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Cassava wastewater as ecofriendly and low-cost alternative to produce lettuce: impacts on soil organic carbon, microbial biomass, and enzymatic activities

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Abstract

The processing of cassava roots for starch extraction in factories generates a large amount of cassava wastewater (CW), which is rich in nutrients that are beneficial to plants. The reuse of this agro-industrial by-product is important for farmers and the environment as an alternative means to support soil fertility and plant production. Lettuce is the most important salad vegetable grown in the world. The objective of this study was to evaluate the impact of CW doses on soil organic carbon, basal respiration, microbial biomass, enzymatic activities, and lettuce growth in an Entisol. The experiment was conducted in pots in a greenhouse at room temperature in a completely randomized design with ten replications. The treatments consisted of the application of CW in increasing volumes that corresponded to fractions of the field capacity (FC) of the soil (100% FC = 206.6 ml dm^{-3}): 0% (negative control), 10%, 20%, 30%, 40%, and 50% of field capacity (FC) per pot. An additional treatment without CW was also included, applying only 1.0 g of mineral fertilizer per pot (20% N, 10% P, and 20% K). After 28 days of emergence, the 10% FC treatment responded similarly to mineral fertilizer, increasing the length of the branches (+42%), number of leaves (+45%), aerial fresh matter (+202%) and the concentrations of Chlorophyll A (+ 33%), and Chlorophyll B (+40%), in addition to soil organic carbon (+15%), all compared to the control. The enzymatic activities in the soil were shown to be sensitive to CW dosage, especially for urease, which grew linearly as a function of the increased pH and K^+ ions in the soil with the application of CW. The dose 20.7 ml CW dm^{-3} (10% FC) was that most increased plant variables, but the nutritional status of the soil and microbial activities benefited more from higher doses, starting from 30% FC, a scenario that could benefit plants more in future phenological stages, when there will be greater nutritional demand.

Keywords: *Manihot esculenta* residue; *Lactuca sativa*; soil conservation; multivariate analysis.
Abbreviations: CW_cassava wastewater; FC_field capacity; BL_lettuce main branch length; NL_lettuce number of leaves; SD_lettuce stalk diameter; AFM_lettuce aerial fresh matter; RFM_lettuce fresh root matter; ADM_lettuce aerial dry matter; RDM_lettuce root dry matter; CA_chlorophyll A; CB_chlorophyll B; TOC_total organic carbon; MBC_microbial biomass carbon; BRS_basal respiration of the soil; Paci_soil acid phosphatase; Palk_soil alkaline phosphatase; Ure_soil urease activity.

Introduction

Cassava (*Manihot esculenta* Crantz.) is the most important tuberous root crop in the world, corresponding to the main source of carbohydrates for the population of many countries (Zainuddin et al., 2018). The by-products from its production and processing have gained wide prominence in the scenario of agricultural sustainability, mainly through reuse to produce bioethanol, biodiesel and biogas (Ratnadewi et al., 2016) and use as a biofertilizer in tropical soils that are poor in nutrients (Moura et al., 2018). Research carried out in China has shown how promising and renewable these activities are, also highlighting the use of liquid waste to mitigating the emission of greenhouse gases (Ye et al., 2017; Jiao et al., 2018).

Given the progress and increase of cassava-producing areas, problems have arisen due to the incorrect disposal of its waste, which has caused serious impacts to the environment and its organisms (Oliveira and Jucá, 2004). Cassava wastewater (CW) is the main by-product from the production of manioc flour and starch, corresponding to about 30% of its total mass (Andrade et al., 2017). This residue is rich in proteins, lipids, carbohydrates, sugars (fructose and glucose), organic matter and hydrocyanic acid (Rosa et al., 2016; Gomes et al., 2016), in addition to a large amount of macro- and micronutrients essential to plants (Dantas et al., 2014; Ribas et al., 2010).

In this context, many studies have evaluated the potential use of CW in agriculture for various purposes, such as plant nutrition (Barreto et al., 2014; Dantas et al., 2017), biological defense (Nasu et al., 2015; Santos et al., 2016) and for improving soil quality, since it promotes an increase in microbial biomass and organic carbon content (Moura et al., 2018). The agricultural use of CW is also desirable due to its low cost, which could be a viable way to promote crop development through the partial replacement of synthetic commercial fertilizers (Santos et al., 2010). However, there is still a gap in our knowledge regarding CW application to sandy soils and the growth of horticultural crops, such as lettuce (*Lactuca sativa* L.), the most widely cultivated and consumed vegetable in Brazil (Scherer et al., 2016).

Considering the factors listed above, the hypothesis was raised that the application of CW doses in a sandy soil cultivated with lettuce would increase significantly the soil base saturation and enzyme activity, both important variables in lettuce good nutrition. The purpose of this study was to test the correlations between these variables and find the main benefits and disadvantages of CW for the soil and lettuce cultivation.

Results

Lettuce growth

The lettuce main branch length (BL) and number of leaves (NL) were the phenotypic attributes that most demonstrated a significant response to CW application (Fig 1A and Fig 1B). For these variables, a dosage of around 10% FC showed the best efficiency for providing significantly higher results than the negative control and was not distinguishable from treatment with mineral fertilizer. In these cases, the 10% FC treatment was sufficient to significantly increase the length of the branches and the number of leaves by 42% (8.6 cm to 12.17 cm) and 45% (11 to 16 leaves), respectively with respect to the control (Table 1).

Despite the high variability of some plant developmental data, important gains were observed in treatments with cassava wastewater (Table 1). The results showed that aerial fresh matter (AFM) from plants that received 10% FC weighed on average 28.96 g, while the control weighed only 9.6 g, an increase of 202% (Table 1). This CW dosage was more efficient for AFM, considering that a similar response only occurred at 50% FC, weighing on average 24.6 g (+144%). Similarly, with the 10% FC dosage, chlorophylls A and B showed an increase of 33% and 40%, respectively, with regard to the control and remained practically stable at subsequent CW dosages (Table 1).

After a stationary phase (75 ml kg^{-1} soil or 36% FC), the BL continued to grow significantly more than in the negative control (Fig 1A). Above 10% CW, the NL decreased and was not different from the control (Fig 1B). As the weights did not differ, the results suggest that the 20.7 ml dm^{-3} CW at 10% FC application led to the growth of thinner branches with more leaves, similar to the treatments with NPK, contrasting from the negative control.

Soil chemical and microbial attributes

The soil chemical attributes pH, potential acidity of soil (H^++Al^{3+}), Na^+, K^+, and total organic carbon (TOC) content showed significant differences among CW dosages, as well as soil basal respiration (SBR) and enzymatic activities, according to the F-test ($p < 0.05$) (Table 1).

Soil acidity declined significantly with the addition of CW, especially at 30% and 40% FC, where it practically neutralized the H^++Al^{3+} ions in the soil (Fig 1C). Also, in this range, K^+ content reached its peak, revealing the potential of CW as a tool for soil correction (Fig 1D). Moreover, the CW demonstrated strong potential to increase soil pH (Fig 1E), with cases where it raised the pH from 5.2 to 6.4 at the higher dosage (50% FC), reporting a positive and strongly adjusted correlation ($R^2 \sim 1$).

SBR peaked at 5.2 C-CO_2 g^{-1} soil day^{-1} at 30% FC and was significantly higher than the control and 10% FC treatments (Fig 1F). TOC showed a rapid increase at 10% FC (8.1 g kg^{-1}) with a maximum between 10% to 30% FC (8.2 g kg^{-1}), differing significantly compared to the control (7.0 g kg^{-1}) (Fig 1G). From these intervals, the TOC decreased again, such as microbial biomass carbon (MBC). The results provide consistent evidence of how CW doses equivalent to 30% affect the behavior of the soil microbial communities studied.

Soil enzymatic activities

The activity of both phosphatases decreased with CW, reaching minimum values for treatments with mineral fertilizer (NPK) or 40% FC (Fig 1H, Fig 1I). The alkaline phosphatase activity in the control treatment was significantly higher than in all other treatments (Fig 1H). The average activity of acid phosphatase between 0% and 20% FC was similar, corresponding to a significantly higher average than the range found between 30% and 50% FC (Fig 1I). Urease increased linearly as CW dosages increased (Fig 1J), recording a maximum activity of 337 µg NH_4-N g^{-1} dwt $2h^{-1}$ at a relative dosage of 50% FC, which represented a growth of 666% compared to the control.

Multivariate analysis

The Constrained Analysis of Principal Coordinates (CAP) was based on Euclidean distance, where the two main axes in biplot explained 75% of the total variance and 96% of the constrained (vectors) variance of eigenvalues (Fig 2). The Permutational Multivariate Analysis of Variance (PERMANOVA) revealed a good fit to the model, with R^2 = 0.72 for the plant attributes explanatory model by the Adonis test (Table 2), being a function of soil bio-chemical attributes (constraining variables). This indicated that lettuce development was influenced by chemical properties or soil enzymatic activities, with significant contributions of pH, soil basal respiration (SBR) and Na (Table 3). Other important attributes for lettuce vegetative development were soil acid phosphatase (Pac), soil urease (Ure), CW doses, total organic carbon (TOC), soil alkaline phosphatase (Palk), potassium (K), and microbial biomass carbon (MBC), in decreasing order (Table 3).

PERMANOVA showed the influence of axis 1 on the model was significant ($p < 0.015$), by the pseudo-F statistic. Therefore, the variables with higher scores on this axis are more important to explain the multivariate model. In this case, the plant attributes that responded well to soil attributes were (in decreasing order) AFM, root fresh matter (RFM), number of leaves (NL), main branch length (MBL), chlorophyll A (CA), root dry matter (RDM), aerial dry matter (ADM), chlorophyll B (CB), and stalk diameter (SD) (Table 4).

A multiple correlation was made among all variables to highlight the most important interactions, demonstrating strong positive interactions between pH, CW dosage, K concentrations in the soil and urease activity (Fig 3). The multiple correlation between the most significant variables revealed that lettuce MBL was more closely related to soil pH increase, while SBR was to K^+ contents (Fig 4). In addition, there was a strong positive influence of CW dosage on pH, K^+ ion concentration and soil urease activity, and a negative one on potential soil acidity. The strong correlation between these variables was tested by analysis of variance,

Table 1. Mean, standard deviation (S), coefficient of variation (CV%), and statistical test of the physiological data of lettuce plants (1 - 9), chemical attributes (10 - 16) and enzymatic activities (18 - 19) in soil cultivated with lettuce under increasing doses of cassava wastewater. Treatments that significantly responded to CW doses were submitted to regression analysis and tests on multiple pairs of means.

	Variable		Dosage - % FC (206.6 ml dm^{-3} soil)									
		NPK	*0%*	*10%*	*20%*	*30%*	*40%*	*50%*	mean	S	CV%	test
1	Chlorophyll A - CA (µg cm^{-2})	20.3	15.4	20.5	18.65	17.7	19.7	20.8	19.01	2.02	9.7	ns
2	Chlorophyll B - CB (µg cm^{-2})	3.4	2.75	3.85	3.15	3.1	3.85	3.6	3.39	0.45	13.7	ns
3	Aerial Dry Matter - ADM (g)	2.57	0.7	2.42	0.92	0.63	1.68	2.11	1.58	0.76	64.7	ns
4	Root Dry Matter - RDM (g)	2.57	2.13	3.15	2	1.05	2.32	2.11	2.19	0.67	42.6	ns
5	Aerial Fresh Matter - AFM (g)	22.13	9.6	28.96	13.3	12.11	15.98	24.62	18.10	8.55	65.5	ns
6	Root Fresh Matter - RFM (g)	18.51	10.08	11.93	10.83	6.59	11.82	9.78	11.36	1.96	44.6	ns
7	Main Branch Length - MBL (cm)	11.23	8.57	12.17	10.63	10.1	12.23	12.3	11.03	1.51	13.2	t
8	Number of Leaves – NL	13.67	11	16	12.33	11.33	11	12.33	12.52	1.9	11	t
9	Stalk Diameter - SD (cm)	0.9	0.78	1	0.93	0.8	0.73	0.78	0.85	0.11	16.3	ns
10	H^++Al^{3+} (cmolc dm^{-3})	0.17	0.17	0.17	0.06	0.06	0	0	0.09	0.08	75	t
11	K^+ (cmolc dm^{-3})	0.05	0.06	0.05	0.19	0.23	0.23	0.24	0.15	0.09	28.5	t
12	Na^+ (cmolc dm^{-3})	0.21	0.19	0.3	0.19	0.17	0.26	0.28	0.23	0.05	8.6	ns
13	pH	5.1	5.2	5.48	5.63	5.68	5.96	6.38	5.63	0.41	2.8	t
14	BRS [C-CO_2 (mg CO_2 g^{-1} day^{-1})]	4.25	1.63	3.57	4.02	5.18	3.55	3.95	3.74	1.15	12.1	t
15	Microbial Biomass Carbon (g kg^{-1})	6.25	12.92	11.25	11.04	8.33	6.77	8.33	9.27	2.32	16.2	ns
16	Total Organic Carbon (g kg^{-1})	6.92	7.02	8.1	7.66	8.19	6.95	7.63	7.50	0.52	3.3	t
17	Alkaline Phosphatase (µg PNF g^{-1} soil h^{-1})	6.41	74.38	30.75	31.5	12.59	4.16	9.96	24.25	25.69	34.5	d
18	Acid Phosphatase (µg PNF g^{-1} soil h^{-1})	5.64	46.86	41.99	30	5.85	1.71	11.65	20.53	19.26	42.4	d
19	Urease - Ure (µg NH_4-N g^{-1} dwt 2h^{-1})	101.01	44.06	91.76	216.87	173.05	292.8	337.34	179.56	113.31	56.5	d

t - Significant regression due to increased dosages by One-way ANOVA (ANalysis Of VAriance) with Tukey HSD (Honestly Significant Difference) and Duncan post-hoc test ($p < 0.05$); d - Significant regression due to increased dosages by One-way ANOVA (ANalysis Of VAriance) with Duncan post-hoc test ($p < 0.05$); ns - not significant regression by F-test ($p < 0.05$); SBR – soil basal respiration; dwt - dry weight of 1.0 g moist soil. The Duncan test was done for the enzyme data, as they showed greater variability than the others, being less rigorous than the Tukey test.

Table 2. The permutational multivariate analysis of variance (PERMANOVA) of the explanatory model of lettuce plant development as a function of soil biochemical attributes. For this purpose, Euclidean distance matrices were used in the Adonis test.

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)
Variables*	11	2217.92	201.629	3.0354	0.78768	0.0282
Residuals	9	597.84	66.427	0.21232		
Total	20	2815.76	1			

*Variables corresponding to CAP vectors (Fig 1). Free permutations: 10000. Df - degrees of freedom.

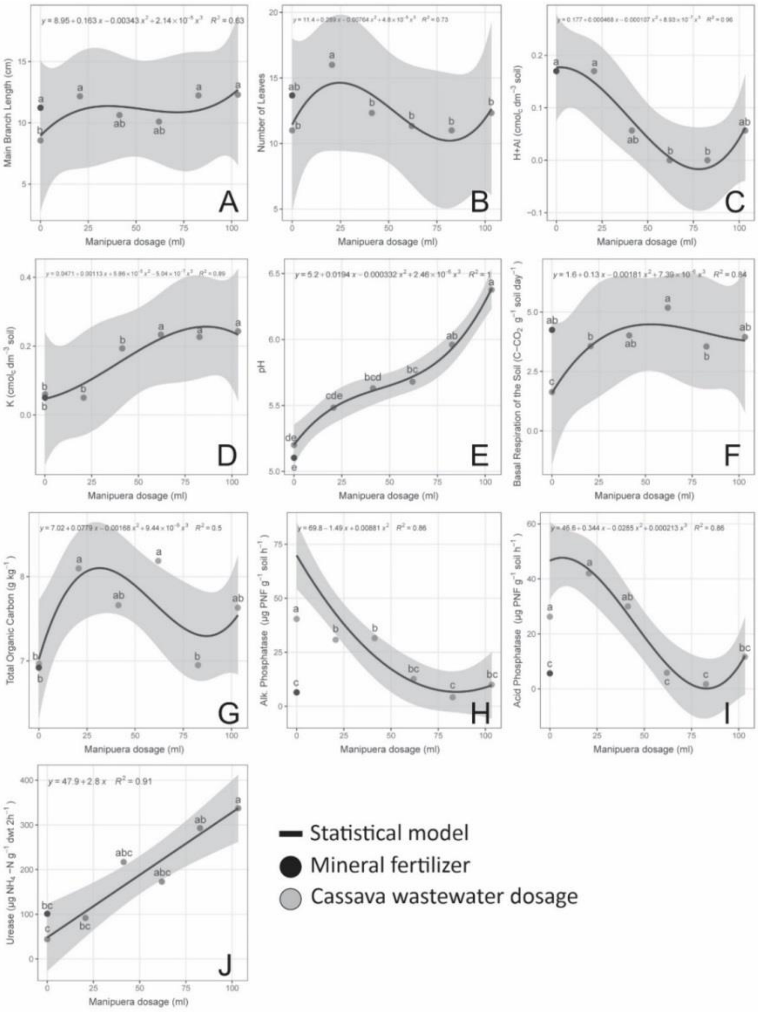

Fig 1. Main contributions of cassava wastewater to the soil and lettuce plants, showing regression curves of variables showing significant responses to cassava wastewater dosage. Averages followed with the same small letters are similar, according to the tests in Table 1 ($p \leq 0.05$). Black dots (out of the curve) represent the averages of additional treatment (NPK mineral fertilizer) while the gray dots are the progressive dosages of cassava wastewater in soil: 0%, 10%, 20%, 30%, 40% and 50% Field Capacity (206.6 ml kg^{-1} soil). The gray area around the loess smoothed-fit curve represents the confidence region (95%).

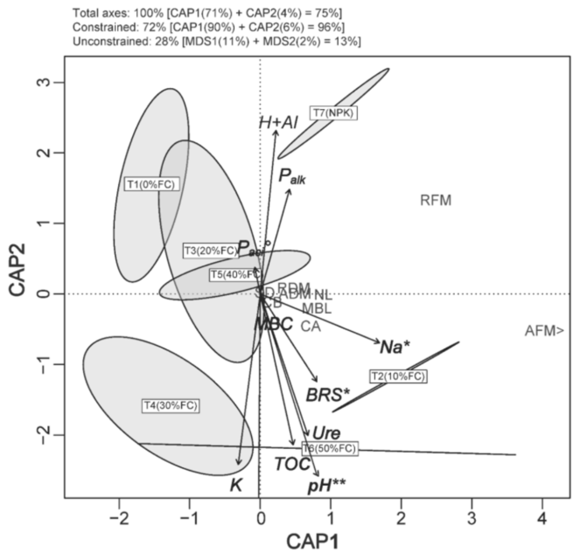

Fig 2. Canonical Analysis of Principal coordinates (CAP). Enzymatic activity and chemical attributes formed the explanatory matrix (Constraining) of the plant development model (Unconstraining) with the ellipses representing 30% of the observations. **Variables:** acid phosphatase (Pac), alkaline phosphatase (Palk), basal respiration of the soil (BRS), chlorophyll A (CA), chlorophyll B (CB), aerial dry matter (ADM), root dry matter (RDM), field capacity (FC), aerial fresh matter (AFM), root fresh matter (RFM), main branch length (MBL), microbial biomass carbon (MBC), number of leaves (NL), stalk diameter (SD), total organic carbon (TOC). Significance of the vectors by the permutation test in multivariate analysis (p-value): 0.001 '**' 0.01 '*' 0.05 '°' 0.1.

Table 3. The permutational multivariate analysis of variance (PERMANOVA) of the explanatory model of plant attributes as a function of soil bio-chemical attributes (constraining variables).

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	Pr(>F)	Pr(>F)
Dosage	1	77.8	77.8	1.147	0.028	0.305	
pH	1	891.4	891.4	13.145	0.317	0.001	***
Na	1	321.6	321.6	4.743	0.114	0.036	*
K	1	11.9	11.9	0.175	0.004	0.818	
TOC	1	44.3	44.3	0.653	0.016	0.462	
MBC	1	6.3	6.3	0.093	0.002	0.924	
Ure	1	88.1	88.1	1.299	0.031	0.277	
Palk	1	33.3	33.3	0.490	0.012	0.544	
Paci	1	208.3	208.3	3.072	0.074	0.091	.
BRS	1	454.8	454.8	6.706	0.162	0.017	*
Residuals	10	678.1	67.8	0.241			
Total	20	2815.8	1				

Df = degrees of freedom. TOC = total organic carbon; MBC= microbial biomass carbon; BRS = basal respiration of the soil; Paci = soil acid phosphatase; Palk = soil alkaline phosphatase; Ure = soil urease activity. Signif. Codes (p-values): 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1.

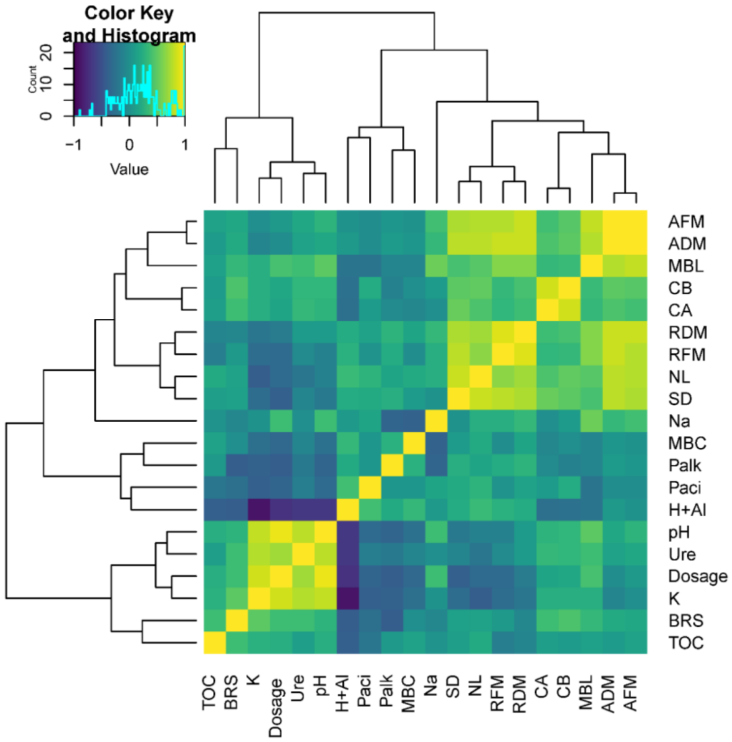

Fig 3. Heat map with correlation matrix of all variables and UPGMA dendrogram based on Pearson's R^2, the Pearson product-moment correlation coefficient (PPMCC). Darker shades represent more negative correlations while lighter shades are the most positive. It is possible to observe stronger correlations ($R^2 \rightarrow [1]$) between pH, urease activity and K contents (positive), followed by the potential acidity ($H^+ + Al^{3+}$) and the Basal Respiration of the Soil (negative). See detailed abbreviations of the variables in Figure 2.

Table 4. Scores of plant variables in the main axes of the CAP ordering. Axis 1 (CAP1) was significant at or below 5% to reject the null hypothesis by forward F-tests for axes, with variance equal to 111 and an F-value of 3.03. Therefore, the variables with the highest CAP1 scores better explained the proposed model, and those that best respond to the biochemical changes studied in this soil.

	CAP1*	**CAP2**	**CAP3**
MBL	0.805	-0.2	-0.334
NL	0.896	-0.01	0.408
SD	0.065	0.021	0.036
AFM	5.412	-0.53	-0.172
RFM	2.49	1.332	0.126
ADM	0.493	0.009	0.015
RDM	0.475	0.085	0.006
CA	0.717	-0.45	0.668
CB	0.168	-0.12	0.168

*Significant by permutation test for CAP scale under reduced model (p = 0.029). Abbreviations are as shown in legend of the figure 1. Free permutations: 1000. MBL = lettuce main branch length; NL = lettuce number of leaves; SD = lettuce stalk diameter; AFM = lettuce aerial fresh matter; RFM = lettuce root fresh matter; ADM = lettuce aerial dry matter; RDM = lettuce root dry matter; CA = chlorophyll A; CB= chlorophyll B.

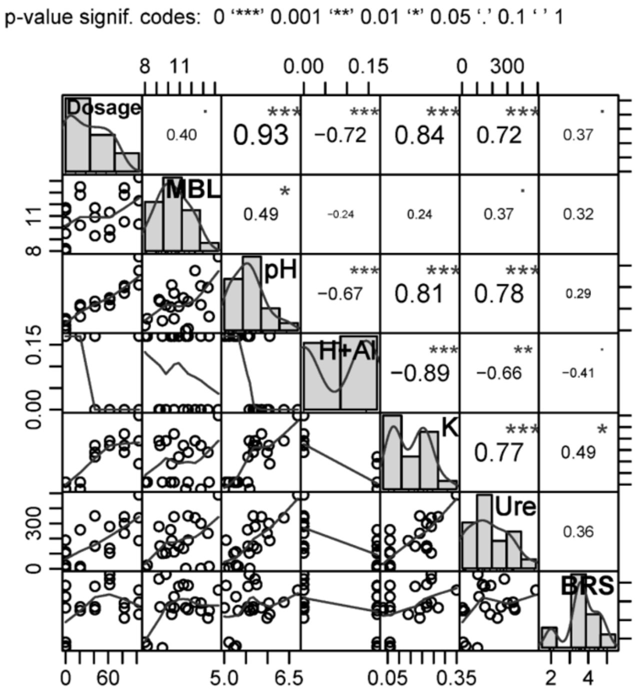

Fig 4. Chart of the correlation matrix (Pearson's R^2) of the main variables studied, showing the bivariate dispersion graphs (below the bar graphs) and the respective adjusted correlations of each model (R_2, above the bar graphs) followed by the indicators of significance, according to the multivariate permutation test. With progressive doses of CW there was a significant increase in pH, K levels and urease activity in the soil, in addition to a significant reduction in total acidity (H^++Al^{3+}). These variables showed a close interaction with each other, supporting the existence of a statistical model that explains the fact (Fig 5). See detailed abbreviations of the variables in Figure 2.

Table 5. Estimates of coefficients and analysis of variance of the proposed linear model for urease activity as a function of pH and soil K^+ concentrations.

	Estimate	Std. Error	t-value	Pr(>|t|)	
α (intercept)	-569.6	241.5	-2.359	0.036	*
$\boldsymbol{\beta_1}$	943.5	237.0	3.981	0.002	**
$\boldsymbol{\beta_2}$	105.2	47.7	2.205	0.048	*
$R^2_{ajs.}$ = 0.91	$Y_{Urease} = \boldsymbol{\alpha} + \boldsymbol{\beta_1}$ (K) + $\boldsymbol{\beta_2}$ (pH)				
Signif. codes: 0.001 '**' 0.01 '*' 0.05					

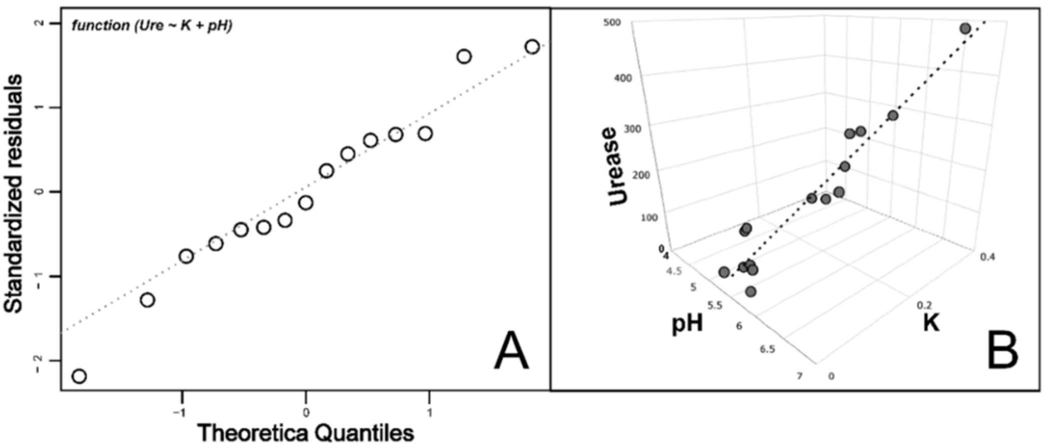

Fig 5. Q-Q plot showing strong evidence of residue normality (A) and three-dimensional graphical representation (B) of the model with estimated regression curve for urease activity (µg NH_4-N g^{-1} dwt $2h^{-1}$) as a function of pH and soil K^+ ion concentrations ($cmol_c$ dm^{-3}).

revealing a significant response of urease activity as a function of pH and K^+ ions in the soil (Table 5). The distribution of standardized residuals showed a linear trend with respect to the theoretical quantiles of the standard normal distribution (Fig 5A). The model suggests that the higher the pH and K^+ ion concentrations, the higher the urease activity in soils with lettuce crop (Fig 5B).

Discussion

Lettuce plants showed significant signs of plant development as a result of CW application, highlighting the increase in fresh mass and the height of aerial parts at higher doses. Cassava wastewater is a macronutrient-rich by-product, especially in terms of N, K_2O, Mg, P_2O_5, Ca and S, in addition to the micronutrients Zn, Cu, Fe, Mn, having also a low C:N ratio (6.5:1) (Ribas et al., 2010). These characteristics partly explain the positive response of the crop to its application in the soil and encourage studies on its use as a biofertilizer.

Dantas et al. (2014) have demonstrated that increasing doses of CW (between 8.5 and 136 m^3 ha^{-1}) resulted in a progressive and significant increase in the fresh mass of sunflower plants cultivated in a Dystrophic Red-yellow Latosol (oxisol), and also in K^+, Ca^{2+}, Mg^{2+}, Na^+, and pH levels, indicating an optimum dose of about 68 m^3 ha^{-1} to improve the chemical attributes of the soil. These results were similar to those of the present study, where the interval between 10% and 50% FC (the minimum and maximum dosages) corresponded to the application of CW between 15.7 and 78.5 m3 ha^{-1}, considering the soil layer of 20 cm (Dantas et al., 2014).

The number of lettuce leaves was the plant parameter that varied most significantly (Fig 1B), reaching a maximum number between 10% and 15% FC (15.7 to 23.5 m^3 ha^{-1}). A similar result was observed by Duarte et al. (2012), who, using different doses of CW in a Dystrophic Regolithic Neosol (Entisol) cultivated with lettuce, observed a maximum number of leaves at the 25 m^3 ha^{-1} application. In this case, it was shown that NL decreased quadratically with increased dosage and after doses from 45 m^3 ha^{-1} also significantly reduced the leaf area, height, and the fresh and dry matter of the aerial parts. In the present study, a similar result was observed for the length of the main branch (Fig 1A), with a maximum of 15.7 m^3 ha^{-1} (10% FC) and a minimum of 47.1 m^3 ha^{-1} (30% FC), growing again after this dosage, which indicates efficient sensitivity of the lettuce to CW applications.

Overall, from multivariate analysis, CW was observed to be a viable alternative to increase pH, exchangeable potassium, TOC, SBR and Ure activity in the soil. This is justified by the fact that these vectors closely follow the dosage vector, pointing to the highest concentration of CW (50% FC) while moving away from the negative control and mineral fertilizer (Fig 2). A similar result was found by Bezerra et al. (2019) in a sandy soil cultivated with *Brachiaria brizantha* in northeastern Brazil, who showed that increasing CW doses in the 0-20 cm layer resulted in increasing quadratic and linear responses to pH and K^+, respectively, reaching up to 120 m^3 ha^{-1}, representing a volume of about 76% FC of soil used in the present study.

pH is the isolated variable that most influences the availability of nutrients in soil, having an optimal range between 6.0 and 6.5, where there is also a reduction of one of the main limitations of agricultural production, exchangeable aluminum (Malavolta et al., 1997). In the present study, the pH started to reach this interval from the CW dosage of 87.3 ml kg^{-1} of soil, about 42.2% FC, reaching pH 6.4 at 50% FC (Fig 1E).

The introduction of bases into the soil, from the CW, also favors an increase in pH due to the adsorption of ions, which displaces the Al^{3+} and H^+ cations in the soil solution. In this case, the aluminum precipitates, mainly in the $Al_2(SO_4)^3$ form, reducing the potential acidity of the soil (Souza et al., 2007).

The effects of acidity on plants are mainly related to the activities of K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, and H_2PO^{4-} anions (Alleoni et al., 2010; Hashimoto et al., 2010). Under these conditions, the application of CW at 30% FC was the treatment that showed the most significant benefits for soil fertility, annulling the effects of potential acidity (Fig 1C) and promoting significant increases in K_2O content, going from 0.05 in the control treatment to 0.24 $cmol_c$ dm^{-3} at the dosage of 50% FC (Fig 2D). According to Malavolta et al. (1997), an exchangeable potassium content above 0.2 $cmol_c$ dm^{-3} is considered high and adequate to meet the demand of most crops, showing the potential of CW as a natural fertilizer with multiple benefits for plants.

In addition to improvements in soil fertility, CW proves to be a potential natural stimulant of the metabolic activity of microorganisms, a fact evidenced by the quadratic increase in the basal breathing rate with a peak of 5.2 mg CO_2 g^{-1} day^{-1} at a dosage of 30% FC (Fig 1F). This same condition was observed for the total organic carbon content (TOC), reaching a maximum value of 8.2 g kg^{-1} at 30% FC (Fig 1G), strengthening this argument.

The relationship between TOC and potential acidity is not coincidental, since the TOC content also controls the activity of aluminum in the soil solution (Ferro-Vázquez et al., 2014; Hagvall et al., 2015). According to Ritchie et al. (1988), the complexation of aluminum with organic molecules dissolved in solution is generally stronger than that of inorganic binders. Guibaud et al. (2000) have demonstrated that even at the pH range favorable to less soluble forms of aluminum (5.5-6.0), organic matter acts to prevent its precipitation.

However, CW applied at high quantities to the soil can show indirect deleterious effects of nutrients, such as potassium. The high biochemical oxygen demand (BOD) and the concentrations of hydrocyanic acid and micronutrients are challenges for the treatment of these effluents, being the main limiting factors of their use as biofertilizer (Ribas et al., 2010). These characteristics emphasize the need for studies on the mitigation of the deleterious effects of CW, allowing its application to soil in greater quantities.

Studying the effect of cassava plant effluents in Nigeria, Ukaegbu-Obi et al. (2018) reported deleterious effects of excess cassava wastewater on soil structure and quality, affecting its microbiota and inducing the predominance of the bacterial genera *Aspergillus* spp., *Penicillium* spp., *Pseudomonas* spp., and *Bacillus* spp. Izah et al. (2017) also identified in abundance these genera in cassava effluent soils, including also the species *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobater* sp., *Micrococus* sp., *Proteus* sp., and the fungi *Saccharomyces cerevisiae*, *Penicillin* sp., *Aspergillus* sp., *Rhizopus* sp., and *Mucor* sp.

These results give rise to the possibility of selecting tolerant microorganisms for bioremediation of soils contaminated by cassava wastewater and the use of CW in large quantities for irrigation of crops after appropriate treatment, such as in anaerobic reactors also intended for biogas production (Watthier et al., 2019). A study by Izah et al. (2017) also

demonstrated a significant reduction in the abundance of microbial communities in soils that received CW, although the diversity did not change. Those authors also recommended the treatment of effluents from factories to mitigate their harmful effects on microflora.
Another relevant fact was the reduction in the activities of acid and alkaline phosphatase due to CW dosage, with minimum values being reached between 30% and 50% FC (Fig 1H and Fig 1I). Ibe et al. (2014), studying the effect of cassava effluents on Nigerian soil, condemned their excessive use in both the dry and wet seasons, observing a negative effect on the activities of acid and alkaline phosphatases in the 15 cm layer. On the other hand, these authors reported a significant increase in urease activity in soils with excessive cassava wastewater, which also corroborated the present study (Fig 1J).
One of the most important points of this study was the high simultaneous correlation between the CW dosages, pH, K_2O content and urease activity in the soil (Fig 5), a result endorsed by regression analysis (Table 2). Fisher et al. (2017), quantifying genes that encode Ure in soils with pH gradients between 3.1 and 7.1, demonstrated that the rate of urea hydrolysis correlated positively and significantly with the pH and number of copies of 16S rRNA and ureC genes, indicating that soils with pH closer to neutral selected more microorganisms capable of producing urease, especially bacteria. Corroborating these data, Ribas et al. (2010), studying the use of urea by corn plants, concluded that CW addition to soil significantly increased the absorption of nitrogen by plants, being another strong indication that the addition of cassava wastewater potentiates the activity of urease in soil.
The exchangeable potassium concentration in the soil was the only significant variable capable of complementing these results (Fig 5). Hess et al. (2006), studying the effect of NH^{4+} toxicity on *Saccharomyces cerevisiae*, demonstrated that genes related to nitrogen metabolism are affected by potassium limitation, highlighting the GAP1 and MEP2 genes, which encode the main carriers of NH^{4+} and amino acids in cells. This result is a strong signal that the high potassium content contained in CW is one of the main factors that potentiates the activity of ureases in the soil.

Materials and methods

Characterization of soil and cassava wastewater

For the execution of this experiment, Entisol (Soil Survey Staff, 2014) was collected from the 0-20 cm layer in a native forest of the semiarid region in Pernambuco state, Brazil. Preliminary soil analysis demonstrated the following characteristics: pH 4.5; P (16.6 mg dm^{-3}); Ca (0.80 $cmol_c$ dm^{-3}); Al (0.15 $cmol_c$ dm^{-3}); Na (0.28 $cmol_c$ dm^{-3}); Mg (0.80 cmolc dm^{-3}); K (0.15 $cmol_c$ dm^{-3}); and H+Al (1.8 $cmol_c$ dm^{-3}); field capacity (206.6 mL dm^{-3}); and density of soil (1.52 kg dm^{-3}).
The chemical composition of CW was characterized and described in Moura et al. (2018) as the following: pH 5.2, organic matter (1.2 %); soil humidity (98.2 %); N (7.0 mg L^{-1}); P (6.0 mg L^{-1}); K (4.3 g L^{-1}); Ca (21.2 mg L^{-1}); Mg (26.2 mg L^{-1}); S (3.0 mg L^{-1}); Cu (0.06 mg L^{-1}); Fe (4.81 mg L^{-1}); Mn (0.88 mg L^{-1}); Zn (0.16 mg L^{-1}); and negligible traces of sodium.

Experimental design

Pots containing 4.0 kg of soil were cultivated with a commercial variety of lettuce plants (*Lactuca sativa* L.), namely Simpson. Three seeds were sown per pot, thinning to two plants five days after emergence.
The experimental design was completely randomized, with seven treatments and ten repetitions. Based on maximum soil water retention capacity, Field Capacity (100% FC = 206.6 ml dm-3), the treatments corresponded to increasing dosage of Cassava Wastewater (CW): T1 - negative control (0% FC), T2 - 20.7 ml (10% FC); T3 - 41.3 mL (20% FC); T4 - 62.0 mL (30% FC); T5 - 82.6 mL (40% FC), T6- 103.3 mL (50% FC), and T7 (additional treatment) - Application of 1.0 g of mineral fertilizer (20% N, 10% P, and 20% K) per pot. Sowing occurred 15 days after treatment and the plants were grown for 28 days after emergence in a greenhouse at room temperature and irrigated daily with distilled water to keep the soil at field capacity.

Plant analysis and soil chemical attributes

The chlorophyll A (CA) and chlorophyll B (CB) content was determined with a clorofiLOG electronic chlorophyll meter, (model CFL1030, FALKER – Brazil). After removal, plants were measured to determine aerial fresh matter (AFM), root fresh matter (RFM), main branch length (MBL), number of leaves (NL), and stalk diameter (SD); aerial dry matter (ADM) and root dry matter (RDM) were measured after drying in a forced ventilation oven at 65°C until their weights remained stable.
The soil pH was determined in water (proportion 1:2.5) and P, Na^+, K^+, Ca^{2+}, Mg^{2+}, and Al^{3+} content, as well soil potential acidity (H^++Al^{3+}), following the methodology proposed by Silva (2009).
The inorganic labile P, Na^+, and K^+ were extracted using Mehlich-1 solution (0.0125 mol H_2SO_4 L^{-1} + 0.05 mol HCl L^{-1}). P was quantified using a spectrophotometer, and Na^+ and K^+ were determined by flame photometry. Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with 1.0 mol KCl L^{-1} solution, where the first two were determined by atomic absorption spectrometry. Potential acidity was determined using calcium acetate (Ca $(CH_3OO)_2$ H_2O) at pH 7.0. In addition, Al^{3+} was determined by titration with 0.025 mol NaOH L^{-1}, using bromothymol blue as indicator. In all extractions, 5.0 g of soil was used for 50 mL of extractive solution (relation 1:10).

Total organic carbon, microbial biomass, basal respiration, and enzymatic activities of soil cultivated with lettuce and treated with cassava wastewater

Soil from 2 mm around the roots (rhizosphere) was used to determine the total organic carbon (TOC) and microbial biomass carbon (MBC) content, in addition to soil basal respiration (SBR), and the urease activity (Ure) (EC 3.5.1.5) and acid (Pac) (EC 3.1.3.2) and alkaline (Palk) (EC 3.1.3.1) phosphatases.
The methodology proposed by Yeomans and Bremner (1988) was used to quantify TOC and MBC by irradiation and extraction adding 80 mL of 0.5 M K_2SO_4 in 20 g of soil. The MBC in extract values were obtained by colorimetry (Bartlett and Ross, 1988). The phosphatase activity was measured according to the methodology of Eivazi and Tabatabai (1977), using p-Nitrophenyl Phosphate as a substrate. Urease activity was measured according to Kandeler and Gerber (1988), using urea as a substrate. The absorbance was measured by spectrophotometer (Libra S22, Biochrom, Cambridge - England), at wavelengths (λ) of 400 nm and 690 nm for phosphatases and urease, respectively.

Statistical analyses

Exploratory analyses, graphs and multivariate statistics were computed using the R software (v 3.4.3). The Constrained Analysis of Principal Coordinates (CAP) and Permutational Multivariate Analysis of Variance (PERMANOVA) were executed through the 'vegan' library using the Euclidean distance matrix data.

The correlations were calculated based on Pearson's algorithm, where the heatmaps were constructed with the 'heatmaply' library, and chart correlation by the 'PerformanceAnalytics' and 'ggplot2' libraries. The ANOVA of the paired data was done through the library 'ExpDes' with Tukey HSD (Honestly Significant Difference) and Duncan post-hoc tests done for possible significance in F-test ($p \leq 0.05$), with the regression graphs traced in these cases, using the resources of the 'ggplot2' library. The three-dimensional models were designed with the help of the 'plotly' library.

Conclusion

Cassava wastewater (CW) favored the development of lettuce plants in relation to the length of the branches, number of leaves and chlorophyll concentrations when it is applied from 10% of the field capacity (FC). The most expressive gains were in primary growth and in the aerial fresh mass, corresponding to a 40% increase compared to the control. This condition also raised the pH and the exchangeable K content to the optimal range and simultaneously neutralized the potential soil acidity. The application of 30% FC was optimal dosage for basal respiration and total organic carbon. Urease activity grew linearly with CW dosage, presenting a significant and directly proportional relationship with the pH and the exchangeable potassium of the soil. This study endorses the use of CW as a low-dose biofertilizer to improve the quality of sandy soil. Given these considerations, 20.7 ml CW dm^{-3} (10% FC) was the dose that improved the greatest number of phenotypic variables of the plants. However, higher doses contributed more to increases in pH (reducing H^{+}+Al^{3+}), K levels, urease activity and basal respiration in the soil, especially 62 ml CW dm^{-3} (30% FC). These soil benefits might be better reflected in the crop at a later phenological stage, when it has greater nutritional demand, a hypothesis that will be tested in the future by reproducing this experiment in the field.

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Declaration of interest

There is no conflict of interest by the authors.

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