S1P1   | 3.22 c         | 0.93 a       | 0.42 a     | 2.99 c         | 1.84 b | 2.91 a   |
| S2     | 3.72 bc        | 0.65 a       | 0.24 a     | 3.41 bc        | 2.08 b | 2.58 a   |
| S2P2   | 4.86 ab        | 0.54 a       | 0.40 a     | 4.40 bc        | 2.21 ab| 4.03 a   |
| S3     | 5.64 a         | 1.19 a       | 0.26 a     | 7.26 a         | 2.47 a | 3.59 a   |

Note: C – Control; MF – Mineral fertilizers; S1P1 – SS (50%) + P (83%) + B + K; S2 – SS (100%) - P + B + K; S2P2 – SS (100%) + P (66%) + B + K; S3 – SS (150%) - P + B + K; SOM – Soil organic matter; CEC – Cation exchange capacity. Means followed by the same letter did not differ by Duncan test (p < 0.05).

Sequential extraction of Ba in the soil showed statistical differences were limited to the organic matter fraction, where the highest levels were obtained in the S2P2 and S3 treatments, which were 71% higher than the other (Table 2). Cadmium was not detected by the analytic method used (< 0.01 mg kg\(^{-1}\)).
in the carbonated and residual fractions, and there were no differences among treatments in the exchangeable, organic matter, and oxides fractions. There were no statistical differences among treatments for Cu in the exchangeable, carbonated, oxides and residual fractions. However, in the organic matter fraction, Cu concentration was 77% higher in the S2, S2P2, and S3 treatments in relation to the control and S1P1. Soil Zn bound to the organic matter and oxides fraction had the highest level in the S3 treatment and was similar to the MF and S2P2 treatments, respectively.

There were no differences among treatments for Ba, Cd, Cu, and Zn concentration and accumulation in the trunk, branches, and leaves of Eucalyptus treated with SS, except for Zn accumulation in leaves, which was 23% higher in the S2 and S2P2 treatments in relation to the other (Table 3).

### Table 3. Heavy metals concentration and accumulation in Eucalyptus components after 36 months of sewage sludge application

| Treat. | Ba mg kg⁻¹ | Cd mg kg⁻¹ | Cu mg kg⁻¹ | Zn mg kg⁻¹ | Ba g ha⁻¹ | Cd g ha⁻¹ | Cu g ha⁻¹ | Zn g ha⁻¹ |
|--------|-------------|-------------|-------------|-------------|-----------|-----------|-----------|-----------|
|        | Concentration | Accumulation |
|        | Trunk | Branches | Leaves | Trunk | Branches | Leaves | Trunk | Branches | Leaves |
| C      | 2.39 a    | 0.0027 a  | 0.41 a    | 0.88 a  | 167.6 a  | 0.180 a  | 27.6 a  | 58.2 a  |        |
| MF     | 1.74 a    | 0.0018 a  | 0.37 a    | 0.53 a  | 134.6 a  | 0.132 a  | 26.4 a  | 41.4 a  |        |
| S1P1   | 1.68 a    | 0.0017 a  | 0.33 a    | 1.62 a  | 123.3 a  | 0.122 a  | 24.4 a  | 123.4 a |        |
| S2     | 1.58 a    | 0.0018 a  | 0.43 a    | 0.98 a  | 115.2 a  | 0.128 a  | 31.2 a  | 68.8 a  |        |
| S2P2   | 2.03 a    | 0.0024 a  | 0.51 a    | 2.29 a  | 162.8 a  | 0.191 a  | 29.5 a  | 190.7 a |        |
| S3     | 1.82 a    | 0.0022 a  | 0.33 a    | 1.05 a  | 138.5 a  | 0.160 a  | 25.2 a  | 83.2 a  |        |
| CV (%) | 30.1       | 38.2       | 36.3       | 82.8     | 38.4      | 38.8      | 38.5      | 90.1     |

Note: C – Control; MF – Mineral fertilizers; S1P1 – SS (50%) + P (83%) + B + K; S2 – SS (100%) - P + B + K; S2P2 – SS (100%) + P (66%) + B + K; S3 – SS (150%) - P + B + K; SOM – Soil organic matter; CEC – Cation exchange capacity. Means followed by the same letter did not differ by Duncan test (p < 0.05).

### 4. Discussion

After 36 months of SS application, there was an increase in SOM and P-resin, although this last one was not significant, with SS doses, without changing the soil pH and CEC (Table 1). With the improvement of soil fertility, the production of Eucalyptus components increased with the SS doses in relation to the control, mainly that one providing 100 and 66% of recommended N and P (S2P2), respectively, and similar production to the mineral fertilization (Table 1), corroborating the results obtained by Abreu-Junior et al. (2017, 2020) [2].
However, together with the input of macro and micronutrients, SS application also furnishes heavy metals to the soil, which can be absorbed by plants, possibly affecting crop development [10]. Despite the increased soil heavy metal concentrations provided by the application of the highest dose of SS (S3; Figure 1), concentrations of Ba, Cd, Cu, and Zn obtained in the soil were below the reference values of soil quality recommended by the Environmental Company of the State of São Paulo [7], which are 75, <0.5, 35, and 60 mg kg\(^{-1}\), respectively. The levels, obtained in this study, were also within the natural concentration of heavy metals in the soils of the state of São Paulo, which are from 2.9 to 548 mg kg\(^{-1}\) for Ba; 0.01 to 0.48 mg kg\(^{-1}\) for Cd; and 4 to 68 mg kg\(^{-1}\) for Zn [15]. These authors also suggest updated levels for the reference values of soil quality, being 54, 0.10, and 22 mg kg\(^{-1}\) for Ba, Cd, and Zn, respectively (Cu was not evaluated). Even with the restriction of the values, the levels obtained in the S3 treatment are below indicated levels, showing the lack of environmental contamination of the soil by these elements through the application of the SS.

The sequential extraction of heavy metals in the soil shows that the fraction bounded to organic matter was the most influenced by the LE application, except for Cd (Table 2). The sorption of Zn and, mainly, Cu is dependent on its interaction with organic matter [5]. Because of this, higher levels of these elements were observed in the fraction bound to organic matter in treatments with greater SS application. Furthermore, the proportion in the fractions showed the following order: organic matter (44%) > residual (34%) > exchangeable (9%) > oxides (7%) > carbonated (6%) for Cu; and organic matter (40%) > residual (30%) > oxides (19%) > exchangeable (8%) > carbonated (3%) for Zn. This demonstrates the greater interaction of Cu and Zn with SOM.

For the Ba and Cd fractionation, despite the Ba increase in the fraction bounded to organic matter with SS doses, the oxide fraction was the one with the highest proportion of these elements, representing 76 and 85% of the quantified Ba and Cd (Table 2), respectively. These results are important because these elements are more harmful to plants in relation to Cu and Zn, which are micronutrients. Barium is associated with feldspar minerals and when free it can precipitate and adsorb to clays or oxy and hydroxides (Lu et al., 2019), as observed in this study. Cadmium has high mobility in the soil and around 55 to 90% is in the ionic form (Cd\(^{2+}\)). The forms of Cd in the soil are dependent on pH, which controls the complexation of the metal by the SOM, influencing its ionic form [12].

In plants, the lack of differences in the heavy metal concentrations in different components (Table 2) may suggest the use of some mechanism to protect Eucalyptus, either complexing the elements in organic acids released by plants, and/or storing the elements in the cell walls or vacuoles of root cells (Fan et al., 2020). On leaves, where the main metabolic processes for plant development occur, there was a higher Ba, Cu, and Zn concentration, with no symptoms of Ba phytotoxicity and the levels of Cu and Zn were below and within the recommended values of 7-10 and 10-18 mg kg\(^{-1}\) [11], respectively, for the purpose of assessing the nutritional status of plants. Ba, Cd, and Cu accumulation were higher in the Eucalyptus trunk relative to canopy (branches and leaves), as evidenced by a high exportation of these elements by the trunk. However, a high proportion of Ba and Cu still remain in the field after harvest from the branches and foliar residues. In general, Zn accumulation was higher in the leaves in relation to the trunk, with this nutrient remaining in the field after the harvest.

5. Conclusions

The application of SS to supply 100 and 150% of recommend N for the cultivation of Eucalyptus provided an increase heavy metal concentrations in the soil, however, at levels lower than those established by current regulation. By sequential extraction, the levels of heavy metals were higher in the fractions: oxides > organic matter > residual > exchangeable > carbonated, showing low availability for plants. On the plant, heavy metal accumulation was similar in the wood and canopy. Sewage sludge application at a rate to provide 100% of recommended N, supplemented with P, also increased soil fertility, which provided Eucalyptus production at levels similar to mineral fertilization for high wood production. Therefore, SS can be a sustainable source of nutrients for Eucalyptus cultivation, without risks of environmental contamination by heavy metals.
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