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Note

Agronomic and environmental implications of using a By-Product of the Intermediate Tanning Processes as Nitrogen Fertilizer

Silvia Maria de Oliveira-Longatti[1], Marcele Gabriel Cannata[1], Júlia Costa Ferrazani[2], Teotonio Soares de Carvalho[1], Claudio Ciavatta[3], Luiz Roberto Guimarães Guilherme[1]*

[1]Federal University of Lavras – Dept of Soil Science, Campus Universitário, C.P. 3037 – 37200-000 – Lavras, MG – Brazil.
[2]Federal University of Lavras – Dept. of Chemistry.
[3]University of Bologna/Institute of Agricultural Chemistry, Via Zamboni, 33 – 40126 – Bologna – Italy.
*Corresponding author <guilherm@dcs.ufla.br>

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ABSTRACT: Nitrogen (N) is an important nutrient for agriculture, and Brazil is heavily dependent on N imports. A by-product of the intermediate processes of tanning (BPIPT) may be used as an N fertilizer which will reduce this dependency, but its chromium (Cr) content is a matter of concern. This work assessed Cr (III, VI) and N (total, inorganic) contents in four soil samples with contrasting characteristics (especially with respect to their content of manganese (Mn), a potential Cr(III) oxidant), following the addition of the BPIPT. Chemical and microbiological indicators of soil quality were measured to assess the agronomic and environmental implications of the BPIPT addition in Brazilian soils. Our results indicate that the BPIPT is a promising source of N. The originally available Mn content in the soil did not influence the effect of the BPIPT on soil Cr(VI) content. Finally, microbial activity was generally stimulated after BPIPT addition to the soil. This information is relevant because: 1) it shows that the beneficial use of the BPIPT as an N fertilizer is important for adding value to a by-product with agronomic potential; and 2) it indicates that, at the dosage of the BPIPT used in this study (2.5 g kg_{soil}^{-1}), the typical increases in the soil concentration of labile Cr (0-25 mg kg_{soil}^{-1}) and Cr(VI) (0-0.8 mg kg_{soil}^{-1}) due to the application of the BPIPT are not detrimental to biological activity in the soil. However, further investigations are still necessary to evaluate the mobility of these Cr species in the soil and possible risks of groundwater contamination, which were not addressed in this study.

Keywords: high-Mn soils, agronomic efficiency, trivalent chromium, hexavalent chromium, microbiological parameters

Introduction

Brazil is a worldwide leader in leather exportation (CICB, 2013) and is increasingly more dependent on fertilizer imports (FAO, 2015). The leather tanning process releases large amounts of organic residues rich in C (average contents between 38 % and 50 %) and organic N (average content between 8 % and 13 %) (Ciavatta and Sequi, 1989), which can be converted into hydrolyzed leather, and utilized as an organic fertilizer, a common practice in several countries in Asia and Europe (Ciavatta and Gessa, 2007).

However, there is concern about the Cr contained in this by-product of the intermediate tanning process (BPIPT), which is mostly in a non-toxic form, i.e., Cr(III), with values between 0.5 % and 3 % and smaller amounts (usually < 0.01 mg kg^{-1}) of Cr(VI) (Ciavatta and Gessa, 2007). A regulation for the maximum concentration of Cr(VI) in organic manure (2 mg kg^{-1}) was recently approved in Brazil by MAPA (2016). Because typical concentrations of Cr(VI) in the BPIPT are usually much below this value, the production and utilization of the BPIPT in Brazil is likely to increase.

Cr(III) is the most stable Cr species in the soil and its conversion into Cr(VI) is generally not thermodynamically favorable under natural environmental conditions. Yet, the oxidation of Cr(III) to Cr(VI) may be favored by high levels of oxidants, such as manganese dioxide (Stern et al., 2010). Cr(VI) is more soluble and mobile than Cr(III) under environmental conditions. In addition, Cr(VI) is mutagenic and carcinogenic to animals and humans. Therefore, it is necessary to monitor this Cr species, instead of focusing only on the total Cr content in the soil.

Previous studies have addressed the use of BPIPT as an N fertilizer (Ciavatta and Sequi, 1989; Ciavatta and Gessa, 2007), but none has focused on the use of this by-product in high-Mn soils, which is relevant not only to agronomic but also to environmental purposes. This work aims to clarify how high native contents of Mn and/or Cr affect the liquid mineralization of N from the BPIPT and the content of Cr(VI) and easily oxidizable Cr(III). Owing to the need to complement information of agronomic and environmental relevance linked to the use of the BPIPT as an N fertilizer, a number of properties related to Cr and N transformations in soils were evaluated, as well as those biological attributes relevant to soil quality.

Materials and Methods

Soil sampling and characterization

Two different soils were collected in the Minas Gerais Quadrilátero Ferrífero, MG, Brazil, an area lo-

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cated basically in the range of latitudes 19º to 21ºS and longitudes 43º to 45º W. One was classified as Latosolic Concretionary Petric Plinthosol - FFc (A and B horizons) and the other as Typic Perferic Red-Yellow Latosol - LVAj (A and B horizons) (Embrapa, 2006). Information about these soils is shown in Table 1.

The choice of soils and horizons to be studied was based on their contents of Cr, Mn, and organic matter. Even though these soils have little agricultural representativeness, their native content of manganese are among the highest worldwide, which characterize them as worst case scenario (highest risk) in terms of possibility of conversion of Cr(III) into Cr(VI). Similarly, even though B horizons cannot also be used as representative soil samples that may receive the addition of fertilizers (for example, hydrolyzed leather), they could represent situations in which the formation of Cr(VI) would be facilitated due to their low organic matter content. The chemical characterization of these soil samples followed the procedure recommended by Embrapa (1997) (Table 2). Soil phytoavailable Cr was extracted by Mehlich-1 (Mehlich, 1978); the Cr semitotal was determined using the USEPA 3051a method (USEPA, 1998); and the Cr total was determined by Reflection X-Ray Fluorescence (Marguí et al., 2010) (Table 2).

Material used in the experiment

Fertilizers based on leather are obtained through hydrolysis of the raw materials, which involves bringing the materials to a temperature of 160-165 °C for at least 10 min and to a pressure of about 5-6 bars (Ciavatta and Gessa, 2007). The final product presents a granular form at the end of the process (1-3 mm diameter particles). In order to verify the behavior and effect of Cr and N in the soil, a product was employed with the following properties: 25,000 mg kg^{-1} of total Cr; 2,632 mg kg^{-1} labile Cr; 0.8 mg kg^{-1} of Cr(VI); 120 g kg^{-1} of N; and 400 g kg^{-1} of C. Analyses were performed according to the methodologies proposed respectively by: Marguí et al. (2010), Barlett and Kimble (1976a), Barlett and Kimble (1976b), and the Kjeldahl method (ISO 11261). Carbon was determined using a TOC analyzer

Experimental design and treatments

The experiment was conducted in a controlled-temperature greenhouse (at 25 to 35 °C), disposed in an entirely randomized design, with three replicates, and consisted of a dose of the by-product of the intermediate processes of tanning (BPIPT) applied to two soils in two horizons (A and B) and the respective control treatment without the BPIPT for each soil and horizon. Each experimental plot contained 250 g of soil packed in funnels under vacuum. In the plots that received the BPIPT, 0.625 g of the product was applied, corresponding to the application of 300 mg N kg^{-1} of soil. Samples were kept at 50 % of the soil field capacity. Soil samplings for further analyses were done at 0, 15, 45, and 75 days after incubation (DAI) with the BPIPT. Out of a total of 96 experimental plots, 24 were used at each sampling time.

Chemical analyses done after incubation of soil with the BPIPT

The following chemical analyses were performed after the incubation of soil with the BPIPT: total nitrogen (N_{total}), quantified from 0.1 g of soil by the Kjeldahl method (ISO 11261:1995); nitrate (NO_3-N) and ammonium (NH_4-N), quantified from 10 g of soil using the ISO 2005 Soil quality method; labile Cr(III), determined in 0.6 g of soil (Barlett and Kimble, 1976a); Cr(VI) extracted in water (Barlett and Kimble, 1976b).

The mineralization rate and N mineralization fraction were calculated following the method recommended by the Environmental Sanitation Technology Company (CETESB, 1999).

Table 1 – Coordinates, location, altitude, native vegetation, and parent material of soils used in the experiment.

Soils	Coordinate	Localization	Altitude	Native Vegetation	Parent Material	Horizon Depth	
						A	B
			m			cm	
FFc	20º11'42" S and 43º52'8.05" W	Nova Lima (MG)	1260	Cerrado field (altimontano)	Ferruginous Dolomite	0 - 20	171 - 200
LVAj	20º06' S and 43º52'30" W	Nova Lima (MG)	1020	Tropical field	Serpentinite	0 - 20	80 - 110

Table 2 – Chemical analyses, phytoavailable, semitotal and total chromium (Cr) in the soils used in the experiment.

Soils	pH		Organic Matter	Mn[1]	Al[1]	Fe[1]	Clay	Sand	Cr[1] Phyto-available	Cr[2] Semitotal	Cr[3] Total
	H_2O	$CaCl_2$									
			g kg^{-1}	dag kg^{-1}					mg kg^{-1}		
FFc (A horizon)	6.0	5.5	24.2	0.8	2.1	22	11	24	0.04	61.24	647
FFc (B horizon)	6.3	5.8	4.4	0.15	2.6	20	48	12	< DL	52.15	265
LVAj (A horizon)	6.3	5.9	39.4	0.13	0.8	24	34	13	4.43	7,629.54	17,126
LVAj (B horizon)	6.5	6.3	8.1	1.05	0.9	25	26	21	27.2	7,013.38	23,169

[1]Soluble Mn, Al, Fe and Phytoavailable Cr – Mehlich-1 extractant; [2]Semitotal Cr USEPA 3051; [3]Total Cr - X-Ray Fluorescence; DL = Detection Limit.

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Microbiological soil parameters

After the incubation, the following biological parameters were evaluated: carbon content of the microbial biomass (CMB) by the fumigation-extraction method in 20 g of soil as described in Vance et al. (1987); soil basal respiration in 20 g of soil as described in Alef (1995); microbial quotient (Q_{Micro}), calculated as the ratio between CMB and organic carbon (C_{org}) (Sparling, 1992); metabolic quotient (qCO_2), calculated as the ratio between respiration and CMB, which represents the amount of C-CO_2 released by unit of microbial C (Anderson and Domsch, 1993); β-glucosidase activity, measured as described by Eivazi and Tabatabai (1988); urease activity, quantified from 5 g of soil as described in Tabatabai and Bremner (1972).

Statistical analyses

Models for chemical and biological soil attributes were estimated by generalized least squares because these parameters were not homoscedastic across combinations of time, treatment, and horizons (Pinheiros and Bates, 2000). After verifying the significance of the models by likelihood ratio tests, a contrast between control and the BPIPT-treated samples for each time and horizon was tested and, in order to avoid the inflation of Type-I error due to multiple testing, the obtained *p*-values were then corrected using the false discovery rate method (Benjamini and Hochberg, 1995).

Redundancy analyses were performed separately for each horizon in each soil using the combinations of treatment (the BPIPT and the control) and time after incubation as explanatory variables. This multivariate analysis is a constrained version of principal components analysis that allows for studying how a matrix of response variables (soil chemical/biological properties in this study) varies in response to a matrix of explanatory variables (in this case a set of dummy variables indicating the combination of treatment and time) (Bocard et al., 2011). All analyses were done in the R 3.0.1.

Results and Discussion

Figure 1A-D shows the ordination of soil parameters evaluated as a function of the treatments and time based on redundancy analysis (RDA). It clearly shows the separation between the control and the BPIPT-treated samples along the horizontal axis for all soils and horizons (Figure 1A-D). In all cases, parameters related to soil nitrogen, especially N_{inorg}, NH_4^+, and NO_3^- contributed the most to the separation between treatments. For the Typic Perferic Red-Yellow Latosol (LVAj) A-horizon, this separation was predominantly due to increased N_{total} and respiration in the BPIPT-treated samples (Figure 1C).

As shown in Figure 2, the BPIPT application increased the N mineralization rate in both soils, with this effect being more pronounced in the A horizon due to higher microbial activity in this soil horizon (Table 5 and Table 6). In fact, Figure 3 shows that more than 50 % of the N originally added to the soil with the BPIPT was completely mineralized 45 days after incubation, whereas less than 13 % of the N added with the BPIPT was mineralized in the B horizon after 75 days. A peak of mineralization of the N in the BPIPT was observed at 45 days for the A-horizon of the LVAj followed by immobilization of inorganic N at 75 days after incubation. Application of the BPIPT generally increased all measured N parameters (N_{total}, NH_4^+, NO_3^-, and N_{inorg}) as shown in Table 3 and 4, indicating the effectiveness of this product as an N-fertilizer, especially in the top soil horizon, where it is more likely to be applied in

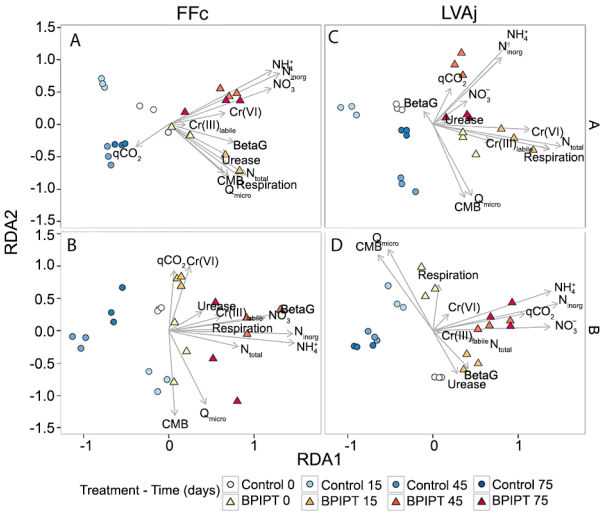

Figure 1 – Redundancy Analysis (RDA) of chemical parameters (N_{total}, NH_4^+, NO_3^-, Cr(III) labile, and Cr(VI)) and microbiological parameters (CMB = carbon of the microbial biomass; Respiration, qCO_2 = metabolic quotient; Q_{micro} = microbial quotient; BetaG = β-glucosidase and Urease). A) soil FFc A-horizon and B) B-horizon; C) soil LVAj A-horizon and D) B-horizon. BPIPT = by-product of intermediate tanning process.

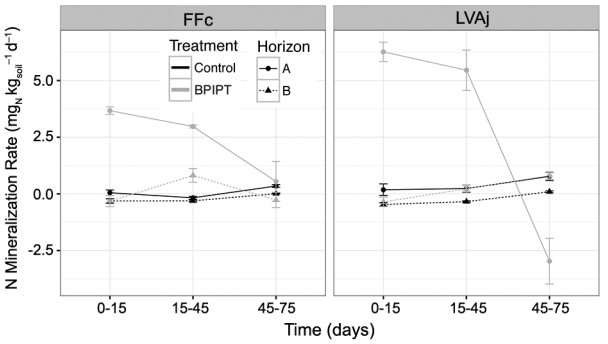

Figure 2 – Nitrogen mineralization rate in the A and B horizons of two soils (FFc and LVAj) with and without application of by-product of intermediate tanning process (BPIPT).

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Table 3 – Chemical analyses of nitrogen in samples of the FFc soil after different incubation times with by-product of intermediate tanning process (BPIPT).

Parameter	Time	FF_C Soil			
		A Horizon		B Horizon	
		Control	BPIPT	Control	BPIPT
N_{total}	0	0.90 ± 0.00	1.13 ± 0.09	0.30 ± 0.00	0.70 ± 0.06***
	15	1.40 ± 0.06	2.90 ± 0.06***	0.41 ± 0.05	0.72 ± 0.08*
	45	1.05 ± 0.04	1.23 ± 0.05	0.52 ± 0.05	0.60 ± 0.02
	75	1.00 ± 0.06	1.53 ± 0.09***	0.10 ± 0.00	0.70 ± 0.00***
NH_4-N	0	10.70 ± 0.62	14.68 ± 0.92**	12.43 ± 0.60	14.85 ± 1.99
	15	11.74 ± 0.75	57.49 ± 1.08***	8.97 ± 0.75	11.74 ± 1.20
	45	6.91 ± 0.62	126.56 ± 0.75***	0.00 ± 0.00	29.70 ± 4.54***
	75	20.10 ± 1.28	161.13 ± 13.98**	0.00 ± 0.00	26.44 ± 3.59***
	0	4.06 ± 0.23	5.61 ± 0.65	3.31 ± 0.26	4.21 ± 0.08*
NO_3-N	15	3.71 ± 0.13	15.34 ± 0.62***	2.26 ± 0.23	2.70 ± 0.08
	45	3.61 ± 0.08	31.38 ± 0.53**	2.51 ± 0.26	8.12 ± 0.87***
	75	4.70 ± 0.64	11.18 ± 3.10	2.82 ± 0.26	3.54 ± 0.42
N_{inorg}	0	15.39 ± 0.81	21.15 ± 1.35**	16.47 ± 0.37	19.93 ± 2.03
	15	16.14 ± 0.90	76.22 ± 0.52***	11.76 ± 0.81	15.14 ± 1.23
	45	10.92 ± 0.72	165.39 ± 1.13***	2.51 ± 0.27	39.57 ± 5.18***
	75	21.26 ± 0.96	181.79 ± 15.36***	2.81 ± 0.27	31.54 ± 3.76***

N_{total} = total nitrogen (g kg^{-1}); NH_4-N, (mg kg^{-1}); NO_3-N, (mg kg^{-1}); N_{inorg} = inorganic nitrogen (mg kg^{-1}); In each line, for each parameter, the asterisks indicate the significance of the contrast between BPIPT and control treatments in each incubation time, in the respective soil horizon; *significant at $p = 0.05$; **significant at $p = 0.01$, and ***significant at $p = 0.001$.

Table 4 – Chemical analyses of nitrogen in samples of the LVAj soil after different incubation times with by-product of intermediate tanning process (BPIPT).

Parameter	Time	LVAj Soil			
		A Horizon		B Horizon	
		Control	BPIPT	Control	BPIPT
N_{total}	0	1.50 ± 0.00	1.87 ± 0.03***	0.40 ± 0.06	0.43 ± 0.03
	15	1.01 ± 0.11	3.13 ± 0.14***	0.63 ± 0.04	0.87 ± 0.07*
	45	1.98 ± 0.09	2.11 ± 0.07	0.49 ± 0.04	0.56 ± 0.04
	75	1.93 ± 0.03	2.40 ± 0.12**	0.10 ± 0.00	0.27 ± 0.03***
NH_4-N	0	27.28 ± 2.71	29.87 ± 1.65	14.51 ± 0.00	17.79 ± 1.20
	15	22.10 ± 1.05	102.04 ± 2.55***	8.80 ± 0.60	11.05 ± 1.41
	45	25.04 ± 2.70	248.47 ±14.51**	0.00 ± 0.00	16.92 ± 2.25***
	75	18.92 ± 0.93	147.39 ± 10.55**	0.00 ± 0.00	38.17 ± 0.36***
NO_3-N	0	8.62 ± 0.67	10.27 ± 0.35	4.01 ± 0.28	4.86 ± 0.22
	15	16.84 ± 0.35	27.87 ± 0.76**	3.00 ± 0.15	6.67 ± 0.22***
	45	20.75 ± 0.83	36.54 ± 0.23**	2.05 ± 0.10	7.17 ± 0.49***
	75	50.49 ±3.55	54.49 ± 2.90	4.86 ± 0.66	8.95 ± 0.46***
N_{inorg}	0	37.51 ± 2.21	41.90 ± 2.01	19.37 ± 0.28	23.69 ± 1.24*
	15	40.25 ± 0.96	135.92 ± 3.43***	12.33 ± 0.65	18.37 ± 1.70*
	45	47.26 ± 2.67	299.63 ± 15.32***	2.06 ± 0.10	25.09 ± 2.85***
	75	70.53 ± 2.67	210.55 ± 10.46***	4.86 ± 0.66	49.37 ± 0.62***

N_{total} = total nitrogen (g kg^{-1}); NH_4-N, (mg kg^{-1}); NO_3-N, (mg kg^{-1}); N_{inorg} = inorganic nitrogen (mg kg^{-1}); In each line, for each parameter, the asterisks indicate the significance of the contrast between BPIPT and control treatments in each incubation time, in the respective soil horizon; *significant at $p = 0.05$; **significant at $p = 0.01$, and ***significant at $p = 0.001$.

practice. Contents of NH_4^+-N and NO_3^--N were variable throughout the experiment as a function of the dynamic behavior of these ions, causing peaks of elevation and suppression in the soils, except for Latosolic Concretionary Petric Plinthosol (FFc) A-horizon, which continued to increase constantly for NH_4^+ (Table 3) and the LVAj for NO_3^- (Table 4). The variations in the contents of these elements are justified due to the complexity of these ions and the biochemical and microbiological processes in which they are involved.

In addition to increasing the N mineralization rate, the BPIPT application generally stimulated biological activity in both soils as indicated by the overall increases in respiration and enzymatic activities (Table

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Table 5 – Microbiological analyses in samples of the FFc soil after different incubation times with by-product of intermediate tanning process (BPIPT).

Parameter	Time	FF_C Soil			
		A Horizon		B Horizon	
		Control	BPIPT	Control	BPIPT
CMB	0	111.51 ± 42.58	94.18 ± 12.51	70.71 ± 0.70	87.00 ± 16.90
	15	113.91 ± 1.04	293.53 ± 20.36**	92.64 ± 5.16	22.46 ± 0.16***
	45	17.61 ± 0.92	49.54 ± 4.02***	39.44 ± 3.82	40.25 ± 4.29
	75	61.95 ± 3.55	60.22 ± 16.50	21.49 ± 4.32	41.85 ± 15.97
Respiration	0	94.40 ± 3.19	121.20 ± 1.24***	28.53 ± 1.68	21.12 ± 2.74
	15	123.15 ± 6.00	243.13 ± 6.22***	78.50 ± 1.56	74.69 ± 4.54
	45	64.72 ± 11.30	96.54 ± 7.12	31.07 ± 0.72	68.70 ± 4.23***
	75	134.53 ± 6.27	139.95 ± 10.90	69.19 ± 5.71	81.21 ± 3.21
qCO_2	0	5.17 ± 2.37	5.57 ± 0.80	1.68 ± 0.09	1.07 ± 0.21
	15	4.51 ± 0.25	3.48 ± 0.22*	3.55 ± 0.21	13.85 ± 0.78***
	45	15.28 ± 2.34	8.22 ± 0.86*	3.33 ± 0.22	7.26 ± 0.80***
	75	9.53 ± 1.24	10.37 ± 1.94	17.07 ± 3.58	11.13 ± 5.84
Q_{micro}	0	1.04 ± 0.40	0.97 ± 0.13	2.56 ± 0.03	4.94 ± 0.96
	15	1.07 ± 0.01	3.03 ± 0.21***	3.36 ± 0.19	1.27 ± 0.01***
	45	0.16 ± 0.01	0.51 ± 0.04***	1.43 ± 0.14	2.29 ± 0.24*
	75	0.58 ± 0.03	0.62 ± 0.17	0.78 ± 0.16	2.38 ± 0.91
β-Glucosidase	0	17.45 ± 0.57	24.55 ± 0.84***	1.49 ± 0.24	0.34 ± 0.09***
	15	10.44 ± 1.06	16.10 ± 0.52**	1.27 ± 0.10	1.37 ± 0.07
	45	18.39 ± 1.14	18.65 ± 1.61	0.00 ± 0.00	3.24 ± 0.12***
	75	12.58 ± 1.37	20.54 ± 2.78	1.32 ± 0.08	2.64 ± 0.26***
Urease	0	2.68 ± 0.12	7.65 ± 0.20***	4.01 ± 0.51	3.11 ± 0.35
	15	6.81 ± 0.53	13.30 ± 0.31***	6.99 ± 0.41	7.89 ± 0.06
	45	0.08 ± 0.08	2.65 ± 0.21***	0.09 ± 0.09	3.27 ± 0.06***
	75	0.37 ± 0.07	1.75 ± 0.42*	0.34 ± 0.34	0.41 ± 0.22

CMB = carbon of the microbial biomass (μg C g_{soil}^{-1}); Respiration, (mg_{CO2} kg_{soil}^{-1} d^{-1}); qCO2 = metabolic quotient ($\mu g_{C\text{-}CO2}$ h^{-1} 10^2/ μg_{CMB} g_{soil}^{-1}); Q_{micro} = microbial quotient (%); β-glucosidase activity, ($\mu g_{p\text{-}nitrophenol}$ g_{soil}^{-1} h^{-1}); Urease activity, ($\mu g_{N\text{-}NH4+}$ g_{soil}^{-1} h^{-1}); In each line, for each parameter, the asterisks indicate the significance of the contrast between BPIPT and control treatments in each incubation time, in the respective soil horizon; *significant at $p = 0.05$; **significant at $p = 0.01$, and ***significant at $p = 0.001$.

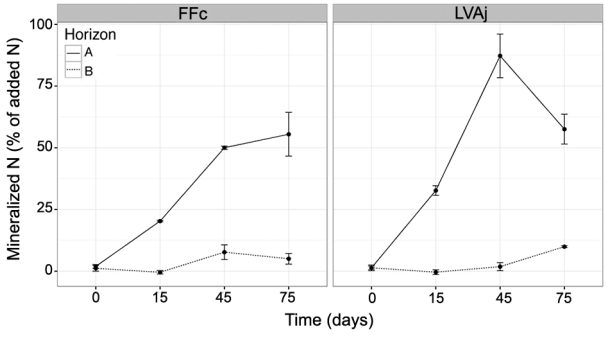

Figure 3 – Fraction of organic nitrogen from by-product of intermediate tanning process (BPIPT) mineralized at each incubation time in the A and B horizons of two soils (FFc and LVAj).

5 and 6). The carbon of the microbial biomass (CMB) fluctuated considerably (more than 4-fold) throughout the experiment, even for the control treatment, and it was generally responsive to the BPIPT. For the LVAj A-horizon, the BPIPT application increased the CMB at 15 and 75 days after incubation, but decreased CMB at 45 days in relation to the control treatment (Table 6). For the B-horizon of this soil, CMB increased immediately after the BPIPT application, but decreased at all the remaining incubation times (Table 6). For the FFc soil, the BPIPT application increased CMB at both 15 and 45 days after incubation in the A-horizon, but decreased CMB at 15 days after incubation in the B-horizon (Table 5).

The metabolic quotient (qCO_2) was also responsive to the BPIPT application. For the FFc at 15 and 45 days after incubation, the BPIPT application decreased the qCO_2 in the A-horizon, but increased it in the B-horizon (Table 5). For the LVAj A-horizon, the BPIPT application increased the qCO_2 from 0-45 days after incubation, but decreased it at 75 days (Table 6). For the B-horizon of this soil, the BPIPT application increased the qCO_2 from 15 to 75 days after incubation. The addition of organic C to the soil can stimulate microbial respiration without necessarily increasing the microbial biomass, resulting in higher qCO_2. In that case, increased qCO_2 should not be interpreted as an indicator of stressing conditions for soil biota (Fernandes et al., 2005; Thirukkumaran and Parkinson, 2000).

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Table 6 – Microbiological analyses in samples of the LVAj soil after different incubation times with by-product of intermediate tanning process (BPIPT).

Parameter	Time	LVAj Soil			
		A Horizon		B Horizon	
		Control	BPIPT	Control	BPIPT
CMB	0	187.03 ± 7.95	273.85 ± 34.95	97.69 ± 3.38	263.45 ± 22.04**
	15	178.94 ± 9.27	303.03 ± 21.97**	335.64 ± 18.77	103.05 ± 4.67***
	45	267.32 ± 10.56	68.55 ± 2.31***	72.01 ± 9.65	10.14 ± 0.03***
	75	37.13 ± 4.31	62.17 ± 2.34***	75.97 ± 0.60	23.40 ± 4.55***
Respiration	0	72.72 ± 1.83	196.62 ± 6.06***	73.36 ± 8.86	176.64 ± 5.47***
	15	54.94 ± 2.19	195.41 ± 3.42***	189.22 ± 5.70	201.28 ± 2.69
	45	65.65 ± 4.26	90.45 ± 3.04***	154.65 ± 3.01	167.35 ± 4.34
	75	86.52 ± 3.64	93.07 ± 8.84	264.67 ± 6.19	269.84 ± 4.26
qCO_2	0	1.62 ± 0.03	3.06 ± 0.27***	3.14 ± 0.38	2.82 ± 0.19
	15	1.29 ± 0.12	2.71 ± 0.19***	2.36 ± 0.09	8.18 ± 0.47***
	45	1.02 ± 0.04	5.50 ± 0.00***	9.26 ± 1.18	68.79 ± 1.74***
	75	10.53 ± 0.59	5.83 ± 0.46***	14.80 ± 0.31	52.13 ± 12.96**
Q_{micro}	0	1.12 ± 0.05	1.74 ± 0.22	2.31 ± 0.08	8.13 ± 0.68***
	15	1.07 ± 0.06	1.93 ± 0.14***	7.92 ± 0.44	3.18 ± 0.14***
	45	1.60 ± 0.06	0.43 ± 0.01***	1.70 ± 0.23	0.31 ± 0.00***
	75	0.22 ± 0.02	0.39 ±0.01***	1.79 ± 0.01	0.72 ± 0.14***
β-glucosidase	0	58.40 ± 1.32	59.00 ± 4.38	3.97± 0.23	1.88 ± 0.08***
	15	55.15 ± 2.21	52.17 ± 1.46	0.28 ± 0.17	0.24 ± 0.14
	45	45.06 ± 2.62	47.88 ± 7.71	1.14 ± 0.03	2.14 ± 0.22***
	75	46.02 ± 2.24	45.00 ± 1.66	1.12 ± 0.07	1.89 ± 0.95
Urease	0	4.15 ± 0.26	2.70 ± 0.36*	7.17 ± 0.32	4.83 ± 0.24***
	15	9.65 ± 0.45	11.90 ± 0.41**	7.84 ± 0.68	17.47 ± 1.42***
	45	0.61 ± 0.11	1.07 ± 0.70	0.18 ± 0.10	0.72 ± 0.14*
	75	0.18 ± 0.18	0.44 ± 0.09	0.16 ± 0.16	0.08 ± 0.08

CMB = carbon of the microbial biomass (μg C g_{soil}^{-1}); Respiration, (mg_{CO2} kg_{soil}^{-1} d^{-1}); qCO2 = metabolic quotient ($\mu g_{C\text{-}CO2}$ h^{-1} 10^2/ μg_{CMB} g_{soil}^{-1}); Q_{micro} = microbial quotient (%); β-glucosidase activity, ($\mu g_{p\text{-}nitrophenol}$ g_{soil}^{-1} h^{-1}); Urease activity, ($\mu g_{N\text{-}NH4+}$ g_{soil}^{-1} h^{-1}). In each line, for each parameter, the asterisks indicate the significance of the contrast between BPIPT and control treatments in each incubation time, in the respective soil horizon. *significant at p = 0.05; **significant at p = 0.01, and ***significant at p = 0.001.

Cr(VI) was detected in the studied soils, irrespective of the treatments, throughout all the incubation period (Figure 4A-D). In general, the effect of the BPIPT on the Cr(VI) concentration decreased toward the end of the incubation period (75 days after incubation) as had also been reported by Aquino Neto and Camargo (2000), working with solid tanning residues and $CrCl_3$ applied in Dusky-Red eutrophic Oxisol and a Yellow-Red Oxisol. According to these authors, Cr(VI) contents increased immediately after the application of hydrolyzed leather, possibly due to oxidation of Cr(III), but this effect decreased over time as was also observed in our study (Figure 4A-D). According to Trebien et al. (2011), decreases in the formation of Cr(VI) can be attributed to progressive decreases in the concentration of labile Cr(III) because of its precipitation in less soluble forms. In general, Cr(III) contents in the soil decreased over time, regardless of the treatment, except for the B-horizon of LVAj (Figure 5A-D). Bartlett and Kimble (1976a) observed a complete precipitation of trivalent chromium from a soil pH above 4.5.

Studies with soil samples kept under natural humidity indicate that Cr(III) can be oxidized to Cr(VI), especially in the presence of manganese in the oxidized form (Mn IV), which acts as an electron receptor (Trebien et al., 2011). On the other hand, electron donors abundant in the soil such as organic matter (Wittbrodt and Palmer, 1995) and Fe (II) in solution or in iron minerals (Fendorf and Li, 1996) can reduce Cr(VI) to Cr(III). Lower concentrations of Cr(VI) are expected in the A-horizon in relation to the B-horizon due to the usually higher organic matter content in the upper soil layers (Xiao et al., 2012). Yet, this attenuating effect of organic matter on the concentration of Cr(VI) was not observed in our study since, in both soils, Cr(VI) was generally more abundant in the A-horizon. Finally, with regard to the hypothesized effect of Mn on Cr oxidation, we did not observe higher concentrations of Cr(VI) in the soils with the highest Mn contents (FFc A-horizon, LVAj B-horizon) after the BPIPT application, indicating that the conversion of Cr(III) to Cr(VI) from the BPIPT is not influenced by the Mn content in the studied soils (Figure 4A-D).

Taken together, our results demonstrate that BPIPT can be an effective N fertilizer, aggregating value to a by-product with agronomic potential. At the dosage

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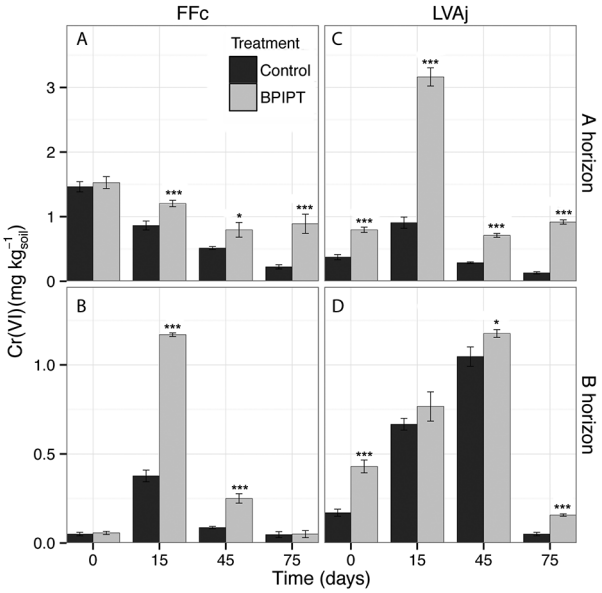

Figure 4 – Chromium (VI) (water-extracted) in different soil samples following the addition of by-product of intermediate tanning process (BPIPT). A) soil FFc A-horizon and B) B-horizon; C) soil LVAj A-horizon and D) B-horizon. Quantification limit of 0.35 mg kg^{-1}; *significant at p = 0.05; **significant at p = 0.01; and ***significant at p = 0.001.

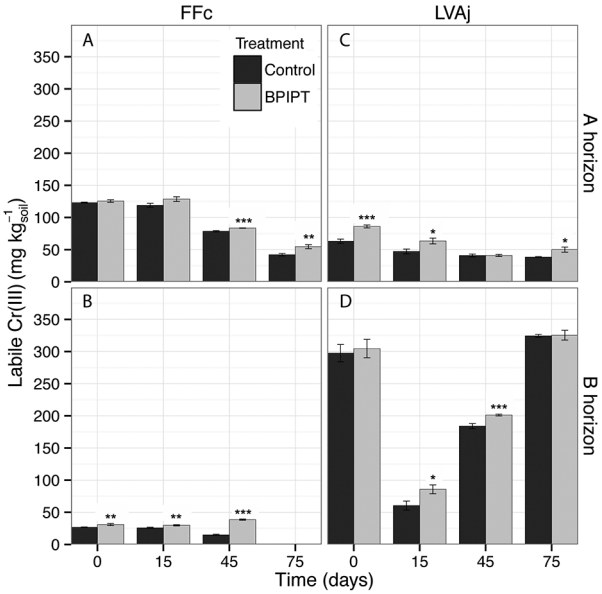

Figure 5 – Chromium (III) (labile) in different soil samples following the addition of by-product of intermediate tanning process (BPIPT). A) soil FFc A-horizon and B) B-horizon; C) soil LVAj A-horizon and D) B-horizon. *significant at p = 0.05; **significant at p = 0.01; and ***significant at p = 0.001.

tested in this study, the BPIPT application containing trace amounts of Cr species is not harmful to biological activity in the soil. However, further studies addressing the impact of repeated BPIPT applications on Cr concentration in the soil as well as the mobility of the applied Cr are still necessary to evaluate the environmental risk of this by-product.

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