Mitigation of Cadmium Uptake in Cocoa: Efficacy of Soil Application Methods of Lime and Biochar

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Abstract

Although mitigation approaches have been developed to reduce Cd in cocoa beans the efficacy of the approaches have been inadequate to make them economically viable. A field study was conducted in a cocoa farm in Biche, Trinidad using two soil amendments, lime and biochar, at recommended rates using three methods of application, soil surface application with incorporation (SA), soil injection (SI) or deep placement using an auger (AA) along with a control. The objective was to determine the application method that would be most efficacious with respect to rapidity of effect, magnitude of reduction and persistency of effect on leaf Cd. The experiment was arranged in randomized complete block design with three replications with 15 trees per replication. Phytoavailable soil Cd, soil pH, CEC and total leaf Cd concentration were monitored monthly on three guarded trees per plot over a one-year period. The results showed that both lime and biochar were effective in reducing leaf cadmium levels albeit at different levels. The efficacy of SI was significantly better than SA in terms of rapidity of the effect on leaf Cd in comparison to the control (40% compared to 30%) as well as the effect was more persistent in SI. With biochar, again the SI was significantly better than SA with regards to reducing leaf Cd levels in comparison to the control (35% compared to 20%) but the time taken to action and the persistency were lower compared to lime application. AA did not significantly reduce Cd level in the leaf with lime or biochar application.

1. Introduction

Bioaccumulation of cadmium (Cd) in the human body from consumption of Cd contaminated food over the human lifetime presents numerous health risks including damage to the kidney, liver, bones and the neurological system (Ashizawa et al. 2012). With the detection of high levels of Cd in chocolate and other cocoa products, food safety regulations stipulating maximum permissible limits for Cd in chocolate and cocoa based products have been established. These include regulations by the European Union (European Food Safety 2012), the State of California Proposition-6, (World Cocoa Foundation 2019) and proposed regulations of the Codex Alimentarius (Joint FAO/WHO Codex Alimentarius Commission 2018).

There is considerable evidence that the Cd concentration in chocolate products is a function of the level of Cd found in cocoa beans (Mounicou et al. 2002). Elevated levels of bean Cd concentrations in cacao have been detected in a number of cocoa-producing countries within the Latin America and Caribbean region (Arévalo-Gardini et al. 2017; Argüello et al. 2019; Barraza et al. 2017; Chavez et al. 2015; Gramlich et al. 2018; Lewis et al. 2019; Ramtahal et al. 2016; Zug et al. 2019). These countries and by extension the chocolate manufacturing industry are currently faced with the challenge of ensuring that their beans and intermediary products from these countries meet the stringent Cd food safety regulations imposed on chocolates. The potential negative economic and social impact of this issue on cocoa producing countries in Latin America and the Caribbean has been well documented (Maddela et al. 2020; Meter et al. 2020).

The levels of Cd found in cocoa beans have been shown to be correlated to the phytoavailable Cd pool in contaminated soils (Arévalo-Gardini et al. 2017; Argüello et al. 2019; Barraza et al. 2017; Chavez et al. 2016; Lewis et al. 2019; Ramtahal et al. 2015, 2019); which in turn has been shown to be influenced by a number of soil physicochemical properties such as low soil pH, organic matter content and certain trace elements as well as an open soil texture (Adriano 2001; He et al. 2015; Shahid et al. 2016; Violante et al. 2010). Studies have shown that understanding the soil factors that contribute to Cd bioavailability is critical in developing appropriate strategies to mitigate Cd levels in cocoa beans. Many studies have used leaf Cd concentrations as a proxy to study bean Cd concentration due to the established strong correlation between the two (Arévalo-Gardini et al. 2017; Argüello et al. 2019; Lewis et al. 2019; Ramtahal et al. 2016). In a study of cocoa genotypes Lewis et al. (2019) reported that on average the bean concentration is around 56% of that of the leaf Cd concentration.

Soil ameliorants such as lime, biochar, inorganic and organic-rich materials and soil microbial consortia have been shown to reduce the soil phytoavailable Cd and consequently the Cd concentrations in cocoa beans (Meter et al. 2020). In a field trial, Ramtahal et al. (2019) demonstrated the effectiveness of both lime and biochar as soil ameliorants in reducing Cd uptake in cocoa but noted that their effectiveness diminished over time. This was attributed to the heavy rainfall and the sloping terrain where cocoa is grown and the inability to effectively incorporate the ameliorants into the soil.

There are many ways in which amendments can be incorporated into soils including broadcasting, soil injection and localized placement. Broadcasting otherwise known as surface application of the material can be done with or without subsequent incorporation by tillage and is considered to be typically the fastest and most economical method of application of amendments, but is prone to amendment losses due to environmental factors (Brady and Weil 2004). Ramtahal et al. (2019) employing surface application with light tilling into the top 20-cm of the top soil layer, while taking care not to damage the lateral feeder roots of the cacao tree, showed this to be an improvement over surface application without tilling (Ramtahal et al. 2018). Another recent pot study, Argüello et al. (2020) highlighted the importance of subsurface liming to improve the effectiveness of lime applications in reducing Cd uptake. Localized or spot placement involves the placement of the material in specific locations (spots or band) or in depressions created by various tools including drills or auger within the dripline of the tree (Jain et al. 2018) and has been used successfully over the years for fertilizer application. Amendment application via soil injection typically as pressure-injected sludge into the soil has been shown to be very efficient in getting the material into the rooting zone of the plant to promote amelioration in crops (Faber et al. 2015). Although soil injection or deep placement via augered holes can be more effective as methods of application, these have not been tested for the mitigation of Cd in cocoa.

The overall objective of this study was to evaluate the effectiveness of three soil application methods, soil injection, deep placement and surface application with tilling, of two amendments, biochar and lime, in reducing the phytoavailable pool of soil Cd, its uptake and bioaccumulation in leaves under field conditions.

2. Materials And Methods

2.1. Location of field study

This investigation was conducted at a cacao estate in Biche, East-Trinidad (10.41'62.96" – 61.12'61.65") during a one-year period from February 2018. The site consisted of 40-year-old trees of a mix of cross compatible TSH varieties grafted onto TSH 911 rootstocks. The landscape was generally flat. The cocoa trees were planted at a spacing of 4 x 4 m with banana and other permanent shade trees.

2.2. Soil characteristics and amendment requirement determination

In order to determine the soil characteristics and required amendment rates for the treatment site, representative soil samples (0–15 cm) were collected, air-dried, ground using a mortar and pestle, sieved to < 2mm and mixed thoroughly. Each dried sample was placed in a clear polyethylene bag, mixed well, labeled and stored until analysis. Sub-samples were taken and subjected to the following analyses:

I. The particle size distribution was determined using the hydrometer method described by Gee and Or (2002).

II. Soil pH was measured in triplicate using three 10 g portions of air-dried soil mixed separately with 10 mL of deionized water, and allowed to equilibrate for 30 min. The pH of the supernatants was then measured with a calibrated pH electrode and meter (Kalra 1995) and averaged over replicates.

III. Organic matter content was measured using the method as described by Walkley and Black (1934).

IV. CEC was also determined according to Sumner and Miller (1996).

V. “Total” Cd, Fe, Mn and Zn soil metal concentrations were determined using the USEPA 3051A method (USEPA 2007). Triplicate samples each containing 0.5 g were digested using 10 mL of concentrated analytical grade 60% HNO_3 (J.T. Baker, USA) in a microwave digester (Multiwave PRO, Anton Paar, Austria). After acid digestion, samples were cooled to room temperature, diluted with 5 mL deionized water in a boiling tube and filtered through Whatman #542 filters into 25 mL volumetric flasks. Each solution was made up to volume with deionized water rinses of the residues and mixed thoroughly.

VI. Phytoavailable soil Cd was determined using the dietheylene triamine penta acetic acid (DTPA) extraction method of Lindsay and Norvell (1978). The method involves extracting 10 g of soil with 20 mL of reagent containing 0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M triethanolamine (TEA) buffered to pH 7.3, with vigorous shaking (240 rpm) for 2 h on an orbital shaker (Precision Scientific Model 360P, USA). Extractions of the soil were performed in triplicate in 50 mL polyethylene containers, centrifuged (centrifuge model Heraeus Megafuge 16R, Thermo Scientific, USA), filtered and analyzed for phytoavailable Cd.

VII. The soil liming requirement was determined using the method of Eckert and Sims (1995). The soil was mixed with a modified triethanolamine-ammonium chloride Mehlich buffer (1:1) of pH 6.60 and the resultant pH measured (BpH). The following equation was then used to calculate the lime requirement (LR) for the soil:

[LR = 0.1(AC) + AC in MT/ha $CaCO_3$, where AC = (6.6-BpH)/0.25].

Since hydrated lime or $Ca(OH)_2$ was used instead of $CaCO_3$, the equivalent LR mass of $Ca(OH)_2$ [Limbux, UK, 95–97% purity] was calculated to be 3000 kg ha^{-1}. However, the application rate used for this study was doubled the calculated amount as recommended by Ramtahal et al. (2019) for optimal amelioration of soils.

VIII. The biochar application rate for the soil was as per instructions of the product, Charcoal Green® Biochar (Charcoal House LLC, NE, USA): 910 g biochar powder in one gallon of water for every 14 m^2 of area (650 kg ha^{-1}). As per recommendations by Ramtahal et al. (2019), the application rate of biochar was also doubled.

2.3. Field Experimentation and treatments

The field trial was carried out at the cocoa estate in Biche, with hydrated lime [$Ca(OH)_2$] applied at a rate of 6000 kg ha^{-1} and biochar (Charcoal Green® Biochar Powder) applied at rate of 326 kg ha^{-1}. Each amendment was applied with three treatments (injector, auger and surface) at the appropriate rates, along with appropriate controls (no application of amendment). The treatments was laid out in a randomised complete block design with three blocks with each plot within a block consisting of three guarded trees.

2.4 Application of treatments

For the soil injection method, the amendment requirement for each plot was calculated, mixed with water using the recommended mixing ratio and injected into the soil using a motorized backpack (Maruyama Model MS75, Fort Worth, TX, U.S.A) equipped with a soil injector (HTI-2000 Soil Injector, Minnetonka, MN, U.S.A). The soil was injected at 15 cm depth using the pressurized injection system at multiple points within the drip-zone of each cocoa tree until all the solution was emptied. With respect to the auger technique, a soil auger (AMC, SST reinforced regular, 6” long, 2 ¾” diameter, Idaho, U.S.A), was used to create ten holes around the drip-zone of each of the tree which was then filled with a slurry of each of the amendment, at the appropriate rate per tree, and covered with soil. The surface application involved the broadcasting of each amendment onto the soil surface within the drip-zone of each tree which was subsequently incorporated using a fork, by gently tilling the 0–15 cm of the upper soil horizon taking care not to damage the surface roots of the cocoa tree. For the control, no amendment was added but the method of applications was applied with water.

2.5 Data collection

The soil and trees within each plots were sampled at monthly intervals for a year. Soil and leaf samples were taken before the treatments were applied and every month thereafter. Three soil cores, 0–15 cm deep were sampled at three locations within the drip zone of each tree using a soil corer (Model A1, 12" long, 3/4" diameter, Oakfield, U.S.A), composited and subsampled for analysis. Thirty leaf samples at the Interflush 2 stage (Greathouse et al. 1971) were obtained from throughout the tree, dried, ground and subsampled. The soil samples were analysed for phytoavailable Cd, pH, CEC and total Cd respectively while the leaf samples were analysed for total cadmium as described below.

2.5.1. Soil and leaf analysis for field study

The preparation and analysis of soil samples for the determination of phytoavailable Cd, pH and CEC in the field study were as described in Sect. 2.3. The leaf samples were carefully washed with deionized water to remove visible surface contaminants such as algae and soil. They were then spread out on paper towels to remove excess water, oven-dried in aluminum foil at 75°C until constant weight and ground to < 0.2 mm using a mortar and pestle. The dried samples were ground and placed in clear plastic bags, labeled and stored until analysis. The 'total' concentrations of Cd in the leaf triplicate samples each containing 0.5 g were digested using 10 mL of concentrated analytical grade 60% HNO_3 (J.T. Baker, USA) in a microwave digester (Multiwave PRO, Anton Paar, Austria). After acid digestion, samples were cooled to room temperature, diluted with 5 mL deionized water in a boiling tube and filtered through Whatman #542 filters into 25 mL volumetric flasks. Each solution was made up to a volume with deionized water rinses of the residues and mixed thoroughly (USEPA 3051A). Total cadmium for the leaf and total and available Cd for the soil were determined by means of Flame Atomic Absorption Spectrometry (FAAS), Varian SpectrAA 880, Australia fitted with a high-sensitivity hollow cathode lamp (UltrAA) to enhance Cd detection in sample solutions (EPA 7000B).

2.5.2 Meteorological data

The rainfall and temperature data for the study period was obtained from the nearest meteorological station.

2.6. Quality control

The water used for preparing samples and cleaning of glassware and other apparatus in this study was glass-distilled and then deionized using a water purification system. In order to avoid trace metal contamination, laboratory glassware and other utensils used in all analyses, were washed with a suitable detergent, soaked in an acid bath of 2 M nitric acid for at least 24 h, rinsed in distilled deionized water and dried in an oven at 50ºC. All reagents used in this study were of analytical grade.

Since no certified soil reference materials were available for the determination of phytoavailable Cd using the selected extractant, an Internal Quality Control Material (IQCM) was developed to act as a surrogate standard in this study. The IQCM was prepared from a bulk soil sample collected from a cacao plantation previously identified as having Cd-contaminated soil. The bulk sample was air-dried for 48 h, then ground and sieved through a 2 mm sieve. It was subsequently analyzed with every batch of soil samples analyzed for phytoavailable Cd, to ensure consistency of Cd levels extracted from the IQCM throughout the laboratory trial. Similarly, to monitor and control the quality of the method of determination of 'total' Cd in soils and leaves, a National Institute for Standards and Technology (NIST) Certified Reference Material (CRM), SRM 2709a, San Joaquin Soil and SRM 1570a, Spinach Leaves were used, respectively, and analyzed with each batch of samples for the experimental period. Recoveries of all reference materials analysed during the investigation fell within the acceptable 95–105 percentile range.

2.7. Statistical Analysis

Data were analyzed using ANOVA through the General Linear Model (GLM) routine. Tukey's test was used to detect differences between means at probability level $P < 0.05$. Analysis was done using the software Number Cruncher Statistical System (NCSS 2007, LLC, USA). Normality of data and variance homoscedasticity were tested prior to carrying out ANOVA. For the field experiment, the initial level of Cd in the soil were used as co-variates in ANOVA to eliminate within field differences affecting the results.

3. Results

3.1 Agro-ecology of the experimental site

The experiment was conducted in cocoa farm situated in the lowlands in Biche, Trinidad and Tobago, with a topography described as flat to slightly undulating. Its topsoil was predominantly clay (65%) as demonstrated by the soil textural analysis, with relatively low organic matter content (Table 1). The soil was acidic in nature with a pH of 4.76 and had a correspondingly low CEC of 5.84 cmol kg^{-1}, typical of acidic soils. The total soil Cd concentration of the study area averaged 1.47 mg kg^{-1} which is above the critical limit (0.43 mg kg^{-1}) for Cd in agricultural soils (USEPA 2002). The concentrations of the other metals Zn, Mn and Fe were within the optimum range for agricultural soils.

Table 1
Selected soil physicochemical properties of the experimental area

Parameter	Mean values
Clay %	65
Silt %	18
Sand %	14
pH	4.76
OM %	2.8
CEC (cmol kg^{-1})	5.84
Total Cd (mg kg^{-1})	1.47
Total Zn (mg kg^{-1})	104.75
Total Mn (mg kg^{-1})	728.85
Total Fe (mg kg^{-1})	52891

The rainfall pattern (Fig. 1) was typical of the humid tropics with a dry period characterised by relatively low rainfall from December to May followed by a period of heavy rains during June to November (wet season). Consequently, the first four months and the last three months of the study period experienced on average 60 ± 13 mm and 19 ± 10 mm rainfall respectively compared to a monthly average of 232 ± 35 mm during June-November, 2018. The monthly average temperature fluctuated between 25.5 to 27.5^{o}C during the study period and dipped below 26.5 ^{o}C between the months of November to March.

3.2 Effect of application methods of lime and biochar on soil pH

Lime application either by soil injection (SI) or surface application (SA) significantly increased ($P < 0.05$) soil pH from an average of 4.7 to pH > 7.0 within a month of application, while the auger method of application (AA) did not significantly ($P > 0.05$) increase soil pH compared to the control (Fig. 2a). Further, lime application by SI maintained the pH above 7 for a period of 7 months after which it showed a modest decline but was above pH 6.5 through the study period. In contrast, surface application showed a steady decline in pH, three months after application (MAA), but the pH was maintained at around pH 6 throughout the study period. Overall, soil injection of lime was significantly better in maintaining a higher soil pH than SA, particularly after 3 months of application. In the case of biochar, neither the application method, auger, injector nor surface, significantly ($P < 0.05$) altered pH levels compared to the control for the duration of the study (Fig. 2b).

3.3 Effect of application methods of lime and biochar on soil phytoavailable Cd

The DTPA-extractable soil Cd (DEC) calculated as proportional change in relation to the control (PCDEC) where the value of 1 is the reference and values above or below represents an increase or decrease in phytoavailable Cd respectively. As expected the PCDEC showed a significant ($P < 0.05$) decline with lime application for SI and SA; while that for AA was not significantly different ($P > 0.05$) from 1 throughout the study period (Fig. 3a). There was also a significant ($P < 0.05$) PCDEC x month interaction. The effect of SA of lime on PCDEC was not significantly different ($P > 0.05$) from the control until 3 MAA following which there was a significant decline (average 23%; $P < 0.05$) which persisted up to 6 MAA. Following 6 months PCDEC for SA increased and was not significantly different ($P > 0.05$) from 1. In contrast, SI of lime resulted in a significant reduction ($P < 0.05$) in PCDEC compared to the control from 2 MAA (av. 11% reduction below 1) with a generally consistent decline until 5 MAA at which time it was 51% below 1. After 5 MAA, there was a gradual increase in PCDEC for SI, however, it remained significantly lower ($P < 0.05$) than the control until the end of the study period.

The PCDEC for SI for biochar treatment showed a steady and significant decline ($P < 0.05$) (av. 11%) below 1 from 3 MAA until 6 MAA then fluctuated. A decline was observed with SA only after 4 MAA and fluctuated following month 5 and remaining mostly not significant ($P < 0.05$) from 1. With respect to the AA, the PCDEC fluctuated and remained at or above 1 and was not significantly different ($P > 0.05$) throughout the duration of the study.

3.4 Effect of application methods of lime and biochar on total leaf Cd

For both lime and biochar amendments (Fig. 4a and 4b), leaf Cd levels for the control remained unchanged (5 mg kg^{-1}) until 3 MAA (not significant $P > 0.05$), following which there was a gradual but significant ($P < 0.05$) increase in Cd levels up to 7 MAA (av. 14%), which then remained quite consistent for the remaining 5 months. Both the effects of 'month', 'application method', 'amendment' and their interactions had a significant ($P < 0.05$) influence on total leaf Cd.

In the case of lime treatment by auger (AA), the leaf concentration of Cd was not significantly different ($P < 0.05$) from the control for the duration of the experiment, except at month 7, when it was significantly ($P < 0.05$) lower than the control (Fig. 4a). In contrast, for SI and SA of lime, there was a declining trend for leaf Cd concentration up to 6 MAA, following which it showed an increase until the end of the study. At 3 MAA, leaf concentration of Cd of SI was significantly lower than that for the control and persisted at that level (average 40% below control) up to 6 MAA. Although there was a significant ($P < 0.05$) increase in leaf concentration of Cd following 6 MAA, the SI treatment remained significantly ($P < 0.05$) below that of the control until month 10. This rate of decrease in leaf Cd concentration for SA of lime was more gradual and was significantly lower than that of the control at 3 MAA (11% lower than control), dropping to an av. of 30% at 6 MAA and remained significantly lower up to 10MAA. Overall, while lime application by SI and SA were both effective in reducing leaf Cd concentration, the SI was effective as indicated by a (a) faster action of lime in reducing leaf Cd levels (b) achieving a higher magnitude of reduction and (c) the effectiveness lasting for a longer period than SA. Lime application by AA was not shown to be effective in this study.

With respect to biochar application, leaf Cd levels for AA were not significantly different ($P < 0.05$) over time, compared to the control (Fig. 4b). For SA, leaf Cd concentrations stayed relatively constant until 5 MAA following which there was on average a significant reduction in Cd concentration (av. 20%; $P < 0.05$). This reduction was short-lived with leaf Cd levels returning to previous levels 6 MAA for the rest of the study period. SI resulted in a more rapid and significant ($P < 0.05$) decline reaching 35% reduction compared to the initial level, 5 MAA. A subsequent loss in effectiveness was observed as leaf levels gradually increased over the remaining months, but remained significantly ($P < 0.05$) lower than the control. There was no significant relationship between biochar application and pH for all methods (Fig. 6a).

As expected, there was a significant correlation (Fig. 5a) between DTPA-extractable Cd (DEC) and soil pH for the lime treatment (SI treatment: $r = -0.879$; $P < 0.05$; SA treatment: $r = -0.578$; $P < 0.05$). The correlation for AA was weak ($r = 0.188$) and not significant ($P > 0.05$). Consequently, as pH of the soil increased in SI and SA treatments, DEC decreased. There was also a significant correlation between DEC and leaf Cd (SI treatment: $r = 0.67$; $P < 0.05$; SA treatment: $r = 0.48$; $P < 0.05$) (Fig. 5b and 5c) for SI and SA treatments indicated by a strong correlation between DEC and Leaf Cd and between pH and leaf Cd. In contrast, biochar application (SI and SA) as expected did not have a significant correlation ($P > 0.05$) with soil pH but had significant effect on reducing DEC and consequently significantly ($P < 0.05$) reducing leaf Cd as indicated by the positive correlation (SI: $r = 0.708$, $P < 0.05$; SA: $r = 0.645$, $P < 0.05$) (Fig. 6b). As expected for biochar by AA, there was no significant correlation ($P > 0.05$) between pH and DEC nor DEC and leaf Cd.

Although no significant relationship ($P > 0.05$) were found between CEC and other measured soil properties, there was a general increasing trend up to 4 MAA followed by a gradual decline when the soil was limed by the injector and surface methods (Fig. 7).

4. Discussion

The application of lime and biochar to Cd contaminated soils have been shown to be effective in mitigating Cd uptake into the cacao tree and into the beans (Ramtahal et al. 2018; 2019). However, a number of studies have recommended that the method of application needs to be improved in order to improve the effectiveness of the soil ameliorants under field conditions (Argüello et al. 2020; Ramtahal et al. 2018, 2019). The effectiveness of the various methods of application were explored in this study based on (a) the time taken for the amendment to take effect (b) the magnitude of reduction in leaf Cd and (c) the duration of effectiveness compared to the control. The monitoring of pH, the soil Cd phytoavailability, CEC and leaf Cd concentrations allowed for the understanding of the mechanisms through which Cd mitigation was achieved. In addition to the typical challenges experienced with environmental conditions that influences the efficacy of Cd mitigation in cacao-growing soils as elucidated in past studies (Ramtahal et al. 2018, 2019), this study highlighted the influence of application methods to efficiently incorporate the amendment as another consideration affecting the effectiveness.

A number of studies have shown that the local agro-ecology is an important consideration in selecting the appropriate amendment, its rate and timing of application to effectively mitigate Cd (Argüello et al. 2020; Ramtahal et al. 2018, 2019). The soil of the experimental site was strongly acidic ($\mathrm{pH} < 5.5$) with a low CEC and OM content which are key factors known to increase the mobility and phytoavailability of soil Cd for uptake and accumulation by cacao trees (Argüello et al., 2018; Barraza et al. 2017; Chavez et al. 2015; Gramlich et al. 2018, 19; Ramtahal et al. 2016, 19; Zug et al. 2019). Under such conditions, application of (a) alkaline materials to increase soil pH thus promoting metal hydrolysis reactions and/or coprecipitation (Mench et al. 1998) or (b) organic-rich materials to increase the soil capacity for Cd ion complexation through improving the CEC, to mitigate Cd levels in the plant (Beesley and Marmiroli 2011; Laird et al. 2010) have been shown to be effective. Previous studies in cocoa have also shown the effectiveness of alkaline and organic-rich materials such as lime (Argüello et al., 2020; Ramtahal et al., 2019) and biochar (Ramtahal et al. 2019), respectively, to be effective in ameliorating Cd-contaminated cacao-growing soils with similar chemical properties. Thus for this investigation, both of these amendments were selected for use to mitigate Cd at recommended rates (Ramtahal et al. 2019) using three application methods, SA, SI and AA.

Even though the field temperature remained relatively constant (25.5 to 27.5^{o}C) for the duration of the experiment, the rainfall varied with an initial period of low precipitation (February-May) followed by an increase until the month of December and a subsequent decline towards the end of the year-long trial. Such marked seasonal differences in rainfall is typical of tropical climates. Ramtahal et al. (2019) showed that the amendments became effective only in the beginning of the rains and that heavy rains tended leach or wash away the amendments. Regardless of the variability in rainfall over the study period, the soil pH remained fairly constant in the control treatment. However, the leaf Cd concentrations in the control showed a gradual but significant increase as the wet season proceeded, mirroring similar increases in the DTPA-extractable Cd. This indicates that the improved soil moisture conditions during the rainy season resulted in enhanced soil Cd phytoavailability which in turn resulted in higher leaf Cd levels in the control. These findings are in conformity with other studies that also show that soil water content influences the mobility and consequently the phytoavailability of Cd ions (Ramtahal et al. 2019; Stafford et al. 2018).

One of the dominant factors which influences Cd phytoavailability in soils is pH (Adriano 2001; Grant and Sheppard 2008; Tsaldilas et al. 2005). When pH increases, there is an increase in CEC which results in the strong chelation of Cd to particles of clay and organic matter making it less phytoavailable (Shahid et al. 2016). The application of lime using the SI and SA treatments were able to effect a significant increase in soil pH, which resulted in a reduction in DEC and significantly lowered leaf Cd concentrations. This was also evident from the strong negative correlation between soil pH and soil DEC and the strong positive correlation between soil DEC and leaf Cd concentration. The effectiveness of lime in reducing leaf Cd concentrations through the reduction in the availability of Cd is well documented in the literature both in cocoa (Ramtahal et al. 2018, 2019; Argüello et al. 2020) and in other crops (Chen et al. 2018; Park et al. 2011; Tlustoš et al. 2006; Woldetsadik et al. 2016. The SI was more effective than SA as evidenced by the rapid increase in soil pH to a level not significantly different from 7, within a month of application and the ability to maintain the pH at 7, up to 8 MAA. Consequently, both DEC and leaf Cd levels started declining 1 MAA and reached the lowest levels that was 50% of the control for DEC and 60% of the control for leaf Cd levels, at 5 MAA and 3 MAA, respectively. Although DEC started increasing following 5 MAA (but remained below the level of the control throughout the study) leaf Cd levels remained at 60% of the control for 5 straight months until 8 MAA before it started increasing.

On the other hand, although SA was able to raise the pH to a level not significantly different from 7 within 1 MAA, the levels started declining 3 MAA but remained at a level above 6 throughout the study period. Consequently, both DEC and leaf Cd concentrations started declining 2 MAA and attained their lowest levels 6 MAA which were 70 % and 75% of the control, respectively, compared to 50% and 60% for SI. Following 6 MAA both DEC and Leaf Cd levels started rising for SA and the leaf Cd levels were not significantly different from the control by 8 MAA. Hence the magnitude of reduction and duration of effectiveness of SA was lower than that of SI. This could be attributed to greater losses due to surface run-off in SA compared to SI. Lime and its ability to effectively increase the pH of the soil is diminished with increasing rainfall, acidity of the rain, soil and site management factors (Goulding and Blake 1998).

In contrast, the AA method was not able to effect a change in soil pH in comparison to the control and consequently was unable to significantly reduce DEC levels in the soil, which resulted in leaf Cd levels not significantly different from that of the control throughout the study period. That lime using the auger method was unable to effectively elevate soil pH indicates that lime placed in auger created soil depressions along the drip line of the tree was unable to migrate laterally to effect soil pH in the clayey soils of that location. Clayey soils are less permeable and are mostly characterized with low infiltration (Aswathanarayana 2001). It is also plausible that the water accumulating in the depressions facilitated the leaching of the lime below the root zone making it ineffective. Other studies have shown that most of the functional roots of cocoa remain within the top 20 cm within the enriched top soil layer (Hartemink 2005; Toxopeus 2008).

Soil injection helps to prevent treatment loss by placing it below the zone subject to erosion (WPHA 2018). Many studies have shown that in order for lime to be effective as a soil amelioration technique, it should be incorporated as much as possible into the soil profile (Kaminski et al. 2007; Mullins et al. 2011; Natale et al., 2020; Wolkowski and Laboski 2011). Overall, SI performed better where a slurry of lime was injected into the subsurface of the soil at high pressure forcing it throughout the soil, resulting in a greater incorporation into the soil profile with reduced chances for loss by run off. The study shows that the greater effectiveness of SI was a function of its ability incorporate the amendment most effectively into the soil compared to SA or AA.

Although it has been suggested that some biochars can induce a liming effect on acidic soils (Ahmad et al. 2014 Hussain et al. 2017), the biochar used in this study regardless of the application technique had no significant effect on soil pH. This was also evident from the non-significant correlation between soil pH and biochar treatment. The type of material used to manufacture biochar and the process by which it is made determine its ability to neutralize soils (Van Zwieten et al. 2010). However, Biochar, in this study, was able to significantly decrease the phytoavailable soil Cd using both SI and SA treatments but not in the AA treatment.

Biochar has the ability to immobilize metal ions through the formation of complexes owing to its physico-chemical properties of an enhanced surface area and increased CEC (Anawar et al. 2015; Lahori et al. 2017; O'Connor et al. 2018; Paloma and De la Rosa 2020). SI again was more effective than SA, possibly due to the same reasons that were identified for the lime treatments. The effect of biochar on lowering DEC for the SI treatment was around 15–20% compared to the control and was achieved at 3 MAA with the effectiveness of the amendment remaining up to 6 MAA, after which the effect diminished. Consequently, the leaf Cd concentration showed a 22% reduction 4 MAA and increased to 35% reduction by 5 MAA but started to increase again. An average reduction of 30% was maintained up to 10 MAA. The variation in CEC mirrored that of leaf Cd indicating the mechanism by which Cd mitigation is achieved with biochar is through its effect on increasing CEC (Jiang et al. 2012; Komkiene and Baltrenaite 2016; Sun et al. 2020). The effect of biochar on lowering DEC for the SA treatment was more gradual than SI resulting in a much smaller reduction (av. 20%) that occurred 1 month after SI and lasted only for 2 months (6 MAA to 7 MAA), This was also mirrored in the changes experienced with CEC. These results suggest that SI achieved a better incorporation of biochar in the soil than SA, resulting in greater treatment effectiveness. Past studies have shown that biochar needs to be thoroughly incorporated in the soil in order for it to be effective as a technique of soil remediation (Ruysschaert et al. 2016; Guo et al. 2020). The application of biochar using the AA had no significant effect on DEC and leaf Cd levels as was demonstrated for lime application.

Overall the application of the amendments using the SI technique was much more efficient than SA in reducing Cd accumulation in cacao leaves. Neither lime nor biochar application by AA was shown to be effective. Further, the study demonstrated the effectiveness of lime over biochar as a soil amendment in reducing Cd uptake in cacao. Overall, lime application by SI had (a) a faster action in reducing leaf Cd levels within 1 MAA compared to biochar that took up to 3 MAA; (b) achieved a higher magnitude of reduction than biochar (40% compared to 35%) and (c) resulted in the effectiveness lasting longer than biochar (4 months compared to 1 month), before leaf Cd levels started to increase. The study also indicated that lime application even with SI required repeat applications, yearly, as was reported by Ramtahal et al. (2019) to achieve effective mitigation of Cd levels. Although the study investigated the effect of amendments and application methods on leaf Cd, the findings could also be extended to cacao bean Cd, as many studies have reported a positive and significant leaf-bean Cd correlation (Argüello et al. 2019; Barraza et al. 2017; Lewis et al. 2018; Ramtahal et al. 2016). Additionally, it is recommended that studies be carried out to evaluate the cost-effectiveness and practicality of these soil application methods under a variety of conditions as terrain, soil and crop characteristics, availability of equipment and associated labor costs can affect the cost-effectiveness of these treatments

Declarations

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Figures

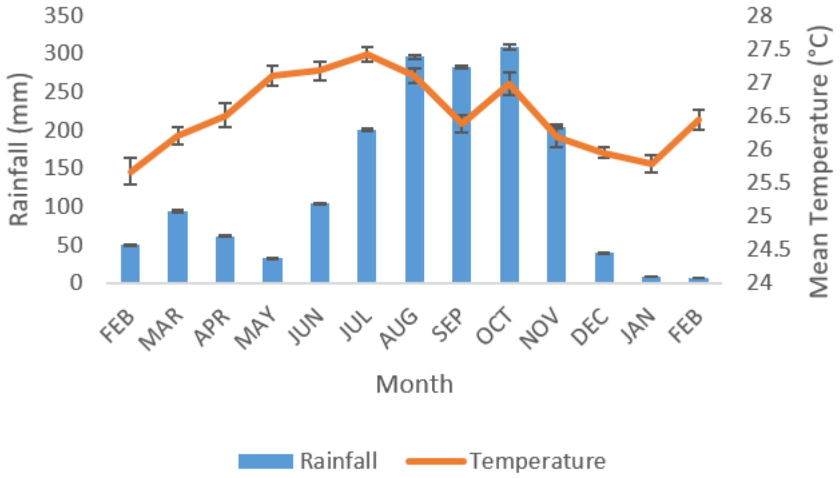

Figure 1

Rainfall and temperature variation over the experimental site over the study period (Feb 2018 to Feb 2019)

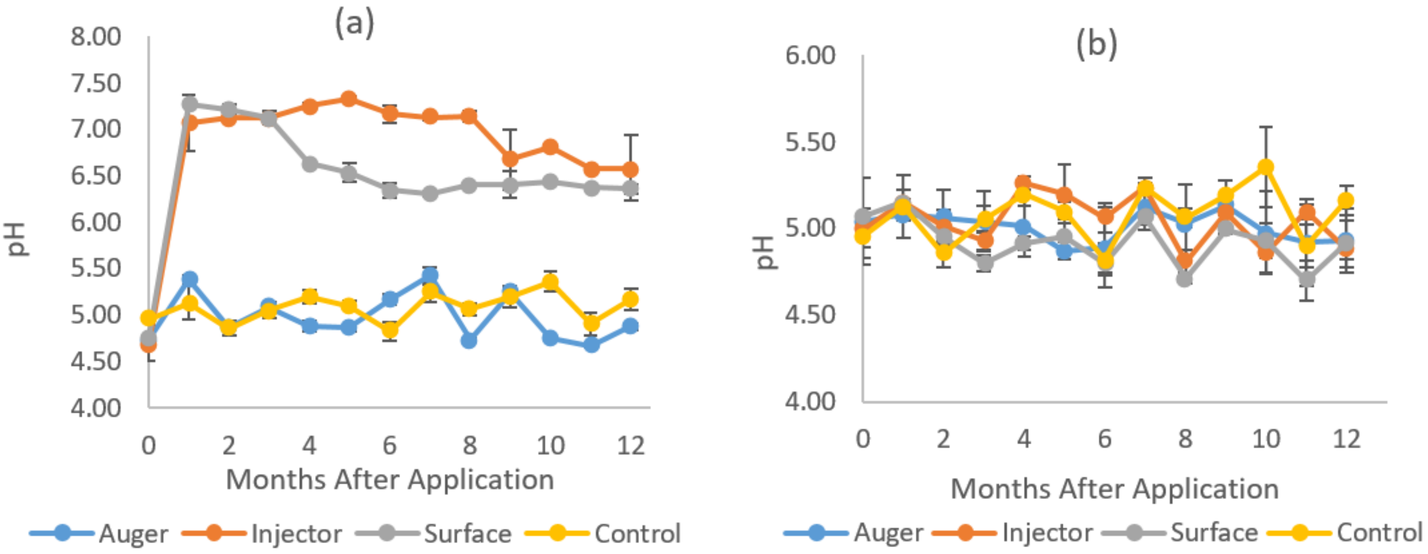

Figure 2

The effect of application methods (auger, injector and surface) of lime (a) and biochar (b) on soil pH in a field study of Theobroma cacao L

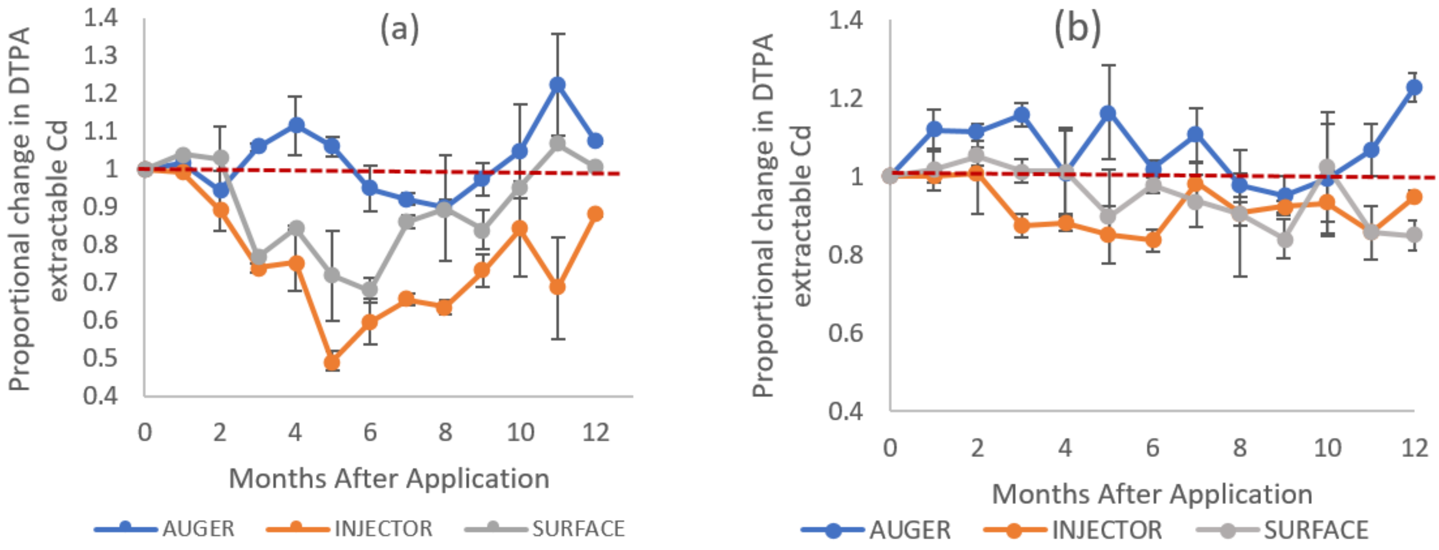

Figure 3

The effect of application methods (auger, injector and surface) of lime (a) and biochar (b) on DTPA-extractable Cd, expressed as a proportion of the control, in a field study of Theobroma cacao L

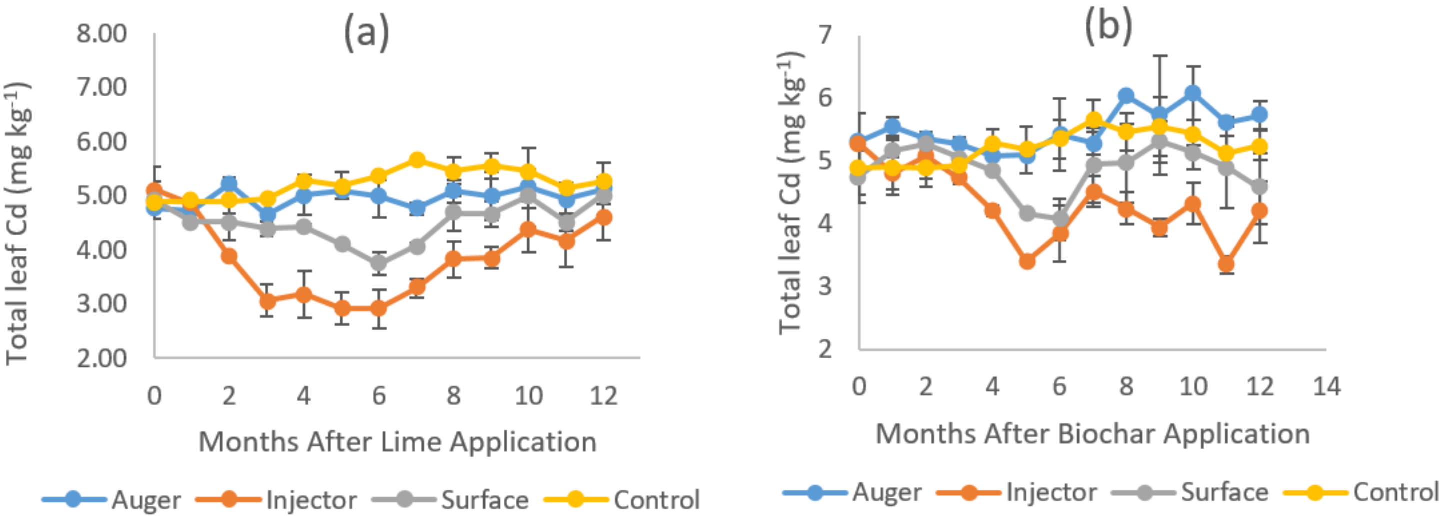

Figure 4

The effect of application methods (auger, injector and surface) of lime (a) and biochar (b) on total leaf Cd, in a field study of Theobroma cacao L

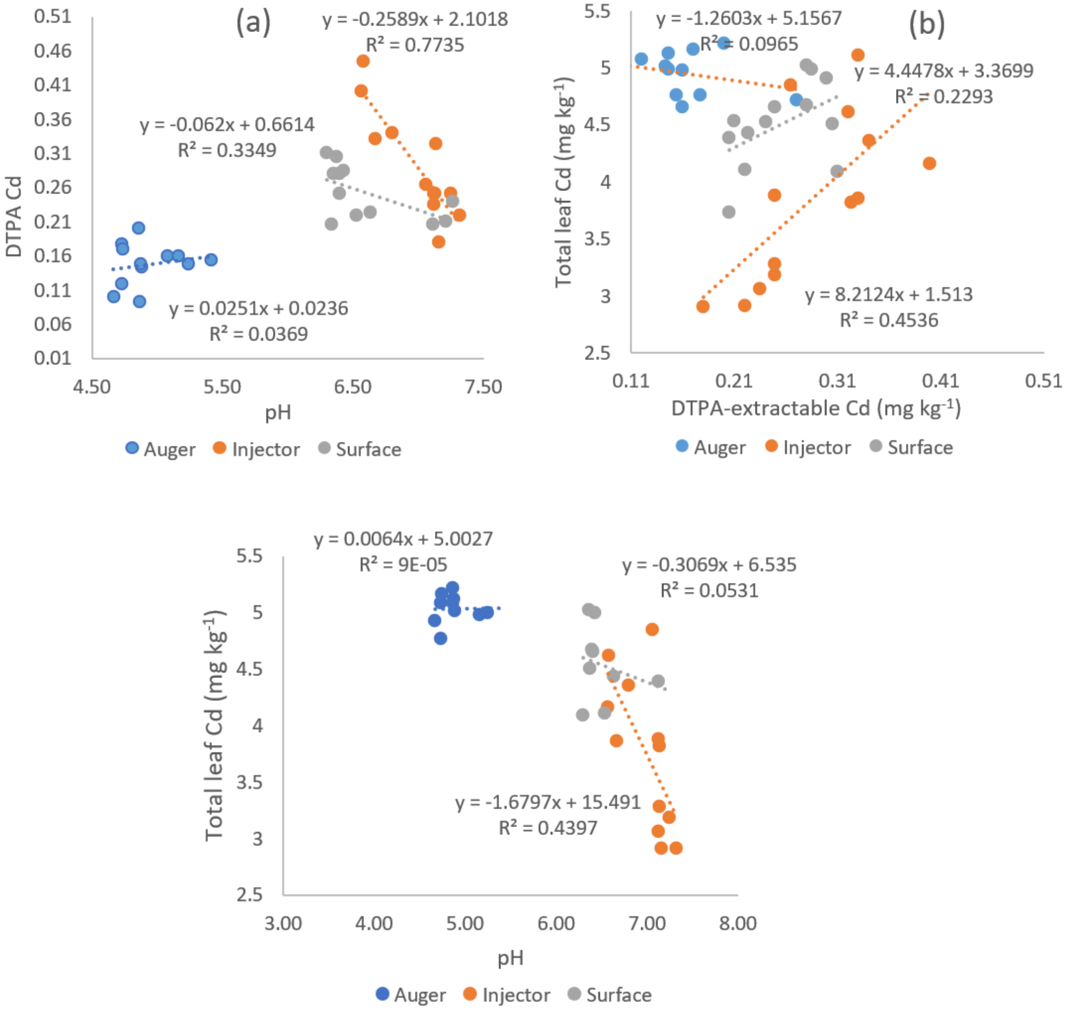

Figure 5

The relationships between DTPA-extractable Cd and pH (a), total leaf Cd and DTPA-extractable Cd (b) and total leaf Cd and pH (c) for different application methods of lime in a field study of Theobroma cacao L

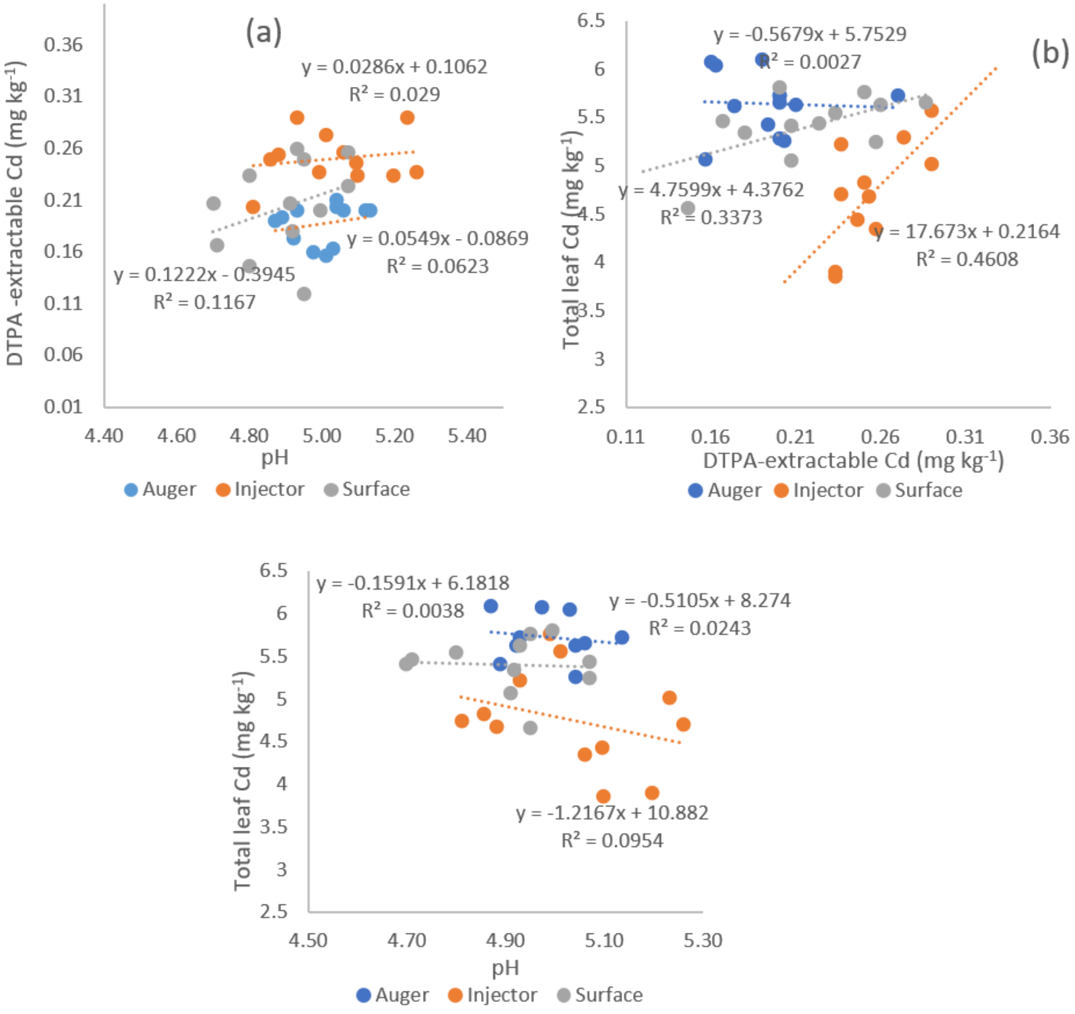

Figure 6

The relationships between DTPA-extractable Cd and pH (a), total leaf Cd and DTPA-extractable Cd (b) and total leaf Cd and pH (c) for different application methods of biochar in a field study of Theobroma cacao L

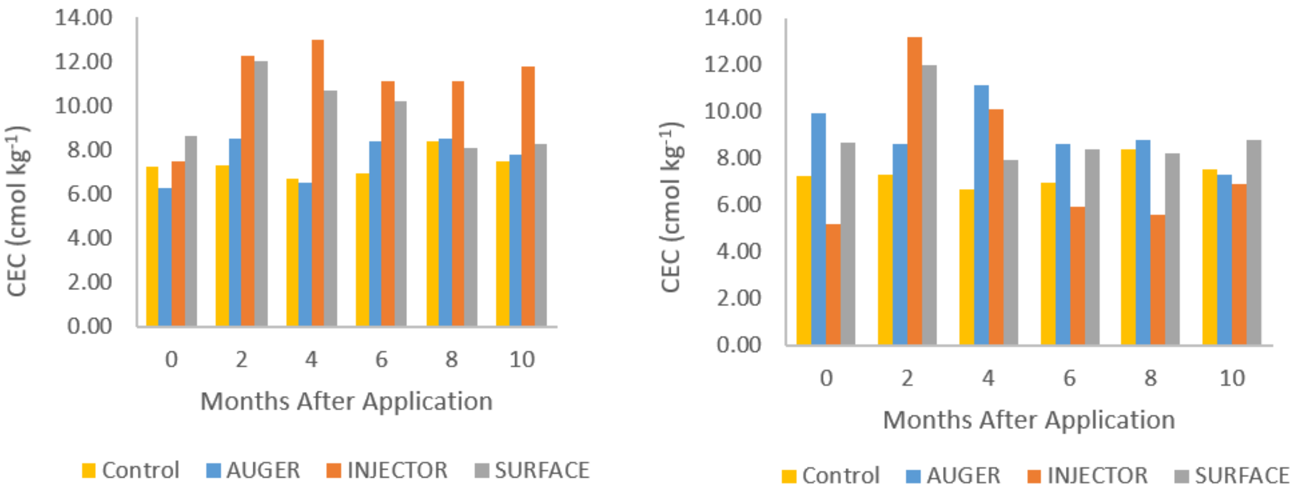

Figure 7

The effect of application methods (auger, injector and surface) of lime (a) and biochar (b) on soil cation exchange capacity (CEC), in a field study of Theobroma cacao L