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(Title Page)

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Abstract

Background: The Diagnosis and Recommendation Integrated System (DRIS) gave valuable indices of the nutritional status of *Eucalyptus* amended with sewage sludge (SS).

Aims: Our objective was to establish a DRIS norms and analytical method for *Eucalyptus* under SS application, by verifying in particular, the influence of potentially toxic elements (PTEs) on the nutritional and plant development.

Method: Data on mean annual increment, nutrient, and PTE concentration were obtained in an experiment at 22, 44, 54, and 76 months after planting *Eucalyptus* amended with SS.

Results: Our results indicated that DRIS can give valuable data on the nutritional balance indices, in which it was possible to verify that Ba was the most limiting element due to its excess present both in the low and high yielding subpopulations, ranging from 10 to 40% of the populations with excess of Ba. The nutritional diagnosis in agreement with the DRIS model ranged from 60 to 98% among the populations. When modeling the DRIS functions with inclusion of PTEs, a consistent evaluation of the *Eucalyptus* nutritional status was observed, which generated more reliable indices that were able to rank the limiting elements for the *Eucalyptus* productivity.

Conclusion: The new approach proved to be an effective tool for interpreting DRIS indices, by presenting reliable data when PTEs are included. Thus, the inclusion of PTEs in DRIS functions can provide valuable information, by determining which element can cause more damage to the plants. The need for specific norms for each region, plant age and sludge management are highlighted.

Keywords: biosolid, circular economy, forest nutrition, organic fertilizer, urban waste.

DECLARATIONS

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Code availability: Not applicable.

Authors' contributions: Conception and design of the work: CA-J and PC. Acquisition of data: PC, BC, and BA. Analysis and interpretation of data: PC. Drafting and revising the work: PC, CA, TN, GC, AJ, and CA-J. All authors contributed to the article and approved the submitted version.

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Abbreviations

Bal.	Balanced
CBH	Circumference at breast height
DRIS	Diagnosis and recommendation integrated system
Ex.	Excess

H	Height
HRMa	Highly responsive macronutrients
HRMi	Highly responsive micronutrients
IA	Nutritional index
ICP-MS	Inductively coupled plasma mass spectrometry
In.	Insufficient
MAI	Mean annual increment
MF	Mineral fertilization
NBIm	Mean nutritional balance index
NBP	Nutritional balance point
PTE	Potentially toxic elements
RRMa	Rarely responsive macronutrients
RRMi	Rarely responsive micronutrients
SS	Sewage sludge

Introduction

Sewage sludge (SS) disposal in cultivated areas has several advantages. This by-product is rich in organic matter and plant nutrients (Nascimento et al., 2020), which results in improvements in soil fertility (Prates et al., 2022; Silva et al., 2022), thus increasing the crop productivity (Athamenth et al., 2015; Bourioug et al., 2014; Marron, 2015; Nogueira et al., 2013; Prates et al., 2022; Xue et al., 2015) and promoting a circular economy approach (Aleisa et al., 2021). However, its use in agriculture and forestry may be limited due to the presence of potentially toxic elements (PTE).

The use of SS in commercial *Eucalyptus* plantations is increasing worldwide (Abreu-Junior et al., 2017, 2020; Cardoso et al., 2022), because wood products are not intended for human or animal consumption. However, the use of SS as amendment/fertilizer in these areas could be unbalanced in relation to *i*) its nutrient concentration; *ii*) soil nutrient concentration, and *iii*) the crop needs. Thus, the SS application could be harmful, either in terms of excess or shortage of nutrients, both limiting factors to productivity. Thus, imbalances between nutrient content can influence plant uptake and translocation (Marschner, 2012).

In a commercial plantation where SS is applied, we must consider even the possible physiological imbalances in plant function, caused by PTE. Thus, we need a nutritional assessment method that considers the possible interference among these elements. The diagnosis and recommendation integrated system (DRIS) method with the inclusion of PTE in its functions and calculations can help in the interpretation of *Eucalyptus* nutritional status.

Originally developed by Beaufils (1973), this method allows for the calculation of indices for each nutrient, using its binary relations with the others (bivariate method) and comparing them with a reference population, aiming to classify the nutrients, regarding the order of limitation to plant growth (Ribeiro et al., 2020). It starts from the premise that the dual relationships between nutrients are more constant compared to their concentration in the plant.

In Brazil, the use of DRIS is widespread and used in several crops, such as 'Pêra' Orange tree (Dias et al., 2017), *Eucalyptus* cuttings (Morais et al., 2019), sugarcane (Calheiros et al., 2018; Silva et al., 2020) soybean and cotton (Kurihara et al., 2015) oil palm (Matos et al., 2018), acai palm (Ribeiro et al., 2020), 'Thompson' atemoya (Santos; Rozane, 2017), mango (Pinto et al., 2010), coffee (Wadt, 2005) and *Eucalyptus* ssp. (Silva et al., 2005; Wadt, 2004). These studies show the relevance of using this tool in the assessment of the nutritional status of plants with the attainment of norms, establishment of sufficiency ranges and order of productivity-limiting nutrients. However, the use of DRIS in areas with SS application has not been investigated. Given the potentially high amounts of PTE in SS, their inclusion in such work is critical.

Therefore, in the assessment of the nutritional status of agricultural and/or forestry crops treated with SS, there is still no tool to reliably assess the influence of PTE on the nutritional status of crops. Thus, the hypothesis of this work is that the inclusion of these elements in the evaluation of the nutritional status of *Eucalyptus* commercial plantation, using the DRIS method, can help to verify the interference among these elements in the plant nutritional aspects. The objectives of this study are to: *i*) establish DRIS norms for *Eucalyptus* grown under SS application; *ii*) evaluate the nutritional status of *Eucalyptus* by the DRIS method, and from its indices, and *iii*) verify the potential influence of these elements on nutrition and plant development with and without r PTE inclusion.

Material and methods

Experimental area

The experiment was installed in February 2015, in a *Eucalyptus* commercial plantation of 2.7 ha (Municipality of Boa Esperança do Sul, SP; 21°59'33" S - 48°23'27"

W; 516 m asl). The experimental area was commercially cultivated with *Eucalyptus* during the last 40 years, without SS application before the installation of the present experiment.

The climate of the region is classified as Cwa – humid subtropical zone with hot summers and dry winters, according to Köppen, with an average annual rainfall of 1074 mm and an average cold temperature of 17.5 °C and a warm temperature of 30.1 °C (Fig. 1). A detailed pedological survey was carried out before the experiment and the soil was classified as Typic Hapludox (Soil Survey Staff, 2014). Chemical analysis was performed according to the method described in Raij et al. (2001) and granulometric analysis by the pipette method (Camargo et al. 1986) (Table S1).

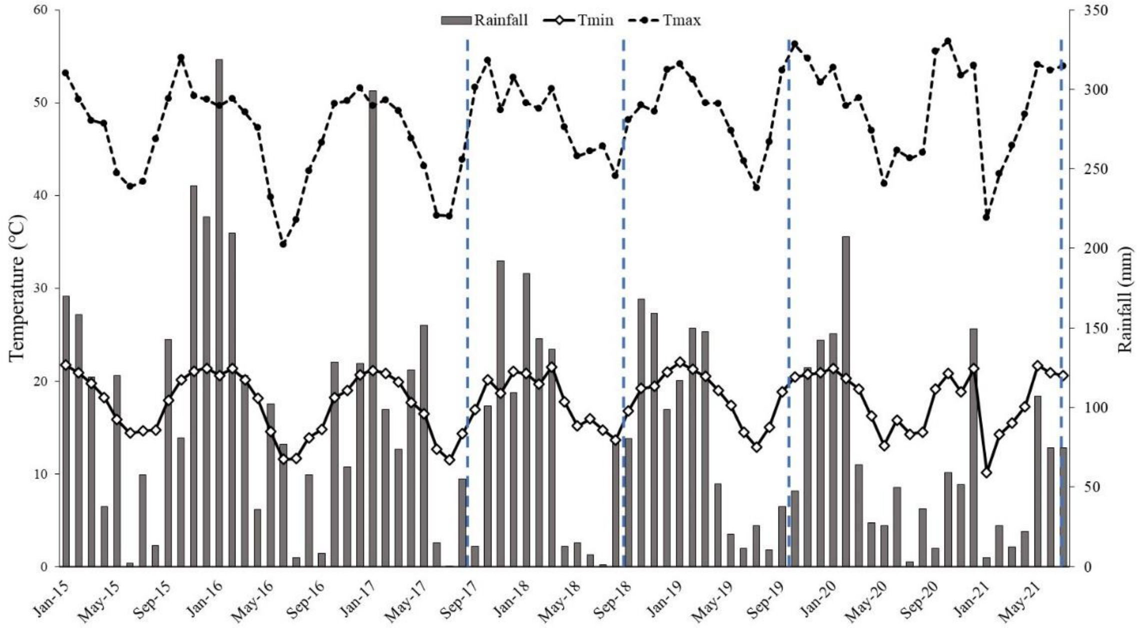

Fig 1. Maximum and minimum temperature (°C) and rainfall (mm) in the municipality of Boa Esperança do Sul, State of São Paulo. Blue and dashed vertical lines represent sampling and inventory times at 22, 44, 54, and 76 months after planting

Sewage sludge characterization

The SS used was generated at the wastewater treatment plant in Jundiaí, SP, where the sewage passes through a system of fully mixed aerated ponds and settling ponds, being dewatered with flocculation based on cationic polymer, followed by centrifugation. The SS chemical composition (Table S2) was obtained according to the methodology proposed by United States Environmental Protection Agency (USEPA, 2007).

Field experiment

Soil preparation took place in January 2015, with subsoiling at a depth of 0.4 m between the rows of the previous planting, where the *Eucalyptus* seedlings were planted. Before planting, 1.8 t ha^{-1} of limestone were applied to provide Ca and Mg to the soil. The genetic material used in the experiment was a hybrid from a cross between *Eucalyptus grandis* and *Eucalyptus urophylla* (*Eucalyptus urograndis*). The seedlings were produced via vegetative propagation for cloning. The planting was carried out manually in February 2015, with a spacing of 3 x 2.25 m, totaling 1,481 trees per hectare.

The experiment was installed in a randomized complete block design with 10 treatments and four replications, totaling 40 experimental units. Each plot consisted of 100 plants (10 x 10 plants), with the 36 central plants corresponding to the useful area and the remaining 64 plants to the border area. The treatments consisted of a control (without SS or mineral fertilizers); two treatments with application of mineral fertilizers at different doses; and seven treatments with sewage sludge application. The amounts of nutrients applied per treatment are shown in Table 1.

Table 1. Amount of nutrients applied per treatment by mineral fertilizers and sewage sludge (SS)

Treatment	N	P	K	B	Zn	Cu
	kg ha^{-1}					
Control (C)	0	0	0	0	0	0
Mineral fertilization level 1 (MF1)	**192**	**26**	**137**	**6.5**	**2.8**	**2.8**
Mineral fertilization level 2 (MF2)	**60**	**17.5**	**84**	**4.2**	**1.8**	**1.8**
SS (50%)[2/] + P (83%) - B + K (S1P1)	96 [1/] (319)	**22** (435)	**137** (290)	**0** (0.015)	**0** (9.7)	**0** (4.5)
SS (50%)[2/] + P (83%) + B + K (S1P1B)	96 [1/] (319)	**22** (435)	**137** (290)	**6.5** (0.015)	**0** (9.7)	**0** (4.5)
SS (100%)[2/] + P (66%) - B + K (S2P2)	192 [1/] (638)	**17.5** (870)	**137** (855)	**0** (0.030)	**0** (19.3)	**0** (9.0)
SS (100%)[2/] + P (66%) + B + K (S2P2B)	192 [1/] (638)	**17.5** (870)	**137** (855)	**6.5** (0.030)	**0** (19.3)	**0** (9)
SS (100%)[2/] - P + B + K (S2B)	192 [1/] (638)	**0** (870)	**137** (855)	**6.5** (0.030)	**0** (19.3)	**0** (9)
SS (150%)[2/] - P - B + K (S3)	288 [1/] (957)	**0** (1,305)	**137** (870)	**0** (0.045)	**0** (29)	**0** (13.5)
SS (150%)[2/] - P + B + K (S3B)	288 [1/] (957)	**0** (1,305)	**137** (870)	**6.5** (0.045)	**0** (29)	**0** (13.5)

[1/] Available N dose applied by sewage sludge, considering a N mineralization rate of 30% for aerobic sewage sludge (Brasil, 2006). Values in parentheses represent the nutrients applied by sewage sludge. Values in bold represent nutrients applied via mineral fertilizer. Adapted from Cardoso et al. (2022).

Mineral fertilizers were applied in the planting furrow and topdressing fertilization was applied in a half crown around the seedling, in doses shown in Table 1. The SS doses to be applied was calculated so that it had the equivalent of 50, 100, and 150% of the recommendation of N (MF1), that is, 192 kg ha^{-1} of N, corresponding to 14.5, 29.0, and 43.5 t ha^{-1} of SS, respectively, on a dry basis. The SS dose was based on the N criterion, as described in Resolution No. 375 (Brasil, 2006). The SS was distributed superficially on the soil in a strip of 0.60 m wide at 0.20 m away from the planting furrow, seven months after planting the seedlings (September 2015). All other silvicultural treatments (weed control) followed the standard adopted by the company in its commercial plantations.

Productivity estimation and sampling of plant material

In September 2017 (22 months after planting), September 2018 (44 months after planting), September 2019 (54 months after planting) and in July 2021 (76 months after planting) the circumference at breast height was measured (CBH – 1.3 m) and height (H) of all trees in the useful plots of the experiment. This was carried out to estimate the wood volume productivity, using the logarithmic model of Schumacher and Hall (1933), adjusted with a biomass inventory carried out at 22 months after planting, and subsequent calculation of the mean annual increment (MAI).

Measured CBH was also used to select trees for thinning and leaf collection for nutritional diagnosis and PTEs. Trees were randomly selected within the range of ±5% of the median CBH, with one tree per plot, except at 76 months after planting, when two trees per plot were felled. To evaluate the nutritional status of *Eucalyptus* using the DRIS method, each experimental plot was considered as a population. After the trees were felled, leaf samples were collected from the four cardinal points in the upper third of the tree canopy, as recommended by Malavolta et al. (1997) and Raij et al. (1997).

Chemical analysis of plant material

In the laboratory, the collected leaves were washed, dried in an oven with forced air circulation at 65 °C for 72h, ground in a Willey-type knife mill, and packed in properly identified paper bags until analysis. The samples were digested according to the procedure described by Araújo et al. (2002). Briefly, 250 mg of plant material was weighed and digested in a solution with 7.5 mL of 3.11 M HNO_3 and 2.5 mL of 30% (v/v) H_2O_2 in a closed microwave system. Leaf nutrient and PTE concentration (except nitrogen) were determined by inductively coupled plasma mass spectrometry (ICP-MS), using an Agilent model 7500ce. Leaf N concentration was quantified by the Kjeldahl method (Malavolta et al., 1997).

Data analysis

First, the mean and standard deviation of the MAI data of the trunk volume (m^3 ha^{-1} per year) and the concentration of the elements determined in the populations were calculated, separating them by planting age (scenario A) and in general, that is, with all the data (scenario B). Then, for scenario A, the populations were divided into low and high productivity (reference population), defined as populations with productivity greater than the general average plus 50% of the standard deviation. In scenario B, the reference population was established by the populations classified as having high productivity at different ages together with those plots that presented MAI above the general average + 50% of the standard deviation (Silva et al., 2005). The mean and standard deviation of the MAI and the concentration of the elements determined in the subpopulations (high and low productivity) were also calculated.

For each relationship between the concentration of two nutrients in the leaves, with and without PTE, the mean and standard deviation of the relationships in the treatments were determined. Because the sewage sludge presents the nutrients in an unbalanced way in relation to the needs of the culture, it was decided to adopt the method proposed by Baldock and Schulte (1996), which provides for the division of nutrients into four groups: *i*) highly responsive macronutrients group (HRMa): N, P, and K; *ii*) rarely responsive macronutrients group (RRMa): Ca, Mg, and S; *iii*) highly responsive micronutrients group (HRMi): B, Cu, and Zn; and *iv*) rarely responsive micronutrients group (RRMi): Fe, Mn, and Mo. Due to the evaluation of potentially toxic elements, another group called PTE will be added, containing the elements: Al, As, Ba, Cd, Cr, and Pb.

We used formulas adapted by Wadt et al. (2007) to overestimate and/or underestimate nutritional deficiencies and excesses, according to the nutrient group so that the formulas will be predisposed as follows. In the HRMa group, the equation aims to overestimate the deficiency when A/B>a/b and underestimate the excess when A/B<a/b:

$$f(A/B) = \left(\frac{A/B - a/b}{S_{(a/b)}}\right) * K * \left(\frac{a/b}{A/B}\right) \tag{1}$$

In the RRMa group, the equation aims to underestimate deficiencies (A/B<a/b) and excesses (A/B>a/b):

If A/B<a/b: $f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{A/B}{a/b}\right)$ (2)

If A/B>a/b: $f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{a/b}{A/B}\right)$ (3)

In the HRMi group, the equation below aims to overestimate deficiencies and excesses:

If A/B<a/b: $f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{a/b}{A/B}\right)$ (4)

If A/B>a/b and for nutrient with potential toxic effect when in excess:

$$f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{A/B}{a/b}\right) \quad (5)$$

For the HRMi group, the adaptation of the equation aims to overestimate only the excess:

If A/B<a/b: $f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * 1$ (6)

if A/B>a/b: $f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{A/B}{a/b}\right)$ (7)

Due to the inclusion of PTE in the DRIS calculation, the equation aims to underestimate the "deficiency" when A/B<a/b and overestimate the excess when A/B>a/b of these elements in the plant:

$$f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{A/B}{a/b}\right) \quad (8)$$

For comparison purposes, the DRIS were also calculated without separating nutrients into groups and, with or without the addition of PTE using the equation proposed by Jones (1981):

$$f(A/B) = \left(\frac{A/B-a/b}{S_{(a/b)}}\right) * K * \left(\frac{a/b}{A/B}\right) \quad (9)$$

Since, the ratio of nutrients in capital letters (A/B) represents the sample under study and in lower case (a/b) represents the reference population and its standard deviation (Sa/b). The "A" nutrients are those that, both in the direct and inverse relationship, represent their respective group. The k value is an adjustment factor for each function, and k=1.5 was used for the HRMa group; k=1.5 for the HRMi group; k=0.5 for the RRMa group; and k=1.0 for the RRMi group; and the k value for the PTE group was determined by the adjustment that presented the best overestimation of the excess and underestimation of the "deficiency" of PTE.

From the values of all the DRIS functions, the DRIS indices (IA) were calculated, according to the equation below, where $\dot{f}$ is the average of the DRIS functions in the direct (f(A/B)) and inverse (f(B/A)) forms; and n is the number of DRIS functions considered.

$$IA = \dot{f} = [f(A/B) - f(B/A) + f(A/C) - f(C/A) + \cdots + f(A/N) - f(N/A)]/n \quad (10)$$

With the indexes of each element, the mean nutritional balance index (NBIm) was calculated. The NBIm was obtained by dividing the absolute value of the nutritional balance index by the number of nutrients evaluated (n):

$$NBIm = (|IA| + |IB| + |IC| + \cdots + |IN|)/n \quad (11)$$

Additionally, to interpret the values of the generated indices, the criterion of potential response to simplified fertilization was adopted, which classifies the nutritional status of the plant for each nutrient as insufficient, balanced and in excess (Wadt, 2005):

- Balanced (Bal.): $|IA| < NBIm$;
- Insufficient (In.): $|IA| > NBIm$ and $IA < 0$; and,
- Excess (Ex.): $|IA| > NBIm$ and $IA > 0$.

After classifying each nutrient in each population, classifications with and without the addition of PTE, as well as the methods used, Wadt et al. (2007) and Jones (1981), were compared by the chi-square test ($p < 0.05$) with the aid of SigmaPlot software version 14.0 (Systat Software, 2020). This analysis aims to verify the agreement of classification of nutritional status when there was or not the addition of PTE in DRIS functions.

The DRIS indices were also used to evaluate the sufficiency range of the concentration of each element in *Eucalyptus* leaves (Souza et al., 2015), based on their correlation with their respective nutritional index (IA). For each element, IA equaled zero and the element concentration called nutritional balance point (NBP) was obtained. The NBP value plus or minus 2/3 of the standard deviation of the element concentration of the entire population represents the lower and upper limits of the sufficiency range, respectively (Kurihara et al., 2013; Souza et al., 2015). The critical level of nutrients corresponds to the lower limit of the sufficiency range and the critical PTE concentration corresponds to the upper limit of the sufficiency range.

Results

Populations productivity

The high productivity subpopulations presented average MAI of 34, 58, 57, and 59 m^3 ha^{-1} per year according to A scenario (Table 2), corresponding to 27, 32, 35 and 24% of the populations at 22, 44, 54, and 76 months after planting, respectively. In B scenario, the high productivity subpopulations had an average MAI of 54 m^3 ha^{-1} per year, corresponding to 47% of the populations. Furthermore, the MAI of the high and low productivity subpopulations were different, showing increments of 8, 9, 10 and 41% at 22, 44, 54, and 76 months after planting, respectively, and of 33% in B scenario.

Table 2. Mean annual increase (m^3 ha^{-1} per year) of *Eucalyptus urograndis*, treated with sewage sludge, in subpopulations of low and high productivity after 22, 44, 54, and 76 months after planting (A scenario) and general (B scenario)

Age	Population			Subpopulation						Sig.
				High productivity			Low productivity			
	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	
22 months	32.3	1.7	40	34.3	0.8	11	31.6	1.3	29	*
44 months	54.9	2.7	40	58.1	1.1	13	53.4	3.0	27	*
54 months	53.6	3.3	40	57.0	1.4	14	51.7	2.4	26	*
76 months	46.0	9.2	79	58.9	7.9	19	41.9	4.6	60	*
General	46.6	10.1	199	53.6	8.2	94	40.3	7.0	105	*

S.D. - Standard deviation; n – Number of populations; Sig. - Significance; * – Significant by the paired t-test ($p < 0.05$).

Evaluation of DRIS norms and functions

The direct and inverse DRIS norms (mean and standard deviation) of the dual relationships of nutrients and PTE were defined from the subpopulation of high productivity at the different ages of plantation evaluation (Table S3). There was a significant variation from 59 to 85% of the bivariate relationships of the elements evaluated between the ages and the general norm (Table S3).

Fig. 2 shows the influence of correction factors proposed by Wadt et al. (2007) on the equation proposed by Jones (1981), enabling the overestimation and/or underestimation of the deficiency or excess of the elements. The values of the DRIS functions by the method proposed by Jones (1981) present a linear behavior with the element relationships, that is, the nutritional imbalance is considered constant and given by the slope of the line (Wadt et al., 2007).

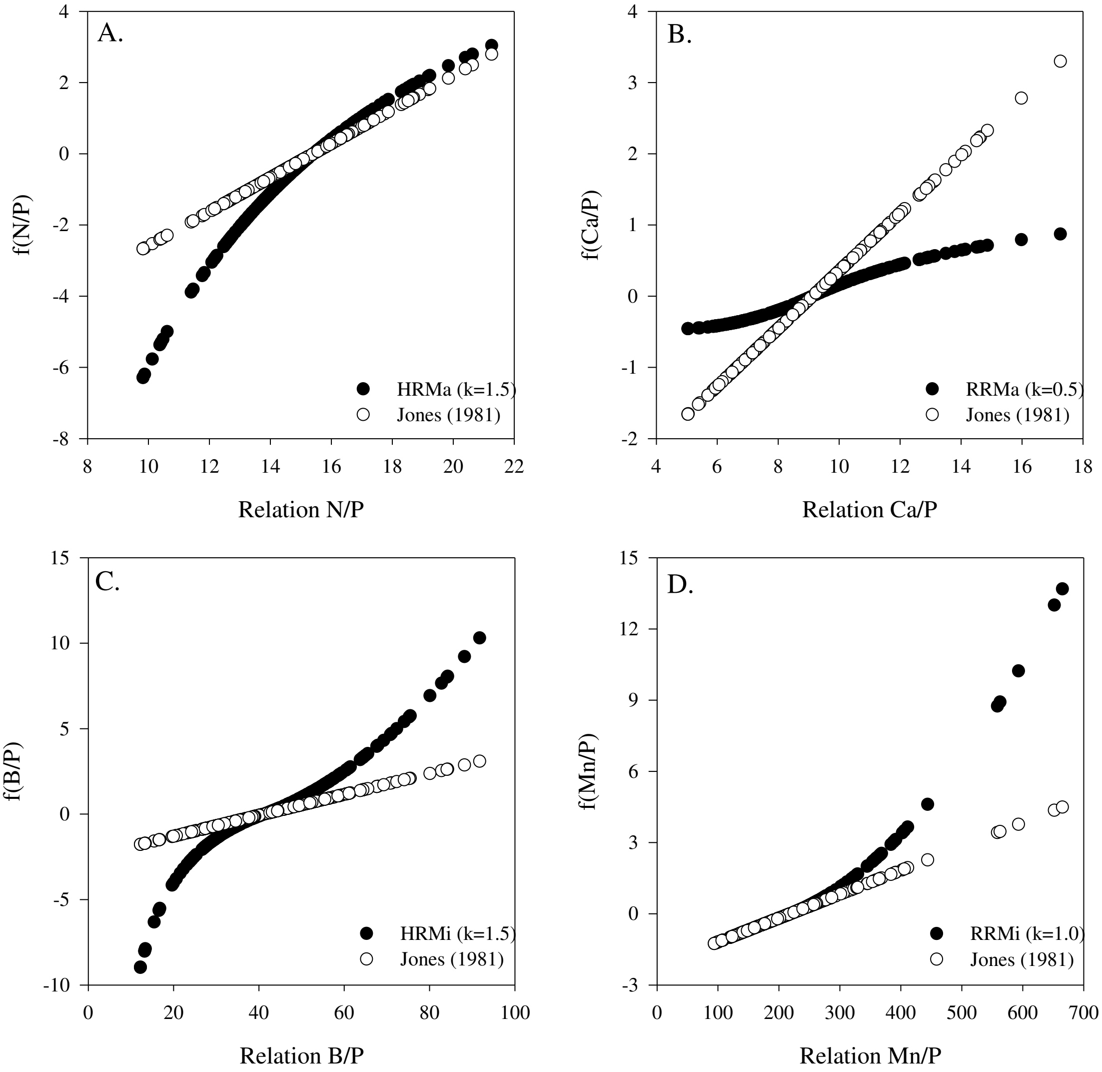

Fig. 2. Values of the DRIS functions in *Eucalyptus*, calculated by the Jones (1981) equation and by the formula for macronutrients with frequent response (A – N/P) and rare (B – Ca/P) and micronutrients with frequent response (C – B/P) and rare (D – Mn/P), regarding the direct relationship of nutrients (n = 199)

Using the Jones (1981) formula, the values of the DRIS functions for the elements of the group of PTE also show linear behavior (Fig. 3a). The adjustment of the sensitivity value (k) can improve the interpretation of the interference of PTE on the nutritional status of plants (Wadt et al., 2007). Therefore, the higher the value of k, the better the adjustment of the model will be to verify the influence of these elements on the nutritional status of the plants, especially in a situation of toxic effect (Fig. 3b). Therefore, the proposed k value for calculating the DRIS function in the PTE group was equal to 1.5 (Fig. 3a).

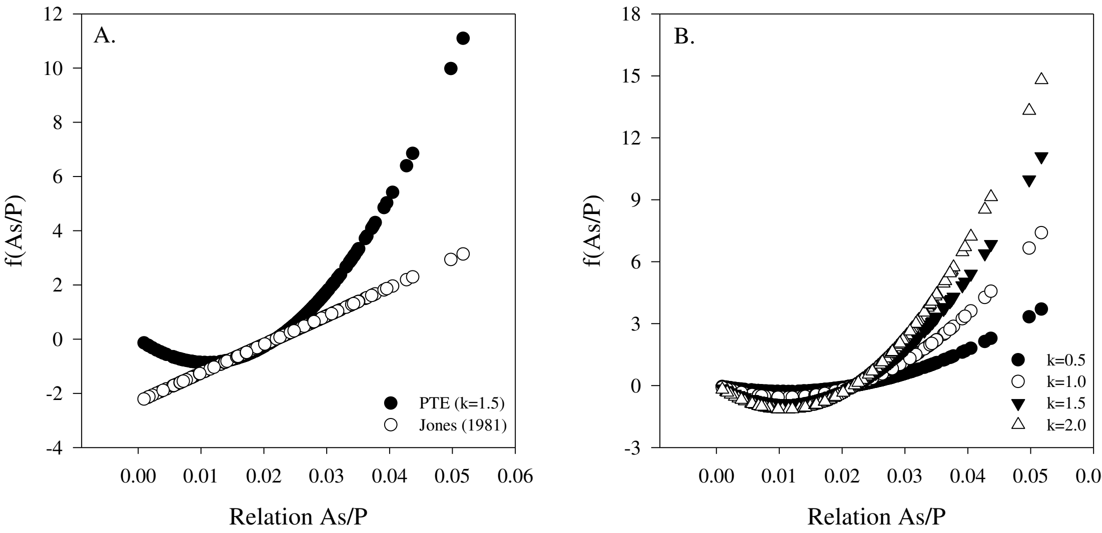

Fig. 3. Values of As/P in *Eucalyptus*, calculated using the Jones (1981) equation and the equation for PTE (A) and with k values between 0.5 and 2.0 (B), regarding the direct As/P ratio (n = 199)

Assessment of nutritional status

The frequency of populations in balance, insufficiency, and excess of each nutrient, with or without the inclusion of PTE, in the low productivity subpopulation is presented in Table 3. In general, most populations were in a situation of nutritional balance, with the exception of B, which presented equivalent frequencies in the assessments of balance, insufficiency, and excess nutritional. The inclusion of PTE in the DRIS calculations did not cause significant changes in the frequency of populations in nutritional balance or imbalance (deficiency or excess) for the elements of the HRMa (N, P, and K) and RRMa (Ca, Mg, and S) group. However, the inclusion of PTE increased the frequency of populations in nutritional balance, and decreased populations in imbalance for the HRMi (B, Cu, and Zn) and RRMi (Fe, Mn, and Mo) groups, mainly, in situations of excess in the RRMi group.

Table 3. Frequency in which low productivity *Eucalyptus* populations showed a balanced (Bal.), insufficient (In.) and excess (Ex.) nutritional status, with or without inclusion of potentially toxic elements (PTE) in DRIS functions

PTE	Interpretation	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn	Al	As	Ba	Cd	Cr	Pb
									22 months										
With	Bal.	66	62	55	100	100	100	31	24	69	48	41	69	48	59	52	52	48	52
	In.	21	38	31	0	0	0	34	45	7	24	31	17	17	0	38	10	41	7
	Ex.	14	0	14	0	0	0	34	31	24	28	28	14	34	41	10	38	10	41

	Bal.	72	69	52	100	100	100	28	34	55	41	45	41						
Out	In.	17	24	24	0	0	0	31	34	10	28	31	21						
	Ex.	10	7	24	0	0	0	41	31	34	31	24	38						
44 months																			
	Bal.	44	70	33	100	100	100	30	56	74	37	70	48	63	52	41	52	74	74
With	In.	15	15	56	0	0	0	41	30	7	37	22	19	4	11	37	41	7	11
	Ex.	41	15	11	0	0	0	30	15	19	26	7	33	33	37	22	7	19	15
	Bal.	44	56	37	96	100	100	26	52	70	33	67	52						
Out	In.	15	11	56	0	0	0	41	30	7	41	22	19						
	Ex.	41	33	7	4	0	0	33	19	22	26	11	30						
54 months																			
	Bal.	54	81	46	100	100	100	42	38	65	42	69	58	58	73	42	62	46	42
With	In.	12	12	19	0	0	0	35	27	12	35	15	15	23	19	15	31	35	50
	Ex.	35	8	35	0	0	0	23	35	23	23	15	27	19	8	42	8	19	8
	Bal.	54	77	46	100	100	100	38	46	50	38	65	42						
Out	In.	12	12	19	0	0	0	38	27	19	38	15	23						
	Ex.	35	12	35	0	0	0	23	27	31	23	19	35						
76 months																			
	Bal.	53	43	62	100	98	100	33	50	77	62	77	52	52	50	37	53	62	57
With	In.	18	2	7	0	2	0	38	37	20	23	13	22	28	30	33	33	25	18
	Ex.	28	55	32	0	0	0	28	13	3	15	10	27	20	20	30	13	13	25
	Bal.	52	35	58	100	100	100	35	38	63	55	67	47						
Out	In.	15	0	8	0	0	0	40	43	32	27	22	32						
	Ex.	33	65	33	0	0	0	25	18	5	18	12	22						

Furthermore, the agreement of nutritional diagnosis (balanced, insufficiency, and excess) when there was or not the inclusion of PTE in the DRIS functions ranged from 57 to 100% of the populations, regardless of the method evaluated and age of planting (Table 4). Among the methods, the frequency of concordant diagnoses varied between 51 and 100% of the populations, except for the RRMa group, where there was no significant association between the methods (Table 5).

Table 4. Frequency (%) of *Eucalyptus* populations with concordant diagnoses of the potential response to fertilization by nutrient between with or without the inclusion of potentially toxic elements in the calculations of DRIS functions, by the methods of Wadt et al. (2007) and Jones (1981)

Age (months)	Method	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn	Mean
22	Wadt et al. (2007)	83*	66*	82*	100^{ns}	100^{ns}	100^{ns}	85*	81*	57*	78*	97*	60*	82
	Jones (1987)	87*	70*	84*	60*	73*	89*	76*	87*	75*	87*	92*	79*	80
44	Wadt et al. (2007)	90*	85*	86*	100^{ns}	100^{ns}	100^{ns}	98*	85*	97*	96*	96*	89*	93
	Jones (1987)	82*	72*	80*	82*	76*	81*	98*	81*	88*	85*	92*	74*	83
54	Wadt et al. (2007)	71*	85*	89*	100*	100^{ns}	100^{ns}	95*	82*	94*	98*	85*	93*	91

	Jones (1987)	60*	82*	86*	94*	90*	91*	84*	94*	80*	95*	96*	95*	87
76	Wadt et al. (2007)	88*	73*	85*	100^{ns}	100^{ns}	100^{ns}	80*	82*	86*	92*	96*	77*	88
	Jones (1987)	79*	68*	87*	85*	77*	87*	91*	93*	75*	93*	89*	85*	84

^{ns} – Not significant by the chi-square test (p > 0.05); * - Significant by chi-square test (*p* < 0.05).

Table 5. Frequency (%) of *Eucalyptus* populations with concordant diagnoses of the response potential to fertilization between the DRIS functions by Wadt et al. (2007) and Jones (1981) methods

Age (months)	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn	Al	As	Ba	Cd	Cr	Pb	Mean
22	86*	93*	90*	75^{ns}	68^{ns}	63^{ns}	84*	86*	94*	86*	95*	75*	94*	95*	89*	98*	98*	100*	87
44	86*	80*	93*	49^{ns}	61^{ns}	68^{ns}	89*	70*	98*	96*	94*	73*	95*	96*	97*	98*	89*	92*	85
54	88*	92*	90*	51*	57^{ns}	71^{ns}	78*	84*	93*	93*	95*	84*	100*	96*	93*	93*	97*	90*	86
76	88*	80*	90*	57^{ns}	52^{ns}	78^{ns}	81*	86*	96*	94*	95*	81*	97*	90*	95*	93*	94*	89*	85

^{ns} – Not significant by the chi-square test (*p* > 0.05); * - Significant by chi-square test (*p* < 0.05).

Order of nutritional limitation

Table 6 shows the order of the most limiting nutrients by deficiency, with and without the addition of PTE, at the different ages of assessment. In both subpopulations, the most limiting element due to deficiency was B, with or without the inclusion of PTE, occurring between 21 and 32% of the populations, except at 22 months after planting, in which there was greater limitation due to Cu deficiency (24–27%) in the low productivity subpopulation and Fe (27%) and N, P, B, and Cu (18%) in the high productivity subpopulation, with and without the inclusion of PTE, respectively.

Table 6. Order of nutrients in deficiency limiting the *Eucalyptus* wood yield in low yield population, and its respective frequency

Subp.	PTE	Deficiency limiting order											
		22 months											
Low	Out	Cu >	Mo >	Mn >	K >	B >	P >	N =	Ca =	Mg =	S =	Fe =	Zn
		27.6	24.1	17.2	13.8	10.3	6.9	0.0	0.0	0.0	0.0	0.0	0.0
	With	Cu >	Mo >	Mn >	K =	B >	P >	Zn >	N =	Ca =	Mg =	S =	Fe
		24.1	20.7	17.2	13.8	13.8	6.9	3.4	0.0	0.0	0.0	0.0	0.0
High	Out	N =	P =	B =	Cu >	K =	Fe =	Mo >	Ca =	Mg =	S =	Mn =	Zn
		18.2	18.2	18.2	18.2	9.1	9.1	9.1	0.0	0.0	0.0	0.0	0.0
	With	Fe >	B =	Cu >	N =	P =	K =	Mo >	Ca =	Mg =	S =	Mn =	Zn
		27.3	18.2	18.2	9.1	9.1	9.1	9.1	0.0	0.0	0.0	0.0	0.0
		44 months											
Low	Out	B >	Mo >	K =	Mn >	Cu =	Fe =	Zn >	N =	P =	Ca =	Mg =	S
		25.9	22.2	14.8	14.8	7.4	7.4	7.4	0.0	0.0	0.0	0.0	0.0
	With	B >	Mo >	K =	Mn >	Cu =	Fe =	Zn >	N =	P =	Ca =	Mg =	S
		25.9	22.2	14.8	14.8	7.4	7.4	7.4	0.0	0.0	0.0	0.0	0.0

High	Out	B >	P =	Fe =	Mo >	K =	Cu =	Zn >	N =	Ca =	Mg =	S =	Mn
		30.8	15.4	15.4	15.4	7.7	7.7	7.7	0.0	0.0	0.0	0.0	0.0
	With	B >	P =	Fe =	Zn >	K =	Cu =	Mo >	N =	Ca =	Mg =	S =	Mn
		30.8	15.4	15.4	15.4	7.7	7.7	7.7	0.0	0.0	0.0	0.0	0.0
		54 months											
Low	Out	B >	Mn >	Fe >	Cu >	K =	Zn >	N =	Mo >	P =	Ca =	Mg =	S
		30.8	19.2	15.4	11.5	7.7	7.7	3.8	3.8	0.0	0.0	0.0	0.0
	With	B >	Mn >	Fe >	Cu >	K =	Zn >	N =	Mo >	P =	Ca =	Mg =	S
		30.8	19.2	15.4	11.5	7.7	7.7	3.8	3.8	0.0	0.0	0.0	0.0
High	Out	B =	Cu >	Zn >	N =	P =	K =	Ca =	Mn =	Mo >	Mg =	S =	Fe
		21.4	21.4	14.3	7.1	7.1	7.1	7.1	7.1	7.1	0.0	0.0	0.0
	With	B =	Cu >	K =	Zn >	N =	P =	Mn =	Mo >	Ca =	Mg =	S =	Fe
		21.4	21.4	14.3	14.3	7.1	7.1	7.1	7.1	0.0	0.0	0.0	0.0
		76 months											
Low	Out	B >	Cu >	Fe =	Mn =	Mo =	Zn >	N >	P =	K =	Ca =	Mg =	S
		30.0	28.3	10.0	8.3	8.3	8.3	6.7	0.0	0.0	0.0	0.0	0.0
	With	B >	Cu >	Zn >	N =	Fe =	Mn >	Mo >	P =	K =	Ca =	Mg =	S
		31.7	28.3	10.0	8.3	8.3	8.3	5.0	0.0	0.0	0.0	0.0	0.0
High	Out	Cu =	Mo >	B >	N =	K =	Mn >	Fe =	Zn >	P =	Ca =	Mg =	S
		21.1	21.1	15.8	10.5	10.5	10.5	5.3	5.3	0.0	0.0	0.0	0.0
	With	B =	Cu >	Mo >	N =	Fe =	Mn >	K =	Zn >	P =	Ca =	Mg =	S
		21.1	21.1	15.8	10.5	10.5	10.5	5.3	5.3	0.0	0.0	0.0	0.0

Subp. – Subpopulation

In the same way as for deficiency, it is possible to verify the most limiting element by excess (Table 7). There was variation in the most limiting nutrients and PTE due to excess at different ages of evaluation. Overall, B (21–27%) was the most limiting nutrient by excess in the high-yielding subpopulation, except at 76 months, where Mo was the most limiting (21%). In the low productivity subpopulation, B (43%), N (26–30%), Zn (15–19%), and P (28–33%) were the most limiting nutrients due to excess at 22, 44, 54, and 76 months after planting, respectively. For PTE, As (24%) and Cd (36%) were the most limiting at 22 months, As (33%) and Ba (31%) at 44 months, Ba (35%) and Cd (29%) at 54 months, and Ba (22–32%) at 76 months after planting, in low and high yield subpopulations, respectively.

Table 7. Order of nutrients in excess limiting the *Eucalyptus* wood yield in low yield population, and its respective frequency

Subpop.	PTE	Excess limiting order											
		22 months											
Low	Out	B >	Cu >	Fe >	Mo =	Zn >	Mn >	N =	K >	P =	Ca =	Mg =	S
		34.5	17.2	13.8	10.3	10.3	6.9	3.4	3.4	0.0	0.0	0.0	0.0
	With	B >	Cu >	Fe =	Mo >	Mn =	Zn >	N =	K >	P =	Ca =	Mg =	S
		34.5	17.2	13.8	13.8	6.9	6.9	3.4	3.4	0.0	0.0	0.0	0.0
		As =	Cd >	Al >	Pb >	Ba >	Cr						

		24.1	24.1	20.7	17.2	10.3	3.4						
High	Out	P =	B >	N =	K =	Cu =	Fe =	Mn =	Mo =	Zn >	Ca =	Mg =	S
		18.2	18.2	9.1	9.1	9.1	9.1	9.1	9.1	9.1	0.0	0.0	0.0
	With	B >	P =	Mn >	K =	Cu =	Fe =	Mo >	N =	Ca =	Mg =	S =	Zn
		27.3	18.2	18.2	9.1	9.1	9.1	9.1	0.0	0.0	0.0	0.0	0.0
		Cd >	As =	Cr >	Al =	Ba =	Pb						
		36.4	18.2	18.2	9.1	9.1	9.1						
						44 months							
Low	Out	N >	B =	Mn =	Zn >	Fe >	Cu =	Mo >	P =	K =	Ca =	Mg =	S
		29.6	18.5	18.5	18.5	7.4	3.7	3.7	0.0	0.0	0.0	0.0	0.0
	With	N >	B =	Mn =	Zn >	Fe >	Cu =	Mo >	P =	K =	Ca =	Mg =	S
		25.9	18.5	18.5	18.5	11.1	3.7	3.7	0.0	0.0	0.0	0.0	0.0
		As >	Al >	Ba >	Cd =	Pb >	Cr						
		33.3	29.6	18.5	7.4	7.4	3.7						
High	Out	B =	Zn >	K =	Mn >	Cu =	Fe =	Mo >	N =	P =	Ca =	Mg =	S
		23.1	23.1	15.4	15.4	7.7	7.7	7.7	0.0	0.0	0.0	0.0	0.0
	With	B =	Zn >	K =	Mn >	Cu =	Fe =	Mo >	N =	P =	Ca =	Mg =	S
		23.1	23.1	15.4	15.4	7.7	7.7	7.7	0.0	0.0	0.0	0.0	0.0
		Ba >	Al =	Cr >	Cd >	As >	Pb						
		30.8	23.1	23.1	15.4	7.7	0.0						
						54 months							
Low	Out	N =	K =	Mn =	Zn >	Cu =	Fe =	Mo >	B >	P =	Ca =	Mg =	S
		15.4	15.4	15.4	15.4	11.5	11.5	11.5	3.8	0.0	0.0	0.0	0.0
	With	Zn >	K =	Mn >	N =	Cu =	Fe =	Mo >	B >	P =	Ca =	Mg =	S
		19.2	15.4	15.4	11.5	11.5	11.5	11.5	3.8	0.0	0.0	0.0	0.0
		Ba >	Al =	As =	Cr >	Cd =	Pb						
		34.6	19.2	19.2	19.2	3.8	3.8						
High	Out	B >	K =	Cu =	Mn =	Mo >	P =	Fe =	Zn >	N =	Ca =	Mg =	S
		21.4	14.3	14.3	14.3	14.3	7.1	7.1	7.1	0.0	0.0	0.0	0.0
	With	B =	Cu >	Mn =	Mo >	P =	K =	Fe =	Zn >	N =	Ca =	Mg =	S
		21.4	21.4	14.3	14.3	7.1	7.1	7.1	7.1	0.0	0.0	0.0	0.0
		Cd >	As =	Pb >	Cr >	Al =	Ba						
		28.6	21.4	21.4	14.3	7.1	7.1						
						76 months							
Low	Out	P >	K =	Zn >	B >	N >	Cu =	Mn =	Mo >	Fe >	Ca =	Mg =	S
		33.3	13.3	13.3	11.7	10.0	5.0	5.0	5.0	3.3	0.0	0.0	0.0
	With	P >	Zn >	K >	B >	N =	Mn >	Mo >	Cu >	Fe >	Ca =	Mg =	S
		28.3	16.7	13.3	11.7	10.0	10.0	5.0	3.3	1.7	0.0	0.0	0.0
		Ba =	Pb >	As =	Cr >	Al =	Cd						
		21.7	21.7	16.7	16.7	11.7	11.7						
High	Out	Mo >	P =	Mn >	K =	Fe =	Zn >	N =	B =	Cu >	Ca =	Mg =	S
		21.1	15.8	15.8	10.5	10.5	10.5	5.3	5.3	5.3	0.0	0.0	0.0
	With	Mo >	P =	Mn >	K =	Fe =	Zn >	N =	B =	Cu >	Ca =	Mg =	S
		21.1	15.8	15.8	10.5	10.5	10.5	5.3	5.3	5.3	0.0	0.0	0.0
		Ba >	Pb >	Cd >	Al =	Cr >	As						
		31.6	26.3	15.8	10.5	10.5	5.3						

Subp. – Subpopulation

Element sufficiency ranges

To determine the sufficiency ranges of each element and its critical concentration (Table 8), from the DRIS indices, the determination coefficients (R^2) of the equations presented values between 0.28 and 0.94, being normally lower for N, P, S, and Zn (Tables

S4 and S5). Despite the low coefficient of determination for these elements, the relationship between nutrient concentration and nutrient indices was significant ($p < 0.05$).

Table 8. Nutrient balance point (NBP) and sufficiency ranges obtained by the DRIS method, with and without the inclusion of potentially toxic elements, to evaluate the nutritional status of *Eucalyptus* treated with class B sewage sludge

Elements	22 months				44 months			
	NBP	Range	NBP	Range	NBP	Range	NBP	Range
N (g kg^{-1})	20.3	18.5-22.0	20.1	18.3-21.8	19.9	17.8-21.9	19.8	17.8-21.8
P (g kg^{-1})	1.46	1.39-1.53	1.45	1.38-1.51	1.29	1.21-1.37	1.29	1.20-1.37
K (g kg^{-1})	7.91	7.28-8.54	7.81	7.18-8.43	9.8	9.0-10.6	9.88	9.11-10.65
Ca (g kg^{-1})	11.0	10.1-11.8	10.8	10.0-11.7	13.7	11.6-15.7	13.6	11.6-15.7
Mg (g kg^{-1})	2.13	1.97-2.30	2.11	1.95-2.28	1.96	1.79-2.13	1.95	1.78-2.12
S (g kg^{-1})	1.06	0.96-1.16	1.05	0.95-1.15	1.05	0.94-1.16	1.04	0.94-1.15
B (mg kg^{-1})	66	52-80	64	50-78	57	48-67	57	48-67
Cu (mg kg^{-1})	3.04	2.70-3.37	2.99	2.65-3.32	2.79	2.55-3.02	2.79	2.56-3.03
Fe (mg kg^{-1})	119	91-147	116	87-144	249	223-275	248	222-274
Mn (mg kg^{-1})	255	215-296	250	209-291	289	241-338	288	240-337
Mo (mg kg^{-1})	0.08	0.00-0.25	0.08	0.00-0.25	0.06	0.05-0.08	0.06	0.05-0.08
Zn (mg kg^{-1})	15.7	14.4-17.0	15.4	14.1-16.7	13.2	12.0-14.5	13.2	12.0-14.5
Al (mg kg^{-1})	147	102-193			336	301-371		
As (mg kg^{-1})	0.01	0.005-0.014			0.06	0.05-0.06		
Ba (mg kg^{-1})	11.2	9.5-12.9			16.4	12.2-20.7		
Cd (mg kg^{-1})	0.003	0.002-0.004			0.01	0.00-0.01		
Cr (mg kg^{-1})	0.16	0.11-0.20			0.20	0.18-0.22		
Pb (mg kg^{-1})	0.09	0.07-0.11			0.15	0.14-0.17		

Elements	54 months				76 months			
	NBP	Range	NBP	Range	NBP	Range	NBP	Range
N (g kg^{-1})	20.9	19.9-21.9	21.0	20.0-21.9	14.3	13.4-15.2	14.3	13.3-15.2
P (g kg^{-1})	1.38	1.32-1.44	1.38	1.32-1.44	0.95	0.86-1.04	0.94	0.84-1.03
K (g kg^{-1})	8.84	8.13-9.54	8.88	8.17-9.58	8.95	8.33-9.56	8.93	8.31-9.55
Ca (g kg^{-1})	9.25	8.54-9.96	9.28	8.57-9.99	10.2	8.8-11.6	10.2	8.8-11.7
Mg (g kg^{-1})	1.99	1.86-2.12	2.01	1.88-2.13	2.37	2.11-2.64	2.42	2.15-2.69
S (g kg^{-1})	1.54	1.46-1.62	1.55	1.47-1.63	1.08	1.01-1.15	1.08	1.01-1.15
B (mg kg^{-1})	37	30-43	37	30-43	55	46-63	55	46-64
Cu (mg kg^{-1})	4.33	3.97-4.69	4.33	3.98-4.69	4.71	4.21-5.20	4.73	4.23-5.22
Fe (mg kg^{-1})	142	131-153	142	131-153	130	113-147	129	112-146
Mn (mg kg^{-1})	223	191-254	224	192-255	306	236-375	305	236-375
Mo (mg kg^{-1})	0.02	0.01-0.02	0.02	0.01-0.02	0.07	0.05-0.09	0.07	0.05-0.09
Zn (mg kg^{-1})	15.9	15.2-16.7	16.0	15.2-16.7	15.5	13.9-17.0	15.5	13.9-17.1
Al (mg kg^{-1})	193	177-209			159	134-184		
As (mg kg^{-1})	0.04	0.03-0.04			0.03	0.03-0.04		
Ba (mg kg^{-1})	7.63	6.54-8.72			12.9	10.4-15.5		
Cd (mg kg^{-1})	0.003	0.002-0.004			0.005	0.003-0.006		
Cr (mg kg^{-1})	0.21	0.20-0.23			0.23	0.19-0.26		

Pb (mg kg^{-1})	0.09	0.08-0.09			0.09	0.07-0.11

Discussion

Populations productivity

Wood production reached an average annual increase above the Brazilian average of 36.8 m^3 ha^{-1} year^{-1}, recorded in 2020 (IBÁ, 2021), at the four evaluation ages, except for 22 months of age (Table 2). In scenario A, the high productivity subpopulations presented 64, 92, 86, and 84% of the populations with SS application, with 45, 54, 50, and 53% with SS application providing 100% of the N recommendation, supplemented with P, with or without B, for *Eucalyptus* at 22, 44, 54, and 76 months after planting, respectively. The high productivity subpopulation in B scenario had 75% of the populations with SS application, with 39% with SS application providing 100% of the N recommendation for *Eucalyptus*. These data demonstrate the representativeness of the SS application in the reference population, especially when applied according to agronomic criteria. Still, studies have already shown that the SS application in forest plantations can be a sustainable alternative for the destination of this residue, increasing soil fertility and increasing wood productivity (Abreu-Junior et al., 2017, 2020; Cardoso et al., 2022).

Evaluation of DRIS norms and functions

To verify the possibility of using a general norm to evaluate the nutritional status of *Eucalyptus* fertilized with SS, a comparison of the norms between the evaluated ages and a general norm was carried out (Table S2). However, there was a significant difference in the dual relationships of the elements between the different ages and between the ages and the general norm. This indicates that preference should be given to the use of specific norms for assessing the *Eucalyptus* nutritional status. A similar result was observed by Silva et al. (2005), evaluating the universality of the DRIS and CND standards for *Eucalyptus* in different regions in the Minas Gerais State.

With the norms defined, the use of different equations for the DRIS functions aiming to overestimate or underestimate the deficiency or excess of nutrient class can help in the interpretation and decision making about the management of nutrients (Wadt et al., 2007). In the HRMa group, the equations used overestimates the deficiency and underestimates the excess (Wadt et al., 2007), this is because the nutrients of this group (N, P, and K) in deficiency can cause great damage to the plants, while in excess there is low impairment of plant development (Fig. 2a). Thus, this method makes it possible to recommend the application of N, P, and K to improve plant nutrition and productivity.

For the RRMa group (Ca, Mg, and S), underestimating the deficiency and excess of nutrients can cause a decrease in the perception of the need to apply these nutrients in a situation of deficiency, due to the approximation of the equilibrium situation with the application of the correction factor (Fig. 2b). In the HRMi group (B, Cu, and Zn), nutrients have a potential toxic effect when in excess for *Eucalyptus* (Matiello et al., 2009; Soares et al., 2000, 2001), so both deficiency and excess were overestimated (Fig. 1c), thus, it is possible to develop an adequate management of these micronutrients at planting. For the RRMi group (Fe, Mn, and Mo), only the excess was overestimated, due to the possibility of toxicity by these elements in *Eucalyptus* (Fig. 2d).

The proposed equation for the PTE group aims to underestimate "deficiency" when $A/B<a/b$ and overestimate excess when $A/B>a/b$ (Fig. 3). This methodology was used due to the non-essentiality and possible toxic effect of PTE. In a situation where the elements of this group are in a lower ratio than the reference population ($A/B<a/b$), called "deficiency", lower concentration are observed, reducing the risks of toxicity, and compromising productivity. In the opposite situation, where the ratio is greater than that of the reference population ($A/B>a/b$), said in excess, the overestimation of the excess can help in the evaluation of the nutritional imbalance caused by these elements in the plant.

Assessment of nutritional status

The frequency of *Eucalyptus* populations in nutritional balance, deficiency, and excess was not significantly altered by the inclusion of PTE in the DRIS norms and functions for both groups of macronutrients (HRMa and RRMa). This occurred even though some evaluated elements show some type of interaction with macronutrients. For example, As can compromise the uptake and transport of P in plants (Singh et al., 2016), as well as Se (not evaluated in this work), shows interaction with S (Zhou et al., 2020). In both situations, there is competition between these elements in terms of absorption and translocation of nutrients in the plant. This occurs due to the low concentration of these elements in the plant, an important factor in the antagonism and synergism interactions between the elements (Safarzadeh et al., 2013).

However, the addition of PTE to the norms changed the frequency of populations in nutritional balance and imbalance for micronutrients. This result may demonstrate more sensitivity in the use of the method to assess the nutritional status of plants in relation to micronutrients with PTE. This can also be observed in the frequency of diagnostic agreement with or without the inclusion of PTE, where the agreements between nutrients in the HRMa group range from 60 to 90% and for micronutrients range from 60 to 98% for the HRMi group and from 57 to 98% for the RRMi group (Table 4).

Thus, the incorporation of these elements in the functions can provide important information as to which element is being more harmful to plants (Tables 6 and 7), without major changes in the interpretation of the plant's nutritional status and consequent fertility and nutrition management to be carried out adopted.

The same can be said when comparing the DRIS methods used in this work, in which the frequency of diagnosis agreement ranged from 51 to 100% of the populations (Table 5), indicating that the method proposed in this work, with the inclusion of PTE to the DRIS calculations, it does not change the interpretation of the results for the evaluation of the SS treated *Eucalyptus* nutritional status.

For PTE, considering that the situation of deficiency and nutritional balance of these elements are desired because they present values lower or similar to the subpopulation of high productivity, the evaluation of situations of these elements in excess is necessary. It can be seen that for the evaluated EPT (Al, As, Ba, Cd, Cr, and Pb), the frequency of excess plots ranged from 7 to 41% with the DRIS calculations (Table 3). Thus, it was observed that As and Pb (41%) had a higher frequency of populations in nutritional excess at 22 months after planting, As (37%) at 44 months after planting and Ba at 54 (42%) and 76 (30 %) months after planting. Based on this, attention should be paid to the concentration of these elements in the sewage sludge to be applied, even in *Eucalyptus* plantations, a large crop, long cycle and low exposure to humans and animals.

Order of nutritional limitation

By ordering the nutrient indexes in increasing order in each population, it is possible to verify the most limiting nutrient by deficiency (most negative index) and by excess (most positive index) (Pinto et al., 2010). Boron was an element of great interest in this study (Cardoso et al., 2022) and presented intriguing results (Tables 6 and 7). The higher frequency of populations in a situation of B deficiency in the low productivity subpopulation was due to the application of SS without supplementation with B, demonstrating the need for the addition of this nutrient in the management of *Eucalyptus* fertilization with SS and mineral fertilizers. In the high productivity subpopulation, B was limiting due to nutritional deficiency and excess. This occurred because the high productivity subpopulations received SS doses supplemented with or without B. Thus, the populations that received B application were in a situation of nutritional excess and those where there was no application, there was a deficiency of B. This result shows the need to adjust the dose of B to be applied when fertilizing with SS in a *Eucalyptus* plantation.

For PTE, in general, Ba was the most limiting element due to excess in the planting of *Eucalyptus* treated with SS. Nascimento et al. (2020), studying sewage sludge from different wastewater treatment plants in the state of São Paulo, observed that SS of mixed (industrial and domestic origin) are less prone to agricultural use due to the high PTE concentration, including Ba, with mean of 668 mg kg^{-1}, concentration similar to that observed in the sewage sludge used in this work (629 mg kg^{-1}; Table S2), but lower than the maximum limit allowed by Brazilian legislation (1.300 mg kg^{-1}; Brazil, 2020).

Element sufficiency ranges

The coefficient of determination (R^2) between the elements concentration and the element indexes developed by the DRIS functions, despite being significant, showed great amplitude, ranging from 0.28 to 0.94 (Tables S4 and S5). Equivalent results were also observed by other authors in distinct cultures (Camacho et al., 2012; Partelli et al., 2014; Santos et al., 2013; Santos; Rozane, 2017; Serra et al., 2010).

The nutrient sufficiency ranges and PTE of *Eucalyptus* treated with SS, determined by the DRIS functions, changed according to the age of planting, once again demonstrating the need for specific rules for each planting situation, such as age and location (Silva et al., 2005). It is also possible to observe a change in the concentration and a reduction in the amplitude of the sufficiency range in relation to the official recommendation of the State of São Paulo (Gonçalves et al., 1997). This occurs due to the specificity of the analysis used in this work and the use of the DRIS functions (Santos; Rozane, 2017).

For PTE, the use of this method can obtain and produce information on the maximum limit concentration of these elements in plants without any loss in productivity and growth of *Eucalyptus* fertilized with SS. Thus, further studies are recommended with the objective of obtaining norms, sufficiency range and maximum concentration of PTE for distinct cultures and management of fertilization with residues and organic fertilizers, such as SS.

The modeling of DRIS functions with the PTE inclusion was able to underestimate or overestimate these elements in situations of “deficiency” and excess, respectively, contributing to the assessment of the nutritional status of *Eucalyptus*. From the indices generated by the DRIS method, it was possible to determine which nutrients were the most limiting due to deficiency and excess, in addition to ordering the PTE limiting the *Eucalyptus* productivity. Barium was the most limiting element in the subpopulations of low and high productivity. The identification of populations in nutritional deficiency, balance, or excess for each nutrient by the concept of potential response to fertilization proved to be an effective tool for interpreting the DRIS indices, presenting equivalent results when PTE where or not included. Finally, the need for specific norms for each region or situation of analysis is highlighted.

References

Abreu-Junior CH, Firma LP, Maldonado CAB et al (2017) Fertilization using sewage sludge in unfertile tropical soils increased wood production in *Eucalyptus* plantations. J Environ Manage 203:51–58. https://doi.org/10.1016/j.jenvman.2017.07.074

Abreu-Junior CH, Oliveira MG, Cardoso PHS et al (2020) Sewage sludge application in *Eucalyptus urograndis* plantation: availability of phosphorus in soil and wood production. Front Environ Sci 8:116. https://doi.org/10.3389/fenvs.2020.00116

Aleisa E, Alsulaili A, Almuzaini Y (2021) Recirculating treated sewage sludge for agricultural use: Life cycle assessment for a circular economy. Waste Manage 135:79-89. https://doi.org/10.1016/j.wasman.2021.08.035

Araújo GCL, Gonzalez MH, Ferreira AG, Nogueira AR, Nóbrega JA (2002) Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials. Spectrochim Acta B 57:2121-2132. https://doi.org/10.1016/S0584-8547(02)00164-7

Athamenh B, Salem N, Et-Zuraiqi S, Suleiman W, Rusan M (2015) Combined land application of treated wastewater and biosolids enhances crop production and soil fertility. Desalin Water Treat 53:3283–3294. https://doi.org/10.1080/19443994.2014.933037

Baldock JO, Schulte EE (1996) Plant analysis with standardized scores combines DRIS and sufficiency range approaches for corn. J Agron 88:448-456. https://doi.org/10.2134/agronj1996.00021962008800030015x

Beaufils ER (1973) Diagnosis and recommendation integrated system (DRIS): a general scheme of experimentation and calibration based on principles developed from research in plant nutrition. Pietermaritzburg: University of Natal.

Bourioug M, Alaoui-Sossé L, Laffray X et al (2014) Evaluation of sewage sludge effects on soil properties, plant growth, mineral nutrition state, and heavy metal distribution in European Larch Seedlings (*Larix decidua*). Arab J Sci Eng 39:5325-5335. https://doi.org/10.1007/s13369-014-1100-0

Brazil (2006) Conselho Nacional Do Meio Ambiente - CONAMA. Resolução 375. Brasília, 31p. Available via: http://www.mma.gov.br/port/conama/legiano1.cfm?ano=todos&codlegitipo=3. Accessed Jul 2017.

Brazil (2020) Conselho Nacional Do Meio Ambiente - CONAMA. Resolução 498. Brasília, 8p. Available via: https://www.in.gov.br/en/web/dou/-/resolucao-n-498-de-19-de-agosto-de-2020-273467970. Accessed Dec 2020.

Calheiros LCS, Freire FJ, Moura Filho et al (2018) Different criteria for determining DRIS standards influencing the nutritional diagnosis and potential fertilization response of sugarcane. Aust J Crop Sci 12:995-1007. https://doi.org/10.21475/ajcs.18.12.06.PNE1147

Camacho MA, Silveira MV, Camargo RA, Natale W (2012) Faixas normais de nutrientes pelos métodos ChM, DRIS e CND e nível crítico pelo método de distribuição normal reduzida para laranjeira-pera. Rev Bras Cienc Solo 46:193-200. https://doi.org/10.1590/S0100-06832012000100020

Camargo AO, Moniz AC, Jorge JÁ, Valadares JMAS (1986) Método de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas. Campinas: Instituto Agronômico.

Cardoso PHS, Deboni Neto A, Oliveira FC et al (2022) Fertilization management with sewage sludge sustains *Eucalyptus* productivity in Cerrado infertile soil. For Ecol Manag 519:120338. https://doi.org/10.1016/j.foreco.2022.120338

Dias JRM, Wadt PGS, Partelli FL et al (2017) Normal nutrient ranges and nutritional monitoring of 'Pêra' orange trees based on the CND method in different fruiting stages. Pesq Agropec Bras 52:776-785. https://doi.org/10.1590/S0100-204X2017000900010

Gonçalves JLM, Raij B, Gonçalves JC (1997) Florestais. In: Raij B, Cantarella H, Quaggio JA, Furlani AMC (ed) Recomendações de adubação e calagem para o Estado de São Paulo. Instituto Agronômico de Campinas e Fundação IAC, Campinas, 285p.

IBA (2021) Indústria Brasileira de Árvores. Relatório IBA 2021. IBA: São Paulo – SP. Available via https://www.iba.org/datafiles/publicacoes/relatorios/relatorioiba2021-compactado.pdf. Accessed Jul 2022.

Kurihara CH, Venegas VHA, Neves JCL, Novais RF, Staut LA (2013) Faixas de suficiência para teores foliares de nutrientes em algodão e em soja, definidas em função de índices DRIS. Rev Ceres 60:412-419. https://doi.org/10.1590/S0034-737X2013000300015

Jones WW (1981) Proposed modifications of the diagnosis and recommendation integrated system (DRIS) for interpreting plant analyses. Commun Soil Sci Plant Anal 12:785-794. https://doi.org/10.1080/00103628109367194

Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do estado nutricional das plantas: princípios e aplicações. 2ed. POTAFOS, Piracicaba.

Marron N (2015) Agronomic and environmental effects of land application of residues in short-rotation tree plantations: A literature review. Biomass Bioenerg 81:378-400. https://doi.org/10.1016/j.biombioe.2015.07.025

Marschner P (2012) Marschner's Mineral Nutrition of Higher Plants (Third Edition), Academic Press.

Mattiello EM, Ruiz HA, Silva IR et al (2009) Transporte de boro no solo e sua absorção por eucalipto. Rev Bras Cienc Solo 33:1281-1290. https://doi.org/10.1590/S0100-06832009000500021

Matos GSB, Fernandes AR, Wadt PGS et al (2018) DRIS calculation methods for evaluating the nutritional status of oil palm in the Eastern Amazon. J Plant Nutr 10:1240–1251. https://doi.org/10.1080/01904167.2018.1434199

Morais TCB, Prado RM, Traspadini EIF et al (2019) Efficiency of CI, DRIS and CND methods in assessing the nutritional status of *Eucalyptus* spp. Rooted cuttings. Forests 10:786. https://doi.org/10.3390/f10090786

Nascimento AL, Souza AJ, Oliveira FC et al (2020) Chemical attributes of sewage sludges: Relationships to sources and treatments, and implications for sludge usage in agriculture. J Clean Prod 258:120746. https://doi.org/10.1016/j.jclepro.2020.120746

Nogueira TAR, Franco A, He Z et al (2013) Short-term usage of sewage sludge as organic fertilizer to sugarcane in a tropical soil bears little threat of heavy metal contamination. J Environ Manage 114:168-177. https://doi.org/10.1016/j.jenvman.2012.09.012

Partelli FL, Dias JRM, Vieira HD, Wadt PGS, Paiva Júnior E (2014) Avaliação nutricional de feijoeiro irrigado pelos métodos CND, DRIS e faixas de suficiência. Rev Bras Cienc Solo 38:858-866. https://doi.org/10.1590/S0100-06832014000300017

Pinto PAC, Dias LE, Alvarez V VH, Choudhury MM, Vieira G (2010) Avaliação do estado nutricional da mangueira Tommy Atkins no submédio do vale do rio São Francisco: cálculo dos índices DRIS. Recursos Rurais 6:5-13. https://doi.org/10.15304/rr.id5292

Prates AR, Kawakami KC, Coscione AR et al (2022) Composted sewage sludge sustains high maize productivity on an infertile Oxisol in the Brazilian cerrado. Land 11:1246. https://doi.org/10.3390/land11081246

Raij B, Andrade JC, Cantarella H, Quaggio JA (2001) Análise Química para Avaliação da Fertilidade de Solos Tropicais, 1st Edn. Campinas: IAC.

Raij B, Cantarella H, Quaggio JÁ, Furlani AMC (1997) Recomendações de adubação e calagem para o Estado de São Paulo. 2ed. Instituto Agronômico de Campinas, Campinas.

Ribeiro FO, Fernandes AR, Galvão JR et al (2020) DRIS and geostatistics indices for nutritional diagnosis and enhanced yield of fertirrigated acai palm. J Plant Nutr 43:1875-1886. https://doi.org/10.1080/01904167.2020.1750643

Safarzadeh S, Ronaghi A, Karimian N (2013). Effect of cadmium toxicity on micronutrient concentration, uptake and partitioning in seven rice cultivars. Arch Agron Soil Sci 59:231–245. https://doi.org/10.1080/03650340.2011.622752

Santos EF, Donha RMA, Magno CM (2013) Faixas normais de nutrientes em cana-de-açúcar pelos métodos CHM, DRIS e CND e nível crítico pela distribuição normal reduzida. Rev Bras Cienc Solo 37:1651-1658. https://doi.org/10.1590/S0100-06832013000600021

Santos EMH, Rozane DE (2017) DRIS standard and normal ranges of foliar nutrients for the culture of ‘Thompson’ atemoya. Cienc Rural 47:e20160613. https://doi.org/10.1590/0103-8478cr20160613

Schumacher FX, Hall FS (1933) Logarithmic expression of timber-tree volume. J Agric Res 47:719-734.

Serra AP, Marchetti ME, Vitorino ACT, Novelino JO, Camacho MA (2010) Determinação de faixas normais de nutrientes no algodoeiro pelos métodos ChM, CND e DRIS. Rev Bras Cienc Solo 34: 97-104. https://doi.org/10.1590/S0100-06832010000100011

Silva GGC, Neves JCL, Alvarez V VH, Leite FP (2005) Avaliação da universalidade das normas DRIS, M-DRIS E CND. Rev Bras Cienc Solo 29:755-761. https://doi.org/10.1590/S0100-06832005000500011

Silva GP, Prado RM, Wadt PGS et al (2020) Accuracy measures for phosphorus in assessing the nutritional status of sugarcane using the comprehensive integrated diagnosis and recommendation system (DRIS). J Plant Nutr 44: 1287-1299. https://doi.org/10.1080/01904167.2020.1849299

Silva RDS, Jalal A, Nascimento REN et al (2022) Composted sewage sludge application reduces mineral fertilization requirements and improves soil fertility in sugarcane seedling nurseries. Sustainability 14:4684. https://doi.org/10.3390/su14084684

Singh S, Parihar P, Singh R, Singh VP, Prasad (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomic. Front Plant Sci 6:1143.

https://doi.org/10.3389/fpls.2015.01143

Soares CRFS, Grazziotti PH, Siqueira JO, Carvalho JG, Moreira FMS (2001) Toxidez de zinco no crescimento e nutrição de *Eucalyptus maculata* e *Eucalyptus urophylla* em solução nutritiva. Pesq Agropec Bras 36:339-348. https://doi.org/10.1590/S0100-204X2001000200018

Soares CRFS, Siqueira JO, Carvalho JG, Moreira FMS, Grazziotti PH (2000) Crescimento e nutrição mineral de *Eucalyptus maculata* e *Eucalyptus urophylla* em solução nutritiva com concentração crescente de cobre. R Bras Fisiol Veg 12:213-225. https://doi.org/10.1590/S0103-31312000000300005

Soil Survey Staff (2014). Keys to Soil Taxonomy, 12th, Edn. Washington, DC: USDA-NRCS. https://doi.org/10.1109/TIP.2005.854494

Souza HA, Rozane DE, Amorim DA et al (2015) Assessment of nutritional status of guava seedlings using preliminary DRIS norms and sufficiency ranges. J Plant Nutr 38:1611-1618. https://doi.org/10.1080/01904167.2015.1017050

Systat Software. SigmaPlot. San Jose, California USA, 2020.

USEPA (2007) Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils and oils. Revision 1. Washington, DC.

Wadt PGS (2004) Nutritional status of *Eucalyptus grandis* clones evaluated by critical level and DRIS methods. Rev Arvore 28:15-20. https://doi.org/10.1590/S0100-67622004000100003

Wadt PGS (2005) Relationship between soil class and nutrition status of coffee plantations. Rev Bras Cienc Solo 29:227-234. https://doi.org/10.1590/S0100-06832005000200008

Wadt PGS, Silva DJ, Maia CE et al (2007) Modelagem de funções no cálculo do índice DRIS. Pesqui Agropecu Bras 42:57-64, 2007. https://doi.org/10.1590/S0100-204X2007000100008

Xue J, Kimberley MO, Ross C (2015) Ecological impacts of long-term application of biosolids to a radiata pine plantation. Sci Total Environ 530:233-240. https://doi.org/10.1016/j.scitotenv.2015.05.096

Zhou X, Yang J, Kronzucker HJ, Shi W (2020) Selenium biofortification and interaction with other elements in plants: A review. Front Plant Sci 11:586421. https://doi.org/10.3389/fpls.2020.586421

Supplementary Files

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